Morphological evaluation of *Trichodina heterodentata* Duncan, 1977 (Ciliophora: Peritricha) from tadpoles and fish

by

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Index

List of **F**igures

List of **F**igures

List of **F**igures

List of **F**igures

List of **T**ables

Chapter **1**:
Introduction

'…men love to wonder, and that is the seed of science…"

Ralph Emerson

A range of trichodinid species are hosted by anurans, either temporary or on a more permanent basis. For example, endotrichodinids such as *Trichodina urinicola* Fulton, 1923, *T. dampanula* Van der Bank, Basson and Van As, 1989**,** *T. entzii* Bretschneider, 1935, *T. ranae* Da Cuncha, 1950, *T. vesicola* Suzuki, 1950 as well as *T. xenopodos* Fantham, 1924 infect the bladder of adult frogs during amplexus (Kruger 1992). These trichodinids usually feed on the debris within the bladder of their hosts (Kruger 1992). Endotrichodinids do not need to leave the bladder of the frogs and translocate to other individual hosts although some may leave the host during amplexus to infect another frog. Therefore, endotrichodinds may utilise adult anurans permanently.

As endotrichodinids infect anurans during amplexus, it is not anticipated that tadpoles will ever host any endotrichodinid species. However, ectotrichodinids are hosted on the skin and / or gills of a number of tadpole species in various countries (Diller 1928; Lom 1961 and Kruger *et al.* 1993). Lom (1961) suggested that these trichodinids are typically not host specific as they are usually fish trichodinids that utilise tadpoles as temporary hosts. For example, *T. nigra* Lom, 1961 and *T. reticulata* Hirschmann and Partsch, 1955 are encountered on the skin and / or gills of fishes as well as various tadpole species in many countries (Lom 1961).

Only a few studies concentrated on the collection and / or description of trichodinids from tadpoles in southern Africa (Thurston 1970, Kruger 1992, Kruger *et al.* 1993). Thurston (1970) could not identify the tadpole trichodinids collected in Uganda to species level. Kruger (1992) and Kruger *et al.* (1993) conducted a study on the morphological characteristics of tadpole trichodinids in southern Africa and although *T. nigra* and *T. reticulata* are encountered on fishes in southern Africa, no tadpoles examined hosted *T. nigra* or *T. reticulata* infestations.

Presently, most trichodinid studies are concerned with the taxonomy of the group, especially with the re-investigating or re-description of known species using modern techniques as well as the description of new species. A broad overview of the literature on the taxonomical status as well as morphological adaptations of trichodinids is presented in Chapter 2.

The present study focussed mostly on the collection and description of tadpole trichodinids in southern Africa and by correlating the findings of *T. heterodentata* Duncan, 1977 from fishes as this species corresponded to the morphological characteristics of the collected tadpole trichodinids. This study contributes to the ongoing aquatic biodiversity project of the Aquatic Research Group (ARG), University of the Free State as the re-evaluation of tadpole and fish trichodinids collected by ARG over the past 30

years was also undertaken during the present study. Chapters 3 and 4 focus on the tadpole and fish hosts examined for the presence of trichodinid infestations.

The material and methods applied, including the preparation of smears for microscopic examination and the morphological characteristics measured to study morphological similarities and dissimilarities (if present) of the various trichodinid populations are presented in Chapter 5. The localities where host populations were collected are also included in this chapter.

Thompson *et al*. (1947) and Lom (1961) mentioned that external trichodinid infestations cannot be kept on fishes or tadpoles for a long period of time under experimental conditions. The degree of trichodinid infestation as well as cross-transmission experiments undertaken with both fish and tadpoles under unnatural conditions are provided in Chapter 6 and 7 respectively.

The degree of trichodinid infestation on tadpoles of various body sizes was also compared and the findings thereof are presented in Chapter 8. The following three chapters focus on three known methods used to describe the morphological characteristics of trichodinids. The first method was proposed by Lom (1958) that suggested that a number of fixed characteristics should be measured for all future trichodinid descriptions (Chapter 9). Chapter 10 includes the findings and discussions on the area occupied by the blades, rays and central parts of the denticles within the denticle loop as proposed by Gong *et al.* (2005). The last method used to compare the morphological characteristics of the various trichodinid populations in this study was by means of a method proposed by Van As and Basson (1989, 1992) as three consecutive denticles of various trichodinids were compared with the results obtained for other tadpole and / or fish trichodinid populations (Chapter 11).

A general discussion is provided in Chapter 12, followed by the references that were used throughout the study (Chapter 13) and an appendix containing supplementary information.

The main objectives of the current study were:

- To determine if the trichodinids hosted on tadpoles in southern Africa is represented by only one species
- To determine if the tadpole trichodinids are known fish trichodinids or trichodinids that can only be found on tadpoles
- To investigate the morphological differentiation (if any) of fish trichodinids and tadpole trichodinids
- To determine if trichodinids prefer younger or older tadpoles of the same species for attachment
- To determine the degree of infestation of trichodinids on various hosts under unnatural conditions.

Trichodinids collected from various fish and tadpole populations in southern Africa, as well as two populations from South America were used to resolve the above by means of:

- Cross-transmission experiments
- Studying the infestation rate of trichodinids on fish and tadpole populations under unnatural conditions
- Comparing the morphological characteristics of the trichodinid populations with one another by means of three recognised methods.

Chapter **2**:
Literature **O**verview

'...I was mazed to see such a diversity of structures; and each too had its own proper motion, wherefore I many times looked upon these delightsome and wondrous little creatures, which quite escape the bare eye...'

Antonie van Leewenhoek, 1702

Antonie van Leewenhoek was born on 24 October 1632, Delft, Holland (Dobell 1960). Although Van Leewenhoek had no formal training as he never went to a university, his curiosity led him to build his own microscopes and he examined anything he could lay his hands on with the aid of these microscopes (Dobell 1960). In 1673, he wrote his first letter to the Royal Society in London for publication in the *Philosophical Transactions*.

In 1692, a member of the Royal Society by the name of Robert Hooke persisted that the function of microscopes in the scientific world has become almost out of use. He further stated that Van Leewenhoek studied material under the microscope for diversion and pastime reasons only (Dobell 1960). Van Leewenhoek proved him wrong as he continued to contribute to our knowledge on material such as the muscles, diaphragms as well as plants on a microscopic level (Dobell 1960). He also examined microscopic organisms collected from different water mediums and wrote eight letters on free-living forms of protozoans that he collected in water. He was amazed with what he found and stated that: '*...seeing these things... I said to myself, if the ladies of our country could see such a wonderful and perfect structure, would they not have reason to bewail the time and gifts which they employ in making such a lot of useless knots, in which not the least bit of art or beauty is ever to be seen!...*'

As an example of his discoveries, he submitted the first description and drawing of *Volvox* Linnaeus, 1758 to the Royal Society in 1700. Another noteworthy observation on a protozoological level by Van Leewenhoek includes a shell of a foraminiferan he collected within the stomach of a shrimp. He also made reference to three different Protozoa (Goldfuss, 1818) groups in one of his well-known letters dealing mainly with rotifers: the two phytoflagellates *Haematococcus* J. Von Flotow, 1844 and *Chlamydomonas* Ehrenberg, 1835 and the ciliophoran *Coleps* Nitzsch, 1827. According to Dobell (1960) Van Leewenhoek also wrote a letter containing recognisable descriptions of at least five different ciliophorans – all of which were first observed by him (i.e. *Carchesium* Ehrenberg, 1830, *Cothurnia* Ehrenberg, 1831, *Kerona* Ehrenberg, 1835, *Vorticella* Linnaeus, 1767 and a *Trichodina* Ehrenberg, 1830 species that occurred on *Hydra* Linnaeus, 1758).

Van Leewenhoek described the *Hydra* that hosted the trichodinids as: '*another little animal...had her body laden with many other animalcules (which are flat beneath and roundish above, and which I have discovered in most other kinds of water, and which are hardly a thousandth of the size of the animals which they crawl on with their little feet, and cause annoyance to)...*' Dobell (1960) stated that the trichodinid species observed by Van Leewenhoek was later classified as *Trichodina pediculus* Ehrenberg, 1831.

Trichodina pediculus, being a mobiline ectoparasitic peritrich, belongs to the Kingdom Protozoa.

1. Classification

1.1 Phylum Ciliophora Doflein, 1901

The phylum Ciliophora Doflein, 1901 (Table 2.1) was previously subdivided into three classes, namely Oligohymenophorea De Puytorac, Batisse, Bohatier, Corliss, Deroux, Didier, Dragesco Fryd-Versavel, Grain, Grolière, Hovasse, Iftode, Laval, Rogue, Savoie and Tuffrau, 1974, the Polyhymenophorea Jankowski, 1967 and lastly, the Kinetofragminophorea De Puytorac, Batisse, Bohatier, Corliss, Deroux, Didier, Dragesco Fryd-Versavel, Grain, Grolière, Hovasse, Iftode, Laval, Rogue, Savoie and Tuffrau, 1974. In addition, De Puytorac *et al.* (1974) divided the oligohymenophoreans into five subclasses: Peniculia Fauré-Fremiet, 1956, Hymenostomatida Delage and Hérouard, 1896, Astomatia Schewiakoff, 1896, Apostomatia Chatton and Lwoff, 1928, and the subclass to which trichodinids belong, namely the Peritricha Stein, 1859.

Table 2.1: Distinctive features of the Phylum Ciliophora Doflein, 1901 according to Corliss (1979).

Distinctive Characteristic	Discussion
Manifestation of nuclear dualism*	• Most possess one or more micronuclei • Most possess one or more macronuclei
Possession of simple cilia or compound ciliary organelles in at least one stage in life cycle*	• Opalinid flagellates can be distinguished from true Ciliophora on other bases • Flagella of phyto- and zoomastigophorans typically longer, fewer • Flagella usually located at apical pole of organism
Presence of infraciliature located subpellicularly in cortex	• Kinetosomes or basal bodies in association with microtubules and fibrils form infraciliature structures
Exhibition of basically homothetogenic mode of binary fission*	• Most other protozoan groups (e.g. flagellates) show symmetrogenic type of binary fission • Plane of division is perpendicular to anterior axis of body (perkinetal fission)
Absence of true syngamy	• Sexual reproduction presented solely by conjugation and autogamy
Presence of cytosome*	• Usually associated with well developed atrial, vestibular or buccal cavity containing cilia • Always accompanied with cytopharynx • Some completely astomatous • Suctorians possess sucking tentacles instead of single oral opening (polystomy)

**Not applicable to all species*

Table 2.1 (Continue): Distinctive features of the Phylum Ciliophora Doflein, 1901.

Distinctive Characteristic	Discussion
Axial symmetry Anterioposterior polarity	• Also used to distinguish ciliophorans from other eukaryotic protozoan groups • Exceptions within phylum itself / observed within nonciliate protozoan groups not classified within phylum Ciliophora
Specific 'systemes secants'	
Acentric mitoses, with intranuclear spindle and no dissolution of nuclear envelope	
'Gametic' rather than zygotic or sporic reduction in meiosis	
No micronuclear nucleoli	
Contractile vacuolar system with permanent pores	
Pellicular cytoproct	
Pellicular alveoli	
Numerous specialised cytoplasmic organelles or bodies	

Corliss (1979) stated that even though the peritrichs have been known to scientists for more than 300 years, these protozoans still challenge researchers with their most likely phylogenetic relationship to other ciliophorans. Corliss (1979) further stated that the morphology of this group is dominated by the development of a ciliary band formed by buccal organelles at the adoral end while a stalk or adhesive disc forms at the aboral end of the organism. Most mobiline peritrichs use the mentioned ciliary band to occur as ecto- or endosymbionts on a number of freshwater or marine hosts. Corliss (1979) stated that there are 17 families of peritrichs of which three of these form part of the suborder Mobilina Kahl, 1933.

Davis (1947) and Corliss (1979) described mobiline peritrichs as organisms with a conical, cylindrical or goblet-shaped body. They also mentioned that the basal adoral disc can be seen as a dominant feature to be used to distinguish between mobiline peritrichs and other peritrich forms (Table 2.2).

De Puytorac (1994) re-evaluated the higher systematics of Ciliophora by dividing the group into three subphylums: Filocorticata De Puytorac, Batisse, Deroux, Fleury, Grain, Laval-Peuto and Tuffrau, 1993, Tubulicorticata De Puytorac, Batisse, Deroux, Fleury, Grain, Laval-Peuto and Tuffrau, 1993 as well as Epiplasmata De Puytorac, Batisse, Deroux, Fleury, Grain, Laval-Peuto and Tuffrau, 1993. Epiplasmata consists of two superclasses where the subclass Membranellephora Jankowski, 1975 contains two classes, with the class Oligohymenophorea containing the subclass Peritricha (Table 2.3).

Table 2.2: Classification of the suborder Mobilina Kahl, 1933 according to Davis (1947) and Corliss (1979).

	Name	Synonym	Distinctive characteristics as explained by Davis (1947) and Corliss (1979)
Phylum	Ciliophora Doflein, 1901	Ciliae, Ciliozoa, Cytoidea, Eozoa, Heterocaryota, Heterokaryota, Infusoria Ciliata [Cilatea, Ciliasida, Euciliata] + Suctoria [Suctorea], Gymnostomea + Ciliostomea + Tentaculifera, Kinetodesmatophora + Postciliodesmatophora, Rhabdophora + Cyrtophora	• Eukaryotic, unicellular protists • 10 – 4 500 µm • Free swimming or sessile • Cilia (simple or compound) in at least one stage of life cycle • Complex cortical infraciliature: somatic plus often oral • Pellicular alveoli • Microtubular or microfibrillar structures, often kinetosome-associated and extrusomes common • Fission homothetogenic, often perkinetal, isotomic or anisotomic, occasionally multiple • Nuclear dualism (macronuclei and micronuclei) with acentric mitosis and 'gameti' meiosis • Conjugation, temporary or total, widespread • Generally monostomic, but some groups mouthless or polystomic • Contractile vacuole typically present, often also cytoproct • Feeding modes: osmotrophy to phagotrophy • Broad distribution, diverse aquatic and edaphic habitats, with ecto- and endosymbiosis exhibited by many species
Class	Oligohymenophorea de Puytorac, Batisse, Bohatier, Corliss, Deroux, Didier, Dragesco Fryd-Versavel, Grain, Grolière, Hovasse, Iftode, Laval, Rogue, Savoie and Tuffrau, 1974		

Table 2.2 (Continue): Classification of the suborder Mobilina Kahl, 1933.

	Name	Synonym	Distinctive characteristics as explained by Davis (1947) and Corliss (1979)
Subclass	Peritricha Stein, 1859	Cyclohymenophora, Dexiotricha, Peritrichasina, Peritrichia, Peritrichidea, Peritrichorida, Stomatoda	• Characteristically inverted bell – / goblet-shaped / conical-cylindrical body • Conspicuous buccal ciliature, winding counter clockwise, at apical pole and scopula (plus prominent holdfast derivatives at antapical pole) • Somatic ciliature reduced to subequatorial locomotor fringe • Ciliated infundibulum, into which contractile vacuole empties leads to cytostome • Stomatogenesis buccokinetal, with plane of fission of body parallel to major axis • Dimorphism (with migratory telotroch stage), colonies, loricae or thecae, and cysts common in the life cycle of many species • Conjugation ('total') invariably involves fusion of micro- with macroconjugant • Very widespread aquatic distribution, with species generally free-living or occurring as symphorionts on diverse hosts, but with some as commensals or parasites on or in other organisms such as other protozoans or vertebrates
Order	Peritrichida Stein, 1859		
Suborder	Mobilina Kahl, 1933	Mobilia, Mobilida, Mobiliida, Mobilorina	• Mobile forms, conical, cylindrical or goblet-shaped, sometimes discoidal • Dominant feature: basal aboral disc, holdfast organelle of considerable complexity (denticulate ring, radiating myonemes, etc.) • Trochal band permanently ciliated • Stalkless, with scopula generally vestigial (though producing cilia in some forms) • All species associated with some other organism as 'host'

Table 2.3: Partial classification of the phylum Ciliophora Doflein, 1901 adapted from De Puytorac (1994).

Subphylum	Superclass	Class	Subclass
Epiplasmata De Puytorac, Batisse, Deroux, Fleury, Grain, Laval-Peuto and Tuffrau, 1993	Ciliostomatophora De Puytorac, Batisse, Deroux, Fleury, Grain, Laval-Peuto and Tuffrau, 1993	Phyllopharyngea De Puytorac, Batisse, Deroux, Fleury, Grain, Laval-Peuto and Tuffrau, 1993	
	Membranellephora Jankowski, 1975	Nassophorea Small and Lynn, 1981	
		Oligohymenophorea De Puytorac, Batisse, Bohatier, Corliss, Deroux, Didier, Dragesco Fryd-Versavel, Grain, Grolière, Hovasse, Iftode, Laval, Rogue, Savoie and Tuffrau, 1974	Peritricha Stein, 1859
			Hymenostomatia Delage and Hérouard, 1896
			Scuticociliatia Small, 1967
			Astomatia Schewiakoff, 1896

Noble and Noble (1976) stated that protozoans had a longer time to evolve than any other animal group; thus a probable explanation for the numerous diverse protozoan species that invade nearly all possible ecologic niches. The systematic characteristics of mobiline peritrichs are presented in the following table (Table 2.4).

Table 2.4: Systematic characteristics of mobiline peritrichs according to Corliss (1979) as well as Lom and Dykova (1992).

	Taxonomic group	Characteristics
Phylum	Ciliophora Doflein, 1901	• Heterotrophic / free-swimming or sessile • Simple and compound cilia in at least one stage of life cycle • Somatic and oral infraciliature is complex • Pellicular alveoli • Kinetosome associated microtubular / microfibrillar structures • Homothetogenic fission and often perinetetal • 1^+ diploid micronuclei; 1^+ polyploid macronuclei • Mouthless, mono- or polystomic, generally monostomic • Contractile vacuole present • Osmotrophic / phagotrophic • Broad distribution • Sometimes ecto- or endosymbionts • Diverse aquatic / edaphic habitats in broad distribution areas
Class	Oligohymenophorea De Puytorac, Batisse, Bohatier, Corliss, Deroux, Didier, Dragesco Fryd-Versavel, Grain, Grolière, Hovasse, Iftode, Laval, Rogue, Savoie and Tuffrau, 1974	• Oral apparatus distinctive from somatic ciliophorans • Oral apparatus comprises of well-defined paroral membrane plus membranelles of peniculli, located in buccal cavity or infundibulum situated on ventral side of body • Oral apparatus contain cytostome at base of cavity • Inconspicuous cytopharynx • Parakinetal / buccokinetal stomatogenesis • Some variation in mode of fission • Conjugation temporary, only in one group • Free-living / symbiotic
Subclass	Peritricha Stein, 1859	• Inverted bell- / goblet-shaped / conical-cylindrical body shape • Morphology dominated by adoral ciliary wreath of buccal ciliature • Cilia winding counter clockwise at apical pole and scopula at antapical pole • Somatic ciliature reduced to subequatorial; form trocheal band (locomotor fringe of cilia) • Contractile vacuole empties into ciliated infundibulum that leads to cytostome • Stomatogenesis buccokinetal • Plane of fission parallel to major axis of body • Dimorphism, colonies, loricae, thecae or cysts at many species • Total conjugation involves fusion of micro- and macroconjugant • Free-living / symbionts, commensals / endo- / ectoparasites • Widespread aquatic distribution on diverse host range
Order	Mobilida Kahl, 1933	• Stalkless with vestigial scopula (mobile) • Conical / cylindrical / goblet-shaped body • Aboral disc present • Permanently ciliated trocheal band • All species associated with a host (skin / gills / digestive- / urinary tract of host)

1.2 Order Mobilida Kahl, 1933

Mobiline peritrichs are stalkless protozoans with a conical, cylindrical or goblet-shaped body form with a flattened oral-aboral side in most cases. The free-swimming forms are characterised by a complex thigmotactic apparatus and their strong affinity for living substrata as hosts such as amphibians, coelenterates, crustaceans, echinoderms, fishes, molluscs and turbellarian individuals (Corliss 1979). The denticle ring (Figure 2.1) contains articulated skeletal denticles that are composed of scleroprotein. Note that representatives of the Urceolariidae Dujardin, 1940 (Figure 2.1A) have a ring similar to the central parts of trichodinids, but that these structures do not have protrusions on either side as in the case of members from the family Trichodinidae Claus, 1874 (Figure 2.1 B,C). The denticle ring and the number, organisation and substructure of the denticles within the denticle ring are of utmost importance for taxonomists as these are the key features that are used to describe these peritrich species. Earlier literature (Corliss 1979) proposed that trichodinids in great abundance may cause localised lesions at the area of attachment on the host as it was thought that the denticle ring is used to bore or cut into the epithelium of the host. However, later literature states that the denticle ring is smooth and flexible and mainly functions as a supporting framework for the peritrich (Van As and Basson 1987). In addition, the body of trichodinids, including the denticle ring, is covered by a thin membrane and therefore the hypothesis by Corliss (1979) is erroneous.

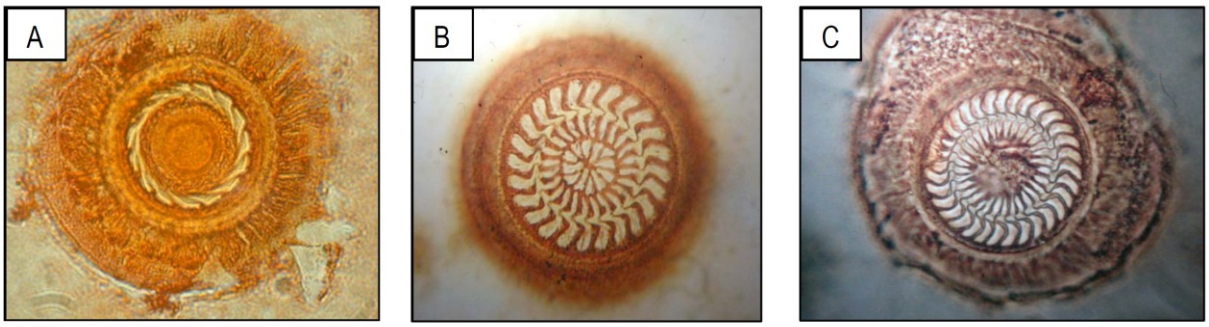


Figure 2.1: Examples of mobiline peritrichs indicating the articulated skeletal system that is composed of scleroprotein. **A**: Representative of the Urceolariidae Dujardin, 1940; **B**:*Trichodina centrostrigeata* Basson, Van As and Paperna, 1983 collected from the skin of *Pseudocrenilabrus philander* Weber, 1897; **C**: *T. dampanula* Van der Bank, Basson and Van As, 1989 collected from the urinary bladder of adult *Amietophrynus gutturalis* Power, 1927 toads.

1.3 Trichodinidae Claus, 1874

Corliss (1979) divided mobiline peritrichs into five families namely Urceolariidae, Trichodinopsidae Kent, 1881, Polycyclidae Poljansky, 1951, Leiotrochidae Johnston, 1938 and Trichodinidae (Table 2.5).

Table 2.5: Classification of the mobiline peritrich families according to Corliss (1979).

Family	Genera	Characteristic	Hosts
Trichodinopsidae Kent, 1881	• *Trichodinopsis* Claparéde and Lachmann, 1858	• Body conical, tapered apically • Adoral spiral of ca. 360º, with greatly reduced radius • Buccal ciliature relatively inconspicuous • Highly specialised infundibular area • Bulbous expansion posteriorly • Denticles (30-40) smoothly, densely linked • Cortical rings, some scopulary cilia present • Macronucleus compact and discoidal	• Intestinal symbionts of a terrestrial prosobranch snail
Trichodinidae Claus, 1874	• *Trichodina* Ehrenberg, 1830 • *Trichodinella* Srámek-Husek, 1953 • *Semitrichodina* Kazubski, 1958 • *Tripartiella* Lom, 1959 • *Dipartiella* G. Stein, Linnaeus • *Paratrichodina* Lom, 1963	• Cylindrical, barrel- or goblet-shaped body, occasionally slightly tapered apically or flattened into discoidal or hemispherical form • Adoral spiral ranges from turn of 180º to two or nearly three full circles with wide radius • Conspicuous buccal ciliature • 15 – 40 or more complex denticles • Marginal cilia often present • Sausage or horseshoe-shaped macronucleus	• Other ciliophorans, integument of various aquatic invertebrates, mantle cavity of land gastropod molluscs, skin, gills, urinary bladder of freshwater and marine fish, amphibians
Leiotrochidae Johnston, 1938	• *Leiotrocha* Fabre-Domergue, 1888	• Cylindrical or barrel-shaped body, slightly bulging apical end • Adoral spiral of ca. 400º with radius corresponding to the aboral adhesive disc • 20 smoothly linked denticles • Macronucleus bulbous with two arms (H-shaped) • Cortical rings present	• Symbionts on gills of marine molluscs, invertebrates (spines of sea urchins)

Table 2.5 (Continue): Classification of the mobiline peritrich families according to Corliss (1979).

Family	Genera	Characteristic	Hosts
Urceolariidae Dujardin, 1940	• *Urceolaria* Stein, 1854	• Cylindrical body, often slightly tipped to one side • Buccal ciliature turns ca. 400º, with wide radius • 20 smoothly linked denticles • Compact macronucleus • Cortical rings absent	• Ectosymbionts of fresh-water turbellarians and marine polychaetes and molluscs
Polycyclidae Poljansky, 1951	• *Polycycla* Poljansky, 1951	• Body conical, tapered apically • Adoral spiral of ca. 360º with greatly reduced radius • Buccal ciliature relatively inconspicuous • 35 – 55 smooth, densely linked denticles • Cortical rings present • Scopulary cilia present • Two trochal bands present • Ribbon-like macronucleus with thick 'nodes'	• Endocommensals of holothurian echinoderms

Corliss (1979) agreed with the protozoan classification of Jones (1974) as the latter author mentioned that trichodinids are classified within the mobiline peritrich cluster (Figure 2.2). Corliss (1979) mentioned that the best known genus from the family Trichodinidae is *Trichodina* as it shows the greatest diversity and is also the most widely distributed. Synonyms of the genus *Trichodina* include *Anhymenia* Fabre-Domergue, 1888; *Cyclochaeta* Jackson, 1875; *Cyclocyrrha* Fabre-Domergue, 1888; *Paravauchomia* Raabe, 1963 and *Poljanskina* Raabe, 1963 (Basson and Van As 2004).

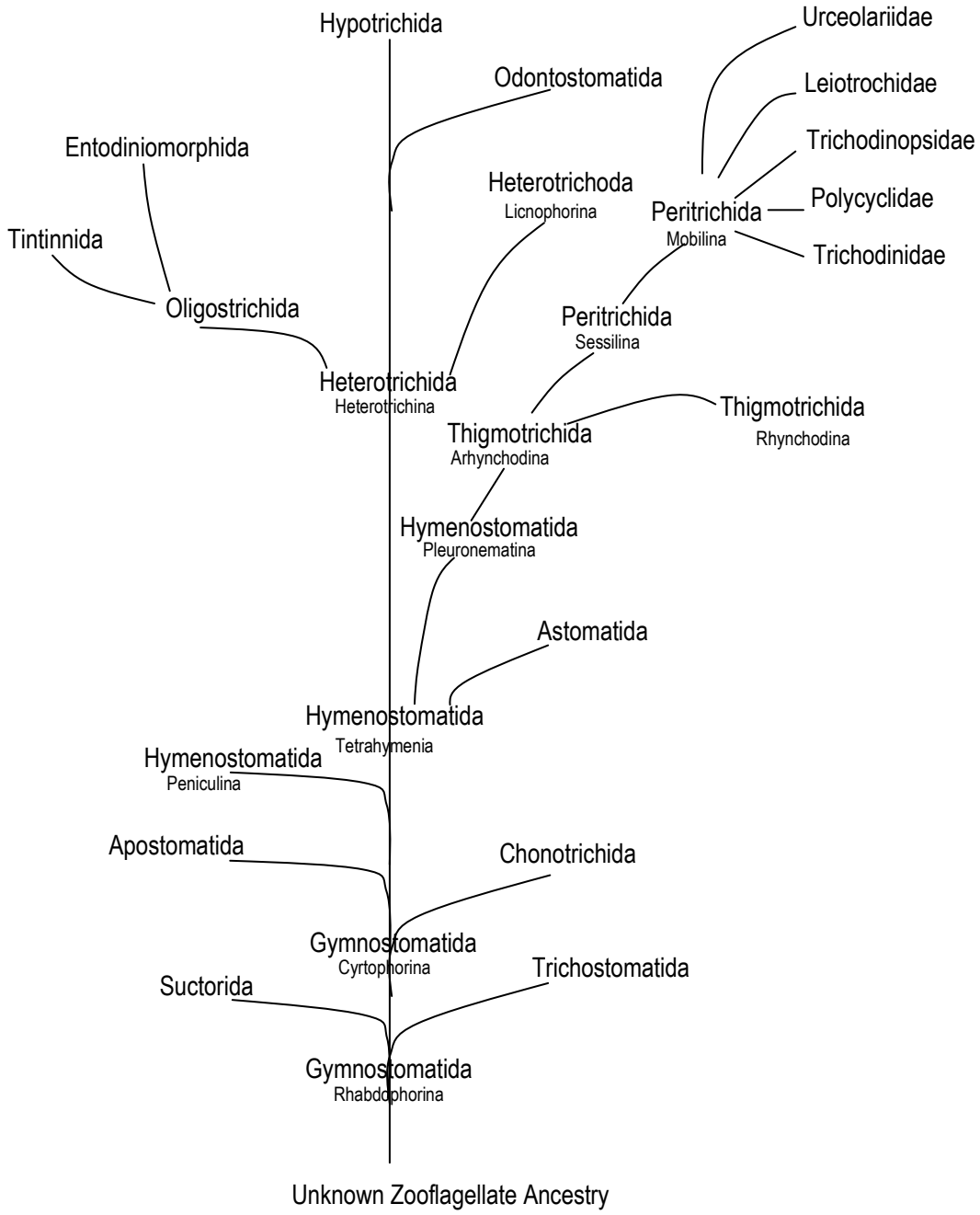


Figure 2.2: Evolutionary tree for ciliophoran orders and suborders as explained by Jones (1974) and Corliss (1979).

Currently, the work by Lom and Dykova (1992) is accepted in which the mobiline peritrichs are divided into three families: Trichodinidae, Urceolariidae and Trichodinopsidae. Urceolariidae and Trichodinopsidae have simple plate-like denticles and are associated with invertebrate hosts. The family Trichodinidae can be distinguished from the other two families due to the presence of blades and rays in the denticle ring (Leer *et al.* 1985). Members of the family Trichodinidae are associated with the body surface of hydras, turbellarians and crustaceans. In addition, these organisms can also be encountered on aquatic and terrestrial molluscs and the skin and gills of fish and tadpoles.

Gong *et al.* (2006) and Zhan *et al.* (2009) studied the phylogenetic tree for trichodinids and other descendants of the group Oligohymenophorea and agreed with Lom and Dykova (1992) that the genera *Trichodina*, *Trichodinella* Srámek-Husek, 1953 and *Urceolaria* Stein, 1854 forms part of the mobiline peritrich cluster (Figure 2.3).

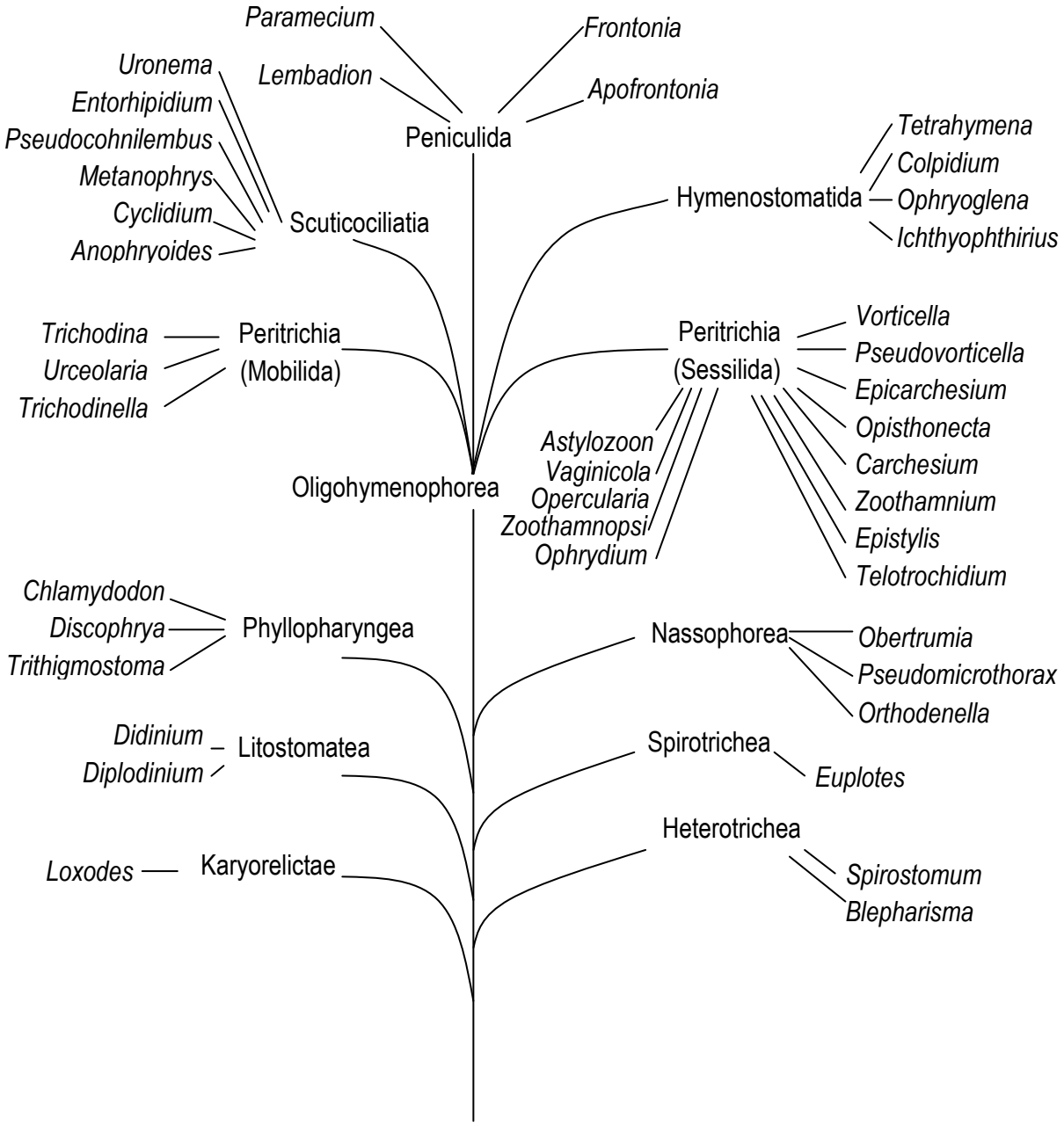


Figure 2.3: Gong *et al.* (2006) and Zhan *et al*. (2009) determined the phylogenetic tree for the genus *Trichodina* Ehrenberg, 1830 inferred from small subunit rRNA sequences.

2. Morphology of the genus *Trichodina* Ehrenberg, 1830

Davis (1947) stated that the concave side of trichodinids is usually referred to as the aboral side. The convex side, which hosts the adoral cilia, is referred to as the adoral side. The trichodinid body is encircled by a band of long cilia (ciliary girdle) that aids the organism with locomotion (Figure 2.4). Lom (1958) stated that the adoral spiral can be used to differentiate between genera as the adoral spiral of *Vauchomia* Mueller, 1938 species makes two to three turns, in contrast to the 360°- 450° that can be observed from species from the genus *Trichodina*.

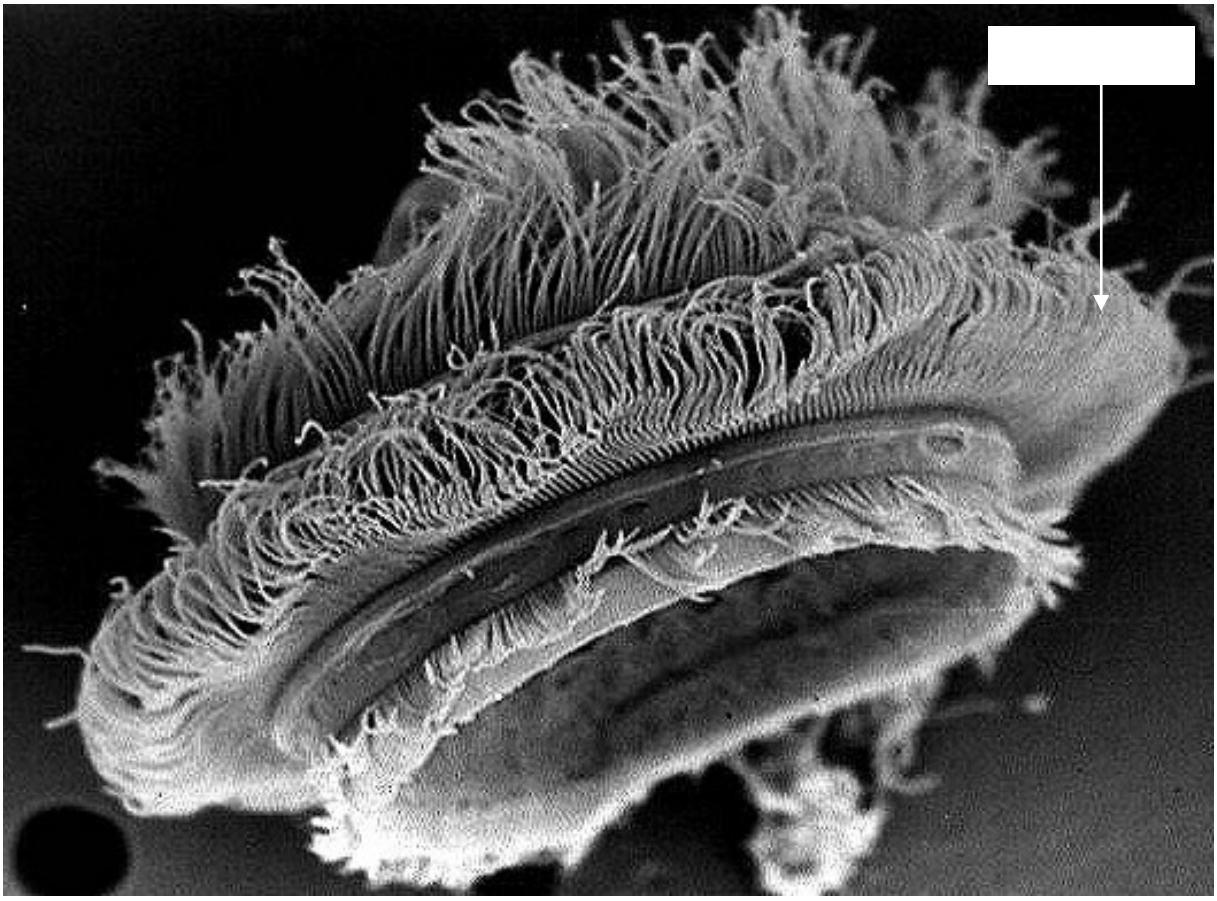


Figure 2.4: Scanning electron micrograph indicating the ciliary girdle of *Trichodina oxystelis* Sandon, 1965 indicating the ciliary girdle that aids in the movement of trichodinids.

The body of trichodinids are covered with a thin transparent membrane (Kruger 1992) and the position of the underlying denticle ring can be observed when viewed with a scanning electron microscope (Figure 2.5). According to Kruger (1992), the denticle ring provides the organism with a certain degree of flexibility as it aids the organism in attaching to the contours of the surface of the host.

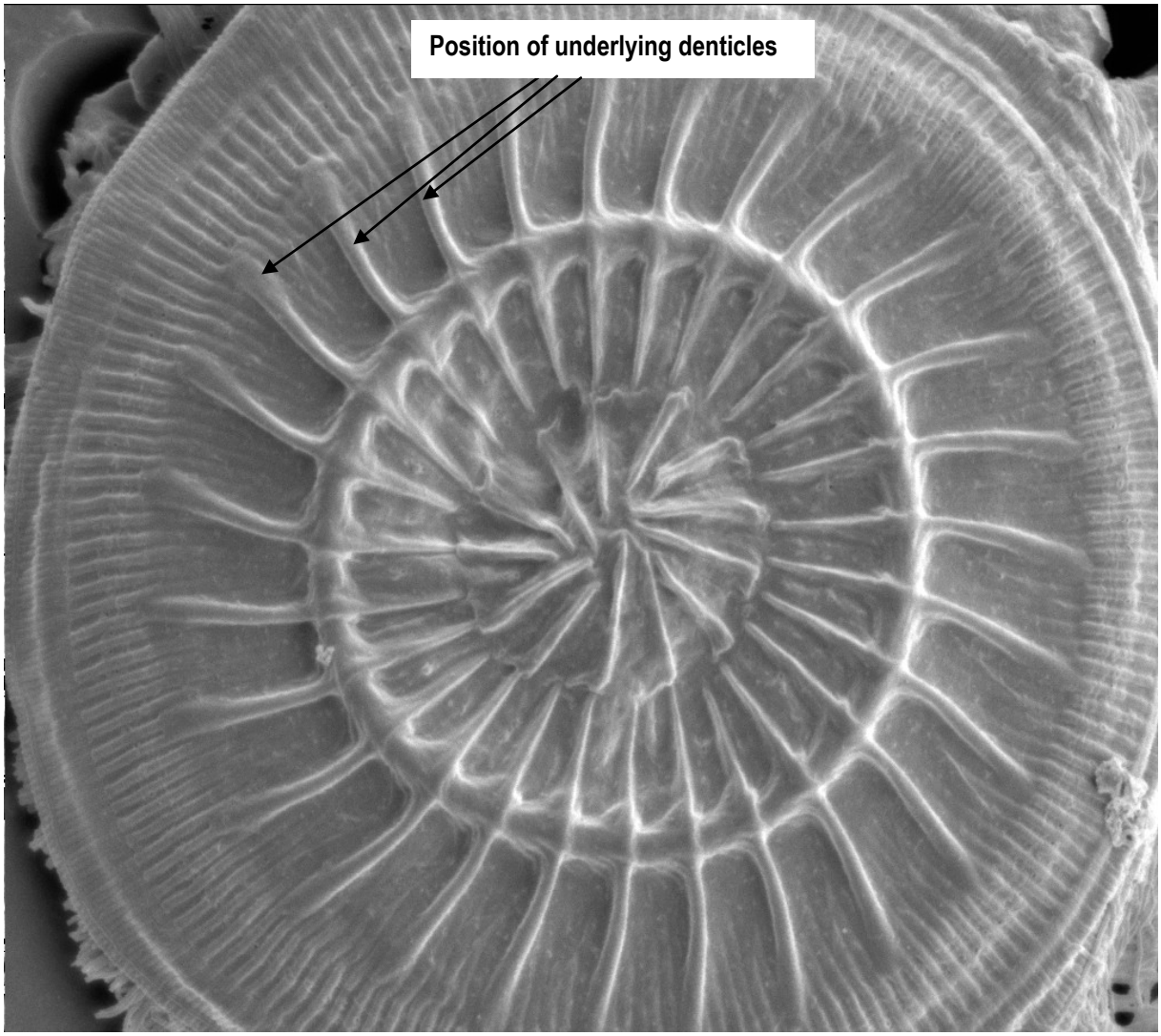


Figure 2.5: Scanning electron micrograph of *Trichodina centrostrigeata* Basson, Van As and Paperna, 1983 to illustrate the visibility of the underlying denticle ring. The denticles are made visible by the contours formed by the membrane.

Trichodinid denticles can be divided into three portions, i.e.: the blade, central part and ray (Figure 2.6A). The denticles function as a supportive structure as the top of the central part (central conical part) of each denticle fits into an adjacent denticle (Kruger 1992). This arrangement is made possible by the fact that the central conical part is shaped like a hollow cone and therefore the smaller end of the cone is able to insert into the cavity of the central part of the following denticle (Figure 2.6B).

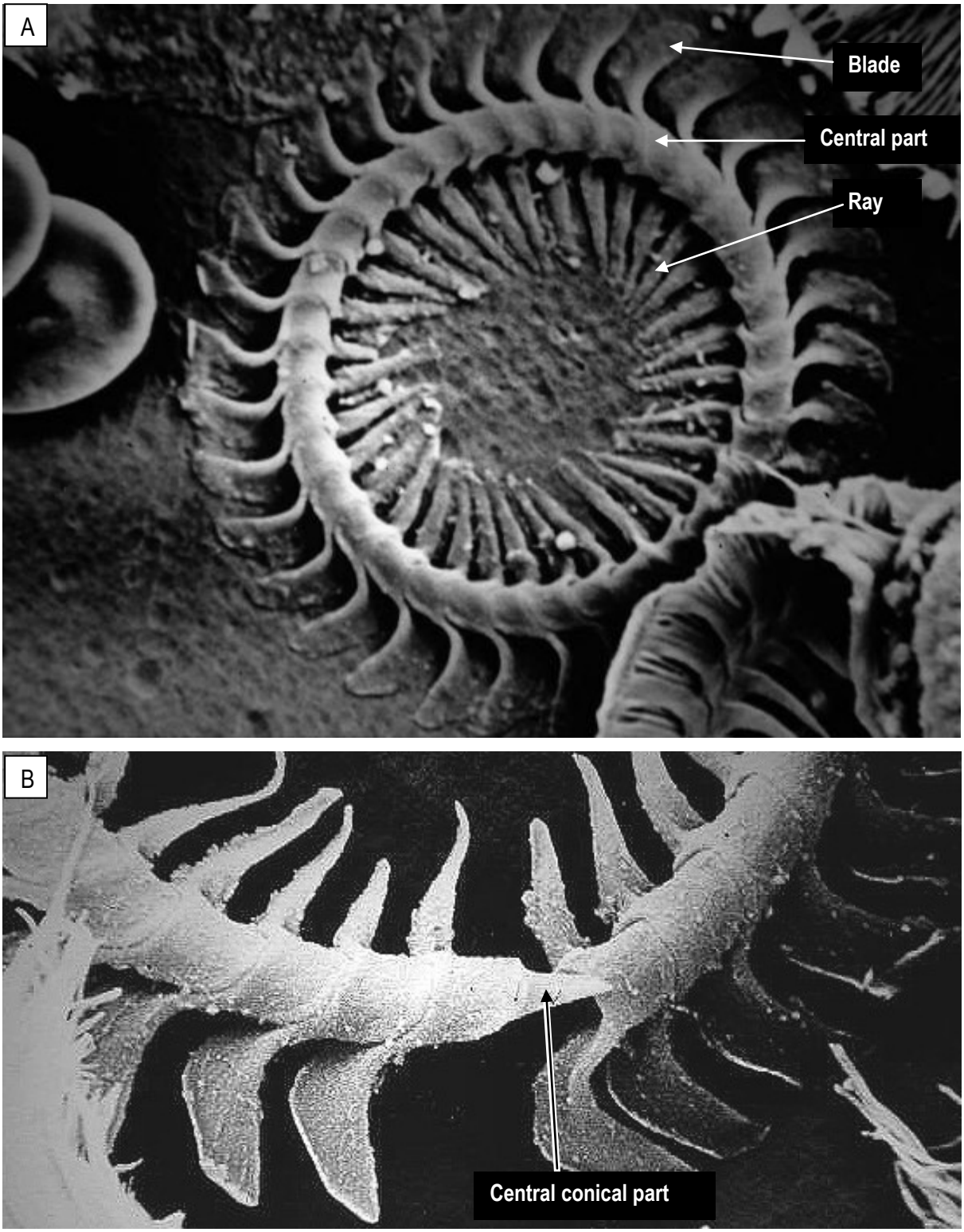


Figure 2.6: Scanning electron micrographs of damaged material of *Trichodina dampanula* Van der Bank, Basson and Van As, 1989 collected from bladders of adult *Amietophrynus gutturalis* (Power, 1927) frogs. **A**: Whole denticle ring with the blade, central part and ray of a denticle; **B**: The cone of the preceding denticle is inserted into the cavity of the adjacent denticle.

The thickness, length and the curvature of the rays depend on the trichodinid species being studied (Kruger 1992). Davis (1947) mentioned that the rays of trichodinids never overlap one another near the centre of the disc. However, Basson and Van As (1994) mentioned that the rays of a trichodinid species collected from the skin, fins and occasionally the gills of various fish species in Taiwan reached the centre of the denticle ring. This species was named *Trichodina mandarin* Basson and Van As, 1994 (Figure 2.7) due to the locality of occurrence.

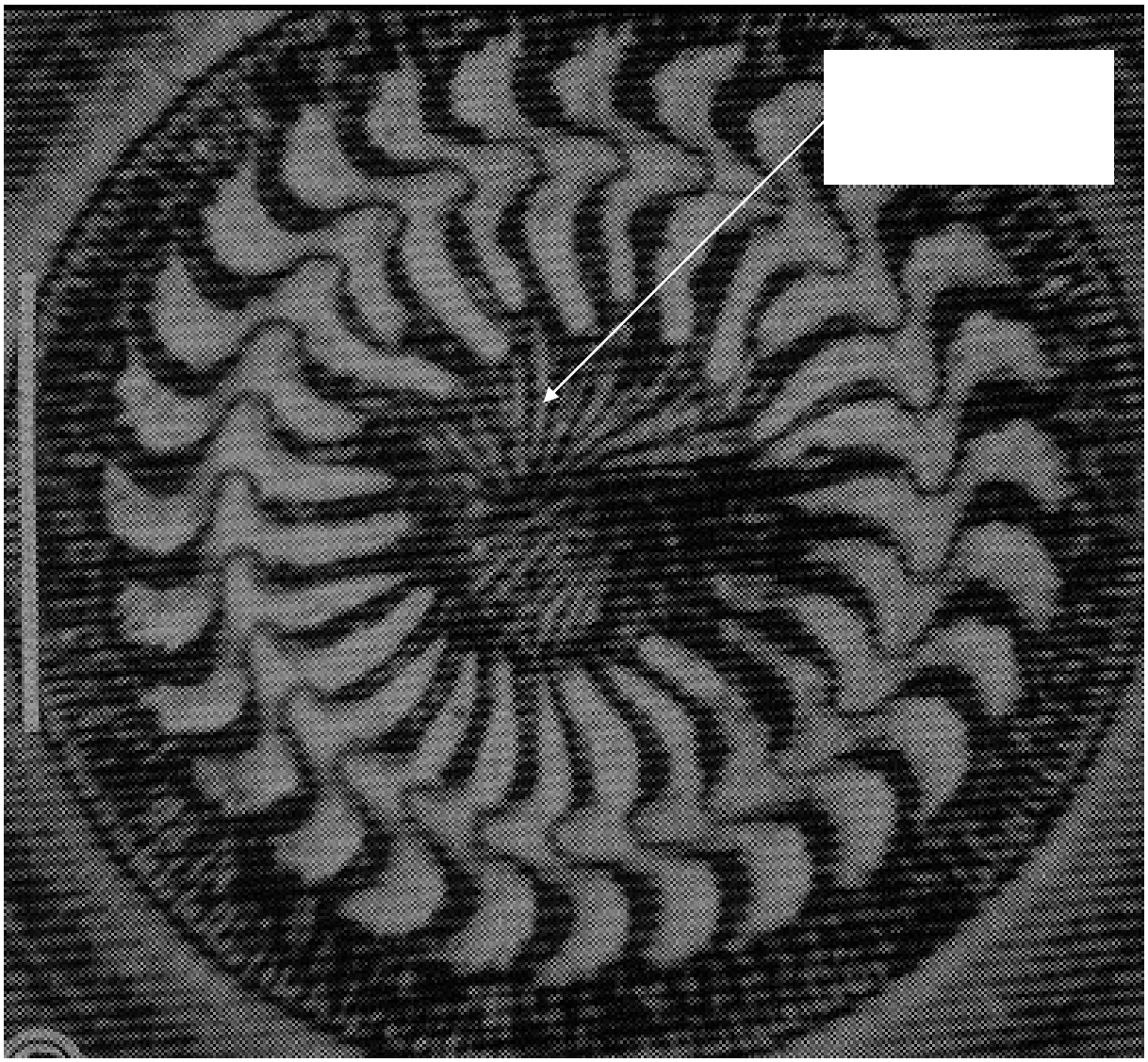


Figure 2.7: Light micrograph of *Trichodina mandarin* Basson and Van As, 1994 collected on the skin of various fish species in Taiwan.

Kruger (1992) found that the radial pins that form the striated band overlap the blades and that the outer end of each radial pin is attached to a membrane that covers the surface of the adhesive disc

(Figure 2.8). Even though the number of radial pins per denticle varies to a certain extent, the variation within a species is constant (Kruger 1992).

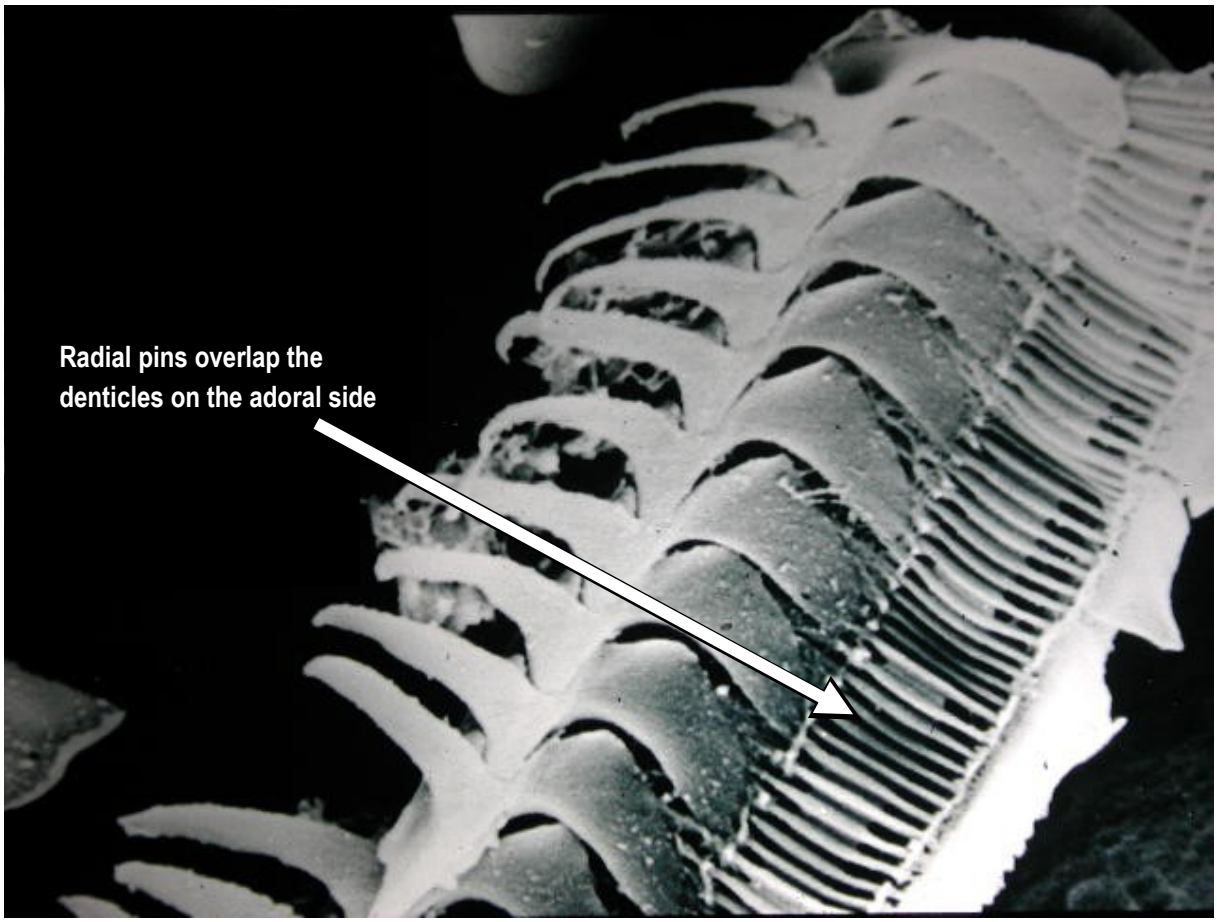


Figure 2.8: Scanning electron micrograph of damaged material of *Trichodina dampanula* Van der Bank, Basson and Van As, 1989 indicating the radial pins that overlap the blade-area of the trichodinid.

The border membrane is a thin, flexible membrane that also contains striations (Figure 2.9). However, these striations are not continuous with those of the striated band and it is not uncommon that these striations outnumber the striations from the radial pins (Kruger 1992). The ciliary girdle is formed by the presence of a series of membranelles that is composed of several cilia. Each cilium is attached to the floor of the groove in a side-way manner (Kruger 1992). The short, delicate marginal cilia can be seen above the membranelles as cilia that are curved upwards while moving in a wave-like motion.

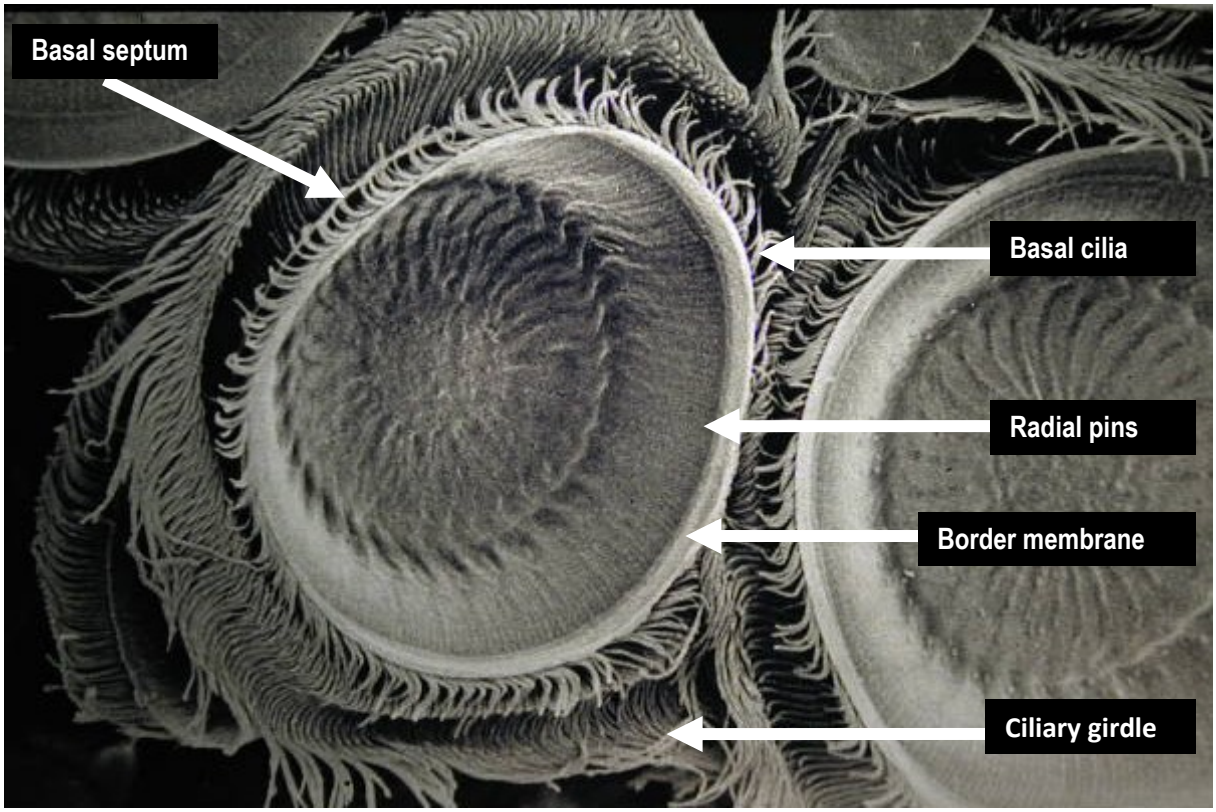


Figure 2.9: Scanning electron micrograph of *Trichodina dampanula* Van der Bank, Basson and Van As, 1989 indicating the position of the border membrane and ciliary girdle.

According to Kruger (1992), trichodinids move on or over the surface of the host such as the skin, gills or urinary bladder by means of the ciliary girdle (Figure 2.10). The mentioned girdle also aids the organism to propel itself through the water in addition to the marginal cilia. The concave adhesive disk is always in the direction of the forward motion of the organism when swimming forward (Davis 1947).

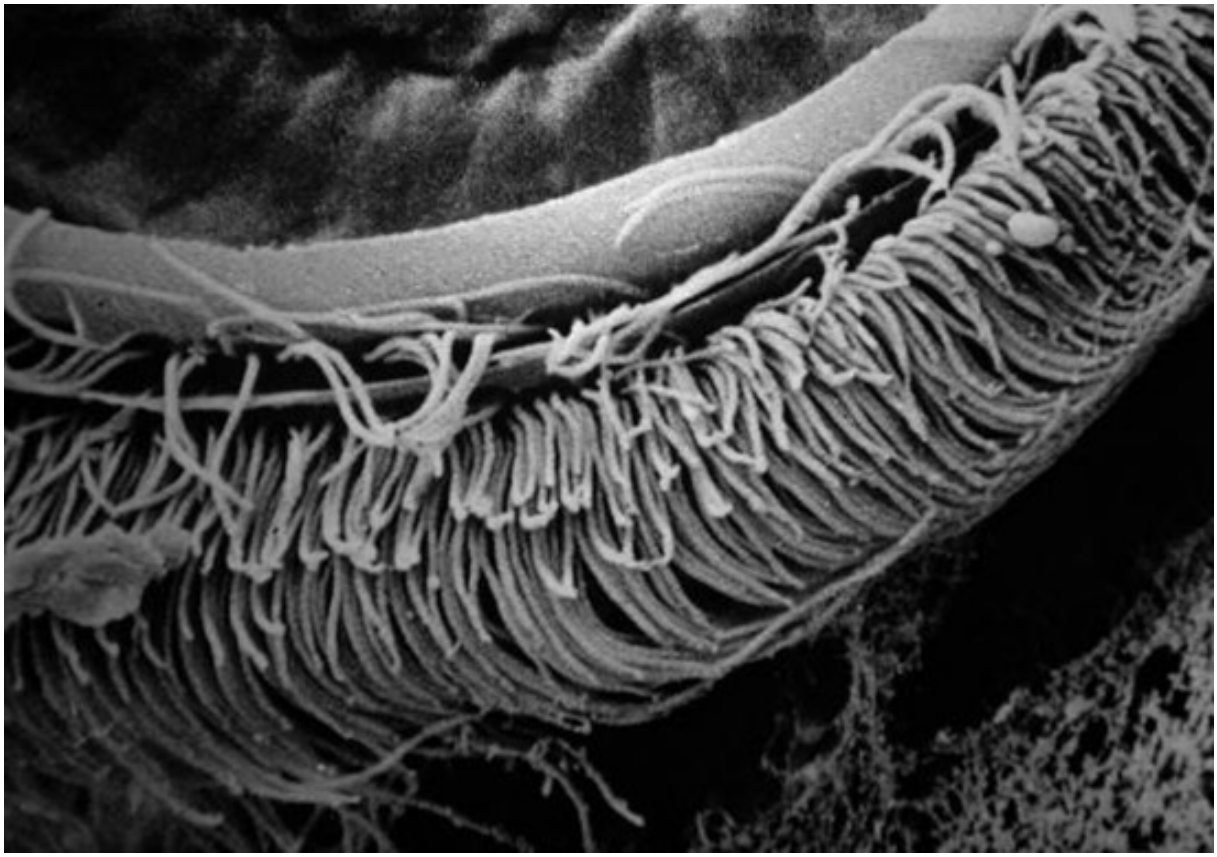


Figure 2.10: Scanning electron micrograph of the ciliary girdle of *Trichodina xenopodos* Fantham 1924.

The mouth opens into the cytopharynx (Figure 2.11). Two parallel rows of cilia are formed at the cytopharynx and a spiral movement of these cilia continue through the mouth opening into a groove (Kruger 1992). This groove (adoral spiral) can be seen as a furrow directed in a clockwise direction around the adoral surface of the trichodinid. The groove is enclosed within two rows of cilia that are usually motionlessly folded over the adoral surface. A large contractile vacuole occurs near the centre of the body and has a duct leading to the exterior of the organism. Smaller vacuoles (i.e. food vacuoles), granular material and fat globules are also present in the endoplasm.

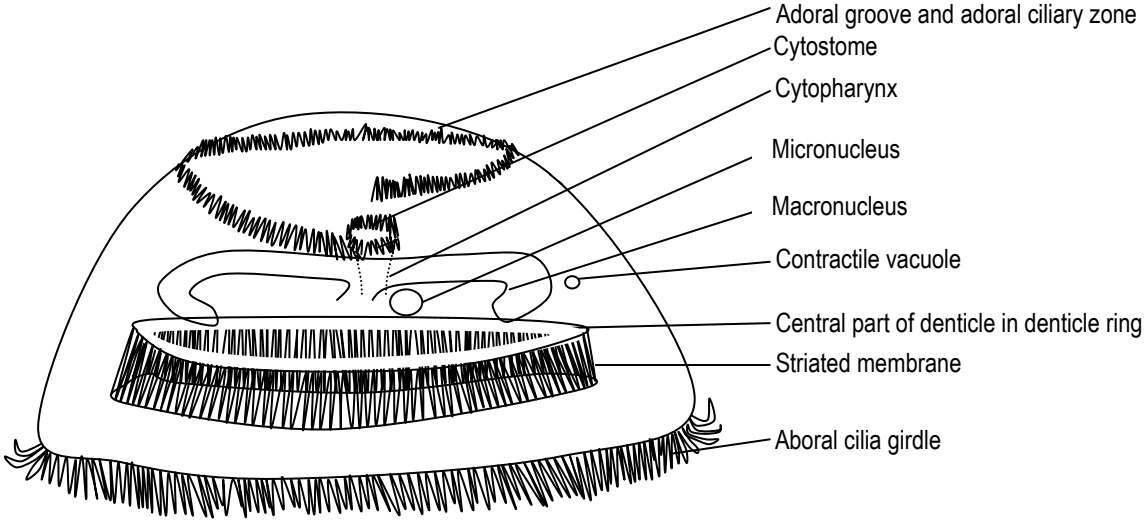


Figure 2.11: Two parallel rows of cilia form a spiral movement that continues through the mouth opening into a groove, redrawn from Kruger (1992).

A horseshoe-shaped macronucleus (Figure 2.12) surrounds the cytopharynx and the duct leading from the contractile vacuole. This structure is mostly filled with granular chromatic material. Rounded bodies of denser material can also be observed in the macronucleus to a lesser extent. Although the micronucleus is always present, it is only sometimes observed as a rounded (or ovoid or elongated) structure near the open ends of the macronucleus during the adult stage of the trichodinid life cycle (Kruger 1992). The micronucleus moves from position during the reproductive stage (Kruger 1992), and will be discussed in the following section.

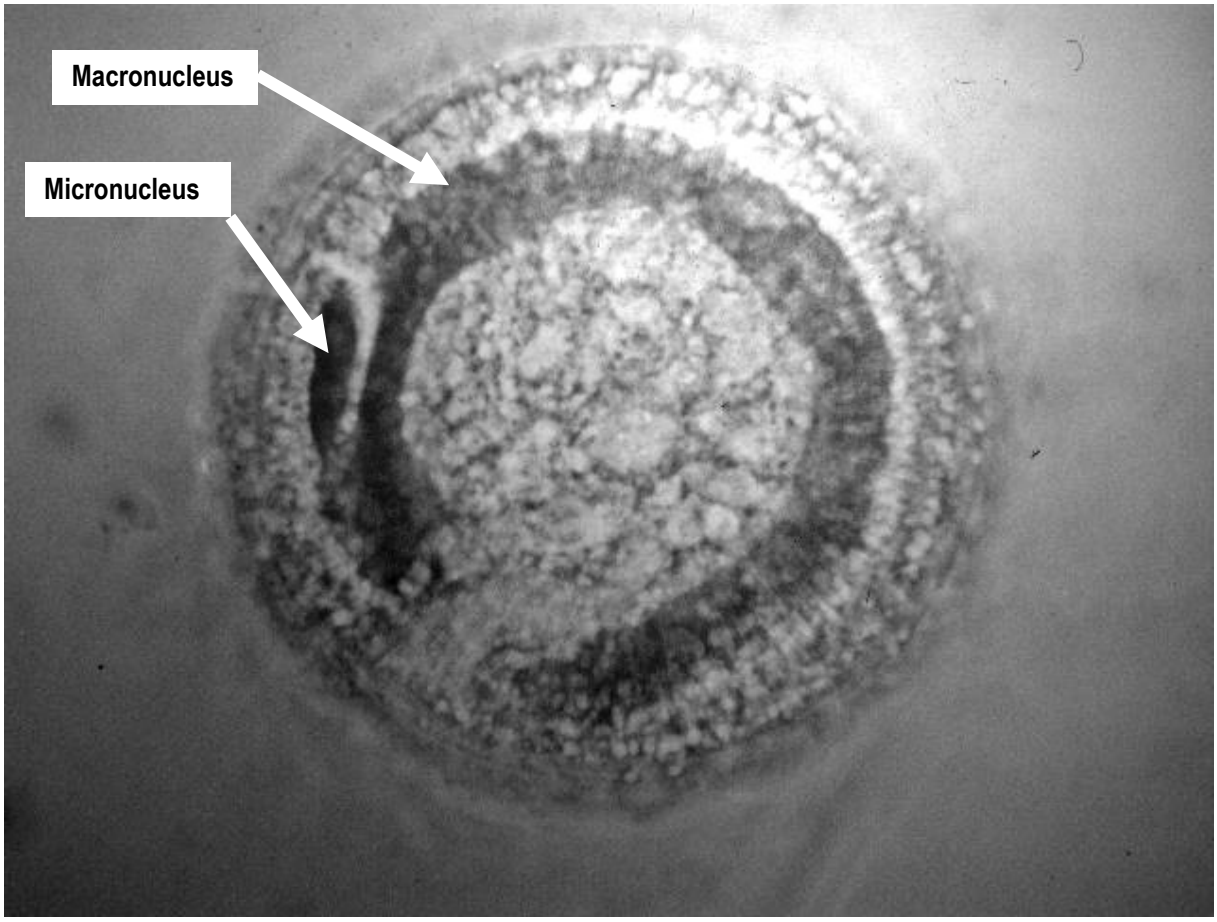


Figure 2.12: Light micrographs of the horseshoe-shaped macro- and micronuclei of *Trichodina mutabilis* Kazubski and Migala, 1968.

3. Reproduction

Reproduction of trichodinids is direct as young individuals look similar than older individuals and no cyst stage is present (Kruger 1992). Trichodinids multiply by means of conjugation, endomyxis, and / or binary fission.

3.1 Endomixis

Endomixis can be described as the reproduction process where the reorganisation of the nucleus occurs by means of the disintegrating process of the macronucleus. During this process, the macronucleus disintegrates into fragments throughout the reproductive process. Eight micronuclei are formed due to the rapidly succeeding mitotic division process and no chromosome reduction is undertaken during these divisions (Diller 1928). A macro anlagen is formed from seven of the micronuclei, while the eighth micronucleus forms the functional micronucleus. The macronuclear

material is distributed to the daughter cells as successive cell divisions occur. The cell division takes place before the micronucleus divides.

3.2 Conjugation

Another reproductive process undertaken by trichodinids where genetic material is exchanged is conjugation. During this process, the aboral surface of the microconjugant is fitted over the adoral surface of the macroconjugant. The micronuclei of both conjugants swell and divide by means of mitosis while the macronucleus breaks down into large, coarse fragments. These fragments continue to break up into smaller fragments until small spherical bodies (containing granules) are formed. The micronuclei move to the centre of the body, underneath the adoral surface. Two micronuclear divisions take place in each conjugant during the final fragmentation process of the macronucleus. The protoplasm of both individuals becomes continuous and the smaller individual passes its content into the larger individual. It is assumed that the gametic nuclei unite at this stage (Padnos and Nigrelli 1942). Protoplasm of the microconjugant passes into the macroconjugant as the protoplasm of the conjugants becomes continuous. The denticles of the macroconjugant become reorganised shortly after fusion of the protoplasmic contents of the two conjugants. Padnos and Nigrelli (1942) assumed that the synkaryon (uniting of the gametic nuclei) forms at this stage while the remaining nuclei are resorbed. Eight micronuclei are formed as the zygotic nucleus divides three times. One of the micronuclei forms the functional micronucleus whilst the others become the macro anlagen. A new ring forms in a similar manner as will be explained during the binary fission section. The micronucleus divides and the macro anlagen distributes between the daughter cells. Each of the daughter cells contains a micronucleus and macro anlagen. The macronucleus increases in size and becomes horseshoe-shaped.

3.3 Binary fission (Figure 2.13)

Although binary fission can be seen as the most common manner of multiplication of trichodinids, previous authors such as Colwin (1936) as well as Padnos and Nigrelli (1942) usually concentrated on the conjugation and endomyxis processes. These authors described the alteration of the macro- and micronucleus during the conjugation and endomyxis processes while the process of binary fission was only briefly described. Diller (1928) studied binary fission and the endomixis process from trichodinids collected from tadpoles and Kruger (1992) used *Trichodina xenopodos* and *T. heterodentata* to study the binary fission process.

Binary fission can be summarised as the division of an individual into two identical daughter cells, without the exchange of any genetic material. This is a complex process and more than one activity takes place at a given interval. Poljanskij and Chejsin (1965) mentioned that the body size of the protozoans usually increases as a result of an extensive feeding period prior to division. Silver impregnated individuals that are preparing for, or in the process of binary fission usually have a thick band (Figure 2.13A) that encircles the adhesive disc (Van As and Basson 1986; Kruger 1992). Kruger (1992) speculated that this band is part of the striated band containing the radial pins that plays a role in the development of a new denticle ring.

During binary fission, the cone of the central part of a denticle will loosen from the cavity of the following denticle in order to break the bond within the denticle ring (Kruger 1992) (Figure 2.13B). The same happens on the opposite side of the denticle ring in order to provide two denticle ring units within the parent cell. The detachment area marks the constriction area that will cause the single organism to divide into two equal parts (i.e. into two individuals).

The macronucleus loses its horseshoe-shape and becomes thicker as it becomes shorter. Lom (1964) mentioned that the micronucleus will be in its prophase stage at the start of the nuclear division process and Padnos and Nigrelli (1942) mentioned that the prophase of the micronucleus takes place with the shortening process of the macronucleus. Diller (1928) stated that the micronucleus moves to one side of the macronucleus and divides by means of mitosis while the macronucleus divides by amitosis and that the division process of the macronucleus takes place after the division process of the micronucleus has started. According to Diller (1928), the nucleus divides into two equal parts after the division of the macro- and micronuclei were undertaken.

Kruger (1992) mentioned that the oral apparatus duplicates before the division of the parent cell takes place. The adoral spiral forms a heart-shaped indentation on the opposite side of the oral opening in order to form an adoral spiral within another. The infundibulum divides to form two buccal openings adjacent to the original opening (Figure 2.13D). The two oral apparatuses turn loose from each other as the haplo- and polykineties are not attached to one another (Raabe 1964).

According to Lom (1964), no cilia are absorbed during the binary fission process and new cilia form after the formation of the nuclei. The oral apparatuses will move away from each other after the formation of the infracilia is completed. In addition, two daughter micronuclei will form at this stage (Padnos and Nigrelli 1942).

The aboral disc now prepares for the formation of two denticle rings by means of an indentation in the denticle ring as well as the presence of thick radial pins on the outer end of the denticle ring (Kruger 1992). Small indentations form within the body of the trichodinid and the striated bands immediately beneath the indentation break (Figure 2.13C). This results in two individual daughter cells containing half the number of denticles and radial pins than the parent cell (Kruger 1992) (Figure 2.13C, E and F).

As soon as the two daughter cells move away from each other, the denticle unit within each cell will once again form a denticle ring. However, it should be kept in mind that this bond is usually not as strong as it was within the parent cell (Kruger 1992) and sometimes this bonding is not successful at all as can be seen in Figure 2.13E.

The cilia of the daughter cells start to multiply as soon as the division process is completed. In addition, the denticle number is restored and can be observed as the 'overlapping plates' that was previously mentioned (Figure 2.13F). Each of these plates enlarges to develop into a central part of a denticle. Kruger (1992) mentioned that this provides stability within the daughter cell. The blades of the new denticle ring form prior to the rays (Figure 2.13G).

Previous authors such as Fulton (1923), Davis (1947) and Lom (1961) suggested that the 'old' denticle ring moves to the middle of the daughter cell. However, Kruger (1992) found the contrary and stated that the 'old' denticle ring remains in position, but slowly disintegrates. The part of the denticle ring that provides support to the organism (the central part) is the last part of the denticle ring that is absorbed by the daughter cell (Figure 2.13H-P).

The central part of the newly developed denticle ring form at a similar position as the 'old' denticle ring and the blade and radial pins develop in a distal direction as the cell increases in diameter (Kruger 1992). The striated band of each of the daughter cells contains half the number of rods of the parent cell. The striated band from the parent cell is not absorbed but new rods form between those of the original band and can be seen as a thin line adjacent to each of the 'old' rods (Kruger 1992) (Figure 2.13L). The thickness and strength of the new rods increase as the trichodinid grows in circumference.

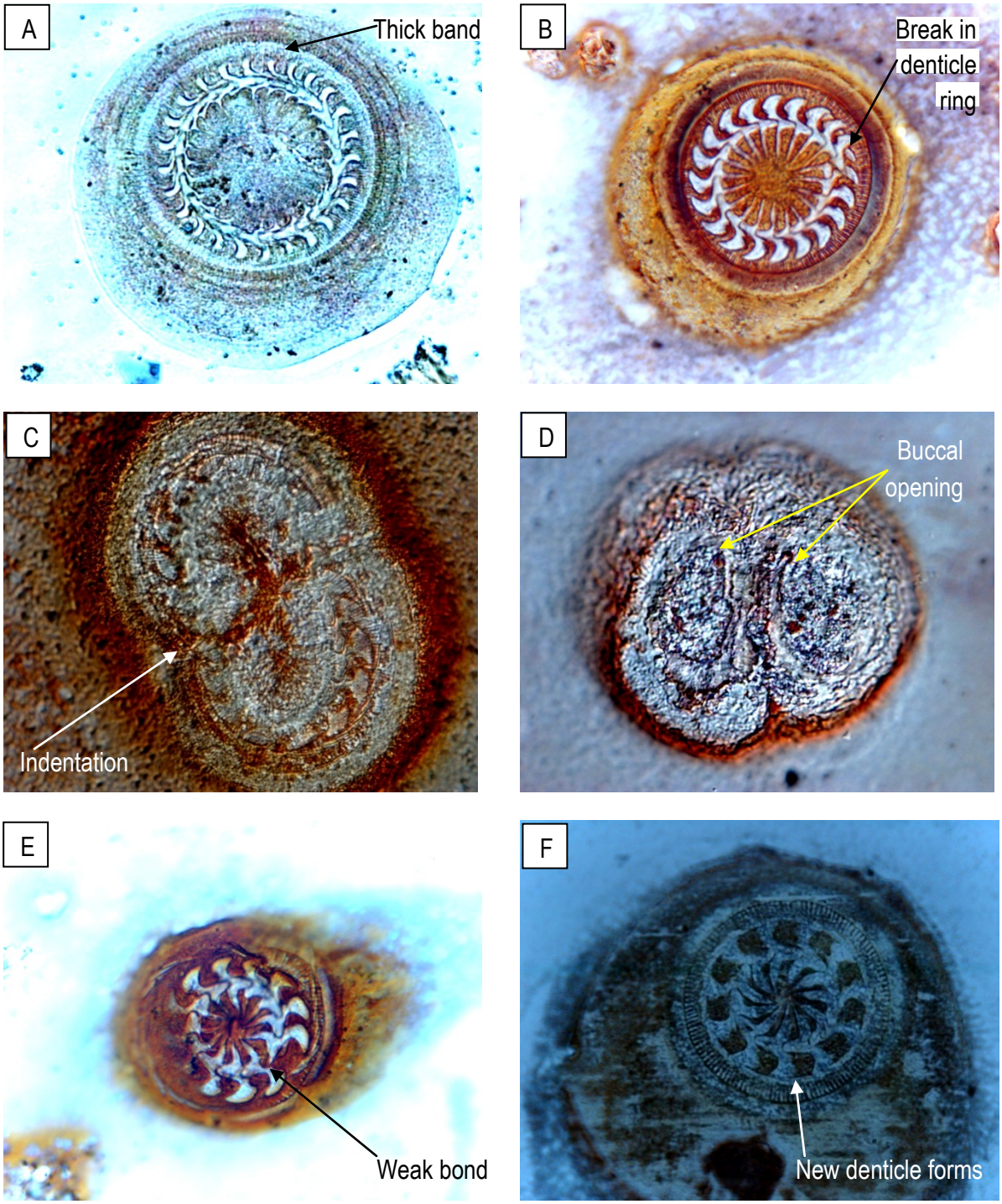


Figure 2.13: Light micrographs of *Trichodina heterodentata* Duncan 1977 collected on the skin and gills of various fish species to illustrate the binary division process of trichodinids. **A**: Start of binary division (note the thick band covering the adhesive disc area); **B**: Overlapping denticles where the denticle ring broke; **C-D**: Formation of two daughter cells (D: view from adoral side); **E**: Daughter cell after division; **F**: Central parts of new denticles start to develop.

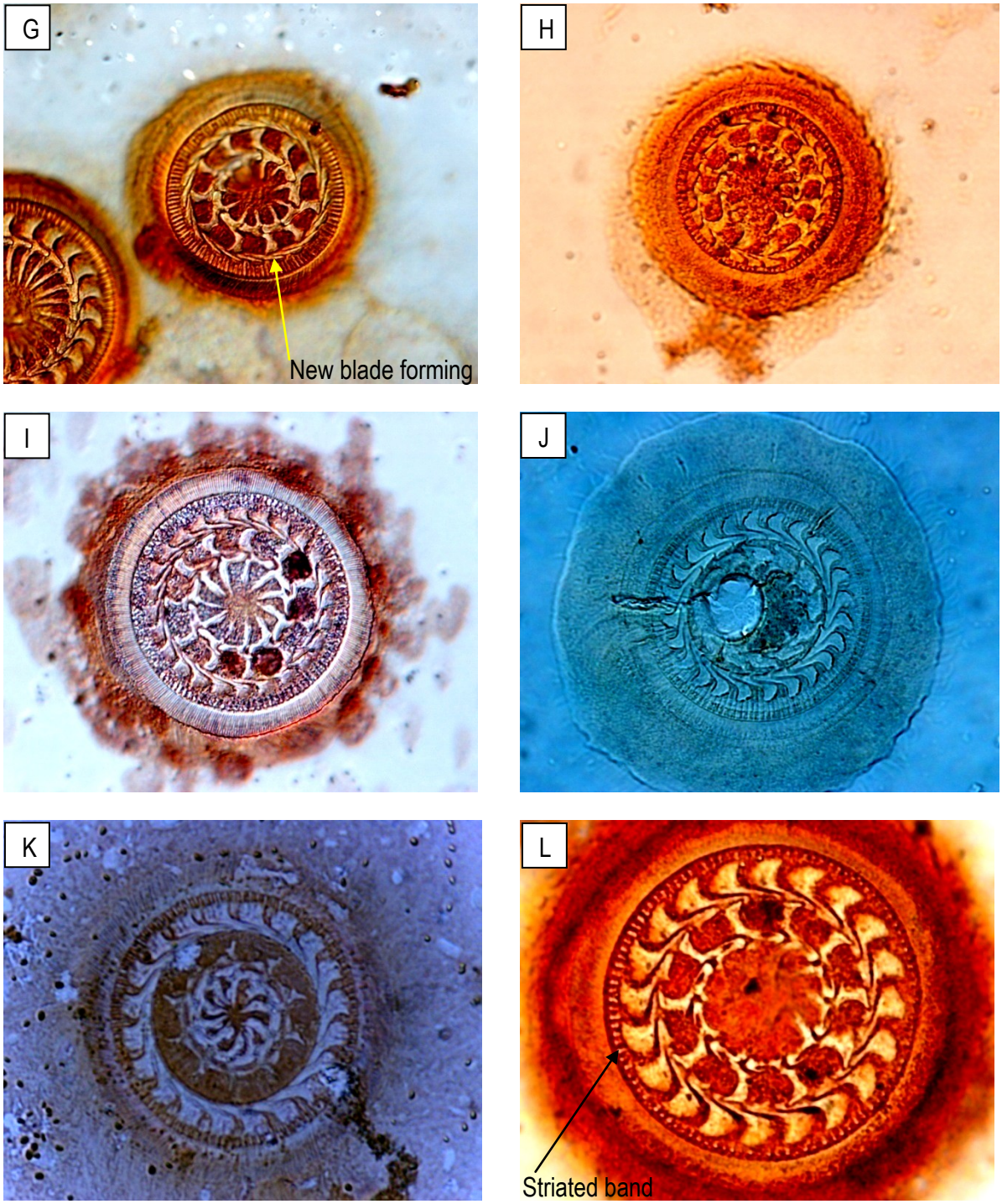


Figure 2.13 (Continue): Light micrographs of *Trichodina heterodentata* Duncan 1977 collected on the skin and gills of various fish species to illustrate the binary division process of trichodinids. **G-L**: Progressive development of blade-area of the new denticles.

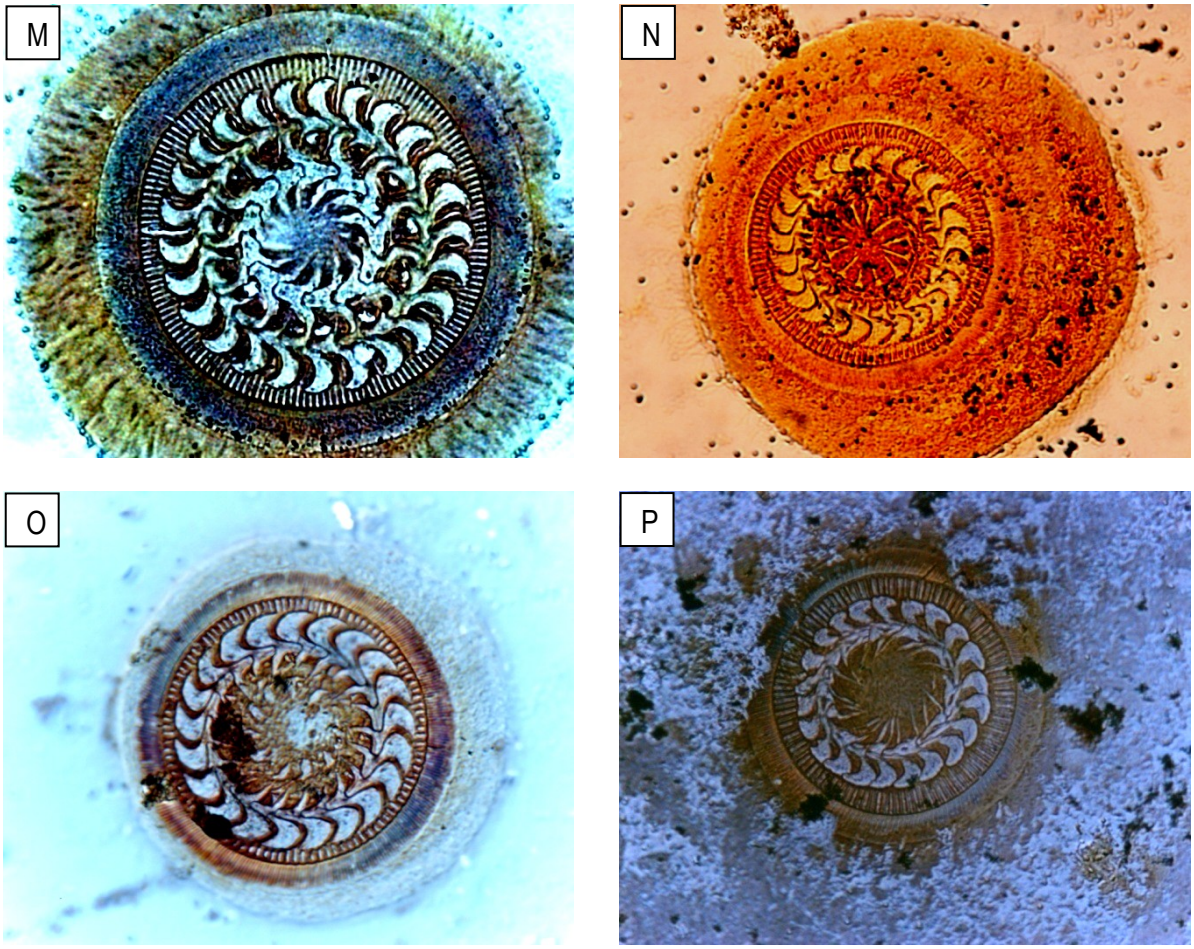


Figure 2.13 (Continue): Light micrographs of *Trichodina heterodentata* Duncan 1977 collected on the skin and gills of various fish species to illustrate the binary division process of trichodinids. **M-N**: Progressive development of blades and rays of the new denticles; **O-P**: Development of new radial pins.

4. Trichodinids and their parasitic / ectcommensal lifestyle

The greater fraction of the more than 270 trichodinid species already described is found associated with freshwater fishes. Trichodinid species utilise many different host species (e.g. fishes, anuran larvae, adult anurans, copepods and molluscs) and Corliss (1979) mentioned that the rim of the denticle ring may apply pressure to some of the underlying cells of the epithelial tissue of the host, causing hyperplasia in heavily infested individuals.

Trichodinids are usually seen as ectocommensals as most trichodinids feed on suspended bacteria and dead cells from its hosts (Diller 1928) while some species are primary pathogens (Noor El Deen and Mohamed 2010). Davis (1947) mentioned that trichodinids are amongst the most common protozoan parasites that cause serious mortalities among trout and pond fishes at hatcheries. In addition, Bruno *et al.* (2006) stated that heavily infested fishes may exhibit epithelial hyperplasia, excessive mucus

secretion and / or epidermal sloughing. The disease caused by ectotrichodinids (i.e. trichodinosis) is mostly known to occur on freshwater fishes (Lom 1970).

Van As and Basson (1987) as well as Kruger (1992) believe that most trichodinid species are not primarily responsible for the epithelial hyperplasia on infested hosts. This is said as other protozoans and / or other parasites such as ich (*Ichthyophthirius multifiliis* Fouquet, 1876) can usually also be found on individuals with hyperplasia and it is known that ich causes severe hyperplasia in infested hosts.

A few authors observed trichodinids representing species commonly associated with fishes on the skin and / or gills of anuran larvae (Table 2.6). However, none of the authors mentioned that the trichodinids seemed to have a negative effect on the tadpoles.

Trichodinid species found on aquatic invertebrates usually comprise different species from those infesting fishes (Van As and Basson 1987). There are, however, a few documented exceptions as *T. pediculus* that was first described on a hydra by Van Leewenhoek (Dobell 1960). The same species is also commonly found to be hosted by many freshwater fish species (Lom 1965). Basson and Van As (1991) noted that *T. diaptomi* Basson and Van As, 1991, a parasite of a calanoid copepod temporarily invaded *Clarias gariepinus* Burchell, 1822 during a fish breeding experiment. However, this cross-transmission experiment was not successful as *T. diaptomi* could not form a viable population on *C. gariepinus*.

Table 2.6: List of some of the trichodinids previously collected from various tadpole species by different authors [information obtained from Diller (1928), Pai (1950), Lom (1961), Chen (1963), Thurston (1970), Kattar (1975), Arthur and Lom (1984), Kazubski (1988), Kruger (1992), Kruger *et al.* (1993), Dias *et al.* (2009) as well as Fernandes *et al.* (2011)].

Trichodinid species	Tadpole host species	Locality	Author
• *T. domerguei* f. *latispina* Dogiel, 1940* • *T. nobillis* Chen, 1963 • *T. reticulata* Hirschmann and Partsch, 1955 • *T. nigra* Lom 1961	• *Rana* species • *Bufo* species	Fish dam in China	Chen (1963)
• *T. pediculus* (O.F. Muller, 1786) Ehrenberg, 1838* • *T. bulbosa* Davis, 1947*	• *Rana* species • *Bufo* species	Shangai	Pai (1950)
• *T. steinii* Claparede and Lachmann, 1858*	• *Bufo ictericus*	Brazil	Kattar (1975)
• Unknown trichodinid species	• *Rana* species • *Bufo* species	Philadelphia Pynnsylvania	Diller (1928)
• *T. acuta* Lom, 1961 (Syn. *T. domerguei* f. *latispina* • *T. nigra*	• *Rana ridibunda* • *R. esculenta* • *R. temporaria* • *Bufo bufo* • *Bombina bombina* • *Hyla arborea*	Czech Republic	Lom (1961)
• *T. hypsilepis* Wellborn, 1967	• Unknown	Cuba	Arthur and Lom (1984)
• Unknown trichodinid species with a denticle number between 19-26	• *Xenopus laevis*	Kajansi, Uganda	Thurston (1970)
• *T. reticulata*	• *Rana temporaria*	Olsztyn (Poland)	Kazubski (1988)
• Unknown trichodinid species	• *Rana esculenta* • *Bufo bufo*	Olsztyn (Poland)	Kazubski (1988)
• *T. heterodentata*	• *Amietia fuscigula* • *Kassina senegalensis* • *Cacosternum boettgeri* • *Xenopus laevis*	Southern Africa	Kruger *et al.* (1993)
	• *Rinella pombali*	Neotropical area	Dias *et al.* (2009) Fernandes *et al.* (2011)

*The validation of these species is questionable as the identification of these species was not undertaken by means of the standard silver impregnation techniques.

4.1 Pathology

The degree of pathological effects of ectotrichodinids usually depends on the number of trichodinids present on the host and the state of the tissue (be it skin or gills) of the host as well as the age of the host. For example, fish populations within hatcheries are usually overcrowded resulting in the reduced mobility of the fish. The reduced mobility facilitates colonisation of the trichodinid population. The limited mobility, competition for food and the occurrence of parasites on the fry thus result in the fry being stressed. Stressed fish are less capable to counteract a trichodinid infestation and heavy infestations of trichodinids are thus mainly diagnosed in young fishes kept within a hostile environment.

To provide evidence for the previous statement, one may turn to work by Paperna and Thurston (1968) and Kazubski (1986) who mentioned that small fishes trapped within residual pools in rivers might be heavily infested with trichodinids. In contrast, fishes (small or large) in larger water bodies might be infested with trichodinids, but that the infestation is usually of a lesser degree on most host individuals. However, a few individuals in the natural habitat may be heavily infested with trichodinids as over dispersion of parasites is known to occur under natural conditions.

4.2 Manner of infestation

According to Davis (1947) and Lom (1961), trichodinids cannot be transmitted by any other means than direct transmission. This is due to the fact that no cyst stage exists (Kruger 1992) and that trichodinids cannot survive for long periods without a suitable host.

4.3 Diagnosis

Davis (1947) mentioned that a severe infested host has a white translucent film covering (i.e. disintegrating epithelial cells) that extends over heavily infested parts of its body. This covering can be observed when the host is viewed under bright light. The covering thickens at certain areas and these can be seen as irregular blotches on the area of infestation (e.g. head, dorsal surface of body, fins, gills) without the aid of a light source. The fins of heavily infested individuals may become badly frayed while the scales may become loosened. A reddish tinge may also be observed on the skin of heavily infested hosts due to obstruction of the blood vessels. Davis (1947) further mentioned that trichodinids are benefited by the reaction of the host as these organisms feed on the epithelial cells formed. The skin is sloughed off in many cases while hemorrhagic areas may also be observed in some cases. The host reacts to the parasitic infestation by becoming sluggish and losing its appetite.

Hyperplasia is not a specific reaction to trichodinids alone as it can also indicate a general irritation that might also be caused by other parasitic groups. As mentioned before, ich may cause a similar reaction to the host as described above and it is not uncommon for trichodinids to occur on a host infested with ich. Therefore, it may not be the trichodinids that causes the white translucent film covering or hyperplasia of the host, but the diagnosis may be a result of other parasitic organisms that irritate the skin of the host (Basson, pers. comm.*).

5. Trichodinids are not free of parasites

Trichodinids are also infected with parasites. For example, Padnos and Nigrelli (1947) mentioned that the suctorian *Endosphaera engelmanni* Entz, 1896 is an endoparasite of *Trichodina spheroidesi* Padnos and Nigrelli, 1942.

5.1 Manner of infestation and reproduction of *Endosphaera* Klebs, 1881

The morphology, infraciliature and life cycle of a member of the genus *Endosphaera* Klebs, 1881 (*E. terebrans* Matthes and Guhl, 1973 hosted by peritrichs such as *Vorticella microstoma* Ehrenberg, 1830, *Epistylis plicatilis* Ehrenberg, 1838 and *E. urceolata* Stiller, 1933 was described by Esteban *et al.* (1991) by means of silver impregnation.

The small larva (swarmer) is attracted by the feeding current of a peritrich and the vortex of the peritrich provides the swarmer with enough impulse to penetrate the peritrich by means of its pointed needle-like cellular projection and cilia (Figure 2.14). The swarmer looses the cilia after penetration and the projection end becomes rounded as the swarmer inserts itself into the host cell. The swarmer turns upside down once it is inserted into the host cell and remains in this position (Esteban *et al.* 1991). The swarmer develops into a sac-shaped form and produces off-spring by means of endogenous budding. The free-swimming larva leaves the host cell through the peristomial disc whereafter the swarmer searches for a new suitable host.

5.2 Diagnosis of an *Endosphaera* Klebs, 1881 infection

One to four *E. terebrans* can occur in deformed host cells (Esteban *et al.* 1991). The infection leads to the enlargement of the infected host cell as well as the displacement and distortion of the macronucleus of the host.

Professor Linda Basson is a professor at the Zoology and Entomology department, UFS where she specialises in aquatic parasitology, including symbiotic associations between ciliophorans such as Trichodinidae and Urceolariidae and various hosts.

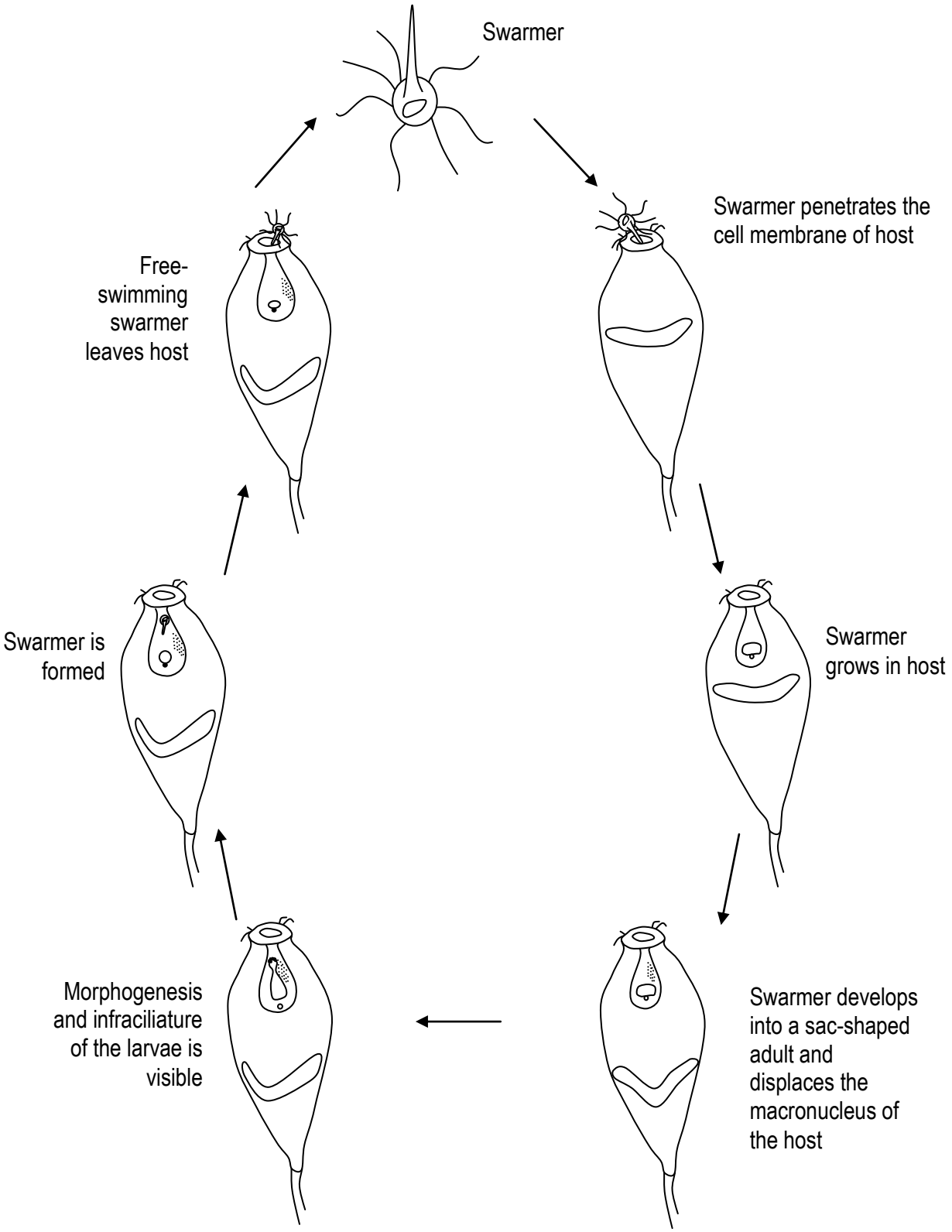


Figure 2.14: Schematic illustration of the life cycle of *Endosphaera terebrans* Matthes and Guhl, 1973 in a protozoan host, redrawn from Esteban *et al.* (1991).

6. Previous research undertaken on trichodinids

The first scientific papers on trichodinids mainly concentrated on the taxonomic or morphological aspects of the organisms. For example, authors such as Dogiel (1940, 1948), Fauré-Fremiet (1943), Fauré-Fremiet and Thaureaux (1944) as well as Uzmann and Stickney (1954) concentrated on the above mentioned aspects. Since the early 1960's, however, mobiline peritrich studies took a turn as authors investigated these organisms on a morphological, systematic, ecological and physiological level (Corliss 1979). As an example, Lom (1958) stated that the taxonomic evaluation of trichodinids could be simplified by using a system containing eleven uniform characteristics. He also proposed that all future trichodinid descriptions should be based on this (now standard) method. Another method used to distinguish between trichodinid species, is worth mentioning here. Van As and Basson (1989, 1992) described a method where three consecutive denticles are drawn and compared with one another. These findings should in turn, be compared with information obtained from other trichodinid populations in order to determine differences within the populations. Please note that the above mentioned methods will be discussed in more detail within the Material and Methods-chapter.

The first scanning electron microscopy (SEM) studies on trichodinid species was conducted by Hamilton-Attwell *et al*. (1981), Sirgel (1983), Arthur and Margolis (1984) and Van As and Basson (1990). However, Kruger (1992) stated that the mentioned studies were relatively unsuccessful as the pellicle that covers trichodinids hindered researchers from examining the internal structures. A method proposed by Van As and Basson (1989) was used by Kruger (1992) to disintegrate the pellicle therefore being able to examine the internal structures of trichodinids without the membrane overlaying the denticle ring.

Fauré-Fremiet and Thaureaux (1944), Fauré-Fremiet (1953), Lom (1973), Hausmann and Hausmann (1981a, 1981b), Maslin-Leny and Bohatier (1984) and Kruger (1992) also made valuable contributions to our knowledge on the ultrastructure of trichodinids. These authors examined the ultrastructure of trichodinids collected from various host species with the aid of transmission electron microscopy (TEM). Another example of an influential study was conducted by Favard *et al.* (1963) who studied trichodinids collected from the urinary bladder of frogs with the aid of TEM.

6.1 Trichodinids from anuran larvae

Favard *et al.* (1963) was not the first to discover that trichodinids make use of anurans as host groups as other authors such as Diller (1928), Pai (1950), Lom (1961), Kazubski (1965), Kattar (1975), Kruger (1992) and lately, Fernandes *et al.* (2011) observed trichodinids on the skin and / or gills of anuran larvae in different countries. For example, *T. pediculus* was first observed on hydra by Van Leewenhoek (Dobell 1960), but is also a common ectosymbiont on some freshwater fishes and also occurs on the skin of various tadpoles (Lom 1960).

Kazubski (1965) found that *T. pediculus* shows a clear preference for the various hosts depending on season as *T. pediculus* appears mainly on hydras during early spring to autumn, but may be transferred to tadpoles or onto fish during these periods. Kazubski (1965) also concluded that these protozoans form an 'open' population on hydras as they move from one hydra to another at any time. It seems as if *T. pediculus* has a preference for hydras, but will also target fishes as hosts if there are no hydras in the water medium. Lom (1961) did not agree with Raabe (1959) who proposed that the vector of transmission of *T. pediculus* onto tadpoles are diaptomids, as the species from crustaceans is not transmissible to either fish or tadpoles. In addition, the trichodinid species hosted by crustaceans is a different species than the trichodinids hosted naturally on tadpoles.

Lom (1961) conducted transmission experiments to determine if trichodinids hosted by fish can form a viable population on tadpoles and vice versa and concluded that the tadpoles developed an infestation, and in most cases a severe one. Lom (1961) also stated that trichodinid species previously collected from tadpoles are trichodinids that are not host specific and that these populations represented species commonly occurring on fishes.

Lom (1961) therefore regarded tadpoles as facultative hosts of fish trichodinids and that the main condition for the development of most ectotrichodinid infestations on fish is the weakening of their host organism through starvation, lack of oxygen, and other unfavourable factors. This takes place especially during winter months so that a greater infestation can be observed on almost all fish during early spring. With the onset of the new season, the health condition of fishes improves, and they cease to be favourable for the reproduction of trichodinids. At this moment, most of the trichodinids detach themselves from fishes in search of a new host. The skin and gills of tadpoles are very suitable mediums for their multiplication as young tadpoles mainly swim in shallower water which may increase

the advantage of warmer water as trichodinids do multiply at a higher rate during these conditions (Lom 1961).

Kruger (1992) stated that trichodinids do not occur on adult amphibian skin and agreed with Lom (1961) that it seems as if the trichodinids detach themselves from tadpole hosts before the metamorphosis process of the host is completed. Kruger (1992) concluded that the trichodinids on tadpoles seems to move to the membranes between the toes as the skin of the tadpole undergoes changes during the metamorphosis process. Lom (1961) and Kruger (1992) further mentioned that the trichodinids eventually leave the host all together.

Interestingly, only a few previous studies concentrated on the collection of trichodinids from tadpole species in southern Africa. The first was by Thurston (1970) who collected infested *Xenopus* Wagler, 1827 tadpoles from Uganda while Sandon (1965) collected trichodinids from the urinary bladder of *X. laevis* Daudin, 1802 but failed to collect any trichodinids from the gills of *Xenopus* larvae in South Africa. On the other hand, Kruger (1992) and Kruger *et al.* (1993) collected various tadpole species infested with trichodinids in South Africa. Even though a few trichodinid species that were previously collected on tadpoles in other countries occur on southern African fish (i.e. *T. nigra*, *T. reticulata*), all the trichodinids hosted on southern African tadpoles were previously described as *T. heterodentata* (Kruger 1992).

6.2 *Trichodina heterodentata* Duncan, 1977

Trichodina heterodentata was first described by Duncan in 1977 as he collected specimens from different cultured fish hosts in the Philippines. The fish hosts were collected from different experimental ponds which received water from an irrigation canal. The collected hosts were placed in concrete tanks by Duncan (1977) for several days before examination took place. This was probably done in order to create a suitable habitat for the multiplication of trichodinids. The holotype sample (Figure 2.15) of *T. heterodentata* was described from trichodinids collected from the skin of *Oreochromis mossambicus* (Peters, 1852) while additional populations were collected and described in the same article from two additional hosts: skin and gills of *Tilapia zillii* Gervais, 1848 and the skin of *Trichogaster trichopterus* Pallas, 1770. Duncan (1977) also stated that a distinctive prominence arising from the central part of the denticle can be observed when the holotype is examined. Duncan (1977) named the species *Trichodina heterodentata* due to the fact that the shape of the denticles showed a considerable variability within the populations and even individuals.

The holotype population of *T. heterodentata* was described by Duncan (1977) as having a:

- Slightly convex body
- Adoral groove that turns ca. 400º
- Body diameter of 85 (71-106) µm
- Centre that impregnates slightly less than rest of disc with poorly to well-defined border
- Denticular ring 32 (26-37) µm
- Denticle number of 23 (20-27)
- Denticle blade that is strongly falcate with notch on anterior edge near base, tip tapers gradually to fine, sharp, backward-directed point, length 4.1 µm
- Slender, sharp rays on smaller individuals
- Length of 6.9 µm
- Width of central part of 3.4 µm
- Denticle length of 8.0 µm
- Prominence present on denticle of 78% of specimens studied arises from centrifugal edge or adoral surface of central part, or from junction of blade and central part, absent or slight on smaller individuals, slight to robust on larger individuals
- 11 radial pins per denticle
- Border membrane width of 2.7 µm
- Macronucleus that is typically U-shaped

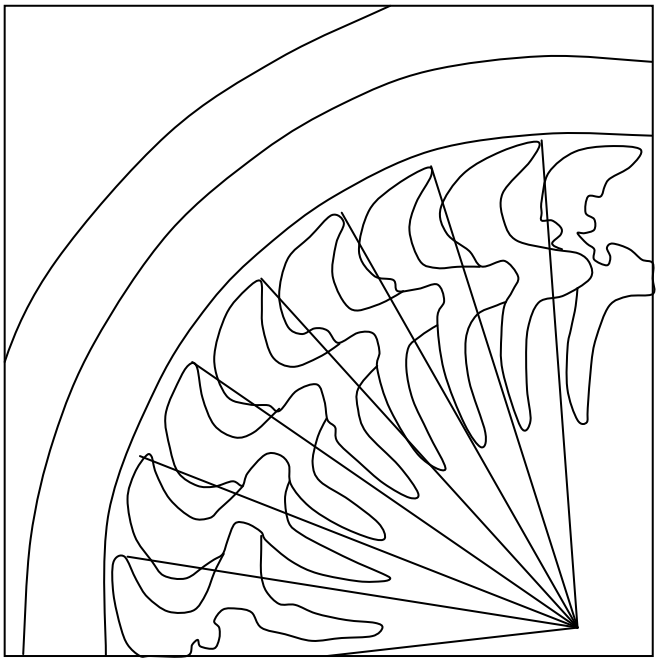


Figure 2.15: *Trichodina heterodentata* Duncan, 1977 as drawn from a light micrograph of the holotype described by Duncan (1977).

In contrast to host specific trichodinids (usually gill parasites) such as *T. centrostrigeata* Basson, Van As and Paperna, 1983 that are associated with Cichlidae Heckel, 1840; *T. reticulata* with goldfish; *T. nobilis* Chen, 1963 and *T. kupermani* Arthur and Lom, 1984 that are usually collected from asian carp (Basson *et al*. 1983; Van As and Basson 1987, 1989; Abaladejo and Arthur 1989), *T. heterodentata* can be found on the skin and gills of many fish species (Dove and O'Donoghue 2005; Martins *et al.* 2010) (Table 2.7).

Table 2.7: *Trichodina heterodentata* Duncan, 1977 was previously collected from various fish hosts [adapted from Dove and O'Donoghue (2005), Martins *et al*. (2010), De Pádua *et al.* (2012) and Miranda *et al.* (2012)].

Host Family	Host species	Reference of first author to collect *Trichodina heterodentata* Duncan, 1977 on the mentioned fish species
Alestidae	*Hydrocynus forskali*	Al-Rasheid *et al*. (2000)
Ambassidae	*Ambassis agassizii*	Dove (2000)
Anabantidae	*Anabas testudineus*	Asmat (2004)
Apogonidae	*Glossamia gilli*	Dove and O'Donoghue (2005)
	G. aprion gilli	Dove (2000)
Atherinidae	*Craterocephalus stercusmuscarum*	Dove (2000)
Balitoridae	*Crassostoma lacustre*	Basson and Van As (1994)
Characidae	*Micralestes acutidens*	Van As and Basson (1989)
	Piaractus mesopotamicus	De Pádua *et al.* (2012)
Cobitidae	*Misgurnus anguillicaudatus*	Basson and Van As (1994)
Cichlidae	*Chetia flaviventris*	Van As and Basson (1989)
	Oreochromis aureus	Van As and Basson (1989)
	O. mossambicus	Duncan (1977)
	O. niloticus	Bondad-Reantaso and Arthur (1989) Van As and Basson (1989)
	Pseudocrenilabrus philander	Van As and Basson (1989)
	Petenia krausi	Kruger (1992)
	Sarotherodon galilaeus	Basson *et al*. (1983)
	Tilapia rendalli	Basson *et al*. (1983)
	T. sparrmanii	Basson *et al*. (1983)
	T. zillii	Duncan (1977)
	Tilapia fry	Basson *et al*. (1983)
	Unknown tilapia species	Basson and Van As (1994)
Clariidae	*Clarias gariepinus*	Kruger (1992)

Table 2.7 (Continue): *Trichodina heterodentata* Duncan, 1977 was previously collected from various fish hosts.

Host Family	Host species	Reference of first author to collect *Trichodina heterodentata* Duncan, 1977 on the mentioned fish species
Cyprinidae	*Acanthobrama* species	Van As and Basson (1989)
	Barbus anoplus	**Current study**
	B. barnardi	Kruger (1992)
	B. eutaenia	Van As and Basson (1989)
	B. marequensis	Van As and Basson (1989)
	B. paludinosus	Basson *et al*. (1983)
	B. trimaculatus	Basson *et al*. (1983)
	Carassius auratus	Basson *et al*. (1983)
	Candida barbata	Basson and Van As (1994)
	Ctenopharyngodon idella	Basson *et al*. (1983)
	Cyprinus carpio	Basson *et al*. (1983)
	Hypophthalmichthys molitrix	Basson and Van As (1994)
	H. nobilis	Albaladejo and Arthur (1989)
	Labeo cylindricus	Van As and Basson (1989)
	Neobola brevianalis	Van As and Basson (1989)
	Puntius gelius	Asmat (2004)
	Sarcocheilichthys nigripinnis	Basson and Van As (1994)
Eleotridae	*Hypseleotris compressa*	Dove and O'Donoghue (2005)
	H. galii	Dove and O'Donoghue (2005)
	H. klunzingeri	Dove and O'Donoghue (2005)
	Philypnodon grandeceps	Dove and O'Donoghue (2005)
Galaziidae	*Galaxias maculates*	Dove and O'Donoghue (2005)
	G. olidus	Dove and O'Donoghue (2005)
Gerreidae	*Gerres* species	Dove and O'Donoghue (2005)
Gobiidae	*Rhinogobius brunneus*	Basson and Van As (1994)
	Glossogobius giurus	Van As and Basson (1989)
Ictaluridae	*Ictalurus punctatus*	Martins *et al.* (2010)
Melantotaeniidae	*Melanotaenia duboulayi*	Dove (2000)
Mugilidae	*Mugil* species	Dove (2000)
	M. cephalus	Kruger (1992)
Osphronemidae	*Trichogaster trichopterus*	Duncan (1977)
Osteoglossidae	*Arapaima gigas*	Miranda *et al.* (2012)
Plotosidae	*Tandanus tandanus*	Dove (2000)

Table 2.7 (Continue): *Trichodina heterodentata* was previously collected from various fish hosts.

Host Family	Host species	Reference of first author to collect *Trichodina heterodentata* Duncan, 1977 on the mentioned fish species
Poeciliidae	*Gambusia holbrooki*	Dove (2000)
	G. affinis	Kruger (1992)
	Poecilia sphenops	Kruger (1992)
	Xiphophorus maculatus	Dove (2000)
Pseudomugilidae	*Pseudomugil signifer*	Dove (2000)
Retropinnidae	*Retropinna semoni*	Dove (2000)
Terapontidae	*Leiopotherapon unicolor*	Dove (2000)

An interesting observation by Basson and Van As (1987) is that this trichodinid species mostly prefer cichlid species as their fish host, but does not appear to be host-specific concerning tadpoles as *T. heterodentata* live on a wide spectrum of tadpoles. For example, *T. heterodentata* seems to be distributed on tadpoles and fishes in the same habitat (Kruger 1992) during spring and early summer months. Therefore, it seems as if there is no preferred host group, when both tadpoles and fishes are available.

6.2.1. Distribution of *Trichodina heterodentata* Duncan, 1977

In addition to its ability to occur on a wide variety of fish and tadpole species, *T. heterodentata* is widely distributed as infested hosts were collected by various authors from many countries, including Venezuela, India and Indonesia (Figure 2.16).

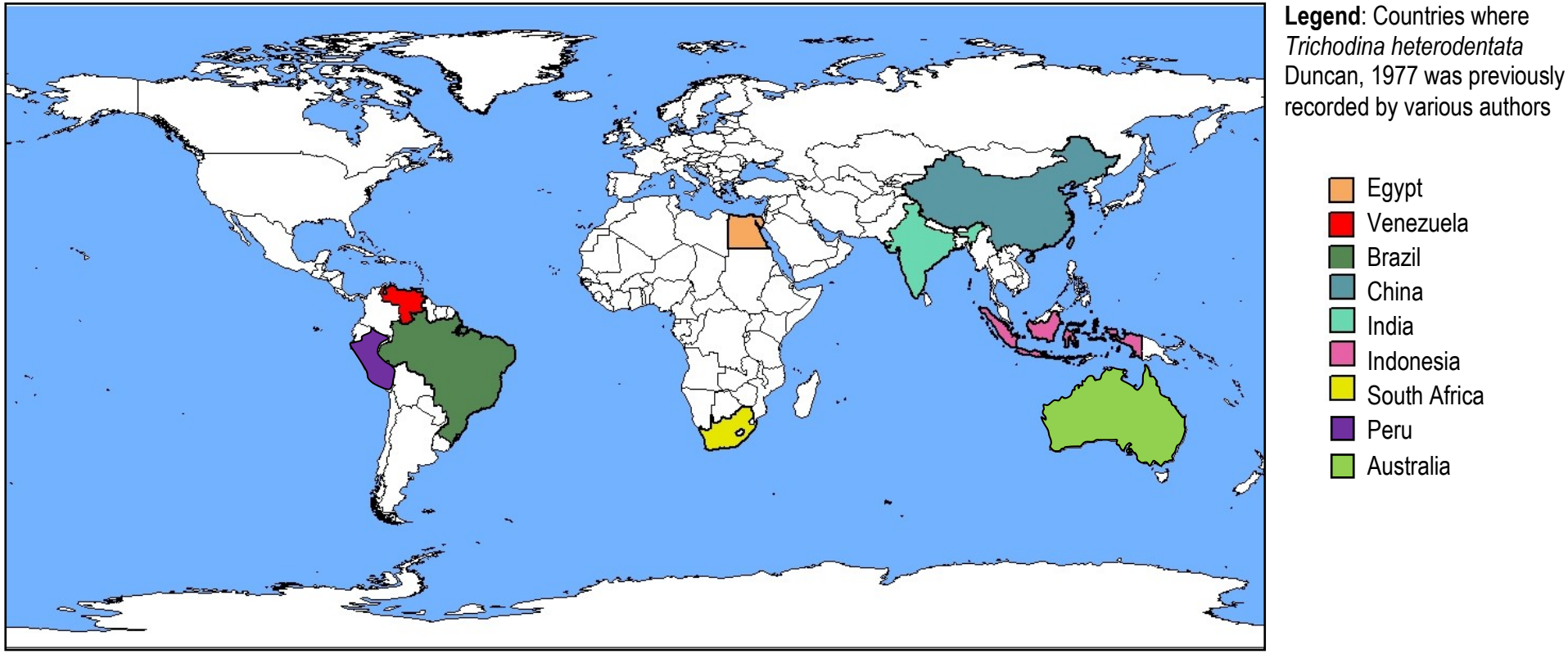


Figure 2.16: *Trichodina heterodentata* Duncan, 1977 was previously collected from various countries, including South Africa, Brazil and Egypt, adapted from Dias *et al.* (2009).

Chapter **3**:

Anuran **H**osts **C**ollected

'…what a wonderful bird the frog are –
When he sit, he stand almost.
When he hop, he fly almost.
He ain't got no sense hardly.
He ain't got no tail hardly either.
When he sit, he sit on what he ain't got – almost…'

From the Rattle Bag, edited by Ted Hughes and Seamus Heaney

Breviceps gibbosus Linnaeus, 1758 was the first African frog to be described as Carolus Linnaeus described a specimen found in the Cape Town region in 1795 (Channing 2001). The first published scientific work on southern African frogs was written by Dr. Andrew Smith and fourteen of the 26 species described in '*The Zoology of South Africa'*, dated 1849, were discovered by Smith himself (Du Preez and Carruthers 2009). According to Channing (2001), Wilhelm Peters described twelve frog species and provided details of frog biology of the specimens primarily collected in Mozambique.

A valuable contribution in South African batrachology was made by a South African scientist, J. Hewitt as he described no less than 24 genera or species of frogs during 1913 and 1937 (Channing 2001). Since then, many scientists gathered an immense amount of information on batrachology in South Africa. A field guide on amphibians in southern Africa worth mentioning was published in 2001 (Channing 2001). This field guide, titled '*Amphibians of Central and Southern Africa'*, contains photographs of adult frogs while including diagrams of tadpoles as a reference for scientists or the public that are interested in southern African batrachology.

One of the first scientists that studied tadpoles in southern Africa was Van Dijk who reviewed tadpoles in southern African (Channing 2001). Du Preez (1996) also made a valuable contribution to our knowledge on tadpoles in southern Africa by publishing a book on the frogs and toads of the Free State as well as his collaboration with Carruthers that led to a book titled '*The complete guide to frogs of southern Africa*' (Du Preez and Carruthers 2009).

1. Classification of frogs in southern Africa

The phylum Chordata (Bateson, 1885) is characterised by the presence of an axial notochord as well as a hollow dorsal nerve cord (Du Preez and Carruthers 2009). The most familiar animals of this phylum belong to the sub-phylum Vertebrata Cuvier, 1812, such as fish, reptiles, birds and mammals. Amphibia Gray, 1825, a class under the sub-phylum Vertebrata usually have two distinct phases in their lives as most of these species have an aquatic larval stage and a terrestrial, adult stage that is characterised by a squat bodied tetrapod that lost its tail upon completion of metamorphosis.

Extant examples of Amphibia can be divided into three orders, namely: Urodela Duméril, 1806 which includes the salamanders and newts, Apoda Pallas, 1775 (caecilians) and the order that includes the frogs and toads of the world, Anura (Fischer von Waldheim, 1813) Gray, 1825. Only specimens of the order Anura occur in southern Africa. Anura is the largest order of amphibians with 32 families known

worldwide, thirteen of these are represented in southern Africa (Du Preez and Carruthers 2009). Even though Africa is known to have a diverse range of animal and plant species, southern Africa have less than 160 species of the world's 5 200 described frog species.

2. Evolution

Amphibians are the evolutionary ancestors of all terrestrial vertebrates and were the first vertebrates to exploit the terrestrial landscape as these creatures emerged from the known water habitat onto dry land more than 350 million years ago (Du Preez and Carruthers 2009) (Figure 3.1). These animals evolved many physical and functional adaptations that aided them in their exploitation of the diverse terrestrial habitats. For example, a skeleton capable of supporting the body's weight in air, evolution of lungs and adaptation in reproductive communication, etc. were necessary in order to accomplish a terrestrial existence (Du Preez and Carruthers 2009).

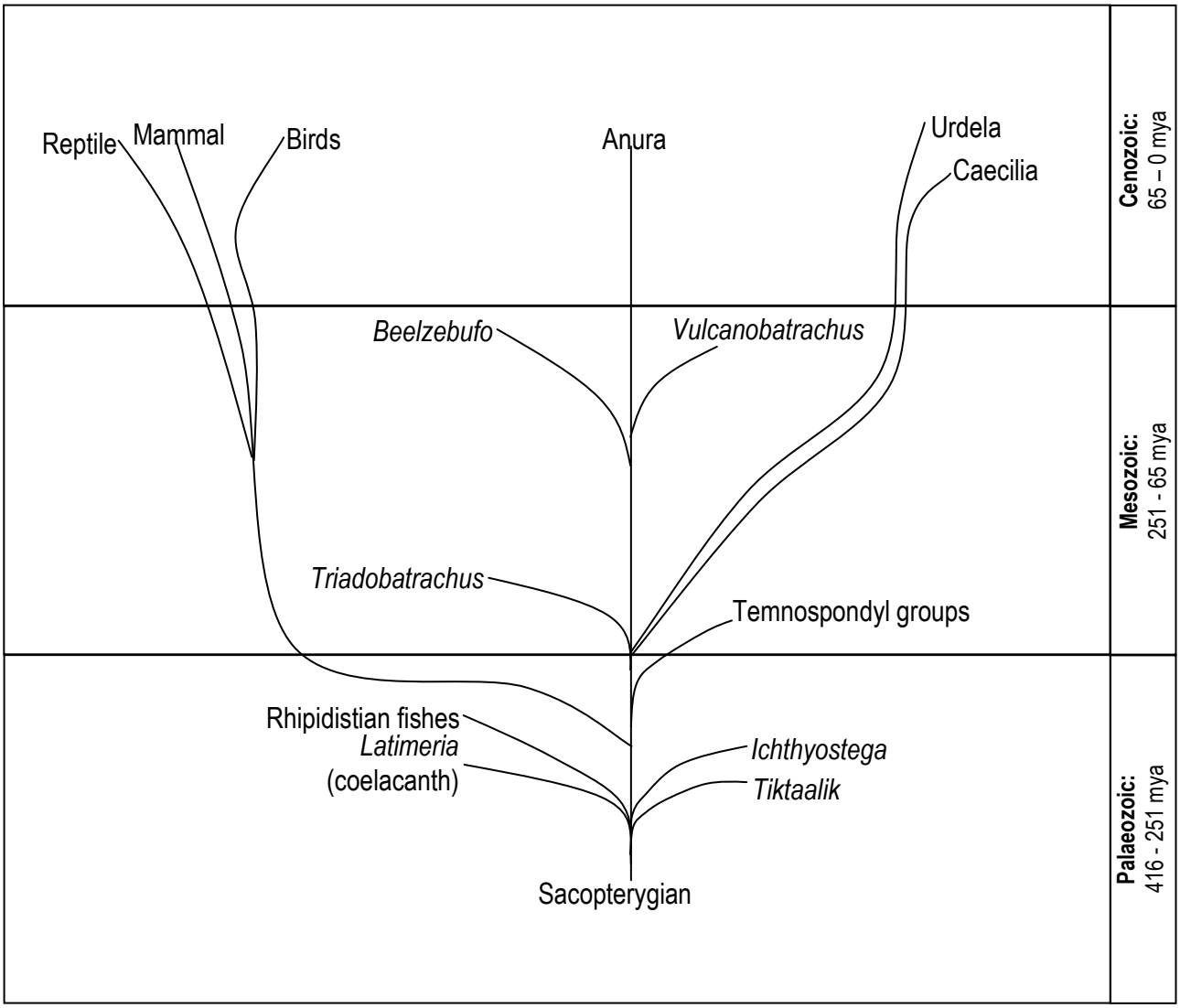


Figure 3.1: The evolution of early tetrapods and the decent of amphibians, adapted from Du Preez and Carruthers (2009).

3. Palaeontological and archaeological discoveries of anurans in southern Africa

One of the oldest fossils found of an anuran in southern Africa is that of *Vulcanobatrachus mandelai* Trueb, Ross and Smith, 2005, collected from Late Cretaceous deposits in the Marydale District, Northern Cape, South Africa (Trueb *et al*. 2005). Van Dijk (1985) described the first specimen collected by diamond prospectors that excavated test pits into a kimberlite pipe on the farm Stompoor, Marydale in the late 1970's. During 1999, Trueb and her team (Trueb *et al.* 2005) recovered more than 200 primarily adult frog specimens of this species from the same farm. In addition, many fossils of tadpoles in various stages of development were also recovered during this period.

On an archaeological level, evidence was found that African bullfrogs (*Pyxicephalus edulis* Peters, 1854) and platannas (*Xenopus laevis*) were used as food resources since remnants of their bones can still be found at various archaeological sites within southern Africa (Channing 2001).

4. Distribution of frogs in southern Africa

According to Du Preez and Carruthers (2009) southern Africa has diverse suitable habitats, climate and topography that contribute to the anuran species richness in the mentioned area. Climate, evolutionary origin of species and range restriction are the main driving forces and / or determinant of distribution patterns of frogs in southern Africa (Figure 3.2).

5. Evolutionary origin of anuran species in southern Africa

Climatic changes during the evolutionary past influenced frog distribution on the southern African subcontinent as periodic warmer temperatures led to the expansion of tropical species to the south, while species already inhabiting the south retreated (Du Preez and Carruthers 2009). In contrast, during cooler periods, many of the tropical species expanded northerly while species inhabiting the north retreated. This led to the isolation of several populations and many of these populations evolved into subspecies or new species.

5.1. Climatic factors

Du Preez and Carruthers (2009) mentioned that climate plays an important role in the distribution pattern of frogs as all amphibians are physiologically dependent on temperature as well as moisture. Therefore, the number of species increases from the dry, western region to the east of southern Africa.

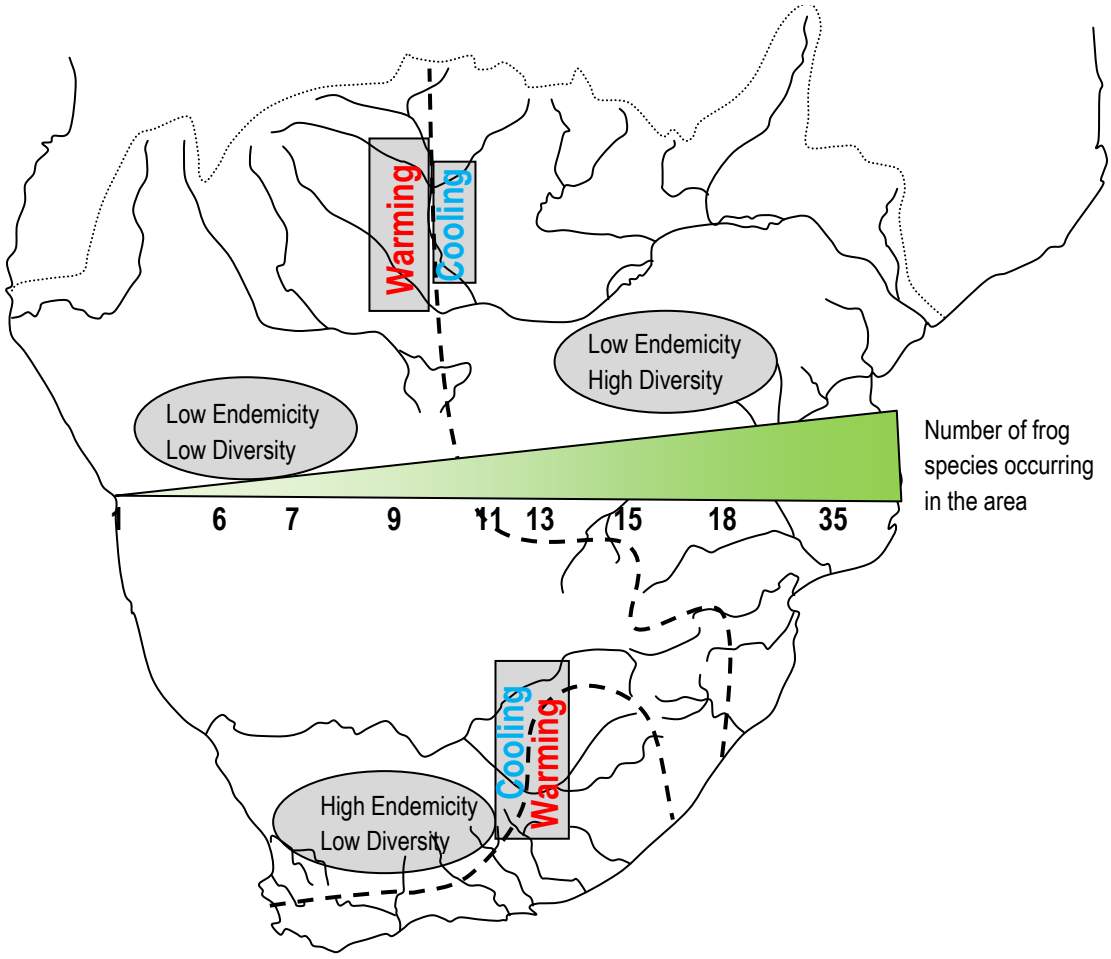


Figure 3.2: The number of frog species that occur within southern Africa increases to the warmer east while a high endemicity and low diversity is to be found in the southern part of southern Africa, adapted from Du Preez and Carruthers (2009).

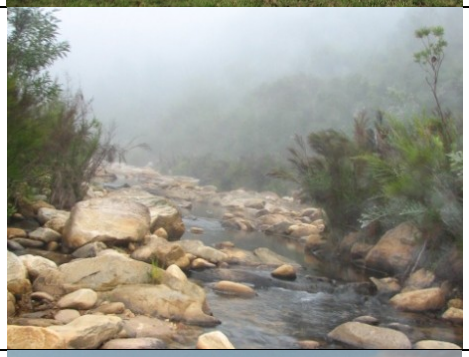
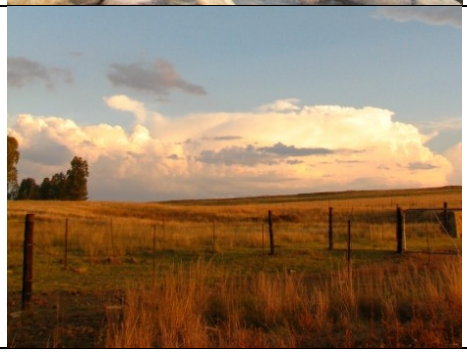
5.2. Range restriction

The topography of southern Africa influences the distribution of frogs as mountains and river valleys act as barriers to the frog populations living in specific areas. As an example, many frog species living in or below the Cape and KwaZulu-Natal escarpments have distribution ranges of less than 20 000 km^2 as these species can be found in isolated topographical areas (Du Preez and Carruthers 2009).

5.3. Habitats

Frogs can be found in a wide range of habitats (Table 3.1) and it is a well-known fact that water is a decisive factor in determining their habitat (Du Preez and Carruthers 2009).

Table 3.1: A list of the habitats utilised by frogs in southern Africa.

Habitat System	
Endorheic systems Some habitats utilised by anurans are endorheic systems. Not fed or drained by water courses as depressions are filled by rainwater or seepage. Evaporation / absorption into substrate deplete water.	
Lacustrine systems Dam or a lake may be classified as lacustrine system. Characterised as water bodies with total area greater than 8 ha in topographic depressions / dammed river channels.	
Palustrine systems Shallow marshland areas with up to 30% covered with emergent hydrophytic vegetation.	
Riverine systems Normally watercourses contained within channel, except when in flood. Examples: rivers, floodplains, perennial streams, mountain torrents.	
Terrestrial systems Ecological systems with no conspicuous standing / flowing water bodies. Examples: sand dunes, open grassveld, forest floors.	

6. Parasites of southern African anurans

Frogs may play hosts to endo- as well as ectoparasitic organisms in southern Africa. One such parasitic group is the mobiline ciliophorans that belongs to the genus *Trichodina* (Du Preez and Carruthers 2009). As an example of a trichodinid hosted by anuran species is *T. xenopodos* that feeds mainly on debris within the urinary bladder of adult *X. laevis* (Kruger 1992). *Trichodina xenopodos* can only be transmitted from one individual to another during amplexus and thus their life cycle is synchronised with those of their host. In contrast, another trichodinid*, T. heterodentata* was previously found on the skin and gills of different tadpoles in southern Africa (Kruger 1992). Kruger (1992) suggested that ecto-trichodinid species cannot survive on adult *X. laevis* skin as no infestation of this trichodinid was previously recorded on adult *X. laevis*.

7. Chytrid

In addition to the mentioned parasites, the fungus *Batrachochytrium dendrobatidis* Longcore, Pessier and Nichols, 1999, also commonly known as chytrid (pronounced 'kit-rid'), can be found on the skin of juvenile and adult anurans. A chytrid infection (chytridiomycosis) destroys the structure and functions of the skin of the host and may eventually lead to the death of the host [Department of Primary Industries, Parks, Water and Environment (DPIPWE) 2012].

7.1. Prevalence / distribution

Weldon *et al*. (2004) examined almost 700 archived specimens of *Xenopus* frogs collected in southern Africa from 1879 to 1999. The authors found a chytrid infection on one of the *X. laevis* specimens collected during 1938 and suggested that the fungus formed a stable infection in southern Africa from the late 1930's before the first chytrid infections were found outside of Africa. The authors further suggested that *B. dendrobatidis* thus originated in Africa and that the trade in *X. laevis* led to the spread of the fungus to other parts of the world.

Chytrid infections are now known to be the cause of mass mortalities of amphibians in North America, South America, Central America, Europe, Australia, Thailand, South Korea and China (Lips 1999, Daszak *et al*. 2003, Herrera *et al.* 2005, Rowley *et al*. 2007, Kusrini *et al*. 2008, Lethinen *et al.* 2008, McLeod *et al*. 2008, Yang *et al*. 2008, Fisher *et al*. 2009 and Wei *et al.* 2010).

7.2. Manner of infection

The fungus is naturally spread from one water medium to the next by means of the translocation of infected individuals (DPIPWE 2012). Johnson and Speare (2005) suggested that the fungus can be introduced to new areas as the fungus can survive and grow in moist soil and on bird feathers.

Humans also play a role in the spread of the disease as the fungus is transported from one habitat to the next by means of infected camping equipment, vehicle tyres, boots and by means of spraying of water containing *B. dendrobatidis* on roads and veld fires. In addition, the international trade of frogs can also be seen as one of the ways that this fungus is spread from one habitat to another. For example, bullfrogs often escape captivity and an infected bullfrog could thus introduce the fungus to new areas (Garner *et al.* 2006).

7.3. Harm from infection

The zoospore-form of the fungus is capable of chemotaxis (directing their movements according to certain chemicals in their environment) and move towards certain molecules (amino acids, proteins and sugars) on the skin of the possible host (Moss *et al*. 2008). According to Symonds *et al*. (2008) the fungus utilises the amphibian skin as a nutrient source by digesting the amphibian cells with the aid of its proteolytic enzymes (aid in digesting long protein chains into short fragments) and esterases (splits esters into acids and alcohol with the aid of water).

A zoospore that reaches a host initiates the reproductive portion of its life cycle as it forms a cyst underneath the surface of the skin. Zoospores that are developed from the zoosporangia may re-infest the host or be released into the surrounding aquatic environment (Berger *et al*. 2005).

7.4. Diagnosis of *Batrachochytrium dendrobatidis* Longcore, Pessier and Nichols, 1999 infected individuals

- Abnormal posture and behaviour
  - Adults sit with hind legs out
  - Wobble or have difficulty when moving
  - Seizures may also occur
- Changes in the skin
  - Discolouration of the skin
  - Swelling of the body
  - Skin may peel

  - Ulcerated skin
- Sudden death
- Abnormal mouthparts in juveniles

7.5. Physiology

Piotrowski *et al.* (2004) suggested that the fungus grows optimally at temperatures ranging between 15-25ºC. However, the fungus can survive at 4ºC, enabling it to survive on the skin of their hosts in environments where the temperature is even lower than 4ºC. It should also be mentioned that the growth tempo of the organism decreases at temperatures above 28ºC.

7.6. Prevention and treatment

Authors such as Brucker *et al*. (2008a) found that amphibians that carry higher levels of the bacterium *Janthinobacterium lividum* Eisenberg, 1891 or *Lysobacter gummosus* Christensen and Cook, 1978 tend to survive a chytrid infection. This is due to the inhibitory effect that these bacteria have on the growth of *B. dendrobatidis* (Brucker *et al*. 2008b) as it produces a sufficient number of violacein that prevent the infection by *B. dendrobatidis (*Harris *et al.* 2009).

There is currently no effective way to treat wild infected frog populations and it is therefore important to prevent further spread of the fungus from infected to uninfected habitats (DPIPWE 2012). This can be achieved by adhering to the following:

- Frogs and tadpoles should never be transferred to another habitat
- If tadpoles or frogs are captured and released, one should release the individuals at the site of collection
- Gear should be kept clean
- All soil should be removed from boots and camping gear and allowed to dry completely before remote areas are visited
- Vehicles (including other equipment and tyres) should be washed and dried before one enters gravel roads within reserved areas
- Never dispose water (drinking water or any type of water) into rivers, creeks, ponds, lakes or moistened muddy areas. It is better to dispose water on a dry stony area
- Do not transfer aquatic organisms (including plants, wet land fill, fish and tadpoles) between frog habitats

The fungus is destroyed on captive frogs by means of effective cleaning and drying of the skin of the infected host. A few disinfectants are also effective in destroying the fungus. Woodhams *et al.* (2003) incubated infected red-eyed tree frogs (*Litoria chloris* Boulenger, 1892) at a temperature of 37ºC and found that the frogs recovered from their infections.

8. The importance of frogs to humans

8.1. Medical importance

Even in the Medieval Medicine time, European medics suggested that frogs were of medical importance (Du Preez and Carruthers 2009). It was believed that tonsillitis could be prevented by hanging a frog around one's neck.

Today, it is still believed that frogs may have a positive influence in the medical world (Channing 2001) as the skin glands of frogs produce a range of substances used to protect themselves from predators and infections. These substances are often analogs of mammalian hormones made of amines, proteins, peptides and steroidal alkaloids. Frogs have an immense potential pharmacological value as their skin secretes antibiotics and painkillers. Most importantly, the animals do not have to be killed to harvest the secretions and these substances can be duplicated in the laboratory once they have been identified.

8.2. Hunting

The poison arrow frogs of South America (Dendrobatidae Cope, 1865) are known for their toxic skin (Du Preez and Carruthers 2009). Indian people of tropical America pierce the frogs with a sharp stick and heat them over a flame. This causes the frogs to exude droplets of poison which are collected and allowed to ferment. Small animals are almost instantaneously paralysed when shot with an arrow previously dipped into the fermented poison.

8.3. Food resource

According to Du Preez and Carruthers (2009), inhabitants from countries such as France, Malaysia and Mozambique utilise frogs as a food source. Du Preez and Carruthers (2009) also stated that the giant bullfrog (*Pyxicephalus adspersus* Tschudi, 1838) and the African bullfrog (*P. edulis*) are eaten in Limpopo as well as in Mozambique.

8.4. Animal trade

During the 1933's, adult *X. laevis* were exported from South Africa throughout the world for its use in pregnancy tests as a female *X. laevis* will produce eggs if injected with urine from a pregnant woman (Du Preez and Carruthers 2009). This test was used until the 1960's when commercial pregnancy tests were made available.

Today, frogs are sometimes kept as exotic pets. This leads to the capture and trade of natural occurring frogs (Du Preez and Carruthers 2009). As rarer species reach the highest prices, threatened or endangered species are under enormous pressure to escape the animal trade.

8.5. Biological indicator

It is a well known fact that amphibians are used as biological indicators of environmental health (Table 3.2). Anurans, in particular, are important for us as human beings in this respect as these animals are very sensitive to environmental deterioration (Du Preez and Carruthers 2009). As an example, the permeable skin of anurans absorbs water and any solvents that the water may contain.

Table 3.2: Morphological, physical and behavioural adaptations of frogs that can be used to study the health state of a given environment, adapted from Du Preez and Carruthers (2009).

Adaptation	Reason
Permeable skin	• Absorbs water, solvents within water
Sequestered tissue contaminants	• Disruption of healthy development of tadpoles (metamorphosis is hormone-driven) may occur if exposed to foreign hormones
Food contamination	• Frogs may swallow heavy metals / contaminated soil / plant material during different stages of their life cycle
Fragmented distribution	• Habitat loss may lead to isolation of frog populations • Higher risk of becoming extinct in area
Climate	• Sensitive to extreme environmental changes • Excessive temperature changes may affect their biology
Lifestyle	• Terrestrial / aquatic environmental changes may be deciphered by examining frogs as frogs are exposed to both environments
Trophic level	• Prey on invertebrates / eaten by wide diversity of predators • Influence wide ecological spectrum

9. Identification of anuran larvae

Tadpoles are generally small and the morphological characteristics of tadpoles within a single genus are usually similar to one another. The identification of tadpoles can be established by rearing the tadpoles and to identify the adult frog after the metamorphosis process has been completed. However, this process is time consuming and it is therefore suggested that a combination of the behaviour,

distribution and morphological characteristics of a specimen is noted in order to identify a tadpole to genus and / or species level.

9.1. Distribution

Field guides such as '*Amphibians of Central and Southern Africa*' by Alan Canning (2001), '*Field guide and key to the frogs and toads of the Free State'* (Du Preez 1996) or '*A complete guide to the frogs of southern Africa'* by Du Preez and Carruthers (2009) can be used to establish which species occurs in a specific area.

9.2. Behaviour and habitat

The behavioural aspects of the tadpoles and adult frogs (if available) in the water medium should not be ignored when one wishes to determine the species of tadpoles that occur within the water medium. Certain species of tadpoles will only be found in certain habitats as most frogs are particular in the type of habitat they lay their eggs. It should also be noted whether the tadpoles school together, live in midwater or are found to be dwelling at the bottom of the water medium as this will also aid in determining species of tadpoles being examined. As an example, *P. adspersus* tadpoles will not occur in deep, fast running water as the adults usually lay eggs within large pans that are filled with water during the rainy season (Du Preez and Carruthers 2009).

During the breeding season, young *P. adspersus* males assemble in a small area in shallow water while the larger (older) males inhabit the centre of the breeding arena. The older males are more aggressive and chase off other males in order to prevent other males from participating in breeding activities. A female will swim along the surface until she reaches a distance of about 3 m from the males. The female will dive and reappear on the surface in the middle of the breeding arena. Amplexus and mating will follow soon after she reappears on the surface. It should be noted that the eggs are fertilised above the surface as a female arches her back to position her vent above the surface. The eggs are then laid in the shallow edge of the pond. It seems as if the eggs hatch almost simultaneously as they usually form large schools of individuals of the same age.

The dominant male practices parental care as these males can usually be seen near the eggs and or tadpoles (Du Preez and Carruthers 2009). These males care for the young by attacking possible predators and digging channels in order to release the young into the main water medium if they were caught up in drying ponds.

9.3. Morphological characteristics of anuran larvae

A hand lens can be used to examine the morphological characteristics of tadpoles collected during a field trip. These characteristics of tadpoles can also be examined by means of a light microscope in the lab (Canning 2001). The latter method is recommended as individuals can be stained by commercial food colouring products. The mentioned colouring process aids in the visibility of the opening of the spiracle as well as the papillae around the mouth (Figure 3.3). It is important to study the mentioned features as an individual can best be assigned to a species by examining its mouthparts.

The mouthparts most often used to describe tadpoles consist of labial teeth rows, jaw sheaths as well as oral papillae. Jaw sheaths are made of serrated keratinised material and it covers the cartilages used to cut food particles. In contrast to the hard jaw sheaths, the fleshy projections at the mouth are called the oral papillae. Each of the labial tooth rows consists of a line of small labial teeth that are imbedded in a tooth ridge (Du Preez and Carruthers 2009).

The labial tooth rows are used to generate the labial tooth row formula (LTRF) for each of anuran species. As part of this formula, the labial tooth rows are numbered from top to bottom. The position (anterior or posterior) of the labial tooth rows is also noted. Gaps in the middle of a labial tooth row (medial gaps) are also indicated by means of paratheses (Du Preez and Carruthers 2009). For example, the LTRF for the tadpole presented by Figure 3.3 is as follows: 3(2-3)/4(1-2). Thus, the formula indicates that the individual has three anterior labial tooth rows of which the two closest to the jaw sheaths have medial gaps and four posterior labial tooth rows of which the two closest to the jaw sheaths have medial gaps.

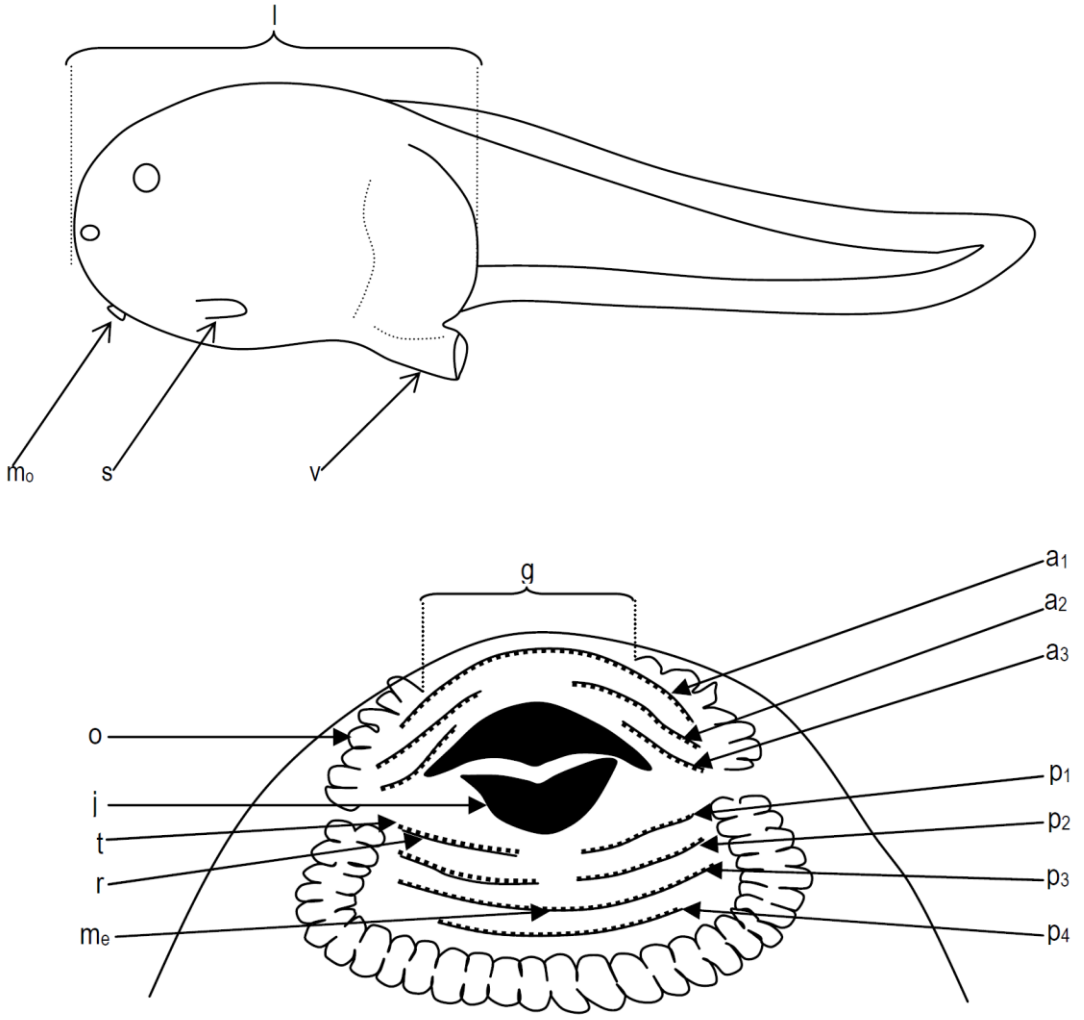


Figure 3.3: Morphological characteristics to be examined to determine the species of tadpole examined, redrawn from Canning (2001).

***Abbreviations*: a_1**: Outer anterior labial teeth row; **a_2**: Second anterior labial teeth row; **a_3**: Third anterior labial teeth row; **g**: Gap in papillae; **j**: Jaw sheath; **l**: Body length; **m_e**: Medial gap; **m_o**: Mouth; **o**: Oral papillae; **p_1**: Inner posterior labial teeth row; **p_2**: Second posterior labial teeth row; **p_3**: Third posterior labial teeth row; **p_4**: Fourth posterior labial teeth row; **r**: Tooth ridge; **s**: Spiracle; **t**: Teeth; **v**: Vent tube

10. Frog tadpoles collected and examined for the presence of trichodinids during the present study

A total of twelve species representing four families of anurans were examined during this study.

10.1. Family: Bufonidae Gray, 1825

Genus: *Amietophrynus* Frost, Grant, Faivovich, Bain, Haas, Haddad, De Sá, Channing, Wilkinson, Raxworthy, Campbell, Blotto, Moler, Drewes, Nussbaum, Lynch, Green and Wheeler, 2006

Amietophrynus Frost, Grant, Faivovich, Bain, Haas, Haddad, De Sá, Channing, Wilkinson, Raxworthy, Campbell, Blotto, Moler, Drewes, Nussbaum, Lynch, Green and Wheeler, 2006, or the typical toads, can be characterised by the adult's dark, horizontal pupils usually with a symmetrical, dorsal pattern. The skin is rough with rounded, wart-like elevations on the back while the underside is granular.

***Amietophrynus gutturalis* Power, 1927 (Figure 3.4)**

- Common name: guttural toad / gorrelskurwepadda
- Occur in suburban gardens, near open pools, vleis, semi-permanent, permanent water bodies in grasslands, farmlands, thickets, savanna areas (Du Preez and Carruthers 2009)

Adult:

- Markings on back arranged in pairs
- Darker brown colour outlines these markings
- Pallid cross marking on head
- Transverse bar runs between eyes (Channing 2001)

Juvenile:

- Hatch within 2-3 days
- Metamorphosis completed within ±75 days (Channing 2001)
- Tadpoles reach a length of 25 mm (Channing 2001)
- Iridescent spots can be observed on black coloured larvae
- Central quarter of tail shaft white in colour (Du Preez and Carruthers 2009)
- Little pigmentation at belly midline
- Sparse / no pigmentation across anterior throat region
- Fairly curved tail; deepest point at middle of tail
- Tail ends in blunt point (Du Preez and Carruthers 2009)

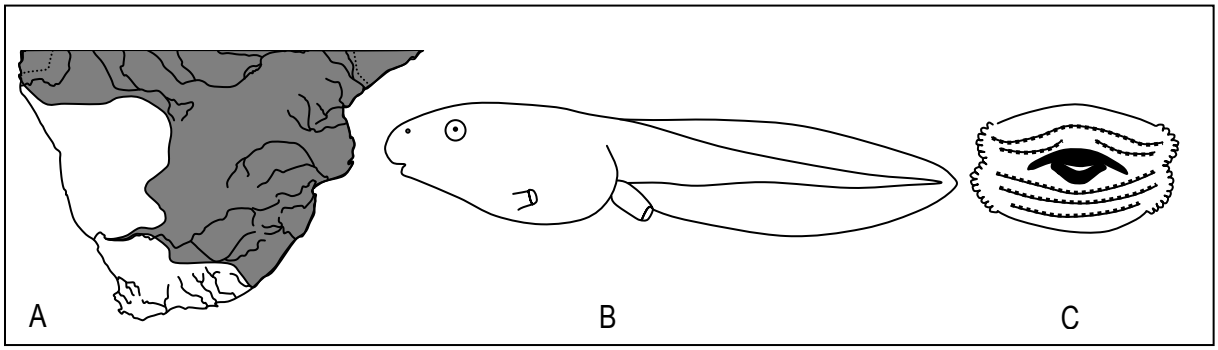


Figure 3.4: Schematic illustration of the distribution (**A**), tadpole (**B**) and mouthparts of the tadpole (**C**) of *Amietophrynus gutturalis* Power, 1927, redrawn from Channing (2001) and Du Preez and Carruthers (2009).

***Amietophrynus poweri* Hewitt, 1935 (Figure 3.5)**

- Common name: western olive toad / Power se skurwepadda
- Adults lives near vleis, pans, thornveld savanna with average rainfall less than 600 mm per annum
- Tadpoles usually found at shallow edges of pools (Channing 2001)

Adult:

- Females reach length of 10 cm
- Back covered with dark tipped warts (Channing 2001)
- Distinguished from *A. gutturalis*: symmetrical paired or almost random pattern of blobs
- Yellow-brown to olive-green background with dark-edged chocolate or reddish-brown patches on back (Du Preez and Carruthers 2009)
- Patches sometimes symmetrical or almost randomly scattered
- Patches not fused at eyes, no patches on snout

Juvenile:

- Metamorphosis period: ± 73 days (Channing 2001)
- Up to 36 mm in length
- Plump, ovoid body (Du Preez and Carruthers 2009)
- Mostly black, lighter or darker variation to match substrate
- Hyaline tail, less than twice body length (Channing 2001)

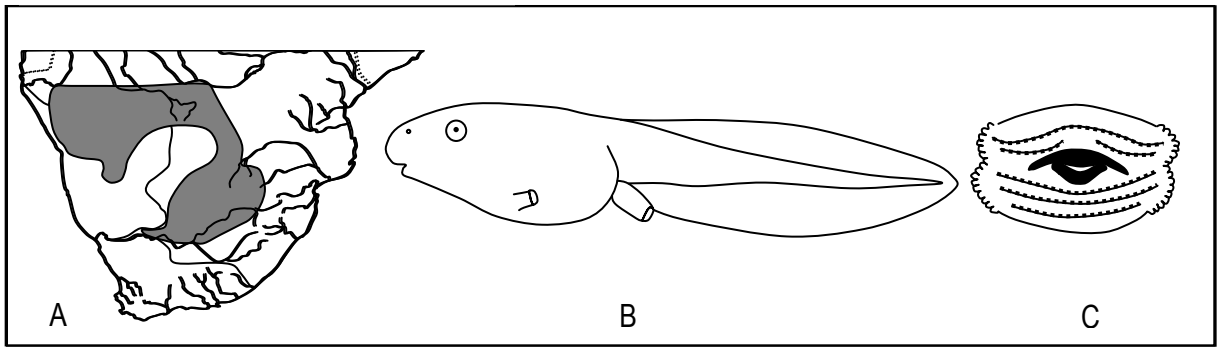


Figure 3.5: Schematic illustration of the distribution (**A**), tadpole (**B**) and mouthparts of the tadpole (**C**) of *Amietophrynus poweri* Hewitt, 1935, redrawn from Channing (2001) and Du Preez and Carruthers (2009).

***Amietophrynus rangeri* Hewitt, 1935 (Figure 3.6)**

- Common name: raucous toad / lawaaiskurwepadda
- Often observed near ornamental fountains, rivers / streams in grassland or fynbos (Du Preez and Carruthers 2009)

Adult:

- Reach body length of 115 mm (Du Preez and Carruthers 2009)
- Back usually olive-grey to brown with irregular shaped patches along the spinal column
- Dark patches behind eyes fused into bar

Juvenile:

- Metamorphosis period: unknown
- 25 mm in length with plump, ovoid body
- Convex tail fin, deepest point anterior to middle of tail
- Tail ends round

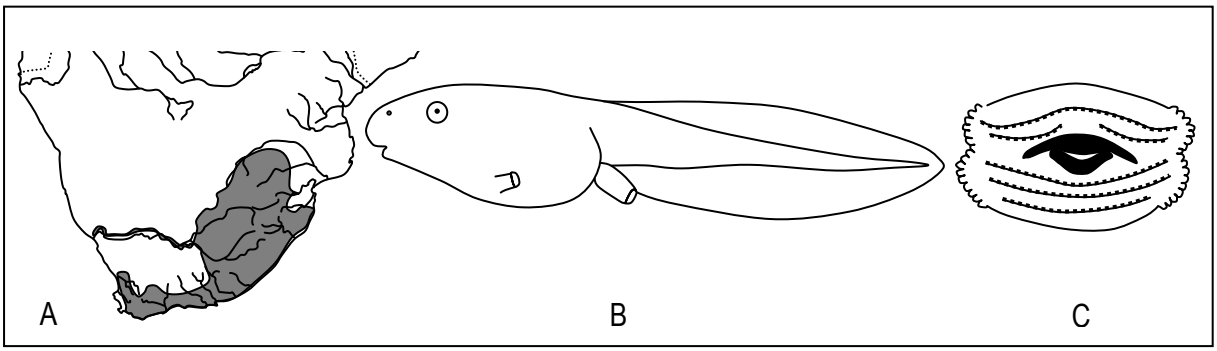


Figure 3.6: Schematic illustration of the distribution (**A**), tadpole (**B**) and mouthparts of the tadpole (**C**) of *Amietophrynus rangeri* Hewitt, 1935, redrawn from Channing (2001) and Du Preez and Carruthers (2009).

Genus: *Schismaderma* Smith, 1848

***Schismaderma carens* Smith, 1848 (Figure 3.7)**
- Common name: red toad / rooiskurwepadda
- One species in genus

Adult:
- Females reach body length of 92 mm
- Glandular ridge runs from tympanum to hind leg (Channing 2001)
- Shoulder markings and brown marks on lower back
- Pale or dark flanks, reddish / light brown back, whitish underside with grey speckles
- Dark glandular ridge on flanks runs from above tympanum to hind leg (Du Preez and Carruthers 2009)
- A pair of small dark markings situated on the lower back, larger markings on shoulder region

Juvenile:
- Metamorphosis period: unknown
- Tadpoles reach length of 35 mm
- Horseshoe-skin flap extends from eyes to middle of body (Du Preez and Carruthers 2009)
- Flap serves as floating mechanism to tadpoles in foul water (Channing 2001)
- Flap contains numerous capillaries, serves as respiratory organ

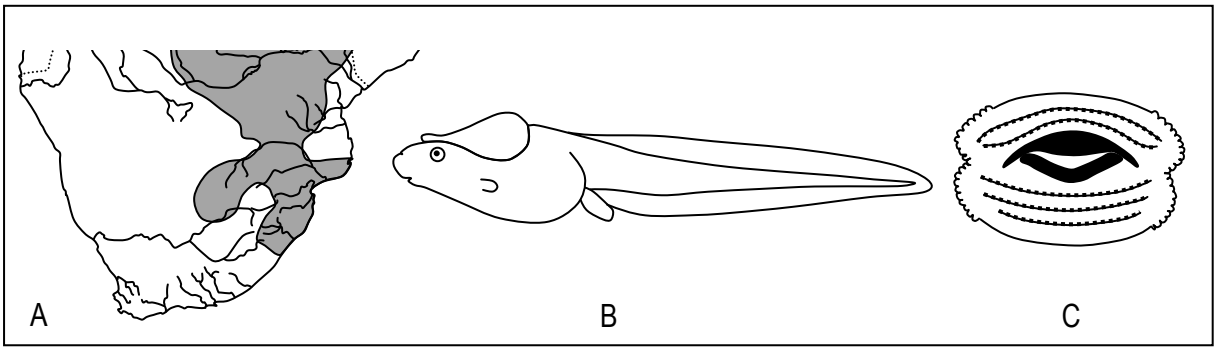


Figure 3.7: Schematic illustration of the distribution (**A**), tadpole (**B**) and mouthparts of the tadpole (**C**) of *Schismaderma carens* Smith, 1848, redrawn from Channing (2001) and Du Preez and Carruthers (2009).

10.2. Family: Pipidae Gray, 1825

Frogs from the family Pipidae are well adapted for an aquatic lifestyle as species from this family spend most of their life in water (Channing 2001). The adaptations include lateral lines on a smooth, streamlined body. Pipidae also have large webbed feet with claws on most of the toes to aid the frog in feeding.

Genus: *Xenopus* Wagler, 1827

According to Channing (2001), six species of *Xenopus* can be found in central and southern Africa, i.e. *Xenopus muelleri* Peters, 1844, *X. gilli* Rose and Herwitt, 1927, *X. petersii* Bocage, 1895, *X. epitropicalis* Fischberg, Colombelli and Picard, 1982, *X. fraseri* Boulenger, 1905 and the common African clawed frog, *X. laevis* Daudin, 1802. The characteristic strong back legs are equipped with large webbed feet that assist the frog in swimming. The claws at the three inner toes are used to shred large food particles that are held in the frog's mouth.

***Xenopus laevis* Daudin, 1802 (Figure 3.8)**
- Common name: clawed frogs / platannas
- Restricted to wetland habitats

Adult:
- Reach length of 147 mm (Du Preez and Carruthers 2009)
- 17 sense organs located around eyes
- Dark brown to grey back, with irregular dark markings
- Underside usually uniform coloured, grey markings on some individuals

Juvenile:
- Metamorphosis period: unknown
- May reach length of 80 mm
- Transparent body, long sensory tentacles (Du Preez and Carruthers 2009)
- Long translucent tail ends sharply
- No keratinised mouthparts
- Maintain head-down position with constant vibration of tail tip (Channing 2001)
- Sensory organs prevent tadpoles from becoming trapped in vegetation due to anterior blind spot

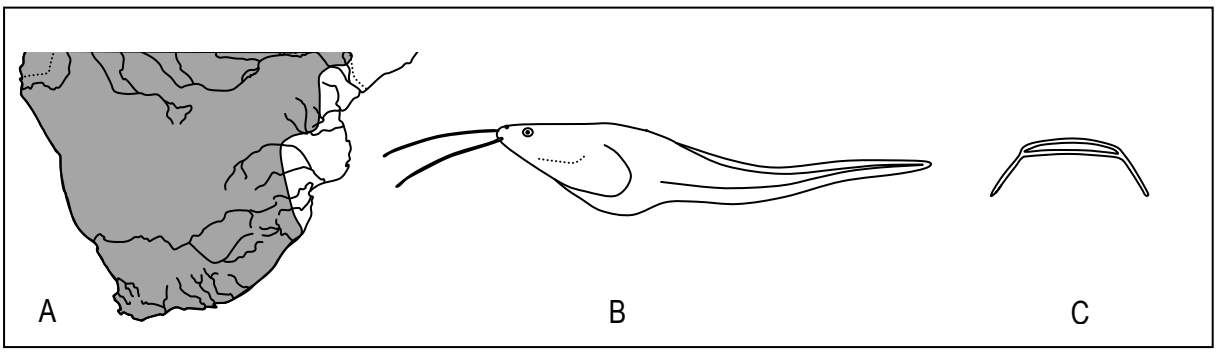


Figure 3.8: Schematic illustration of the distribution (**A**), tadpole (**B**) and mouthparts of the tadpole (**C**) of *Xenopus laevis* Daudin, 1802**,** redrawn from Channing (2001) and Du Preez and Carruthers (2009).

10.3. Family: Hyperoliidae Laurent, 1943

Six genera of Hyperoliidae occur in southern Africa, namely *Hyperolius* Rapp, 1842, *Leptopelis* Günther, 1859, *Afrixalus* Laurent, 1944, *Semnodactylus* Hoffman, 1939, and *Kassina* Girard, 1853. Two species belonging to two genera were collected during this study, namely *Hyperolius parallelus* Günther, 1865 and *Kassina senegalensis* Duméril and Bibron, 1841.

Genus: *Hyperolius* Rapp, 1842

The reed frogs belong to a large genus with 130 species occurring in Africa. Only 16 of these species can be found in southern Africa (Du Preez and Carruthers 2009). The body of these climbing frogs usually have notable colours. Colour variation may occur in populations of single species as a result of geographic isolation, sex and age. The colour of some species may become more vibrant at night. Reed frogs prefer warm areas with high rainfall and well-vegetated habitats.

***Hyperolius parallelus* Günther, 1865 (Figure 3.9)**
- Common name: Angolan reed frog / Angolese rietpadda
- Inhabits rivers, streams in savana (Du Preez and Carruthers 2009)
- Breeds in deep water bodies

Adult:
- Average length of 35 mm
- Back: cream background with red / brown convolute markings (Du Preez and Carruthers 2009)
- Markings on back may blend to form even red back surface
- White underside
- Red inner legs, toes, fingers
- No webbing between last phalanges of longest toe or last 1.5 phalanges of longest toe

Juvenile:
- Metamorphosis period: unknown
- Reach length of 65 mm (Du Preez and Carruthers 2009)
- Robust body, muscular tail with deepest point in middle of tail
- Most of body brown; tail ends in reddish sharp point

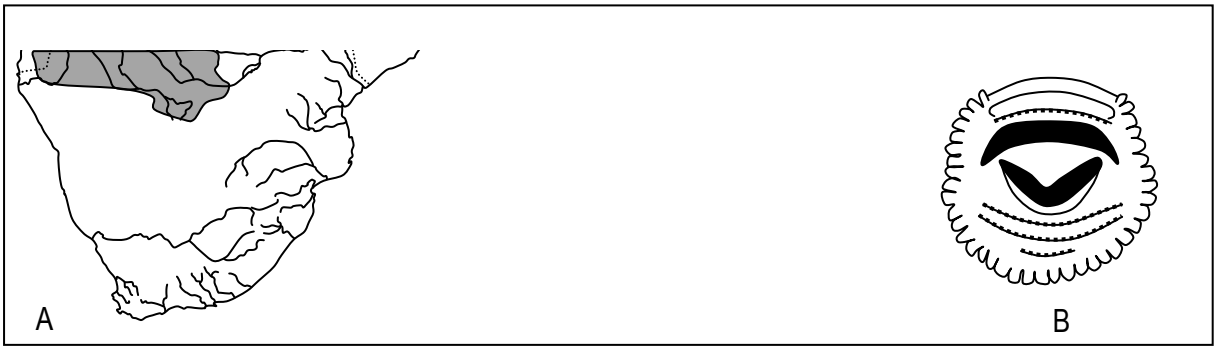


Figure 3.9: Schematic illustration of the distribution (**A**) and mouthparts of the tadpole of *Hyperolius parallelus* Günther, 1865 (**B**), redrawn from Du Preez and Carruthers (2009) and Du Preez (pers. comm.*).

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Genus: *Kassina* Girard, 1853

Two of the 16 *Kassina* species were previously recorded in southern Africa (Du Preez and Carruthers 2009). Kassinas prefer moist regions in the summer rainfall areas.

***Kassina senegalensis* Duméril and Bibron, 1841 (Figure 3.10)**
- Common name: bubbling kassina / borrelvleipadda
- Located in grasslands near vleis, pans
- Deposits 1-20 eggs onto underwater object / dropped to bottom of temporary / permanent water body (Channing 2001)

Adult:
- Relatively large eyes protrude from small body (maximum 49 mm)
- Olive background back colour
- Yellow / silver-beige with solid black band runs along vertebrates
- Black broken band on each side (Du Preez and Carruthers 2009)
- Off-white underside
- Smooth anterior, granular posterior surface

Juvenile:
- Metamorphosis period: unknown
- Dark
- Reach length of 80 mm (Du Preez and Carruthers 2009)
- Upper fin originates perpendicular to eyes
- Reddish tail fin ends in sharp point

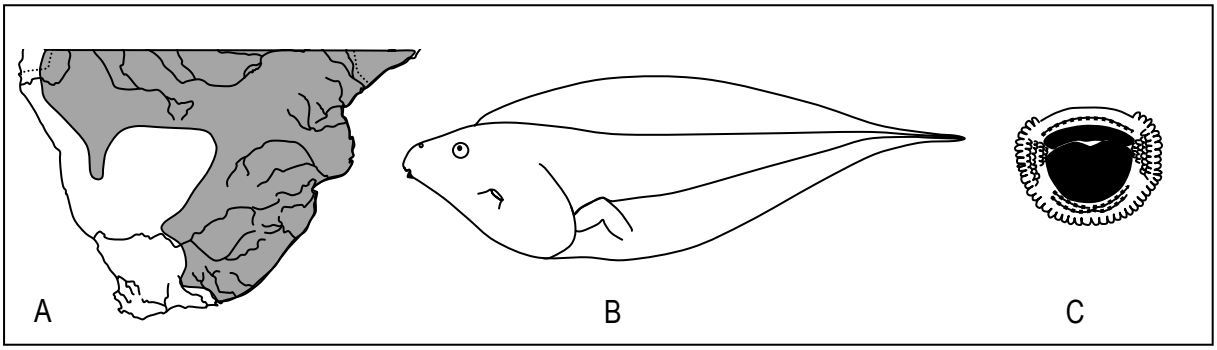

Figure 3.10: Schematic illustration of the distribution (**A**), tadpole (**B**) and mouthparts of the tadpole (**C**) of *Kassina senegalensis* Duméril and Bibron, 1841**,** redrawn from Channing (2001) and Du Preez and Carruthers (2009).

10.4. Family: Pyxicephalidae Bonaparte, 1850

Genus: *Amietia* Dubois, 1987

It should be noted that the genus *Amietia* Dubois, 1987 was previously classified under the family Ranidae Rafinesque, 1814 (Channing 2001). Most *Amietia* species can be observed near water resources (Du Preez and Carruthers 2009). Skin ridges may be observed on the back of adults (Du Preez and Carruthers 2009). However, these skin ridges are broken and do not extend along the full length of the body, as in the case with *Ptychadena* species. *Amietia* species can be distinguished from *Hildebrandtia* and *Hylarana* specimens by examining the length of the tibia as the length of the tibia of *Amietia* species are more than half the length of the body. Tadpoles are usually strong swimmers as they possess muscular tails. The tail is higher than the body (Du Preez and Carruthers 2009). A spiracle can be observed close to the body while the vent is marginal and dextral.

***Amietia angolensis* Bocage, 1866 (Figure 3.11)**

- Common name: common river frog / gewone rivierpadda
- The common river frog inhabits banks of slow-flowing streams or other permanent water resources within grasslands, savannas or forest fringe (Du Preez and Carruthers 2009)

Adult:

- Streamlined body may reach length of 90 mm
- Narrow head houses pointed snout
- Scattered circular dark blotches sometimes observed on green to brown back
- Pale vertebral line often present (Du Preez and Carruthers 2009)

Juvenile:

- Metamorphosis period: unknown
- May reach length of 80 mm
- Body shape oval
- Tail fin widens from behind body, reaches deepest point shortly after middle of tail (Du Preez and Carruthers 2009)
- Tail ends in pointed tip
- Eyes located dorsolateral
- Development usually completed within 9 – 12 months
- When food supply is scarce or water temperature low, development might take longer (up to two years)

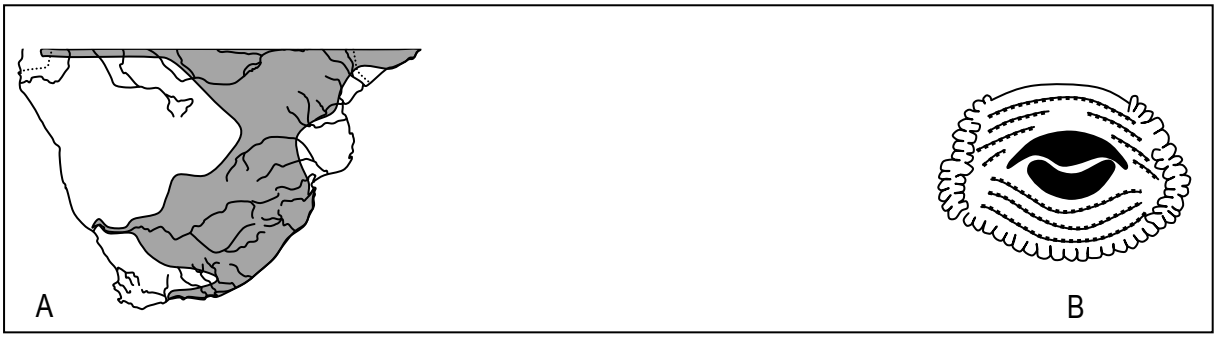

Figure 3.11: Schematic illustration of the distribution (**A**) and mouthparts of the tadpole (**B**) of *Amietia angolensis* Bocage, 1866, redrawn from Channing (2001) and Du Preez and Carruthers (2009).

Genus*:* Cacosternum Boulenger, 1887

***Cacosternum boettgeri* Boulenger, 1882 (Figure 3.12)**

- Common name: Boettger's caco / gewone blikslanertjie
- These frogs occupy habitats in the Nama Karoo, succulent Karoo, grasslands and tickets (Du Preez and Carruthers 2009). These frogs are usually absent from dense forests but may be observed in forest clearings. *Cacosternum boettgeri* aestivate in dry seasons.

Adult:

- Small (max 23 mm) frog, with long neck, narrow head (Du Preez and Carruthers 2009)
- Back colour varies from brown (light or dark) to emerald-green
- Back sometimes coloured stripes, spots or uniformly coloured
- Dark band bordered by skin ridge runs from eye area to base of arm, pale line sometimes observed from below nostrils to base of arm area

Juvenile:

- Metamorphosis period: 37 days in captivity (Channing 2001)
- Metamorphosis completed within more or less three weeks in natural conditions
- Tadpoles may reach length longer than the adults (28 mm)
- Body ovoid, slightly flattened
- Tail fin reaches deepest point in middle of tail (Du Preez and Carruthers 2009)
- Tail ends in fine, rounded tip
- Eyes located dorsolateral
- Small, narrowly spaced nostrils

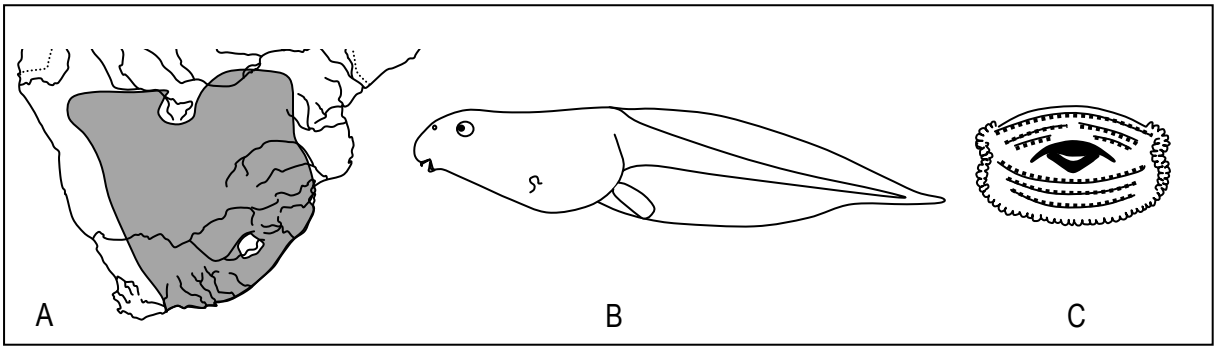

Figure 3.12: Schematic illustration of the distribution (**A**), tadpole (**B**) and mouthparts of the tadpole (**C**) of *Cacosternum boettgeri* Boulenger, 1882, redrawn from Channing (2001) and Du Preez and Carruthers (2009).

Genus*: Natalobatrachus* Hewitt and Methuen, 1912

***Natalobatrachus bonebergi* Hewitt and Methuen, 1912 (Figure 3.13)**

- Common name: kloof frog / kloofpadda
- Only species in genus
- May be observed in dark kloofs, rocky stream-beds in closed canopy areas (Du Preez and Carruthers 2009)

Adult:

- Small (max 37 mm) with slender body (Du Preez and Carruthers 2009)
- Snout pointed, overhanging lower jaw
- Pale triangular parch visible on the snout area

Juvenile:

- Metamorphosis period: within 60 days (Channing 2001)
- Reach length up to 41 mm
- Body oval, streamlined
- Tail fin scarcely higher than body, dorsal fin reaches deepest point in middle of tail (Du Preez and Carruthers 2009)

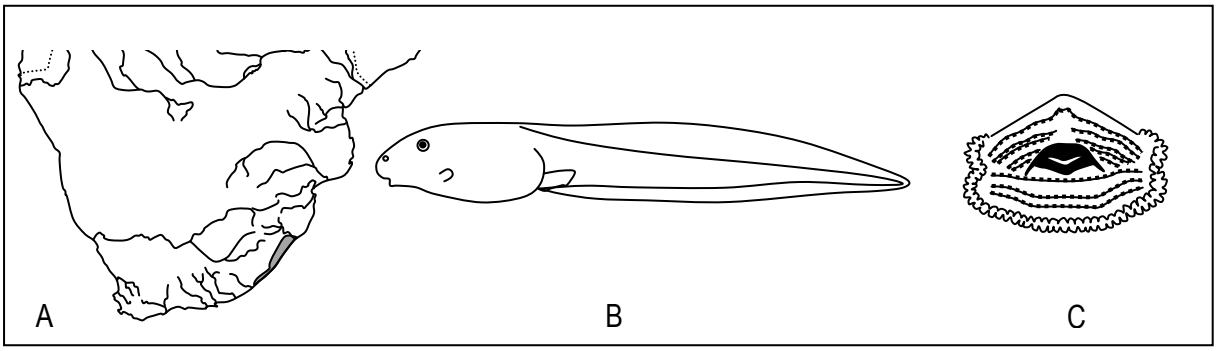

Figure 3.13: Schematic illustration of the distribution (**A**), tadpole (**B**) and mouthparts of the tadpole (**C**) of *Natalobatrachus bonebergi* Hewitt and Methuen, 1912, redrawn from Channing (2001) and Du Preez and Carruthers (2009).

Genus*: Pyxicephalus* Tschudi, 1838

***Pyxicephalus adspersus* Tschudi, 1838 (Figure 3.14)**

- Common name: giant bullfrog / grootbrulpadda
- The giant bullfrog aestivates underground for most of the year (Du Preez and Carruthers 2009)
- Can be found in seasonal shallow grassy pans, vleis or other rain-filled depressions in open, flat areas

Adult:

- Largest frog in southern Africa (up to 245 mm long; 1.4 kg in weight)
- Back usually olive-green
- Grey or brown specimens also common (Du Preez and Carruthers 2009)
- Bluish specimens rare
- *Pyxicephalus adspersus* distinguished from *P. edulus*: *P. adspersus* no white spot on tympanum, no pale interorbital bar present

Juvenile:

- Metamorphosis period: between 18-33 days (47 in captivity) (Channing 2001)
- Tadpoles (71 mm) robust, plump body
- Concave tail fin reaches deepest point behind middle of tail
- Eyes located dorsolateral with nostrils close to eye area (Du Preez and Carruthers 2009)

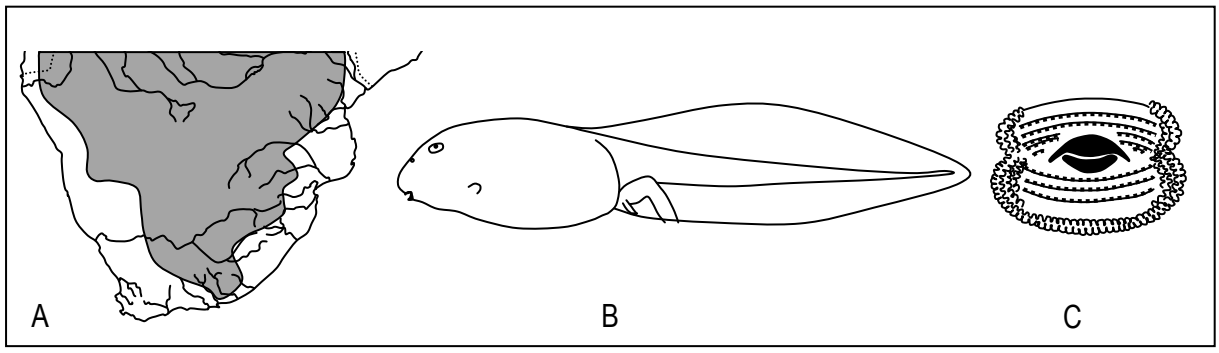

Figure 3.14: Schematic illustration of the distribution (**A**), tadpole (**B**) and mouthparts of the tadpole (**C**) of *Pyxicephalus adspersus* Tschudi, 1838, redrawn from Channing (2001) and Du Preez and Carruthers (2009).

10.5. Family: Ptychadenidae Dubois, 1987

Genus: *Ptychadena* Boulenger, 1918

Grass frogs have a streamlined body with powerful hind legs enabling these animals to jump with giant leaps (Du Preez and Carruthers 2009).

***Ptychadena subpunctata* Bocage, 1866 (Figure 3.15)**

- Common name: speckled-bellied grass frog / spikkelpens-graspadda
- Prefers deep permanent water mediums such as pools, swamps, river backwaters in savana (Du Preez and Carruthers 2009)

Adult:

- May reach 68 mm
- Light to dark brown with pale triangular patch on snout
- Sometimes red infusions behind eyes
- Green or pale cream vertebral band sometimes visible from snout to vent
- Back of thighs two or more longitudinal dark lines below vent that runs from knee to knee (Du Preez and Carruthers 2009)
- Retreat to water when disturbed

Juvenile:

- Tadpoles (55 mm) have shallow body with moderately curved tailfin
- Eyes located dorsal to dorsolateral
- Nostrils narrowly spaced, face anteriolaterally
- Spiracle located below body axis at 45° backwards

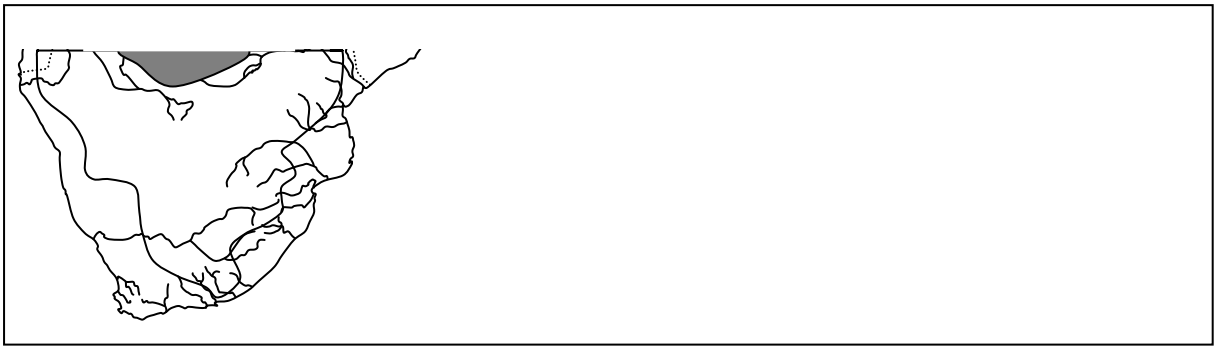

Figure 3.15: Schematic illustration of the distribution of *Ptychadena subpunctata* Bocage, 1866, redrawn from Du Preez and Carruthers (2009).

Chapter **4**:

Fish **H**osts **C**ollected

'…the earth provides enough to satisfy every man's needs,
but not every man's greed…'

Mohatma Gandhi

McCarthy and Rubidge (2005) proposed that the cut off between the Kalahari-Zimbabwe Axis caused the formation of the interior Lake Makgadikgadi. The Kalahari River captured the Karoo River as the Transvaal-Griqualand Axis was uplifted during the same era as the formation of Lake Makgadikgadi. McCarthy and Rubidge (2005) mentioned that this process led to the formation of the Orange River system.

Skelton and Cambray (1981) suggested that the Orange River was formerly two separate river systems as the upper Orange drained the south western area via the Olifants River. The second river system (i.e. the lower Orange) had an enlarged northern drainage. The current Molopo River and its tributaries are remnants of the lower Orange River System.

Tweddle *et al.* (2009) proposed that arches started forming in the interior of the African continent and that it was followed by a series of events resulting in the formation of the Kalahari Basin, a depression in the interior of southern Africa. Tweddle *et al*. (2009) further mentioned that it is generally accepted that an early large river system flowed south-west from the Lake Bangweulu region in Zambia, into the Kafue, Upper Zambezi, Okavango and Kunene Rivers. The Okavango Delta-Makgadikgadi region received water from the above mentioned rivers, forming a large central lake.

The upwelling of cold water on the west coast caused arid conditions in the western parts of southern Africa. Together with the foresaid, the progressive capture of inflow by the Zambezi River caused the drying up of lakes in the Kalahari Basin 14 m.y.a. During this period, the Zambezi captured rivers such as the Kafue as well as the Kwando. However, the Kunene River and the Kaufe River broke away from the central complex of rivers (Tweddle *et al*. 2009). The rift related faults blocks water from the northern parts of Botswana and thus prevents another diversion by the Zambezi River (McCarthy and Rubidge 2005), leading to the formation of the current river basins of southern Africa (Figure 4.1)

The above description on the evolution of the southern African river systems partly explains the distribution of fishes within the current study area.

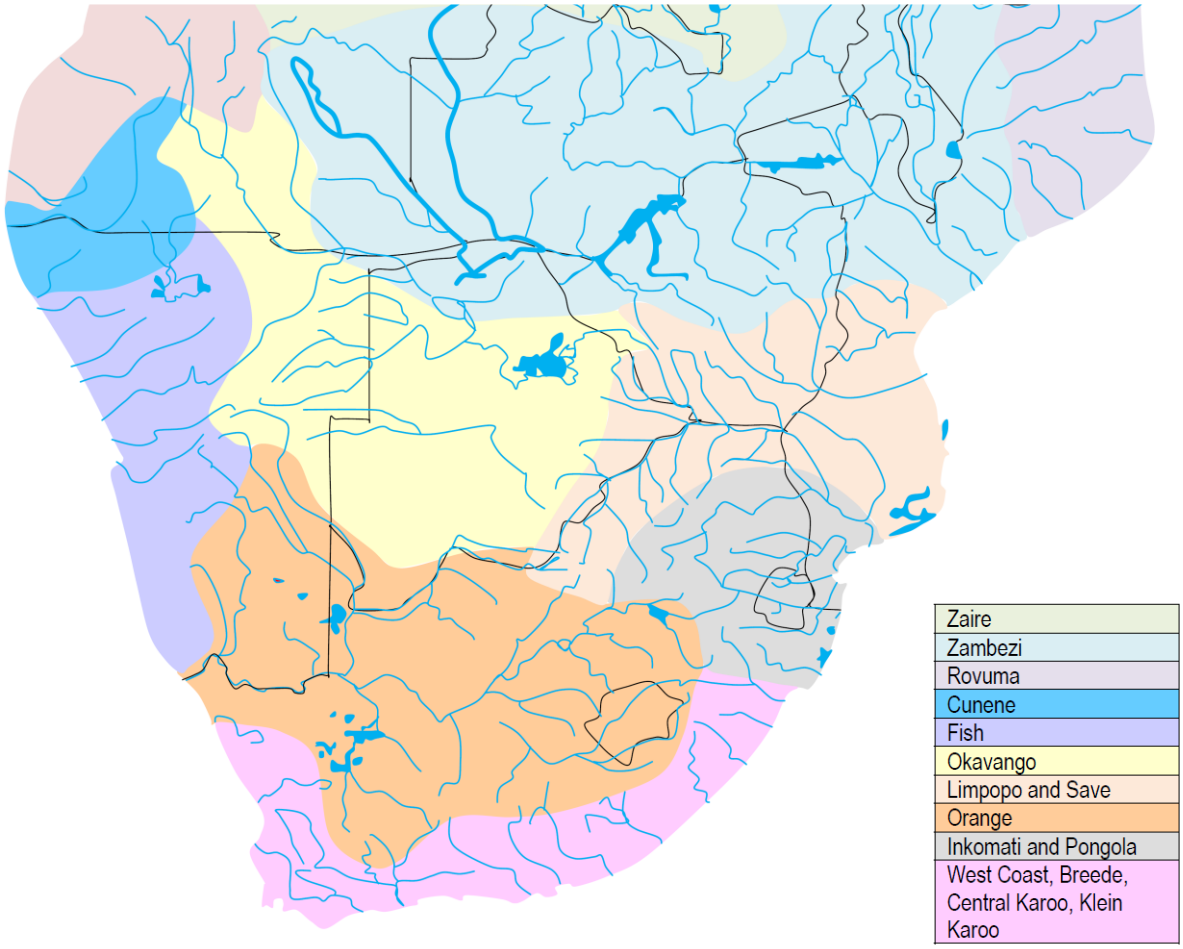

Figure 4.1: Map indicating the current major river basins of southern Africa, redrawn from Conley (1996).

It is interesting that the first freshwater fish from southern African waters was described more than 26 years after the first description of a frog in this area was noted. William Burchell was the first to publish information on the freshwater fishes in southern Africa as the smallmouth yellowfish [*Labeobarbus aeneus* (Burchell 1822)] and the sharptooth catfish (*Clarias gariepinus*) were described in his book '*Travels in the interior of southern Africa'* (Skelton 2001). In addition to Smith's valuable contribution to our knowledge on frogs in southern Africa, he also described many freshwater fish species in southern Africa. Other noteworthy contributions on freshwater ichthyology in southern Africa were made by Castelnau and Peters (Skelton 2001). Gilchrist and Thompson wrote the first comprehensive catalogue on southern African freshwater fishes (Gilchrist and Thompson 1917). The first field guide on freshwater and marine fishes in South Africa was published in 1947 (Skelton 2001). Twenty years later, Jubb (1967) published a book worth mentioning, namely '*Freshwater fishes of southern Africa*' and he also introduced many scientists such as Skelton to the world of ichthyology. Skelton himself is a well-known

fish taxonomist and the author of the '*Southern African Red Data Book – Fishes*' (Skelton 1987) and the '*Complete guide to the freshwater fishes of southern Africa*' (Skelton 1993; 2001).

1. Classification and composition of freshwater fishes in southern Africa

Southern Africa covers more than a sixth of the African continent (Skelton 2001). However, southern Africa hosts less than 10% of the 3 000 African fish species (Table 4.1) and about 145 of the primary and secondary freshwater species are endemic to southern African water resources.

Table 4.1: Number of fish species occurring in southern African freshwater resources, adapted from Skelton (2001).

Grouping	Number of fish groups occurring in southern Africa freshwater bodies
Families	39
Genera	105
Species	208
Primary freshwater	179
Secondary freshwater	61
Alien fish species	24
Diadromous	5
Peripheral marine species	11
Sporadic marine species	23

According to Skelton (2001) the general pattern of fish distribution in southern Africa is determined by historical events, such as the capturing of one river by another. As an example, it is proposed that the Cape and Karoo fauna reached the Orange and Cape coastal rivers during an invasion in the mid-Pliocene period while another invasion occurred during the late Pliocene when a connection between the Okavango-upper Zambezi and the Limpopo basins most likely existed. A third invasion may have occurred along the coastal region that linked the Zambezi and Limpopo basins during the Pleistocene period. The last invasion linked the Orange and Limpopo basins during the medieval era (Skelton 2001).

The fact that the closest relatives of the Cape galaxia (*Galaxias zebratus* Castelnau, 1861) are found in South America, Australia and New Zealand, can be used as an indication that the southern fish species occurred in southern Africa prior to the separation of the supercontinent Gondwanaland (Skelton 2001). It is also proposed that some species may have occurred in the region before the separation of India.

With the above in mind, it is highly unlikely that the fish species occurring in southern Africa originated further north in Africa. Skelton (2001) also stated that the presence or absence of species on a local level is dependent on ecological factors such as temperature.

The majority of freshwater fishes in southern Africa belong to the Ostariophysi (including the catfishes, barbs, labeos and knerias) (Figure 4.2) even though the more evolutionary advanced Perciformes (including cichlids, gobies and mullets) is worldwide the largest and most diverse group of modern fishes. According to Skelton (2001) the order Perciformes includes no less than 7 800 species, classified within 150 families.

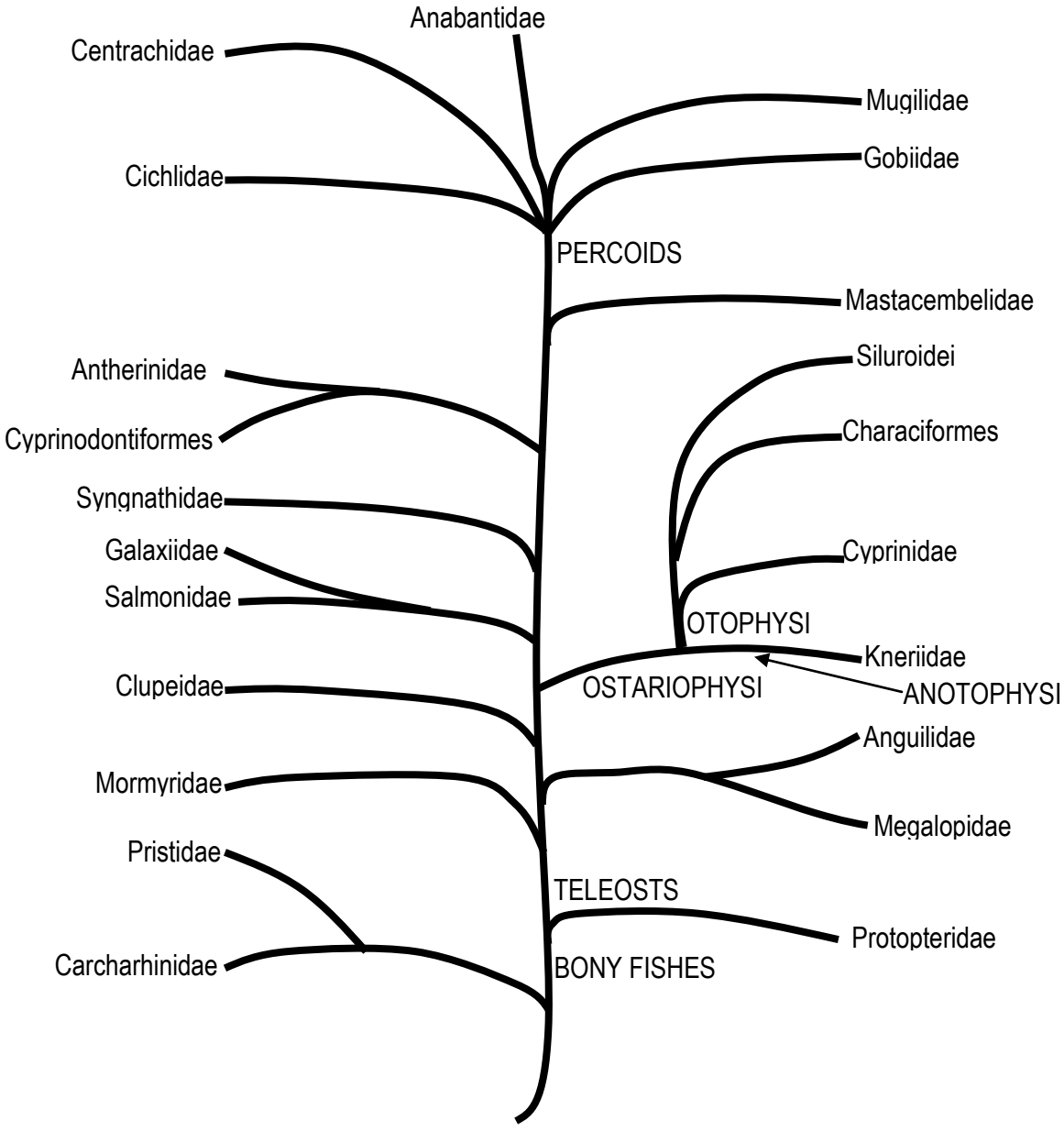

Figure 4.2: Major groups of fishes that occur within southern African freshwater resources, redrawn from Skelton (2001).

2. Fishes and their parasites

Many parasites make use of fishes as hosts during at least one stage of their life cycles. Annually, some of these parasitic species cause great financial losses within the aquaculture industry and aquarium trade within southern Africa (Skelton 2001). As an example, Ich (also known as white-spot disease) is caused by a protozoan parasite named *Ichthyophthirius multifiliis* and debilitates the individual as it burrows itself under the skin of the host. Another well-known protozoan that is hosted by fishes form part of the Trichodinidae family (Kruger 1992). The disease trichodinosis, caused by representatives from the Trichodinidae family was described in Chapter 2.

3. The importance of freshwater fish to humans

3.1 Fish as a food resource

Archaeologists found that the early San and Khoi made use of fishes as a food resource as fish remains are commonly found near the riverside middens (Skelton 2001). Traditional fishing activities in southern Africa takes mostly place in the tropical regions. The fish communities in the large rivers such as the Okavango River in Botswana are sufficiently large. The local communities near the Okavango River make use of the rich fish resources and uses both active and passive methods to capture fish as traps, fences, funnels, rods and lines and woven grass or reed scoops. Plunge baskets are also used for this purpose. All fish species caught, whether big or small, are usually enjoyed by these communities. Skelton (2001) stated that 840 tonnes of river fishes are harvested annually by local communities in the Okavango Delta and the Kavango.

3.2 Recreational purposes

According to Skelton (2001) angling can be seen as the largest participator sport in southern Africa and about 20% of indigenous freshwater fishes are suitable angling species. On the other hand, trout [*Oncorhynchus mykiss* (Walbaum, 1792) and *Salmo trutta* (Linnaeus, 1758)] is popular in the fly-fishing industry.

3.3 Aquaculture

Many fish species, including *O. mykiss* and *Clarias gariepinus* are successfully cultured for agricultural purposes in South Africa (Skelton 2001). About 80 species of 'ornamental' fish (e.g. gouramis and goldfish) are also cultured in South Africa for the local and international markets.

4. Fishes collected and examined for the presence of trichodinid infestations during the present study

Fishes collected during the study was identified by means of a key proposed by Skelton (2001). The fin ray formula (Skelton 2001) played a valuable contribution to the identification of the collected fishes. The mentioned formula collaborate features of the spines, segmented rays, branched rays, dorsal fin, and the anal fin in order to differentiate between different species. It should be mentioned that the spines are designated by means of roman capital letters while the segmented rays are designated by roman lower case letters. Branched rays, in turn, are given by arabic numerals. The letter D is a prefix for the description of the dorsal fin while the anal fin is prefixed A.

In addition, the lateral scales are counted along the lateral line row, if present. In cases where the mentioned row is absent, the lateral scales should be counted along a comparable row. The lateral line count is determined by taking all scales over the base of the caudal fin (at the articulation or line of flexure) into account. Abnormal small scales or peculiar forms are not considered during the above mentioned counting process.

When the lateral line count is to be determined for cichlid and anabantid species, one should take the division of the lateral line into account. In these cases, one should count all the pored scales in the upper line. Pored scales occurring on the lower line should also be counted. However, only pored scales following directly below the last pored scale of the upper line should be counted.

As an example, the mosquetofish *Gambusia affinis* (Baird and Girard, 1853) (Figure 4.3) has the following scale formula: D 6-8; A 9-10; Lateral line scales 29-30. In other words, the dorsal fin has three segmented rays and eight branched rays while the anal fin also has three segmented rays. However, the anal fin contains only five branched rays and 29 – 30 pored scales could be counted on the lateral line.

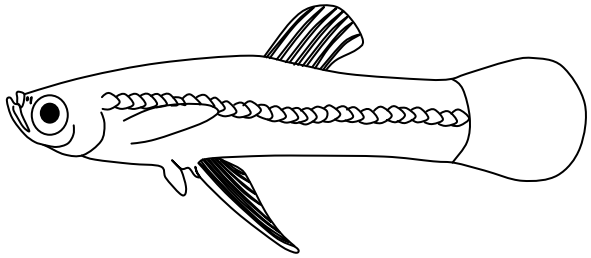

Figure 4.3: Sketch of *Gambusia affinis* (Baird and Girard, 1853) to illustrate the identification process of fishes during the present study, redrawn from Skelton (2001).

During the current study, a total of eleven species representing six families of freshwater fishes were collected and examined for the presence of external trichodinids.

4.1 Family: Cichlidae Heckel, 1840

Skelton (2001) mentioned that cichlids are most closely related to some marine families (e.g. Labridae Cuvier, 1816, Embiotocidae Gibbons, 1854 and Pomacentridae Block and Schneider, 1801 fishes). Although approximately 900 species have been described in Africa, only 42 species representing eight genera are known from southern Africa.

Characteristics of cichlids:

- Scales on head, body
- Spinous, soft-rayed sections along dorsal, anal fins
- Thoracic positioned pelvic fins consist of spine plus five branched rays
- Divided lateral line
- One pair of nostrils
- Breeding:
  - Usually involves pair-formation plus nest building activities
  - Parental care (eggs, young) usually takes place
  - In many species, the female (sometimes male) picks up eggs, incubates eggs in the mouth
- Tilapines:
  - Plant / sediment feeders
  - One dark eye-spot near the base of the dorsal fin (tilapia spot) of all juveniles
- Haplochromines:
  - Predators
  - Series of clear spots on anal fin (egg spots, or egg dummies) typical of adults
  - Males make use of these spots to improve chances to produce offspring
  - Male release milt when female tries to gather ‘eggs’ (spots on male anal fin) in her mouth

Genus: *Oreochromis* Gűnther, 1889

These mouthbrooding cichlids are adapted to live in wide temperature- and salinity ranges. Species from this genus are relatively large in body size. Fine teeth can be observed in several rows on the jaws. Most of the 33 described *Oreochromis* Gűnther, 1889 species utilise water mediums in tropical, eastern and / or southern Africa. Skelton (2001) gave a key to eight *Oreochromis* species that occurs in southern Africa and mentioned that six of these species are indigenous while *O. niloticus* (Linnaeus, 1758) and *O. aureus* (Steindachner, 1864) are introduced to the area.

***Oreochromis mossambicus* (Peters, 1852) (Figure 4.4)**

- Common name: Mozambique tilapia / bloukurper
- D XV-XVII, 10-13; A III, 9-12; Lateral line scales 30-32
- Prefer standing waters, occurs in all but fast-flowing water mediums
- Prefer warm water (above 22˚C), survives below 15˚C
- Tolerates temperatures to 42˚C
- Algae feeder
- Lives / breeds in fresh / seawater

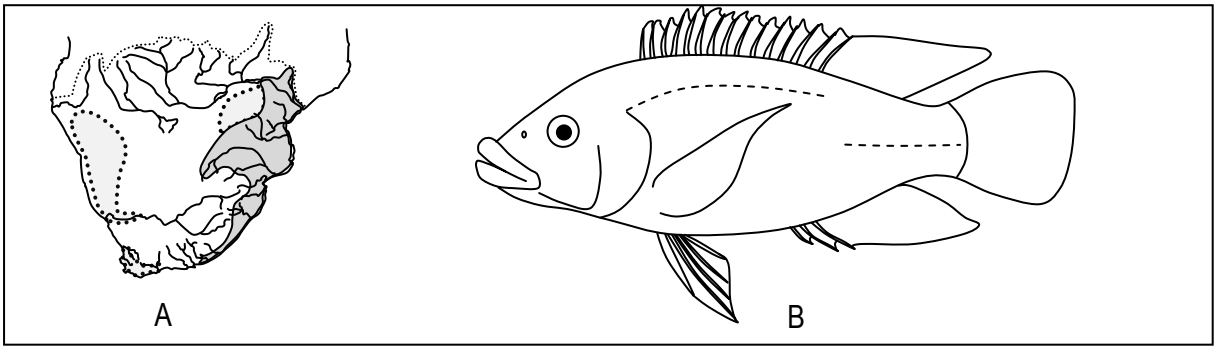

Figure 4.4: Schematic illustration of the distribution (**A**) and an adult *Oreochromis mossambicus* (Peters, 1852) (**B**), redrawn from Skelton (2001).

Genus: *Pseudocrenilabrus* Fowler, 1934

Only one of the three species occurs within the southern African water resources. Two distinctive features from species of this particular genus, is the occurrence of a rounded caudal fin as well as an orange tip of the anal fin of males.

***Pseudocrenilabrus philander* (Weber, 1897) (Figure 4.5)**

- Common name: southern mouthbrooder / suidelike mondbroeier
- D XIII-XVI, 9-11; A III, 7-9; Lateral line scales 27-30
- Black vertical bars visible on light brown skin of females
- Females: yellowish fins
- Males:
  - Colour varies with locality
  - Colour accentuated during breeding season
  - Body usually covered with mesh of iridescent light blue, yellow with oblique bar through eye
  - Red, blue blocks visible on caudal, anal fins
  - Anal tail ends with orange tip
- Utilise wide variety of habitats (flowing waters, lakes, isolated sinkholes)
- Favours vegetated zones
- Food includes insects, shrimps, small fish
- Lifespan: 4-5 years
- Breeding:
  - During early spring to late summer
  - Males construct simple cleared nest to attract ripe females
  - Males defend territory, including cleared nest-area
  - Eggs laid in nest
  - Eggs collected by female after fertilisation
  - Female withdraws to quiet nursery to broods eggs, larvae
  - Several broods raised in one season

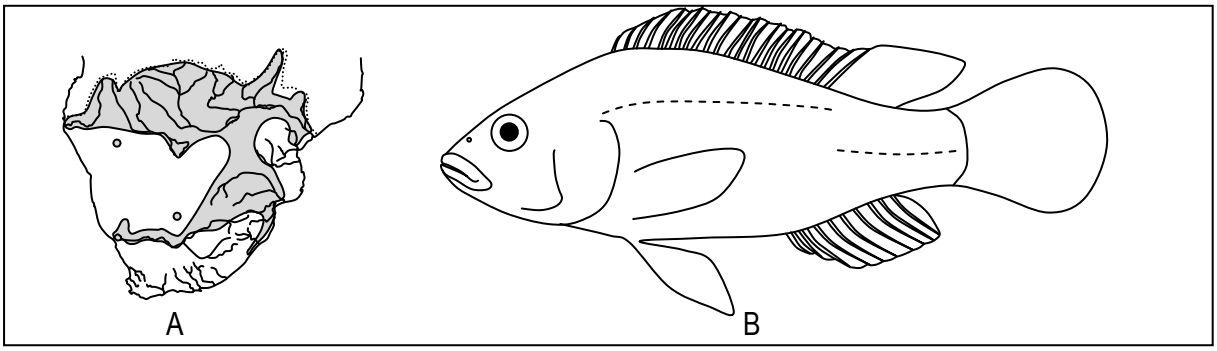

Figure 4.5: Schematic illustration of the distribution (**A**) and an adult *Pseudocrenilabrus philander* (Weber, 1897) (**B**), redrawn from Skelton (2001).

Genus: *Tilapia* A. Smith, 1840

The genus *Tilapia* A. Smith, 1840 is restricted to the substrate spawning species from the tribe Tilapiini. The tribe Tilapiini is characterised by the presence of five pores on the lachrymal bone (for the lateral line). The apophysis on the base of the skull of Tilapiini fishes is part of the paraspenoid bone only. In addition, a clear black spot at the base of the soft dorsal fin is visible at all juveniles (Skelton 2001). This spot (tilapia spot) is also visible in the adult stage of most tilapias.

All members from the genus *Tilapia* retain the tilapia spot, also in adulthood. Although no marked differentiation occurs between the sexes of this genus, a firm pair-bond relationship is formed during the breeding season. Both parents also guard and tend the offspring (Skelton 2001).

***Tilapia sparrmanii* A. Smith, 1840 (Figure 4.6)**

- Common name: banded tilapia / vleikurper
- D XIII-XV, 9-11; A III, 9-10; Lateral line scales 27-29
- Colour varies, mostly deep olive green with 8-9 dark vertical bars on body
- Dark spot on gill cover surrounded by iridescent green / blue scales
- Iridescent blue observed along lower jaw
- Breeding males: bright red margin on caudal, dorsal fin

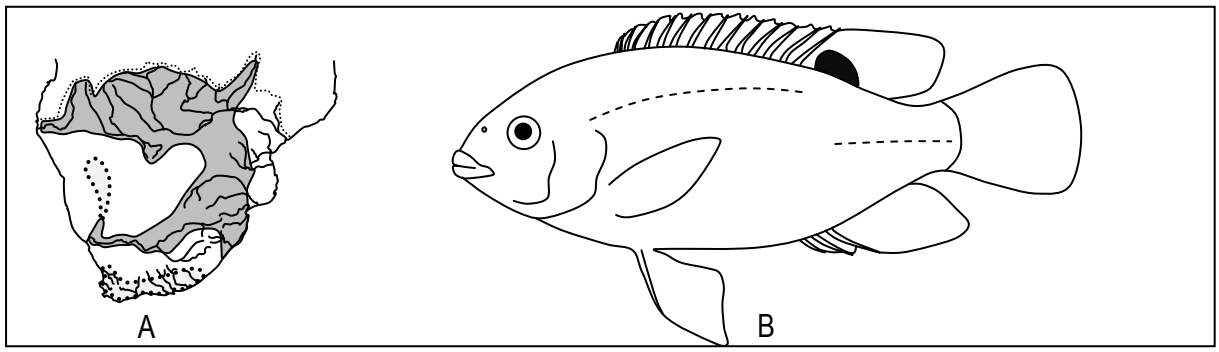

Figure 4.6: Schematic illustration of the distribution (**A**) and an adult *Tilapia sparrmanii* A. Smith, 1840 (**B**), redrawn from Skelton (2001).

4.2 Family: Clariidae Bonaparte, 1846

African and Asian catfish are characterised by their bony, helmet-like heads and elongated bodies. These animals are well known for their hardiness, their ability to breathe air and to survive desiccation of the environment.

Genus: *Clarias* Scopoli, 1777

Species from the genus *Clarias* Scopoli, 1777 are characterised by the presence of a spine at each of the pectoral fins (Skelton 2001). In addition, these animals have long-based soft dorsal and anal fins. These catfish have four pairs of barbels close to their large mouths. The chamber above the gills houses the accessory air-breathing organ.

***Clarias gariepinus* Burchell, 1822 (Figure 4.7)**

- Common name: sharptooth catfish / skerptandbaber / baber
- D61-75; A45-60
- Strongly compressed body
- Dorsal, anal fin consists of soft rays
- Heavy boned, large depressed head
- Back: Black to light brown, marbled in shapes of olive green or grey
- Underside: White, sometimes with red flush to extremities of fins during spawning
- Length: 1.4m

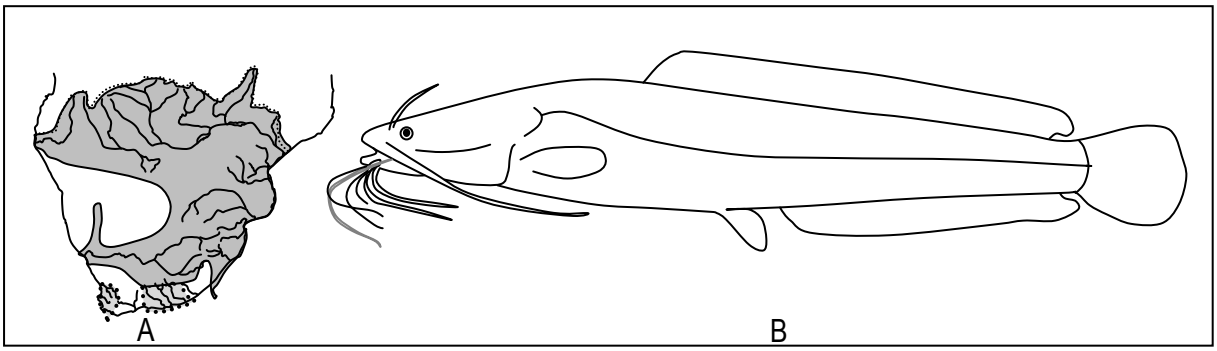

Figure 4.7: Schematic illustration of the distribution (**A**) and an adult *Clarias gariepinus* (Burchell, 1822) (**B**), redrawn from Skelton (2001).

4.3 Family: Cyprinidae Rafinesque, 1815

Members from the cyprinid family lack teeth on the jaws as well as a true stomach. However, these animals have teeth on their strong pharyngeal bones and some species have an extended and convoluted gut. The latter is particularly true for detritus and plant feeders. Although Cyprinidae is the largest family in southern Africa, only eight (consisting of 80 species) of the approximately 275 genera already described occur in southern African freshwater resources (Skelton 2001). The other cyprinids are hosted in freshwater resources in other parts of Africa as well as Asia, Europe and North America.

Genus: *Barbus* Cuvier and Cloquet, 1816

Most of the barbines that occur in Africa is described as *Barbus* Cuvier and Cloquet, 1816 species. However, as more information on their biology, chromosomes, genetics and morphology revealed that only a few species from the Maghreb region of north-west Africa and certain species from Europe should be referred to as a *Barbus* species. Skelton (2001) classified the large yellowfish within the genus *Labeobarbus* Rüppell, 1835 while certain groups of small barbs were also informally described within their possible future classification.

***Barbus anoplus* Weber, 1897 (Figure 4.8)**

- D iii, 7; A iii, 5; Lateral Line Scale 33 – 37
- Simple, flexible primary dorsal ray
- Single pair of short barbels occur near small mouth
- Typically blunt head, otherwise rounded
- Standard length for adult males: 100mm; females: 120mm
- Golden coloured breeding males, other individuals greyish green above with small dot at mid-base of caudal fin
- Broad, dark band along body may also occur on males
- Numerous radial striations on scales
- Prefers cool waters in small streams to large rivers / lakes
- Omnivorous (diatoms, green algae, insects, seeds, zooplankton)
- Larger fish, birds prey on *B. anoplus*
- Breeds in warmer months
- Eggs laid between vegetation and hatches within three days

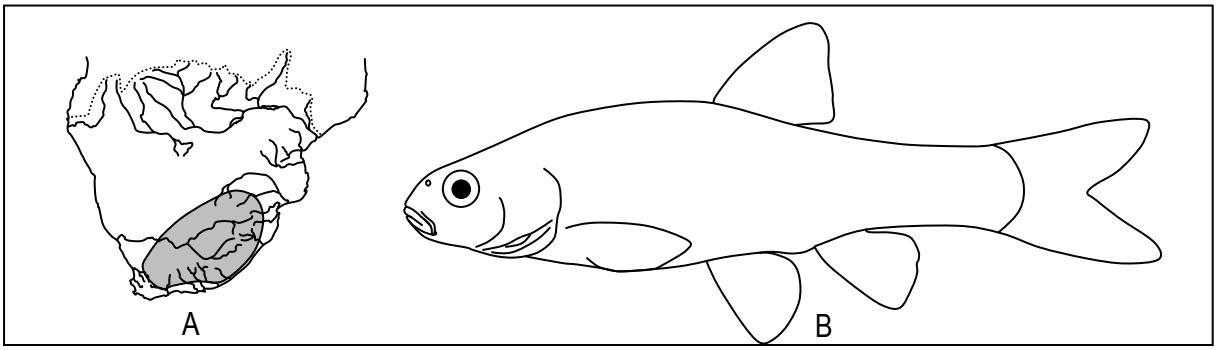

Figure 4.8: Schematic illustration of the distribution (**A**) and an adult *Barbus anoplus* Weber, 1897 (**B**), redrawn from Skelton (2001).

***Barbus barnardi* Jubb, 1965 (Figure 4.9)**

- D iii, 8; A iii, 5; Lateral Line Scale 29 – 33. Caudal peduncle: 12
- Slender, fusiform body
- Small barbel observed on each side of petite mouth
- Colour: translucent brown; silver below sides; pale yellow fins
- Straight black stripe from tip of snout to caudal base
- Black spots: irregular spots along midline of back; spot at base of anal fin
- Reach length of 70mm
- Occupies shallow, well-vegetated water bodies
- Feeds on algae, small aquatic insects
- Breeding: eggs laid in vegetation during summer months

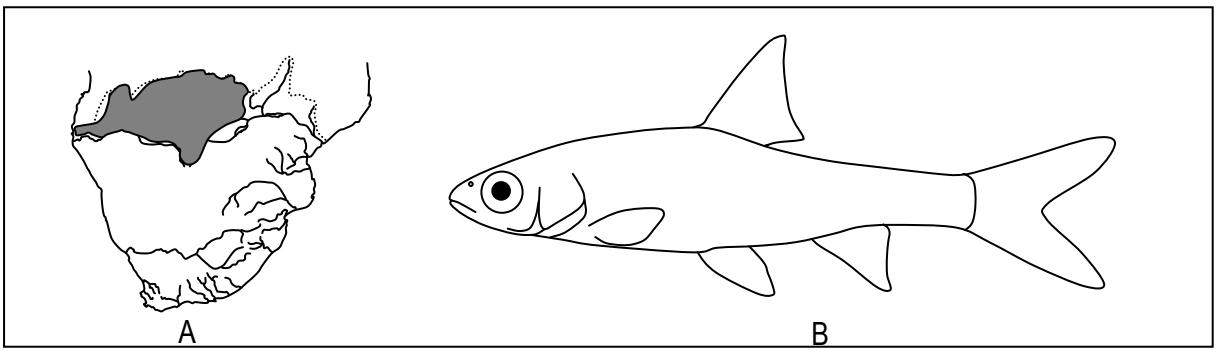

Figure 4.9: Schematic illustration of the distribution (**A**) and an adult *Barbus barnardi* Jubb, 1965 (**B**), redrawn from Skelton (2001).

Genus: *Cyprinus* Linnaeus, 1758

***Cyprinus carpio* Linnaeus, 1758 (Figure 4.10)**

- First importation of this introduced species was reported in 1700's; many importations occurred in 1800's
- D III, 17 – 22; A II-III, 4-5; Lateral Line Scale 35-39; Caudal peduncle: 14
- Fullscale carp: normal scales
- Mirror carp: scattered scales, most of carp without scales
- Leather carp: no scales
- Deep-bodied
- Primary ray on long-based dorsal fin made up of a bony serrated spine
- Single pair of barbels near protrusible mouth
- Colour: olive brown to rich brazen gold
- Dark grey fins

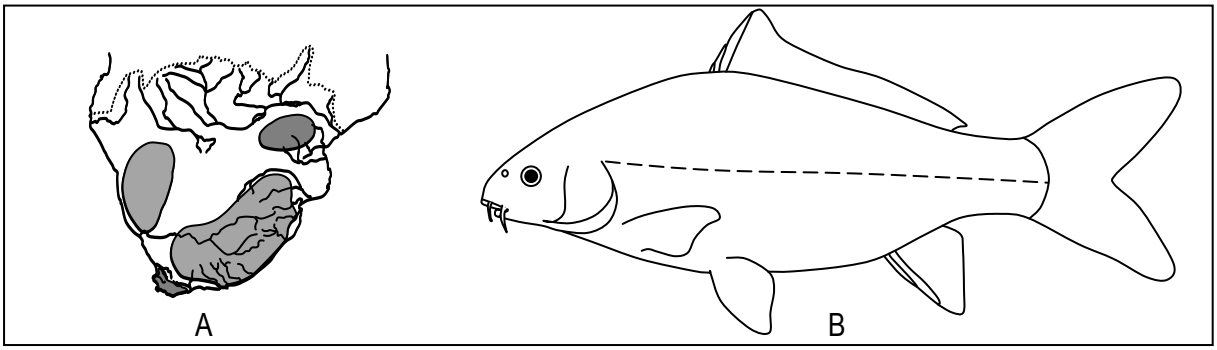

Figure 4.10: Schematic illustration of the distribution (**A**) and an adult *Cyprinus carpio* Linnaeus, 1758 (**B**), redrawn from Skelton (2001).

4.4 Family: Poeciliidae Garman, 1895

These small fish are introduced to southern Africa from Central and South America. Individuals belonging to the subfamily Poeciliinae Garman, 1895 are live-bearers. The anal fin of males is modified to form a gonopodium (intromittent organ). It is said that the populations of these fish established in natural waters of southern Africa as aquarium fish were released in these water mediums.

Genus: *Gambusia* Poey, 1854

The genus *Gambusia* Poey, 1854 comprises more than 40 species, of which most are principally found in freshwater habitats.

***Gambusia affinis* (Baird and Girard, 1853) (Figure 4.11)**

- D 6-8; A 9-10; Lateral Line Scale: 29-30
- Upturned mouth
- Flat head
- Long caudal peduncle with rounded caudal fin
- Colour:
  - Plain translucent light brown; metallic silvery gold gill cover, silvery abdomen, clear fins
  - May have small black spots / iridescent blue reflections on body /dusky sports may occur on fins
  - Females: Black spot may be visible on vent of the gravid female
- Males: slender with short anal fin
- Prefers areas with plant cover in standing waters
- May occur in water temperatures of 4 – 38˚C
- Tolerates wide range of salinity
- Feeds on mosquito, fish larvae, nips on fish fins
- Breeding: eggs fertilised internally; viviparous

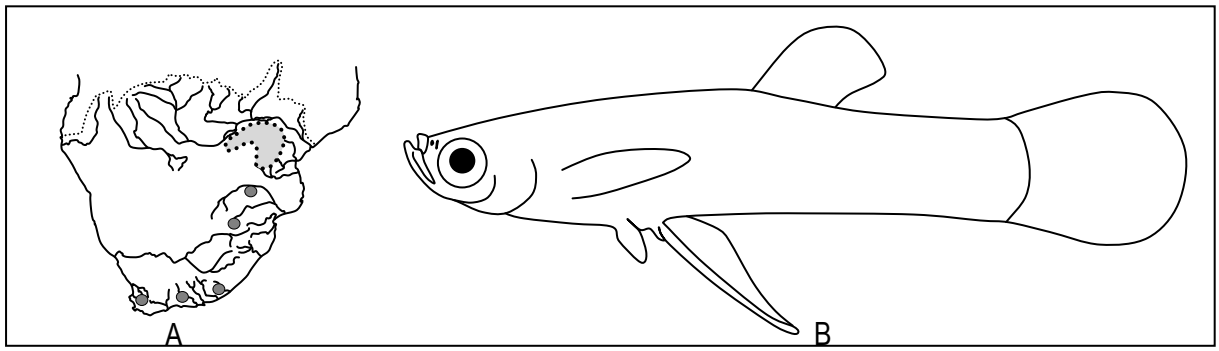

Figure 4.11: Schematic illustration of the distribution (**A**) and an adult *Gambusia affinis* (Baird and Girard, 1853) (**B**), redrawn from Skelton (2001).

4.5 Family: Distichodontidae (Bonnaterre, 1788)

This family consist of at least 17 genera and 90 species that are found in Africa. These fishes can be distinguished from other genera by having two rows of teeth, unusual square shaped mouths and their distinctive ctenoid scales. In addition, many species are marked with bars on the body (Skelton 2001).

Genus: Hemigrammocharax (Pellegrin, 1923)

These fishes are characterised by the presence of prominent vertical bars along the slender body and a caudal 'eye' spot.

***Hemigrammocharax multifasciatus* Boulenger, 1923 (Figure 4.12)**

- D iii, 10-12; A iii, 8-9; Lateral line scales: 40-44
- Incomplete lateral line
- Slender compressed body
- Relatively large eyes on pointed head
- Colour: Translucent light olive with 16-25 dark bars
- Length: 45mm
- Prefers vegetated margins of rivers, oxbow lagoons, shallow lakes
- Feeds on small invertebrates, epiphyton from stems of plants
- Breeds in summer

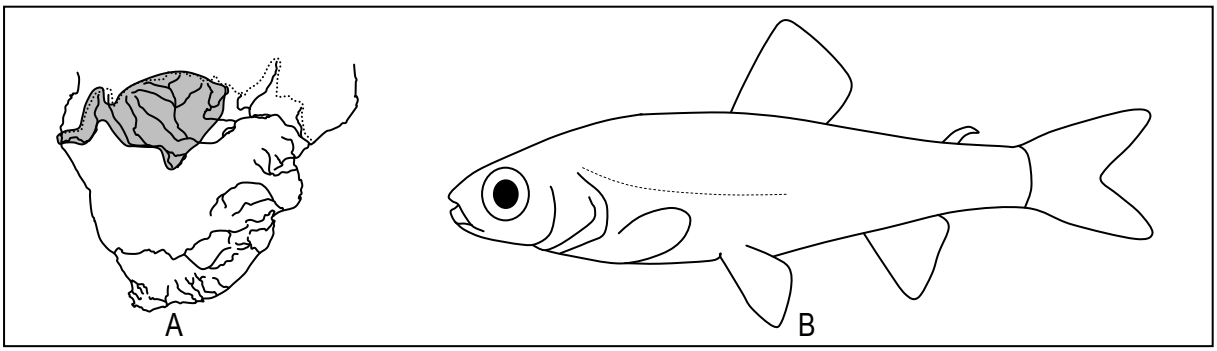

Figure 4.12: Schematic illustration of the distribution (**A**) and an adult *Hemmigrammocharax multifasciatus* Boulenger, 1923 (**B**), redrawn from Skelton (2001).

Trichodinid material was also examined from skin smears prepared from two fish species (Table 4.2) from Lake Valencia. The material was obtained from Professor Ilan Paperna.

Table 4.2: Trichodinids examined during the current study that was collected from fish hosts from other parts of the world.

Family	Genus and species	Locality of collection	Date collected	Collector
Poeciliidae	*Poecilia sphenops*	Lake Valencia	1980	I. Paperna
Cichlidae	*Petenia krausi*	Lake Valencia	1980	I. Paperna

Chapter **5**:

Material and **M**ethods

'...how many creatures are still unbeknown to us,
and how little do we yet understand...'

Antonie van Leewenhoek

1. Host populations were collected from various localities in southern Africa

As part of the present study, preserved material of trichodinids collected from skin and gills of fishes and tadpoles at different localities had to be examined. This was partly achieved by re-evaluating trichodinids previously collected by the Aquatic Research Group of the Department of Zoology and Entomology, UFS since 1980 (Figure 5.1, Table 5.1). Additional material was also collected during the present study from various localities in southern Africa (Figure 5.1, Table 5.1).

Figure 5.1: Map of southern Africa indicating the localities where fish and tadpoles have been collected for the examination of the presence of ectotrichodinids. Please refer to Table 5.1 for more information on the locality numbers. A: Botswana. Source: Google Earth (2013).

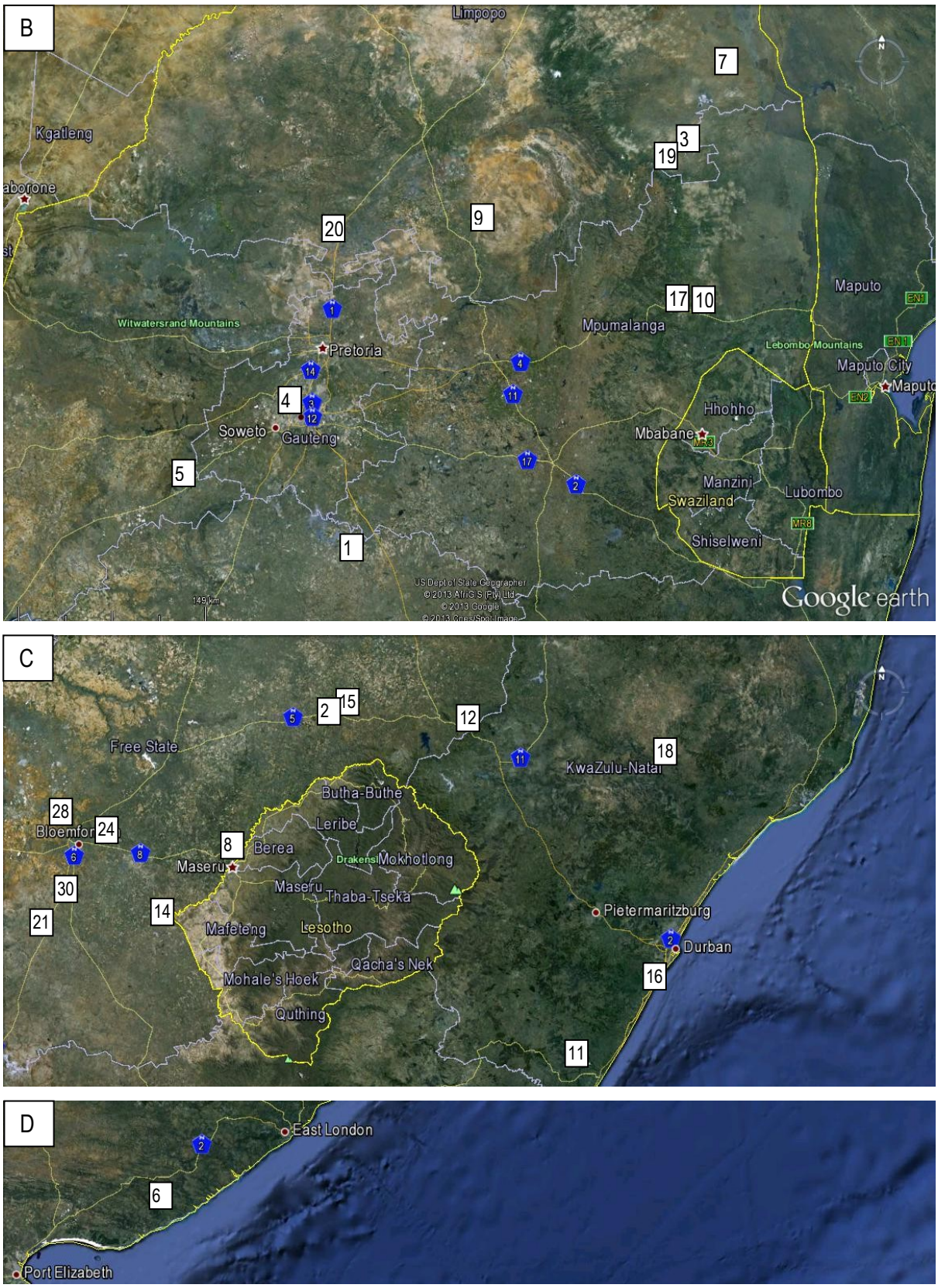

Figure 5.1 (Continue): Map of southern Africa indicating the localities where fish and tadpoles have been collected for the examination of the presence of ectotrichodinids. **B**: Northern part of South Africa; **C**: Central part of South Africa; **D**: Kowie River. Source: Google Earth (2013).

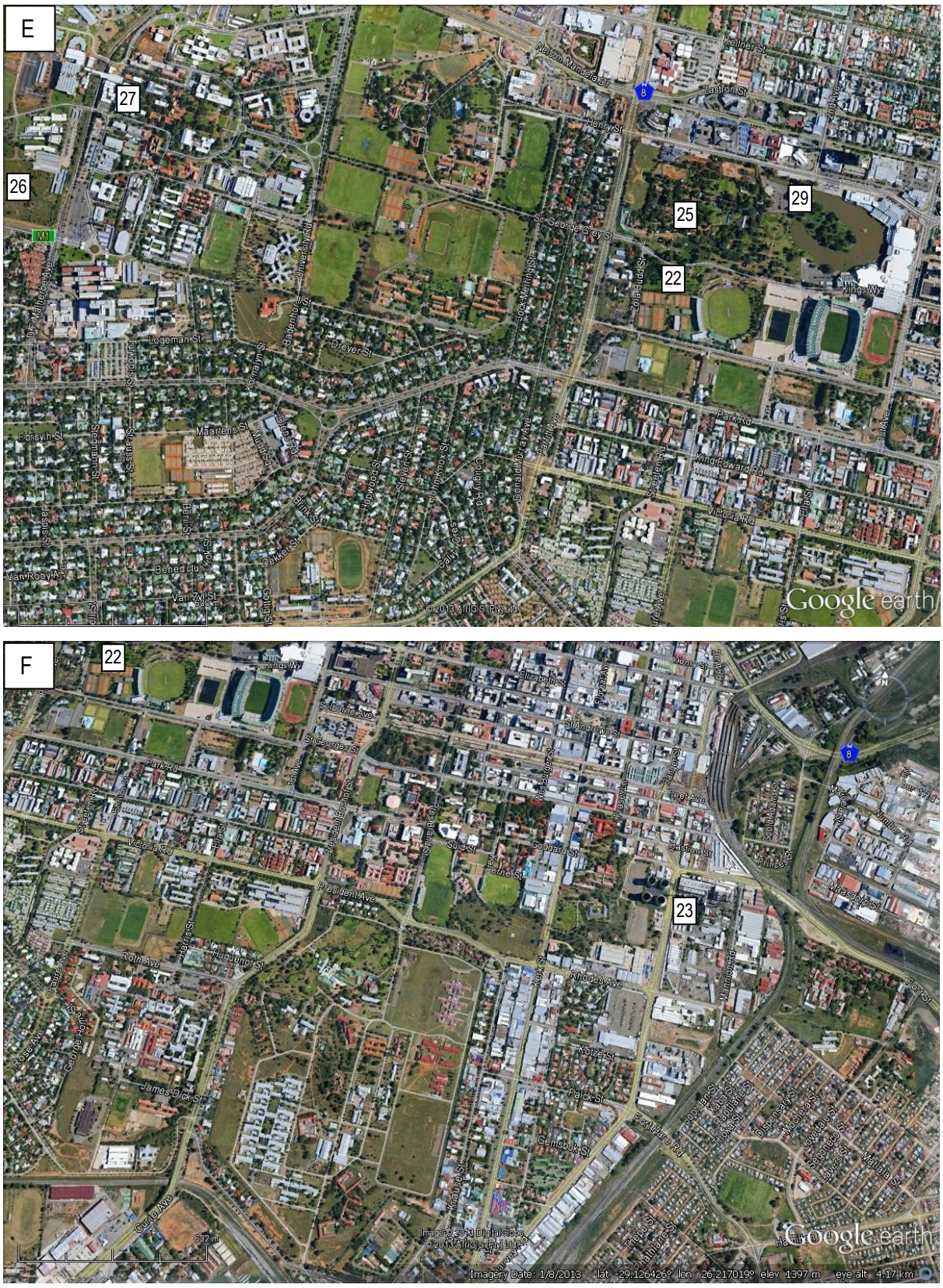

Figure 5.1 (Continue): Map of southern Africa indicating the localities where fish and tadpoles have been collected for the examination of the presence of ectotrichodinids. **E and F**: Localities of collection within Bloemfontein itself. Source: Google Earth (2013).

Figure 5.1 (Continue): Map of southern Africa indicating the localities where fish and tadpoles have been collected for the examination of the presence of ectotrichodinids. **G**: Fish and tadpoles were collected from two localities on the farm Sabella, located 10 km northwest of Wepener. Source: Google Earth (2013).

Table 5.1: Indication of the localities in southern Africa where host species were sampled for the examination of skin and gill trichodinids. Note that the locality number corresponds to the number indicated on the maps in Figure 5.1. Explanation on the population number: The first letter of the population number indicates if the host population is fish (F) or tadpoles (T). The number indicates the number of fish or tadpole population studied, the following two letters indicate the host species while the last two letters indicate the locality of collection. For example, F3BuBV refers to the third fish population studied. This trichodinids were hosted on unknown *Barbus* species collected at the Barrage Vaal. *Abbreviations*: **NA**: No trichodinid population number provided as no trichodinids were observed on the smears prepared from the skin or gills of the possible hosts collected.

Locality number	Locality	Host species	Date collected	Collector	Discussion	Population number
1	Barrage-Vaal	*Barbus* species	Jun-82	L. Basson	Collected with F15PpBV and T35UuBV	F3BuBV
		Pseudocrenilabrus philander	Jun-82	L. Basson	Collected with F3BuBV and T35UuBV	F15PpBV
			Mar-89	L. Basson	Cross-transmission experiment with T31XlBE	F16PpEB
		Unknown tadpole species	Jun-82	L. Basson	Collected with F3BuBV and F15PpBV	T35UuBV
2	Bethlehem	*Amietophrynus* species	Nov-90	J. Kruger	Collected and dissected for the presence of skin and or gill trichodinids	T4AuBH
3	Hoedspruit	*Amietia* species	Aug-89	J. Kruger	Collected and dissected for the presence of skin and or gill trichodinids	T16AMuHS
		Xenopus laevis	Sep-89	J. Kruger	Collected and dissected for the presence of skin and or gill trichodinids	T27XlHS
4	Roodepoort, Johannesburg	*Amietophrynus gutturalis*	Mar-07	Present Study	Collected in fish pond containing three adult koi	NA
5	Boskop Dam	Unknown tadpole species	Jun-05	L. Basson	Collected and dissected for the presence of skin and or gill trichodinids	NA
6	Kowie River	Unknown tadpole species	Unknown	Unknown	Collected and dissected for the presence of skin and or gill trichodinids	U1UuKR
7	Crocodile River	*Pseudocrenilabrus philander*	Sep-89	L. Basson	Collected and dissected for the presence of skin and or gill trichodinids	NA

Table 5.1 (Continue): Indication of the localities in southern Africa where host species were sampled for the examination of skin and gill trichodinids.

Locality number	Locality	Host species	Date collected	Collector	Discussion	Population number
8	Ladybrand	*Amietophrynus rangeri*	Dec-91	J. Kruger	Collected and dissected for the presence of skin and or gill trichodinids	T13ArLB
		Amietia species	Apr-88	J. Kruger	Collected and dissected for the presence of skin and or gill trichodinids	NA
9	Lowveld Fish Breeding Station	*Cyprinus carpio*	Oct-82	L. Basson	Collected with F11OmLF and T36UuLF	F8CcLF
		Oreochromis mossambicus	Oct-82	L. Basson	Collected with F8CcLF and T36UuLF	F11OmLF
		Unknown tadpole species	Oct-82	L. Basson	Collected with F8CcLF and F11OmLF	T36UuLF
10	Nelspruit	*Pseudocrenilabrus philander*	Sep-89	L. Basson	Collected and dissected for the presence of skin and or gill trichodinids	F14PpND
		Oreochromis mossambicus	Sep-89	L. Basson	Collected and dissected for the presence of skin and or gill trichodinids	NA
11	Oribi Gorge (Natal)	*Barbus* species	Sep-89	L. Basson	Collected with T15AMuOG	F4BuOG
		Amietia species	Sep-89	J. Kruger	Collected with F4BuOG	T15AMuOG
		Xenopus laevis	Sep-89	J. Kruger	Collected with F4BuOG	T26XlOG
12	Platberg, Harrismith	Unknown tadpole species	Nov-09	Present Study	Collected and dissected for the presence of skin and or gill trichodinids	NA

Table 5.1 (Continue): Indication of the localities in southern Africa where host species were sampled for the examination of skin and gill trichodinids.

Locality number	Locality	Host species	Date collected	Collector	Discussion	Population number
13	Sabella Dam, 10 km north west of Wepener	*Barbus anoplus*	Nov-09	Present study	Collected with T11AgSD and T34XlSD	F1BaSD
		Amietophrynus gutturals	Nov-09	Present study	Collected with F1BaSD and T34XlSD	T11AgSD
		Xenopus laevis	Nov-09	Present study	Collected with F1BaSD and T11AgSD	T34XlSD
14	Sabella Spruit, 10 km north west of Wepener	*Barbus anoplus*	Nov-09	Present study	Collected with T18AMaSS	F2BaSS
		Amietia angolensis	Nov-09	Present study	Collected with F2BaSS	T18AMaSS
		Xenopus laevis	Nov-09	Present study	Collected with F2BaSS and T18AMaSS	NA
15	Saulspoort Dam	*Labeo umbratus*	Nov-90	L. Basson	Collected and dissected for the presence of skin and or gill trichodinids	NA
		Barbus species	Nov-90	L. Basson	Collected and dissected for the presence of skin and or gill trichodinids	NA
		Barbus holubi	Nov-90	L. Basson	Collected and dissected for the presence of skin and or gill trichodinids	NA
16	Scottsburg	*Amietophrynus* species	Sep-89	J. Kruger	Collected and dissected for the presence of skin and or gill trichodinids	T3AuSB
17	Stads Rivier	*Amietophrynus* species	May-89	J. Kruger	Collected and dissected for the presence of skin and or gill trichodinids	T1AuSR

Table 5.1 (Continue): Indication of the localities in southern Africa where host species were sampled for the examination of skin and gill trichodinids.

Locality number	Locality	Host species	Date collected	Collector	Discussion	Population number
18	Vernon Crookes	*Natalobatrachus bonebergi*	Sep-89	J. Kruger	Collected and dissected for the presence of skin and or gill trichodinids	T22NbVC
19	W. Uys Farm Dam, near Blyde River	*Clarias gariepinus*	Sep-89	L. Basson	Collected and dissected for the presence of skin and or gill trichodinids	F7CgUP
		Amietia species	Sep-89	J. Kruger	Collected and dissected for the presence of skin and or gill trichodinids	T17AMuWU
20	Bela-Bela (Warmbad)	*Schismaderma carens*	Feb-94	L. Basson	Collected and dissected for the presence of skin and or gill trichodinids	T25ScWB
		Amietophrynus garmani	Feb-94	L. Basson	Collected and dissected for the presence of skin and or gill trichodinids	NA
21	Wuras Dam	*Amietia* species	Dec-89	J. Kruger	Collected and dissected for the presence of skin and or gill trichodinids	T14AMuNW
Bloemfontein						
22	Bird Park, Bloemfontein	*Cacosternum boettgeri*	Jan-89	J. Kruger	Collected and dissected for the presence of skin and or gill trichodinids	T19CbBP
23	Bloemfontein	*Xenopus laevis*	Apr-89	J. Kruger	Collected and dissected for the presence of skin and or gill trichodinids	T28XlBB
		Xenopus laevis	Feb-89	J. Kruger	Collected and dissected for the presence of skin and or gill trichodinids	T32XlBB
24	Quarry	*Xenopus laevis*	Mar-89	J. Kruger	Collected and dissected for the presence of skin and or gill trichodinids	T33XlBQ

Table 5.1 (Continue): Indication of the localities in southern Africa where host species were sampled for the examination of skin and gill trichodinids.

Locality number	Locality	Host species	Date collected	Collector	Discussion	Population number
Bloemfontein (continue)						
25	Bloemfontein Zoo	*Xenopus laevis*	Feb-92	J. Kruger	Cross-transmission experiment with F16PpEB	T31XlBE
26	UFS Campus	*Cacosternum boettgeri*	Nov-91	J. Kruger	Collected and dissected for the presence of skin and or gill trichodinids	NA
		Barbus species	Jun-91	L. Basson	Collected and dissected for the presence of skin and or gill trichodinids	F6BuBL
		Kassina senegalensis	May-90	J. Kruger	Collected and dissected for the presence of skin and or gill trichodinids	T21KsZZ
27	Bloemfontein, kept in Laboratory	*Oreochromis mossambicus*	Oct-88	L. Basson	Cross-transmission experiment with F9GaEB and T30XlBE	F10OmEB
		Xenopus laevis	Oct-88	J. Kruger	Cross-transmission experiment with F9GaEB and F10OmE	T30XlBE
28	Pony Club, Woodland Hills	*Xenopus laevis*	Apr-89	J. Kruger	Collected and dissected for the presence of skin and or gill trichodinids	T29XlPC
29	Loch Logan Waterfront	*Gambusia affinis*	Oct-88	L. Basson	Cross-transmission experiment with F10OmEB and T30XlBE	F9GaEB
		Tilapia sparrmanii	Nov-09	Present study	Collected with T23PaBW	F17TsBW
			Jun-07	Present study	Collected and dissected for the presence of skin and or gill trichodinids	F18TsBW
		Pyxicephalus adspersus	Nov-09	Present study	Collected with F17TsBW	T23PaBW

Table 5.1 (Continue): Indication of the localities in southern Africa where host species were sampled for the examination of skin and gill trichodinids.

Locality number	Locality	Host species	Date collected	Collector	Discussion	Population number
Bloemfontein (continue)						
30	Paradys Experimental Farm	*Kassina senegalensis*	Nov-09	Present study	Collected and dissected for the presence of skin and or gill trichodinids	T8KsBP
Okavango Panhandle and Okavango Delta, Botswana						
31	Shakawe	*Amietophrynus* species	Dec -06 and Jan-07	L. van As, J.G. van As	Degree of infestation experiment by Van As and Van As (unpublished)	T2AuSH
32	Leseding Camp	*Amietophrynus poweri*	Oct-07	Present study	Not naturally infested, part of experiment with T7ApTB, F5BbTT, T9ApCF. Tadpoles were collected on a daily basis from the Crocodile Farm Office as a control during the experiment, as a control	T6ApCF
			Oct-07	Present study	Not naturally infested, part of experiment with T7ApTB, F5BbTT, T6ApCF. Uninfested tadpoles were placed in the same tank as the now infested fish (F5). Entire tadpole was squashed on slide	T9ApCF

Table 5.1 (Continue): Indication of the localities in southern Africa where host species were sampled for the examination of skin and gill trichodinids.

Locality number	Locality	Host species	Date collected	Collector	Discussion	Population number
Okavango Panhandle and Okavango Delta, Botswana						
33	Nxamasere	*Hemigrammochorax multifasciatus*	Oct-07	Present study	Collected with T10AgNC	F19HmNA
		Amietophrynus gutturalis	Oct-07	Present study	Collected with F19HmNA and one uninfested catfish	T10AgNC
			Oct-07	Present study	Collected with F19HmNA and one uninfested catfish, (T10AgNC, placed in tank A)	T10AgNC (a)
			Oct-07	Present study	Collected with F19HmNA and one uninfested catfish, (T10AgNC, placed in tank B)	T10AgNC (b)
			Oct-07	Present study	No gill smears of the larger individuals as these individuals escaped from the water periodically (lungs). Larger individuals: most trichodinids on legs and area of attachment of legs to body	T12AgND
			Oct-07	Present study	No gill smears of the larger individuals as these individuals escaped from the water periodically (lungs). Larger individuals: most trichodinids on legs and area of attachment of legs to body (T12AgND, placed in tank D)	T12AgND (d)
			Oct-07	Present study	No gill smears of the larger individuals as these individuals escaped from the water periodically (lungs). Larger individuals: most trichodinids on legs and area of attachment of legs to body (T12AgND, placed in tank C)	T12AgND (c)

Table 5.1 (Continue): Indication of the localities in southern Africa where host species were sampled for the examination of skin and gill trichodinids.

Locality number	Locality	Host species	Date collected	Collector	Discussion	Population number
Okavango Panhandle and Okavango Delta, Botswana						
33	Nxamasere	*Amietophrynus poweri*	Oct-07	Present study	Doggy pond, Lake Nxamasere. Collected with uninfested cichlids. Large school of tadpoles at surface. Water was warm, and dirty. Entire tadpole squashed on slide	T5ApNB
		Hyperolius parallelus	Oct-07	Present study	Collected and dissected for the presence of skin and or gill trichodinids	T20HaNE
		Ptychadena subpunctata	Oct-07	Present study	Collected and dissected for the presence of skin and or gill trichodinids	T24PsNF
34	Thamalakane Bridge	*Barbus barnardi*	Oct-07	Present study	Cross-transmission experiment with T7ApTB, T9ApCF and T6ApCF. Experiment started on 24 October 2007. These uninfested fish were placed in same tank as infested tadpoles (T7)	F5BbTT
35	Toteng Bridge	*Amietophrynus poweri*	Oct-07	Present study	Naturally infested, part of experiment with T6ApCF, F5BbTT, T9ApCF. Infested tadpoles were collected on 16 October 2007. Experiment started on 24 October 2007. These tadpoles were placed in same tank as uninfested fish (F5).	T7ApTB
South America						
NA	Lake Valencia	*Poecilia sphenops*	1980	I. Paperna	Collected and dissected for the presence of skin and or gill trichodinids	F12PsLV
		Petenia krausi	1980	I. Paperna	Collected and dissected for the presence of skin and or gill trichodinids	F13PkLV

2. Collection of possible host species

Small fish nets or commercial fish nets were used to collect tadpoles and fishes in shallow water (Figure 5.2). In addition, snail nets were used when water reached knee depth while scoop nets and hoop nets were used in deeper water bodies. Fishes were also collected by means of a fishing rod in deeper water mediums.

Figure 5.2: Collection of possible trichodinid host species during a field trip in October 2007 by means of different nets. **A**: *Amietophrynus* Frost, Grant, Faivovich, Bain, Haas, Haddad, De Sá, Channing, Wilkinson, Raxworthy, Campbell, Blotto, Moler, Drewes, Nussbaum, Lynch, Green and Wheeler, 2006 species tadpoles were collected near Shakawe; **B**: *Barbus barnardi* Jubb, 1965 fish were collected near the Thamalakane Bridge.

Collected material were either fixed in 70% ethanol, 10% formalin or kept alive upon collection thereof. Individuals that were kept alive were transported in temporary tanks filled with water from point of collection to the laboratory [i.e. Department of Zoology and Entomology, UFS or the Leseding Research Camp (Figure 5.3) that is situated near the Samochima Village, Shakawe, Botswana] as these individuals were either examined for a trichodinid infestation at a later stage or used during the experimental phase of the present study. It should be mentioned that all collected populations were kept separate from one another. For example, different fish or tadpole species collected from the same locality were kept separate from one another and populations from different localities were kept apart. Tadpoles and fishes were provided with fish food flakes on a daily basis.

Figure 5.3: Water tanks at Leseding Research Camp that was used to keep the fish and tadpole populations collected at different localities in Botswana. Note that each possible host population were kept separately from each other in order to prevent any cross-infestation prior to examination of the host groups.

3. Identification of host species

Skelton (2001) was used to identify the collected fish species while the tadpoles were identified by using either a key by Channing (2001) or a key by Du Preez and Carruthers (2009).

4. Determining the degree of trichodinid infestation on a host

Skin and gill smears from the different fish and tadpole populations were prepared. Light microscopes were used to note the degree of infestation on the wet skin and gill smears by means of a similar index (Table 5.2) as proposed by Van As and Basson (1992) where a degree of '0' meant that no trichodinids could be observed on the slide while '5' meant that many trichodinids occurred in most of the visual fields examined.

Table 5.2: The degree of external trichodinid infestation on the fish and tadpole populations were determined by means of a similar index as proposed by Van As and Basson (1992) where a degree of infestation with a value of two (2) indicate that more than ten but less than 51 trichodinids was observed on the smear.

Degree of trichodinid infestation	Number of trichodinids counted on the slide
0	0
1	1-10
2	11 –50
3	51-100
4	101-200
5	201+

5. Trichodinid impregnation technique

As a further preparation for compound light microscopy, air dried smears were impregnated with a modified version of Klein's (1926) sliver nitrate impregnation technique, as proposed by Wellborn (1967). The air dried smears were placed in a 2% silver nitrate solution for 8-15 minutes, before it was washed with distilled water. The slides were exposed to a 254 nm ultraviolet light for a period of 30-45 minutes. Canada Balsam or Eukitt were used to mount cover slips onto the respective stained slides.

6. Morphological and taxonomical evaluation

6.1 Method proposed by Lom (1958)

Lom's (1958) standard method for describing trichodinids (Figure 5.4) was used for the description of the trichodinids collected during this study as well as specimens previously collected by the Aquatic Research Group. This was achieved by using a Zeiss Axiophot compound microscope and a Nikon DXM1200F digital camera to produce digital images of the impregnated specimens. The necessary measurements were made by using the ImageJ software package (ImageJ version 1.40g, 2008). The measurements were presented as the minimum, first quartile, mean, third quartile and maximum and all measurements are in micrometers (µm). The number of specimens measured was also noted.

Biometrical data of the trichodinids from the collected host groups were statistically compared with one another by using the values obtained from the characteristics mentioned in Figure 5.4.

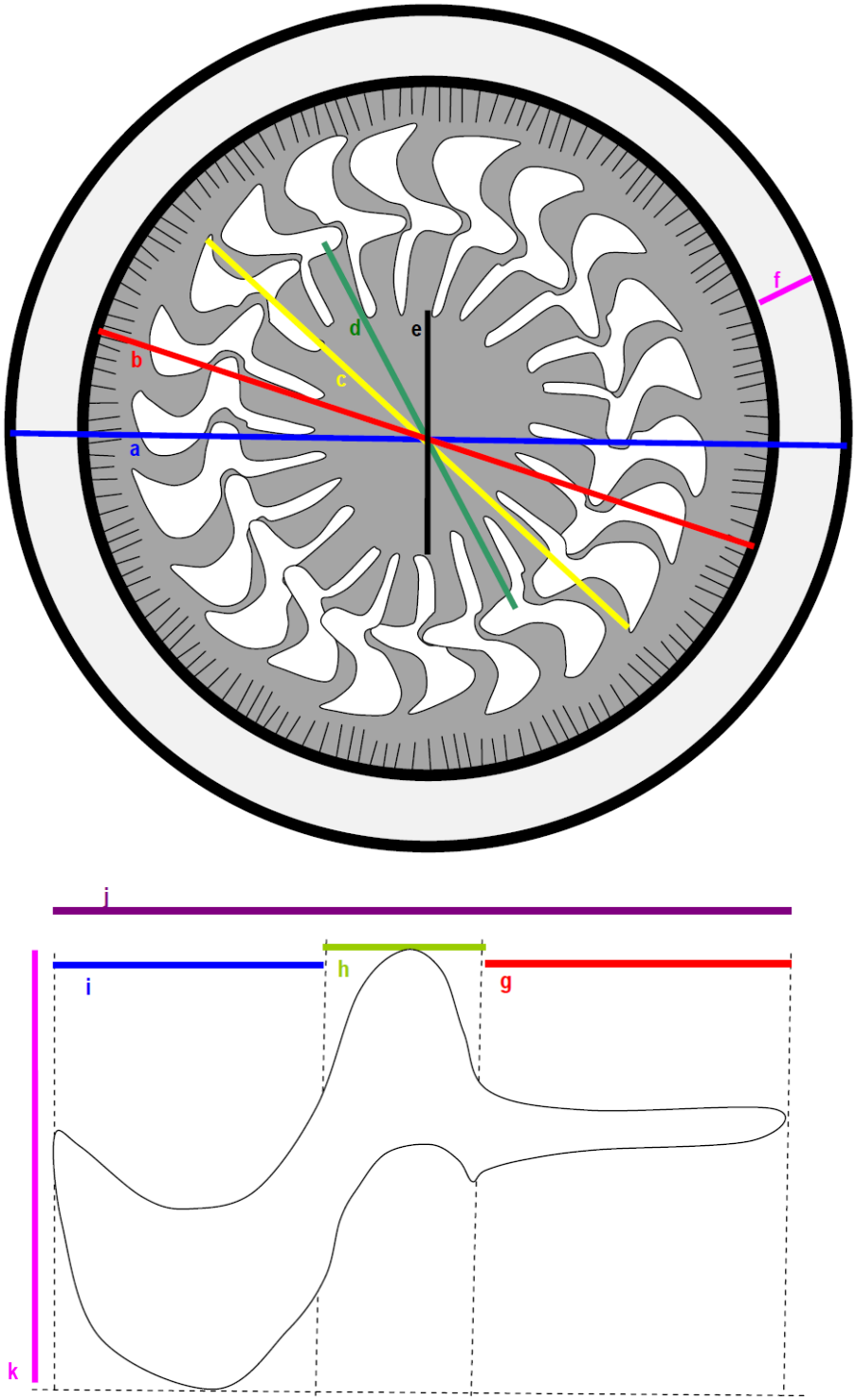

Figure 5.4: Schematic diagram of the aboral disc as well as a denticle of trichodinids to illustrate the features measured during the taxonomic description of different populations of trichodinids evaluated during the current study, as proposed by Lom (1958).

Abbreviations: **a**: Body diameter; **b**: Adhesive disc diameter; **c**: Tangent point diameter*; **d**: Denticle ring diameter; **e**: Tip of ray diameter*; **f**: Width of border membrane; **g**: Ray length; **h**: Central part width; **i**: Blade length; **j**: Denticle span; **k**: Denticle width. The number of denticles within the denticle ring as well as the number of radial pins per denticle was also determined.
*Features not part of the standard method for trichodinid description as proposed by Lom (1958).

6.2 Method proposed by Gong *et al.* (2005)

Further quantitative descriptions for the denticles were achieved by determining the percentages of the areas of the blade, central part and ray respectively, to that of a single denticle (Figure 5.5) and the percentages of the total area of all the denticles in the adhesive disc to that of the loop of the denticulate ring as proposed by Gong *et al.* (2005). The loop of the denticle ring consists of the denticles within the denticle ring as well as the matrix within the denticle ring (Figure 5.5). The necessary measurements were made by using the ImageJ software package. The measurements were presented as the minimum, mean and maximum values where after the average percentages are given. Please note that all measurements are in micrometers (µm). The number of denticles measured as well as the number of specimens measured was also given. The average percentages of the above mentioned characteristics were statistically compared with one another.

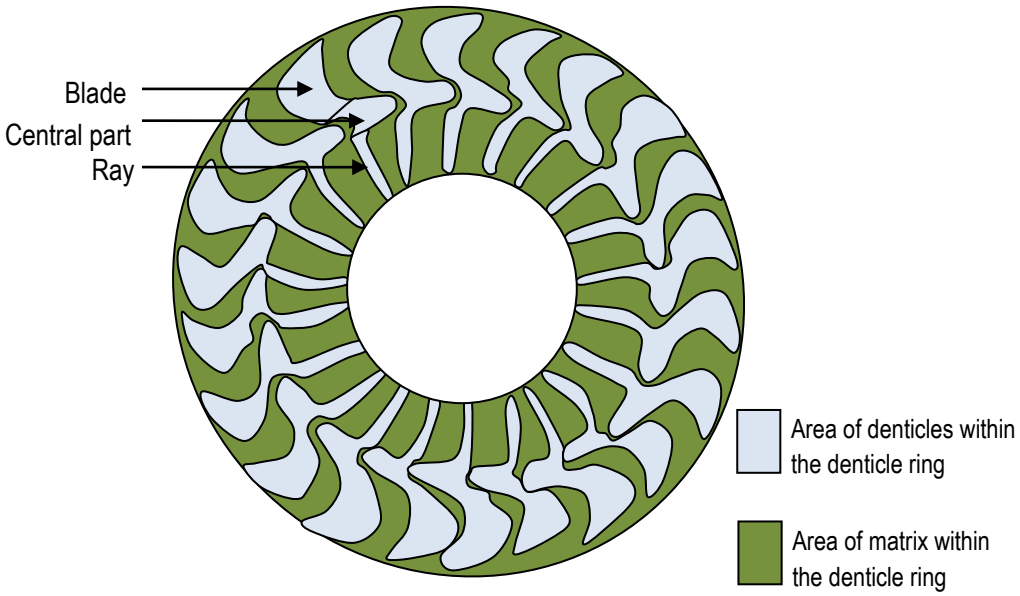

Figure 5.5: Schematic diagram of the loop of the denticle ring of a trichodinid to illustrate the features measured in order to determine the area of a given feature of the trichodinids evaluated during the current study. Note that the area of the loop of the denticle ring consists of the area of the denticles within the denticle ring as well as the area of the matrix within the denticle ring.

6.3 Method proposed by Van As and Basson (1989, 1992)

In addition to the conventional measurements and descriptions proposed by Lom (1958) as well as the method proposed by Gong *et al.* (2005) a method proposed by Van As and Basson (1989, 1992) were also used to accurately describe the characteristics of the trichodinid denticles. Three consecutive denticles were thus drawn from the images taken with the Nikon DXM1200F digital camera (Figure 5.6) and compared with one another as proposed by Van As and Basson (1989, 1992) as this method is known to indicate differences within trichodinid species. Lines were drawn from the centre of the adhesive disc to the tip of the tangent point for each of the drawn denticles. These lines present the y-1 axis, y–axis as well as the y+1 axis. In addition, a line was drawn from the tip of the central part, perpendicular to the y–axis as indicated in the figure (x-axis). These lines were used as lines of reference for the description of the shape of the denticles.

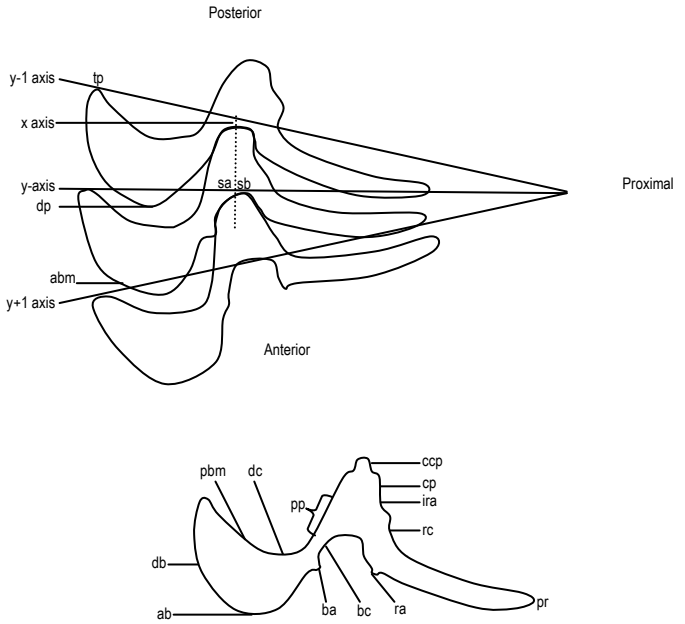

Figure 5.6: Schematic diagram of three consecutive denticles to illustrate the features examined as proposed by Van As and Basson (1989, 1992).

Abbreviations: **ab**: Apex of blade; **abm**: Anterior blade margin; **ba**: Blade apophysis; **bc**: Section connecting blade and central part; **ca**: Centre of adhesive disc; **ccp**: Section connecting central part and ray; **cp**: Central conical part; **dbm**: Distal blade margin; **dp**: Deepest point of curve relative to apex; **ira**: Lower central part indentation; **pbm**: Posterior blade margin; **pp**: Posterior projection; **ra**: Ray apophysis; **rc**: Ray connection; **sa**: Section of central part above x axis; **sb**: Section of central part below x axis; **tp**: Tangent point

Biometrical data of the trichodinids from the collected host groups were statistically compared with one another by using the values obtained from the above mentioned measurements.

7. Cross-transmission experiments

Two cross-transmission experiments were conducted during the present study: the first in December 2006 whilst the last was conducted 10 months later in order to determine if trichodinids collected from one host population (for example fish) are transmissible to another possible host population (for example tadpoles).

7.1 December 2006

Unidentified tadpoles were collected on 18 December 2006 near the China Shop in Shakawe, Botswana. Skin and gills smears verified that these tadpoles were infested with flagellates, sessile ciliophorans and trichodinids. These tadpoles were kept in a temporary aquarium at the Leseding Camp and skin and gills smears were prepared from individuals on a regular basis to determine if the trichodinid infestation persisted on the tadpoles during the next few days.

Eight adult frogs were observed in one of the tanks filled with water a few days earlier at the Leseding camp during the last week of December 2006. No organisms were placed in the tanks prior to the arrival of the adult frogs. These frogs reproduced and hundreds of tadpoles developed from these eggs.

Skin and gill smears were prepared on 30 December 2006 of some of the tadpoles originally collected from Shakawe to determine if the trichodinid infestation persisted. Skin and gill smears were also examined from ten of the smaller, younger tadpoles collected from the Leseding Camp to ensure that no trichodinid infestation occurred on these tadpoles.

One of the objectives of the present study was to determine if a trichodinid population on one host population can be transmitted to individuals from another possible host population. These two host populations were ideal to use to resolve the above as a control group of each of the populations were available.

Therefore, two tanks filled with aged water were used to hold four infested tadpoles as well as ten uninfested tadpoles each. The tadpoles were fed with plankton containing numerous mosquito larvae and rotifer species. Two large (naturally infested) and ten smaller (naturally uninfested) tadpoles were externally examined with the aid of a light microscope to determine the degree of infestation on the skin of these tadpoles during the next few days. The counting process was simplified by the previously mentioned method (Table 5.2) developed by Van As and Basson (1992).

The population size of each of the host groups mentioned in the first part of this experiment was small and an additional experiment conducted with a higher number of possible host individuals was needed. Two tanks filled with aged water were used to keep ten tadpoles infested with trichodinids. These tadpoles were collected from Shakawe. More than 200 younger, smaller, uninfested tadpoles collected from the Leseding Camp were also kept in each of the tanks.

Plankton was added to the water in order to provide a food medium to the tadpoles. A further difference between this and the previous experiment is that skin and gill smears of one tadpole originally from the Shakawe group and ten smaller tadpoles (originally from the Leseding Camp) were made on a daily basis for six consecutive days to determine the possible spread of the trichodinid infestation.

7.2 October 2007

An additional objective of the present study was to determine if uninfested fish hosts can form a viable population of trichodinids from trichodinids that originally occurred on tadpoles, and *vice versa*. Therefore, *Barbus barnardi* Jubb, 1965 fish were collected near the Thamalakane Bridge, Botswana and *Amietophrynus poweri* tadpoles were collected near the Toteng Bridge, Botswana. Both fish and tadpoles were examined for external trichodinids. The tadpoles were used as the infested host group as a high infestation occurred on the skin of *A. poweri*. Even though no trichodinid infestation was observed on the fish, the fish were placed in a 1.5% salt solution for 45 minutes to eradicate any trichodinids that might occur on the fish so that the fish could be used as the uninfested host group during the experiment.

For the first part of the experiment, 100 uninfested *B. barnardi* were placed in the same water medium than 20 heavily infested *Amietophrynus* tadpoles. It should be noted that no fish or tadpoles were kept in the tanks prior to the arrival of the experimental host groups. Skin and gill scrapings of five fish were made daily over a period of seven days to determine the presence (if any) of trichodinids on the fish.

For the second part of the experiment, 100 *Amietophrynus* tadpoles were collected from a pond at the office of the crocodile farm. No fish were observed in the pond and therefore the possibility that these individuals were infested with trichodinids was impossible. Nevertheless, the tadpoles were placed in a 4% solution formalin bath for a period of 10 min in order to render them trichodinid free. These tadpoles were then placed in a clean tank filled with aged filtered water. Twenty of the fish used during the first part of the experiment (now infested with trichodinids previously hosted by tadpoles used in the first part of this experiment), were placed in the tank with the bathed tadpoles. Skin and gill smears were

made from ten of the tadpoles on a daily basis for ten consecutive days. Tadpoles from each control group were also dissected on a daily basis.

The trichodinids on the smears (if any) were counted to determine the multiplication speed of trichodinids and to determine the spread of trichodinids on the different hosts. The same method as mentioned previously was used to determine the trichodinid infestation on the hosts.

8. Reference material

All material is deposited at the Department of Zoology and Entomology, UFS and form part of the collection of the Aquatic Research Group.

Chapter **6**:

Degree of **T**richodinid **I**nfestation on **D**ifferent **H**ost **P**opulations

'…In natural science the principles of truth ought to be confirmed by observation…'

Carolus Linnaeus

Authors such as Thompson *et al*. (1947), Lom (1966), Viljoen (1983) and Kruger (1992) mentioned that external trichodinid infestations cannot be kept on fish and tadpoles in unnatural environments for a long period of time. As an example, Kruger (1992) mentioned that a trichodinid infestation could only be kept until the eleventh day of a cross-transmission experiment conducted on fish and tadpoles at the Zoology and Entomology Department, UFS.

Kruger (1989) also mentioned that the degree of trichodinid infestation on *X. laevis* tadpoles decreased rapidly during a seven day period under laboratory conditions. It should be noted that these tadpoles were kept in a small tank that did not imitate natural conditions.

As part of the present study, it was aimed to determine if the degree of trichodinid infestation is similar on different host populations and / or species under unnatural conditions as this will aid in future laboratory experiments. Therefore, the degree of trichodinid infestation on different host populations was studied by means of an experiment conducted in October 2007.

1. Material and methods

Possible fish and tadpole hosts were collected from various water mediums in October 2007 during a field trip in Botswana (Table 6.1). Please refer to Figure 5.1 and Table 5.1 for more information on the various host species collected. Each host population was kept separate from one another to ensure that no transmission of trichodinids to and from the different possible hosts could occur during the transportation of the possible hosts to the Leseding Research Camp. The tadpoles were identified by using either a key by Channing (2001) or by Du Preez and Carruthers (2009) while Skelton (2001) was used to identify the fish species collected.

Table 6.1: A list of the tadpole and fish populations used to determine the degree of trichodinid infestation on consecutive days in unnatural conditions. Refer to Table 5.1 and Figure 5.1 for an indication on the locality where the host populations were collected. The population number will be used to distinguish between the different populations that was utilised during this experiment.

Host species	Collection locality and additional information	Population number
Amietophrynus poweri	• Collected at the Toteng Bridge, Maun 	T7ApTB
	• Tadpoles collected daily from pond at Krokavango crocodile farm, next to Samochima Lagoon • Note that Leseding research camp is located on premises of Krokavango crocodile farm 	T6ApCF

Table 6.1 (Continue): A list of the tadpole and fish populations used to determine the degree of trichodinid infestation on consecutive days in unnatural conditions.

Host species	Collection locality and additional information	Population number
Amietophrynus poweri	• Tadpoles collected in pond at fossil floodplain: Lake Nxamasere 	T5ApNB
Amietophrynus gutturalis	• Tadpoles collected on second trip to Lake Nxamasere 	T10AgNC(a)

Table 6.1 (Continue): A list of the tadpole and fish populations used to determine the degree of trichodinid infestation on consecutive days in unnatural conditions.

Host species	Collection locality and additional information	Population number
Amietophrynus gutturalis	• Tadpoles collected in same pond as Population T5ApNB a week after collection of Population T5ApNB • No tadpoles collected in middle of pond (30ºC) • Many tadpoles observed in shallow, warmer water near surface of dam (36ºC) • Only skin smears prepared from certain individuals as they already underwent progressive metamorphosis and utilised floating objects in temporary aquariums during experiment • Most trichodinids observed on hind legs of tadpoles 	T12AgND(c)
	• Tadpoles collected with fish (tilapines, mormyrids, mouthbreeders) • No fish hosted trichodinid infestations 	T10AgNC(b)

Table 6.1 (Continue): A list of the tadpole and fish populations used to determine the degree of trichodinid infestation on consecutive days in unnatural conditions.

Host species	Collection locality and additional information	Population number
Amietophrynus gutturalis	• Tadpoles collected with two *Hemigrammochorax multifasciatus* fish • No trichodinid infestations observed on fish 	T12AgND(d)
Hemigrammochorax multifasciatus	• Fish collected with Population T12AgND(d) in pond at Lake Nxamasere	F19HmNA
Hyperolius paralellus	• Tadpoles collected in another pond at Lake Nxamasere during second trip 	T20HaNE
Ptychadena subpunctata	• Single *P. subpunctata* population collected during current study • Individuals collected during second trip to Lake Nxamasere	T24PsNF

Skin and gill smears were made from a number of hosts within each population and a compound microscope was used to determine if the possible hosts were infested with trichodinids. In cases where the tadpoles were too small, an entire tadpole was squashed between a microscope slide and a cover slide. The degree of infestation was noted by means of a similar index (Table 5.2) as proposed by Van As and Basson (1992). The number of trichodinids counted was noted in cases where a small number of trichodinids were observed, rather than the degree of infestation. Each of the populations that hosted a trichodinid infestation was kept separately in order to prevent any cross-transmission of these protozoans.

The degree of trichodinid infestation of each population group was recorded on a daily basis preparing skin and gill smears of a few tadpoles of each population in a similar table as Table 6.2. Note that the last column represents the highest degree of infestation, be it on the skin or gills of the individual host utilised. The highest degree of infestation for the consecutive days between the different host populations was compared by means of statistic evaluation. The number of possible hosts dissected on a daily basis as well as the number of days that a specific population was studied was determined by the number of individual tadpoles or fish within a given population. For example, Population T7ApTB consisted of 80 tadpoles that could be examined during the experiment. In contrast, Populations F19HmNA and T20HaNE both consisted of only two possible host specimens and therefore the degree of infestation within these populations could not be determined for more than two days.

Table 6.2: Example of the table used to note the degree of trichodinid infestation on the examined host individuals.

Population Number	Experimental Day	Number of host individuals	Degree of Infestation on host		
			Skin	Gills	Highest degree of infestation
Example: T20HaNE	1	1	2	1	2
		2	3	2	3
		3	4	4	4
		4	2	3	3
		5	1	4	3
		6	3	2	3
		7	2	3	3
		8	1	2	2
		10	5	3	5
		Minimum	1	1	2
		Maximum	4	4	5
		Average	2	2	3

Abbreviations on the degree of infestation: **0**: No trichodinids observed on the slide; **1**: 1-10 trichodinids observed on the slide; **2**: 11-50 trichodinids observed on the slide; **3**: 51-100 trichodinids observed on the slide; **4**: 101-200 trichodinids observed on the smear; **5**: 201+ trichodinids observed on smear.

2. Results and discussion

2.1. Average degree of trichodinid infestation

Amietophrynus poweri, A. gutturalis, Hyperolius paralellus and *Ptychadena subpunctata* tadpoles were collected and examined for the presence of trichodinids. In addition, two *Hemigrammochorax multifasciatus* fish were also collected and examined for trichodinids during the experiment. The average degree of trichodinid infestation of each of the collected host populations for each of the experimental days is presented in Figure 6.1. Please also refer to Appendix A.

In some instances, no trichodinids were found on the skin and or gill smears prepared from certain individual tadpoles or fish of populations that hosted a trichodinid infestation, and in some cases a severe trichodinid infestation was recorded. For example, no trichodinids were observed on six of the thirteen individuals examined on the second day of the experiment from individuals from Population T24PsNF. In contrast, more than 200 trichodinids were counted on some smears made on the same day from individuals from the same population. This can partly be explained by the fact that some individuals may be more resistant to a trichodinid infestation (Kruger 1992) or due to overdispersion.

No trichodinids occurred on the skin or gills of T6ApCF and F19HmNA throughout the experimental period. Arthur and Lom (1984) collected *Trichodina hypsilepis* Wellborn, 1967 from unidentified tadpoles in Cuba where no fishes were collected in the same water medium that hosted *T. hypsilepis*. This is the only recorded case from published records where infested tadpoles were collected where no infested fish could be found. As previously mentioned, members from Population T6APCF were collected on a daily basis from a pond near the office of the crocodile farm. No fish occurred in this pond and therefore these tadpoles were never in contact with fishes. If a trichodinid infestation was to exist on these tadpoles, the statement by Lom (1963) that tadpoles are infested by trichodinids by means of direct transmission from fishes could be proven to be wrong. However, by examining Figure 6.1, it is evident that no trichodinids were hosted by these tadpoles.

In all other cases [except Population T10AgNC(b)], the degree of infestation was either constant or increased from the on-set of the experiment until the last day of the experiment. Population T10AgNC(b) was collected together with tilapines, mormyrids and mouthbreeders. Interestingly, none of the fishes examined hosted trichodinids and yet a trichodinid infestation was present on the gills of these tadpoles on the first day that these tadpoles were examined. However, the degree of infestation decreased from the fifth day (degree of infestation: 3) to the last day of the experiment when 51 – 100 trichodinids were recorded on some of the slides made from the skin or gills of these tadpoles.

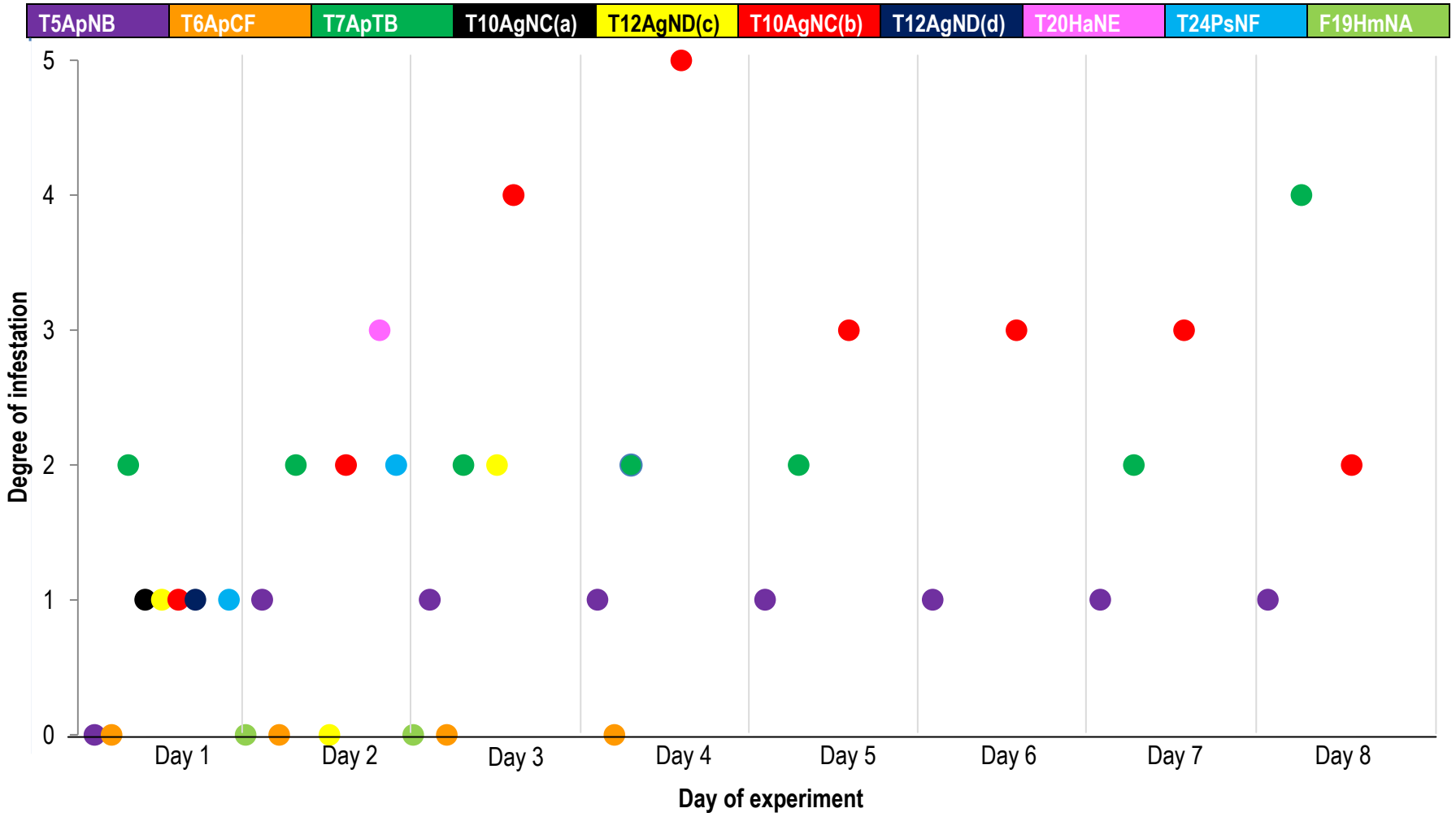

Figure 6.1: Average degree of trichodinid infestation on the examined tadpole and fish populations kept under laboratory conditions to determine the degree of trichodinid infestation on the various host populations during an eight day period (where possible). Refer to Table 5.1 and Figure 5.1 for more information on the host species as well as the locality where these host populations were collected. Refer to Table 5.2 for more information on the degree of trichodinid infestation method proposed by Van As and Basson (1992).

Although none of the collected fishes hosted a trichodinid population, it is not to say that infested fish were not contained in the water medium.

In addition, Kruger (1989) determined that *T. heterodentata* can survive for a period of 30 minutes in a water medium if detached from a suitable host. Trichodinids can be transferred from one water medium to a next via a non-suitable (temporary) host. For example, humans utilising Lake Nxamasere (Figure 6.2) could spread a trichodinid infestation from one pond to the next. Human activities at Lake Nxamasere include the washing of clothes (Figure 6.2A), fishing (Figure 6.2B) and recreational activities (Figure 6.2C) such as swimming (Figure 6.2D). In addition, snails (Figure 6.2E) and birds (Figure 6.2F) also occur in the water bodies at Lake Nxamasere.

If a bird catches an infested fish, some of the trichodinids may be caught on the beak and / or feathers of the bird. If the beak and / or feathers do not dry out before the bird reaches another water medium, the bird may possibly be successful in transporting the trichodinids to the new water medium. It should be kept in mind that the trichodinids have to find a host within a short period in order to survive. Additionally, the possible host species within the new water medium also plays a role in the successful translocation of a trichodinid species as most trichodinid species are host specific. For example, *T. heterodentata* can be found on various fish and tadpole species but cannot survive on hydras, as in the case of *T. pediculus*.

Humans may thus also be responsible for the transfer of trichodinids from one pond to another due to the fact that the ponds are in close proximity to one another and many human activities occurred within the ponds. It is probable that fishes infested with trichodinids were collected by members from the local community in a natural trichodinid infested pond and that their nets / collecting materials could thus contain trichodinids. The community members move from one pond to another to capture fish. It is possible that the wet nets used in the natural infested ponds could aid in the transportation of trichodinids to a naturally uninfested pond.

An introduced trichodinid infestation will firstly infest possible hosts at the surface of the water medium as the human activities usually occur in the shallow waters (Figure 6.2B) and possible hosts in deeper waters may be infested at a later stage. This can also be seen as a possible reason why Population T10AgNC(b) hosted a trichodinid infestation although none of the fishes collected in this pond hosted a trichodinid infestation.

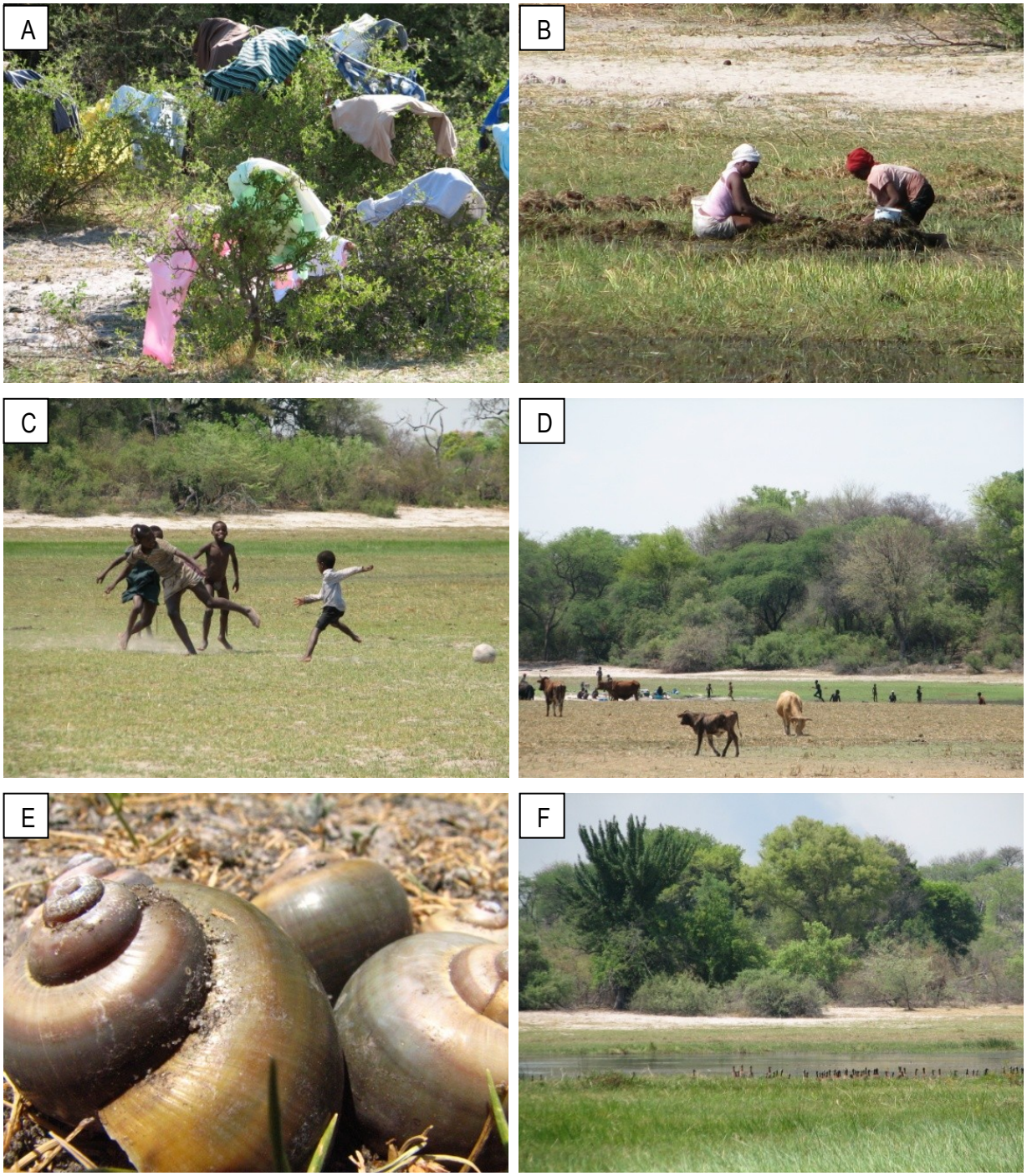

Figure 6.2: Photographs indicating the some of the activities that took place at Lake Nxamasere in October 2007. People from the local community wash their clothes in the ponds (A); Smaller ponds are made to capture fish to collect at a later stage (B); Children play in the open areas (C); Cattle utilise the area for grazing and also for watering (D). In addition, snail shells (E) and birds (F) were also observed.

Population T12AgND(c) was collected a week after Population T5ApNB in the same pond. It is interesting to note that no trichodinids were noted on the skin and or gills of both tadpole populations (with the exception of one trichodinid noted on an individual from Population T5ApNB) on the first day of each of the experiments. It would have been interesting to compare the degree of infestation of these two groups for a longer period. However, only 10 *Amietophrynus gutturalis* tadpoles were collected in this pond during the second trip to Lake Nxamasere.

2.2. The number of trichodinids counted on a smear

The current study also attempted to determine if the same pattern is obtained if the exact number of trichodinids on the respective slides is noted rather than the degree of infestation. Therefore, the number of trichodinids were counted for several of the above mentioned populations (Populations T5ApNB, T10AgNC(a), T20HaNE and T24PsNF), rather than the degree of infestation. In addition, the minimum, first quartile, average, third quartile and maximum of each of the populations were also determined on a daily basis.

A great variation in the number of trichodinids hosted on individuals from the same population was observed for most of the population groups (Figure 6.3). For example, no trichodinids occurred on smears prepared from two tadpoles of Population 5APNB that were examined on the fifth day of the experiment. However, three smears made from other tadpoles of the same population hosted between 31 and 41 trichodinids and 110 trichodinids were counted on a smear prepared from yet another individual of Population T5ApNB on the same day.

Population T5ApNB (nine days, 80 individuals):

The number of trichodinids counted on the smears increased steadily during the first three days of the experiment. However, the number of trichodinids declined on the fourth day only to increase rapidly on the fifth day of the experiment and to return on Day 6 to almost the same number of trichodinids that was counted on Day 4. The number of trichodinids on the prepared smears increased steadily on Day 7 and Day 8, and declined again on the last day of the experiment.

Population T10AgNC(a) (one day, 20 individuals):

Twenty tadpoles were examined a day after the tadpoles were sampled from Lake Nxamasere. Although no trichodinids were observed on the smears prepared from the skin of these tadpoles, a great variation occurred in the number of trichodinids counted on the smears prepared from the gills of these tadpoles. For example, one of the smears contained no trichodinids whilst 48 trichodinids were counted on a smear prepared from another individual.

Population T20HaNE (one day, two individuals):

Almost the same number of trichodinids was counted on the smears prepared from the skin and gills of one of the hosts collected on the second day of the experiment. The average number of trichodinids counted on the skin and gill smears were greatly influenced by the second host as 41 trichodinids were counted on the smear prepared from the skin while 200 trichodinids were counted on the smear prepared from the gills of the host.

Population T24PsNF (two days, two individuals):

Only one trichodinid was observed on the smear prepared from the skin of one of the tadpoles on the first day of the experiment. However, the number of trichodinids counted on the smears increased rapidly on the second day of the experiment as the smears prepared from one individual hosted 210 and 200 trichodinids on the skin and gill smears respectively.

The above discussion on each of the populations indicates that the trichodinid concentration on the hosts increased over the time period. A normal occurrence on individual hosts are also illustrated as some individuals were highly infested while other hosts had a low (or a zero) trichodinid infestation. A deficiency of this experiment is that most of the populations were not kept for a long period of time and this may influence the results.

2.3. The number of trichodinids counted on a smear versus the degree of infestation noted on a smear

A comparison between the degree of infestation and the number of trichodinids counted on the smears were made (Figure 6.4).

The degree of trichodinid infestation is not a clear / true reflection of the increasing (if any) number of trichodinids that was present on the skin and / or gill smears throughout the experiment. For example, the average degree of trichodinid infestation on the smears prepared from Population T5ApNB remained constant from the second day to the last day of the experiment. However, the number of trichodinids on the smears increased rapidly from the fourth to the fifth day, whereafter the number of trichodinids decreased on the sixth day. An average of 25 trichodinids was counted on the smears prepared a week after the experiment started and the number increased to an average of 74 on the eighth day.

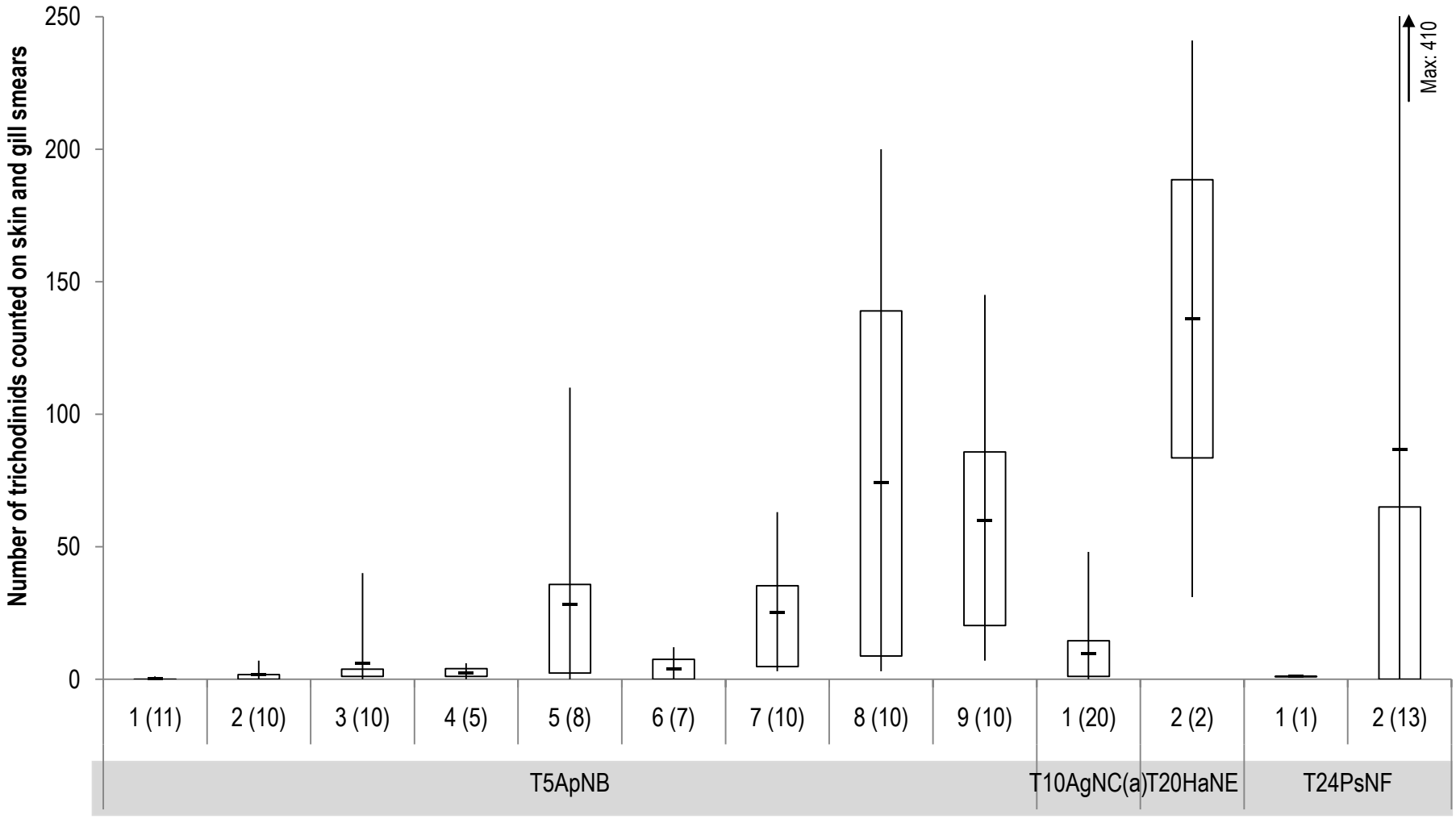

Figure 6.3: The minimum, first quartile, average, third quartile and maximum number of trichodinids counted on the smears prepared from the skin and gills of various host species during the present study. Refer to Figure 5.1 and Table 5.1 for more information on the host populations.

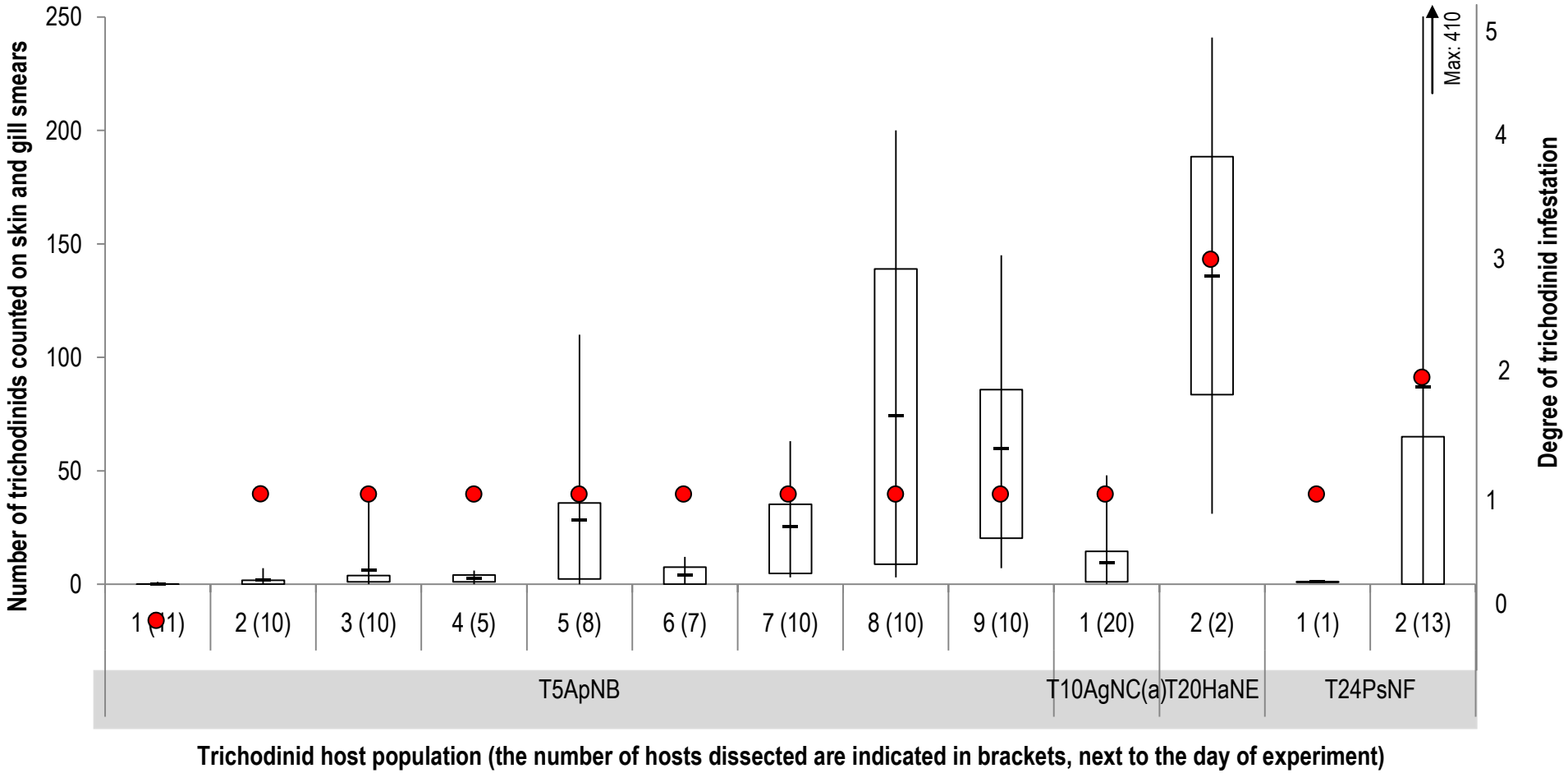

Figure 6.4: The minimum, first quartile, average, third quartile and maximum number of trichodinids counted on the smears prepared from the skin and gills of various host species in comparison with the average degree of trichodinid infestation noted on the same smears. The experimental day as well as the number of hosts used for the preparation of the mentioned slides (in brackets) is presented on the primary x-axis. The average degree was determined by a similar method as proposed by Van As and Basson (1992) where a maximum degree of five can be given. The degree of trichodinid infestation is indicated with a dot. Refer to Figure 5.1 and Table 5.1 for more information on the host populations.

3. Concluding remarks

The degree of trichodinid infestation on various host populations that were kept under experimental conditions was determined by the preparation of skin and / or gill smears of some individuals within each host population on a daily basis. It was found that the trichodinid infestation on all of the infested populations persisted throughout the experiment as the degree of infestation was either constant or increased for each population. It is suggested that the number of trichodinids on the smears should be noted in future for similar experiments as it was found that the degree of infestation does not provide a clear reflection on the trichodinid intensity on the hosts.

Chapter **7**:

Cross-**T**ransmission **E**xperiments

'…in both (history and natural sciences) we start from myths—from traditional prejudices, beset with error—and from these we proceed by criticism: by the critical elimination of errors. In both the role of evidence is, in the main, to correct our mistakes, our prejudices, our tentative theories—that is, to play a part in the critical discussion, in the elimination of error. By correcting our mistakes, we raise new problems. And in order to solve these problems, we invent conjectures, that is, tentative theories, which we submit to critical discussion, directed towards the elimination of error…'

Karl Popper

Raabe (1959) collected a trichodinid species from tadpoles he collected in Warshaw and concluded that the trichodinids belong to the species *Trichodina domerguei* f. *latispina* Dogiel, 1940. Lom (1960) conducted a cross-transmission experiment with *T. domerguei* on crusian carps and tadpoles to support the identity of the trichodinid species described by Raabe on the tadpoles. It was found that these trichodinids could be successfully transferred to naturally uninfested tadpoles, and back to naturally uninfested crucian carps. It was concluded that the trichodinids collected by Raabe from the tadpoles could be assigned to the species *T. domerguei*. This type of experiment was repeated by Lom (1960) with *T. reticulata* and *T. nigra* and found that the trichodinids could form viable populations on the naturally uninfested tadpoles.

Lom (1961) also successfully transferred *T. acuta* Lom, 1961 from *Rana ridibunda* Pallas, 1771 tadpoles to *Cyprinus carpio* Linnaeus, 1758 and *Perca fluviatilis* Linnaeus, 1758 fishes. *Bombina bombina* Linnaeus, 1761 tadpoles and *R. ridibunda*, *R. esculenta* Linnaeus, 1758 tadpoles were also successfully infested with *T. acuta* and *T. nigra* (Lom 1961). These two trichodinid species occurred on the mentioned tadpole species under natural conditions in ponds where infested fishes also occurred. Small tadpoles (1.5 – 2 cm, including tail) hosted the highest intensity of trichodinids during the experiments conducted by Lom (1961).

In addition, Kruger *et al.* (1993) collected naturally infested *X. laevis* tadpoles and placed these tadpoles in containers hosting uninfested tadpoles of *Amietia fuscigula* (Duméril and Bibron, 1841) and *Kassina senegalensis* (Duméril and Bibron, 1841). Uninfested fishes [*Tilapia sparrmanii, Pseudocrenilabrus philander* (Weber, 1897) and *Cyprinus carpio*] were also introduced to the water medium. The originally infested tadpoles were removed from the containers three days after the introduction thereof. Skin and gill smears were prepared from individuals from each of the originally uninfested host populations on the third, sixth and eleventh day of the experiment. It was concluded that all specimens of the originally uninfested host groups hosted medium to high infestations of *T. heterodentata* after three days and that an infestation persisted on these populations until the eleventh day of the experiment.

Cross-transmission experiments were also conducted during the present study to determine if the trichodinid species collected from naturally infested tadpoles could be transferred to uninfested fishes, and vice versa. This was done to determine if the trichodinid species that infested the tadpoles are the same trichodinid species that infested the fish species examined during the present study.

The following experiments were conducted in Botswana during two different field trips, the first being during December 2006 while the latter was conducted during October 2007.

1. December 2006

The first experiment was carried out to determine if uninfested tadpoles could be infested by tadpoles that hosted a natural trichodinid infestation. For this experiment, infested tadpoles were collected near the China Shop, Shakawe, Botswana while uninfested tadpoles were collected from a pond at the crocodile farm near the Leseding Research Camp.

1.1. Part 1

For this part of the experiment, tadpoles collected near the China Shop, Shakawe, Botswana (Population T2AuSH) were kept in a clean tank filled with aged water for a period of ten days before some of the tadpoles were used for the preparation of gill and skin smears. The degree of trichodinid infestation was determined from the skin and gill smears according to the method proposed by Van As and Basson (1992) (Table 7.1).

Table 7.1: Degree of trichodinid infestation on tadpoles collected from the China Shop, Shakawe after 10 days of captivity as well as the tadpoles collected near the Leseding Camp.

Tadpole population	Degree of infestation										
	1	2	3	4	5	6	7	8	9	10	Average
China Shop	4	3	3	4	5	3	3	4	4	3	3.6
Leseding Camp	0	0	0	0	0	0	0	0	0	0	0

The tadpoles collected from the crocodile farm hosted no trichodinids. This group could be distinguished from the infested tadpoles due to their size as the uninfested tadpoles were smaller than those collected near the China Shop.

1.1.1. Material and methods

The infested tadpoles were placed in the same water medium than smaller, uninfested tadpoles. The tank was washed with a salt solution before the water and / or tadpoles were added in order to ensure that the tank was clear of parasitic protozoans. The spread of trichodinids in the closed host system was monitored on a daily basis by means of a method proposed by Van As and Basson (1992) (Table 5.2) as two initially infested hosts and ten initially uninfested hosts were examined during a period of six days. As the tempo of reproduction of trichodinids increases at warmer temperatures as reported by

Kruger (1992), the water temperature was also noted on a regular basis during the experiment. The degree of trichodinid infestation on these host populations were statistically compared with one another.

1.1.2. Results and discussion

It was determined that the water temperature varied between 23°C and 27°C during the experimental period and this temperature is considered to be favourable for the reproduction of trichodinids. No trichodinids were observed on the originally uninfested tadpoles on the day that the experiment started (Day of experiment: 0). However, 80% of the tadpoles from the originally uninfested group hosted a small number of trichodinids within 24 hours (Table 7.2 and Appendix A).

Table 7.2: The degree of trichodinid infestation on the examined host populations. The degree of infestation was determined by means of a method proposed by Van As and Basson (1992) (refer to Table 5.2)

Day of experiment	Degree of infestation in initially infested host population			Degree of infestation on initially uninfested host population										
	Individuals examined		Average	Individuals examined										Average
0	4	3	3.5	0	0	0	0	0	0	0	0	0	0	0
1	4	4	4	0	1*	2*	1*	5*	0	3*	3*	3*	6*	2*
2	4	4	4	1	1	0	0	0	0	0	0	0	0	0.2
3	4	4	4	1	1	0	0	1	1	1	0	1	0	0.6
4	4	4	4	2	3	2	2	2	3	2	1	2	3	2
5	4	3	3.5	3	3	2	3	4	3	2	2	3	2	2.5
6	4	3	3.5	3	4	3	3	2	3	2	3	4	3	2.7

*Only a small number of trichodinids were observed. Therefore, the number of trichodinids observed is given.

From the above table it is evident that a trichodinid population established on the initially uninfested host group (tadpoles previously collected from the crocodile farm office) within 24 hours after the initially uninfested hosts were joined with the initially infested hosts and that the degree of infestation increased rapidly during the experimental period. It is therefore suggested that this trichodinid species that naturally occurred on tadpoles could form a viable population on the uninfested tadpole population.

Only a small number of tadpoles were used during the above experiment and it was suggested that the experiment should be repeated with more tadpoles (more carriers as well as uninfested hosts). This will be discussed as Part 2 of the experiment.

1.2. Part 2

As explained, the above experiment only included a small number of possible host individuals and therefore a similar experiment was conducted a few days later (January 2007) with tadpoles collected from the same initial populations [i.e. infested *Amietophrynus* tadpoles from Shakawe (Population T2AuSH) and uninfested *Amietophrynus* tadpoles collected near the crocodile farm office (no population number was assigned to this group as no trichodinids were collected on these tadpoles)] used during the previous experiment as this will aid in drawing a correlation between the two experiments. In addition, two larger temporary aquariums than those used during the first part of the experiment were used to host the two tadpole populations. The reasoning behind the larger temporary aquariums was that this provided the trichodinids with a larger area to move about. Therefore, the trichodinids had to 'search harder' to find a new host in the larger water medium.

1.2.1. Material and methods

Ten infested tadpoles were placed in each of the larger temporary aquariums. More than 200 uninfested (smaller) tadpoles were added to each of the temporary aquariums. Smears of one initially infested and ten initially uninfested tadpoles were made on a daily basis for the following six days in a similar manner as described during Part 1 of the experiment. The degree of trichodinid infestation on these host populations were statistically compared with one another.

1.2.2. Results and discussion

In both tanks, the initially infested tadpoles hosted a high trichodinid infestation ranging between 3 – 5 degrees throughout the experiment. The degree of infestation on the originally infested population decreased on the fifth day of the experiment in both tanks. However, the infestation increased again on the sixth day in one of the tanks (Tables 7.3, 7.4).

The degree of infestation on the naturally uninfested population increased rapidly in both tanks as 1 – 10 trichodinids were counted on all the skin or gill smears prepared from these tadpoles within 24 hours of the experiment (Tables 7.3, 7.4).

Table 7.3: Degree of trichodinid infestation on originally uninfested tadpoles in one of the two temporary aquariums (Aquarium A).

Day of experiment	Degree of infestation of initially infested host population		Degree of infestation of initially uninfested host population										
	Individuals examined		Individuals examined										Average
0	4	4	0	0	0	0	0	0	0	0	0	0	0
1	4	4	1	1	1	1	1	1	2	1	1	1	1.1
2	4	4	3	2	2	3	3	3	3	3	3	3	2.8
3	4	4	3	3.5	3.5	3	3	3	3.5	3	3	3	3.15
4	4	4	3.5	3.5	3.5	3	3	3.5	4	3.5	3.5	3	3.4
5	3	3	3	3	3	3	3.5	3.5	3	3	3	3	3.1
6	4	4	3.5	4	3.5	3.5	3.5	3	4	4	4	4	3.7

Table 7.4: Degree of trichodinid infestation on originally uninfested tadpoles in the other temporary aquarium (Aquarium B).

Day of experiment	Degree of infestation on initially infested host population		Degree of infestation on initially uninfested host population										
	Individuals examined		Individuals examined										Average
0	4	4	0	0	0	0	0	0	0	0	0	0	0
1	4	4	1	1	1	2	1	1	1	1	1	1	1.1
2	4	4	3	3	3	3	2	3	2	3	3	3	2.8
3	4	4	3	3.5	3	3	3	3	3	3	3.5	3	3.1
4	4	5	3	3	3.5	3.5	3	3.5	3	3.5	3.5	3	3.25
5	3	4	3	3.5	3	3	3	3.5	3.5	3.5	3.5	3.5	3.3
6	3	4	3	3.5	4	3.5	3	3	3	3	3	3.5	3.25

It seems as if the trichodinids formed a viable population on the initially uninfested hosts. It is interesting to note that the degree of infestation is much higher (Figure 7.1) than the infestation during Part 1 of the experiment. As the water temperature variation within the aquariums correlated highly during the two experiments (between 23ºC and 27ºC), the water temperature cannot be seen as the reason behind the increased number of trichodinids on the initially uninfested host population.

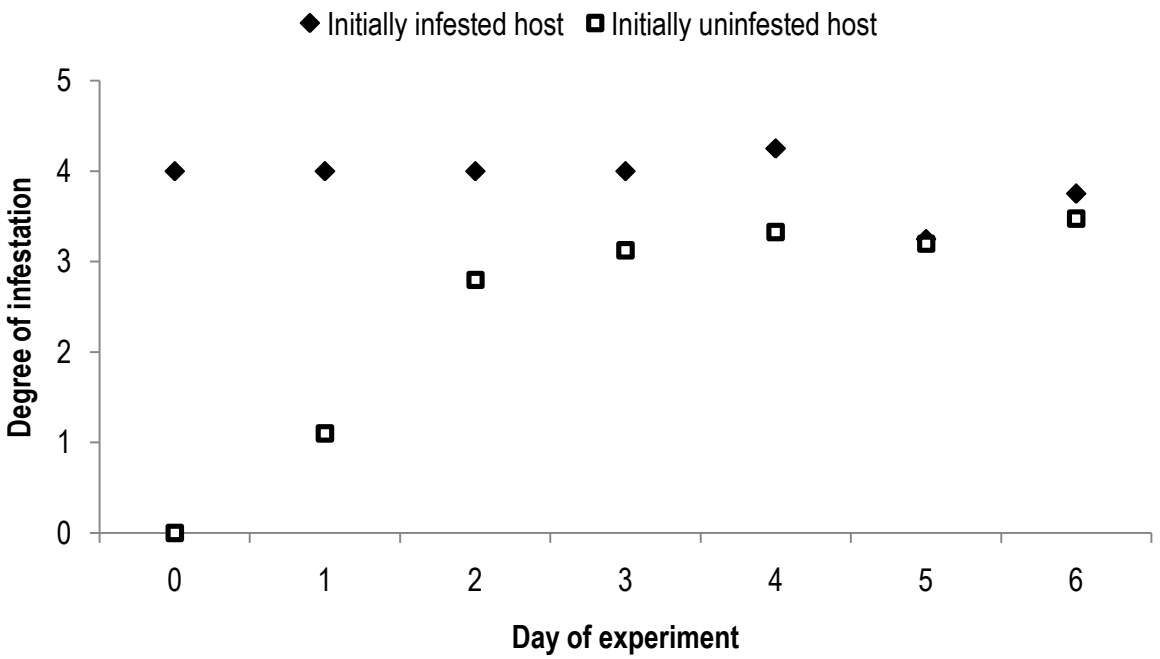

Figure 7.1: Degree of trichodinid infestation on two tadpole populations studied during the present study. Refer to Table 5.2 for an explanation on the degree of trichodinid infestation.

Additional theories are provided for the possible reasoning behind the enormous increase in trichodinids that was observed: the first being the fact that trichodinids increase in number when the host is stressed (Lom 1961). It is proposed that the tadpoles used during this experiment were under less stress than the individuals used during the first part of this experiment as a larger area was available per tadpole during this experiment. It should also be mentioned that the tadpoles were fed with the same plankton material collected from the same water source than used during the first part of this experiment. Therefore, an increase in stress due to food scarcity is also eliminated.

Another potential explanation could be that the larger tadpoles provide a larger area for the attachment / movement of trichodinids on each host. One should refer to the work done by Lom (1961) with the above in mind as he studied the trichodinid infestation on fishes and various tadpoles in the former Czechoslovakia. The author collected various tadpole species [including *Rana ridibunda, R. esculenta, R. temporaria* Linnaeus, 1758; *Bufo bufo* (Linnaeus, 1758); *Bombina bombina* and *Hyla arborea* (Linnaeus, 1758)] infested with *Trichodina nigra and T. acuta* and mentioned that most of the trichodinids were hosted on the thickened central part of the tail or around the rectum of tadpoles with a length of 1.5 – 2cm.

Lom (1961) further mentioned that the infestation was gradually lost as soon as the back legs began to develop. The same observation was made by Kruger (1992) who studied the trichodinid infestations on various tadpole populations in southern Africa. Kruger (1992) mentioned that a smaller number of trichodinids were generally hosted by tadpoles that already developed legs in comparison to the number of trichodinids found on younger tadpoles during her study. A possible reason for the detachment of trichodinids on the older developing tadpoles is twofold:

a) The skin of the tadpoles may be less suitable for the attachment of the protozoa
b) Trichodinids cannot survive in a dry environment and need at least a moistened habitat

The skin of the young toad will become dry as it leaves the water medium to explore its new terrestrial habitat. Therefore, the trichodinids have to detach from the host before it leaves the water medium (Kruger 1992).

Unfortunately, the *Amietophrynus* tadpoles studied during the current study could not be identified to species level. In addition, the body lengths of the tadpoles were not recorded. This said, it is impossible to correlate the findings by Lom (1961) and Kruger (1992) with the information recorded during the current study and therefore no conclusive answer to the reason for the rapid increase in the trichodinid population size during the second experiment can be provided.

Another interesting observation is the fact that the trichodinid population decreased on both the carrier and initially uninfested host groups on the fifth day of the experiment. A possible reason for this remains unresolved.

2. October 2007

Lom (1961) stated that no known trichodinid found on the skin or gills of tadpoles are hosted exclusively by tadpoles and that infested tadpoles can only be found in a water medium containing infested fishes. He further stated that fishes serve as trichodinid reservoirs and that some trichodinid species use tadpoles as a facultative host and disappear from the anuran larvae before the metamorphosis process of the host is completed (Lom 1961).

The last experiment that will be discussed as part of the cross-transmission experiments conducted during the current study was performed to determine if trichodinids hosted on tadpoles in southern Africa can form a viable population on fish hosts on their skin and / or gills, and *vice versa*.

2.1. Part 1

The first part of this experiment was conducted to determine if the trichodinid infestation can be transmitted from naturally infested tadpoles to naturally uninfested fishes. Thus, a large population of heavily infested tadpoles and a large population of uninfested fish had to be collected. This was achieved by collecting *Barbus barnardi* fish at the Thamalakane Bridge and *Amietophrynus poweri* tadpoles near the Toteng Bridge on 16 October 2007. No trichodinids were observed on the skin and / or gill smears prepared from ten fish. Therefore, the fish population was used as the initially uninfested host group. The tadpoles, on the other hand, were heavily infested with trichodinids and could thus be used as the initially infested host group.

2.1.1. Material and methods

Even though no trichodinids could be observed on the skin or gill smears made from ten *B. barnardi* individuals, the fish were bathed in a 1.5% salt solution for a period of 45 minutes in order to render them trichodinid free.

A similar set up as discussed during the previous experiment was used during this experiment as a number of infested hosts (i.e. *A. poweri*) and a larger number of uninfested hosts (i.e. *B. barnardi*) were placed in tanks containing filtered aged water. The number of trichodinids on the skin and gill smears prepared daily from the tadpoles was quantified by the method proposed by Van As and Basson (1992) and the result thereof was simplified as the average degree of infestation for this population was determined on a daily basis. In contrast, the number of trichodinids counted on the smears prepared from the skin and gills of the fish samples was noted, as only a few trichodinids were observed on these smears.

Some of the froglets used during this experiment already developed hind legs. Therefore, small floating objects were placed in the tanks to serve as a temporary refuge for the individuals that wished to escape the water medium at a later stage (Figure 7.2).

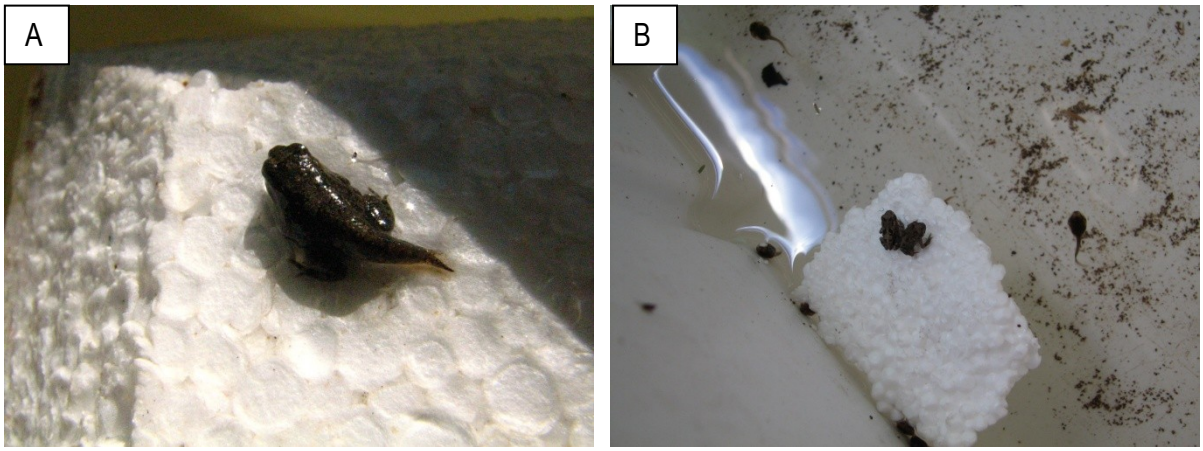

Figure 7.2: Photographs indicating the polystyrene floating objects that were used to serve as a temporary refuge area for froglets that wished to escape the water medium.

2.1.2. Results and discussion

It was found that the tadpoles hosted a naturally heavy trichodinid infestation before the experiment began but that no trichodinids occurred naturally on the skin or gills of the fish collected. Although no trichodinids was observed on the fish, trichodinids such as *Trichodina heterodentata* occur naturally on cichlid fish (Kruger *et al.* 1993).

All individuals (both fish and tadpoles) examined after 24 hours hosted a trichodinid population. One of the tadpoles died during the second day of the experiment. The reason for its mortality is unknown as no external or internal signs indicated a reason for its death. The dead tadpole was examined for the presence of trichodinids and a high infestation on the skin of the tadpole was observed (Degree of infestation: 4).

Although Lom (1961) and Kruger (1992) mentioned that trichodinids will detach from tadpoles before the metamorphosis process is completed, trichodinids were also collected from the skin of individuals that made use of the floating polystyrene objects. Some of these froglets still had tails, while others even developed further (Figure 7.3). A possible explanation for the presence of the trichodinid infestation can be due to the fact that these individuals were kept under unnatural conditions. Tadpoles (and fish) were placed in a closed system and therefore competition between the trichodinids to find a suitable host in this system cannot be ignored. Trichodinids need a moistened habitat to survive (Kruger 1992). The trichodinids could still survive on the skin of individuals that used the floating objects as their skin was still moist. Therefore the trichodinids could still survive on these hosts and could therefore still use these individuals as temporary hosts. It is suggested that the trichodinids would probably have detached from these hosts under natural conditions.

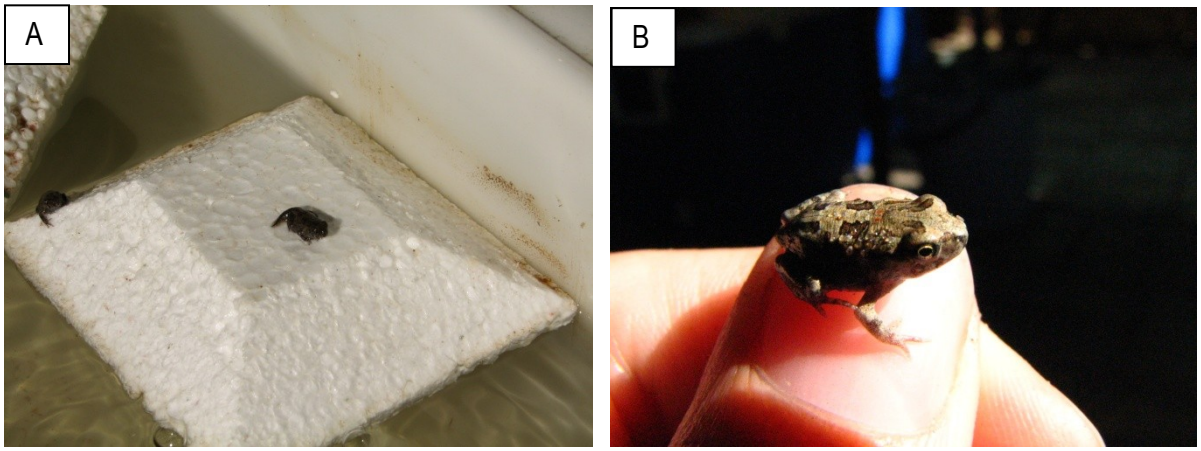

Figure 7.3: Anuran larvae developed into froglets during this experiment.

A trichodinid infestation persisted on the gills of the tadpoles throughout the experiment while no trichodinids were observed on the skin smears prepared from the tadpoles on the first two days of the experiment (Table 7.5).

Table 7.5: Quantified analysis of trichodinid distribution on tadpoles collected from Toteng Bridge. Ten of the collected tadpoles were sampled on a daily basis to determine if the trichodinid population persisted on the tadpoles. Note that no tadpoles were examined on the sixth day.

Day of experiment	Average length (mm)			Average trichodinid infestation	
	Body	Tail	Legs	Skin	Gills
1	5.75	7.05	0	0	1.9
2	5.7	5.80	0	0	2.3
3	5.22	6.39	0	1.4	1.9
4	5.35	6.05	0	1.2	2.4
5	5.85	6.55	0.15	1	1.8
7	5.7	6.25	0	1.5	2.1
8	5.35	6.45	0.1	4	3.4

The number of trichodinids that occurred on *B. barnardi* increased until the fourth day, where after a decrease in the infestation was observed (Table 7.6). It is believed that the fish was under stress during the first part of the experiment and more relaxed during the last part of the experiment as the fish adapted to their new environment and new food source. Another contributor to their stress may include the irritation that the salt solution bath may have had on the fish. As trichodinids multiply at a faster rate when the host is stressed (Lom 1961), the decrease in number of trichodinids observed on the smears made on Day 5 and Day 6 possibly can be ascribed to the fact that *B. barnardi* got used to its new environment and food sources during this period. It is not suggested that the trichodinids decreased in number as they found the fish not to be a suitable host but rather that the general condition of the fish

increased during this period and therefore the skin (and or gills) of the hosts were not that attractive as a host in comparison to the niche available on the tadpole hosts.

Table 7.6: Degree of infestation on initial uninfested *Barbus barnardi* Jubb, 1965 population is given. Please note that the number of trichodinids on the host were small. Therefore, the number of trichodinids counted on the smears is given rather than the degree of infestation.

Day of experiment	Number of trichodinids counted on the smears prepared from the skin and / or gills of the naturally uninfested host population					Average	Number of individuals with a skin infestation	Number of individuals with a gill infestation
0	0	0	0	0	0	0	0	0
1	55	6	5	2	9	15.4	5	2
2	80	38	76	18	35	49.4	5	4
3	34	38	28	37	36	34.6	5	3
4	20	6	150+	30	17	44.6	5	2
5	68	26	3	11	28	27.2	5	3
6	9	6	12	4	21	10.4	5	4
Total number of fish with a trichodinid infestation							30	18

It is suggested that the trichodinids that naturally occurred on the *A. poweri* tadpoles could form a viable population on *B. barnardi.*

2.2. Part 2

For the second part of this experiment, *A. poweri* tadpoles were collected from a pond near the office of the crocodile farm (Figure 7.4A). No fish occurred in the pond. The eggs from which the tadpoles developed, were previously noticed in the pond on the 13th of October 2007 and the first tadpoles were noticed a day later by Makfenya, a lady who worked at the office. Five samples from the pond were collected on the 16th of October to determine if a possible trichodinid infestation occurred on these tadpoles. The tadpoles were still small (6-8mm) (Figure 7.4B) and therefore the whole tadpole was squashed between a slide and a cover slip. One positive outcome of this method is that the host dies almost instantly and that all trichodinids on the host could be counted as the skin and gill smears of larger tadpoles only contained a portion of the trichodinids that occurred on the host.

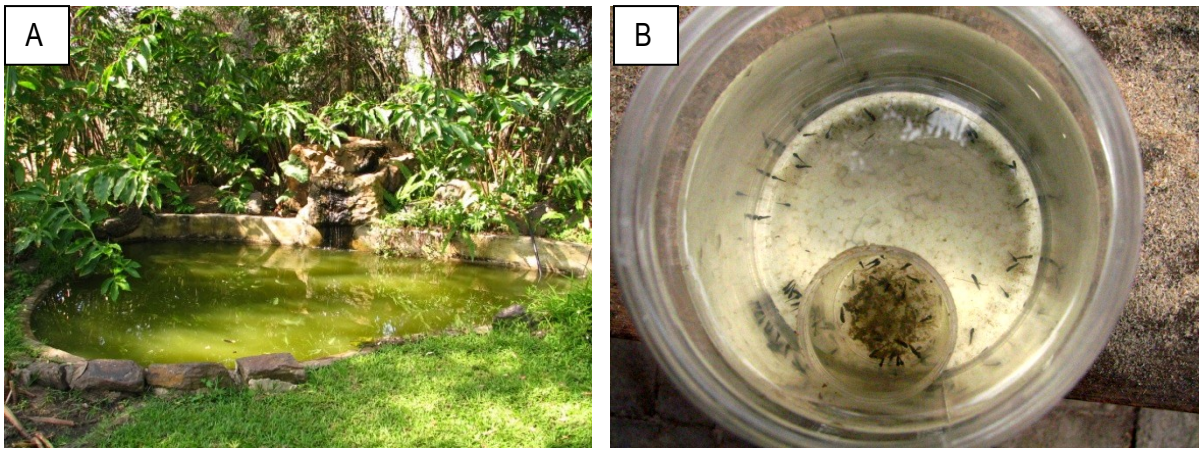

Figure 7.4: Uninfested tadpoles were collected from this pond, near the crocodile farm office (A). The tadpoles were small on the first day of collection (B).

No trichodinids was found on the smears prepared from these tadpoles. This corresponds to the finding by Lom (1961) that a trichodinid infestation can only persist on tadpoles if infested fishes are also to be found in the same water medium.

Lom (1961) conducted a similar experiment and proposed that possible hosts should be washed with trypaflavine to guarantee that no trichodinids survive on the possible hosts. It should be mentioned that Lom treated fish and not tadpoles during his experiment. Lom (1961) kept the fish separately in a clean tank for a period of two weeks before the tadpole infested population was introduced to the fish.

The method by Lom (1961) posed two dissimilarities to the current experiment:
a) Lom treated fish and not tadpoles
b) Time was limited during the current experiment.

The tadpoles were in the process of metamorphosis and therefore the present study couldn't wait another two weeks after the trypaflavine treatment before the infested host group (*B. barnardi*) could be introduced to the tadpole population. Another solution for the problem at hand had to be found and therefore it was decided to play with the concentration of formalin wherein infested tadpoles could be bathed during a certain time interval.

2.2.1. Material and methods

After numerous attempts, it was concluded that tadpoles could be bathed in a 4% formalin solution for a period of ten minutes as this ensured that no trichodinids survived on the skin and or gills (or water medium) of these tadpoles (Figure 7.5). Although the trichodinids died within a shorter period when

higher formalin concentrations were used, the tadpoles seemed to be negatively affected as they moved at a slower, irregular pace even two hours after treatment than untreated individuals.

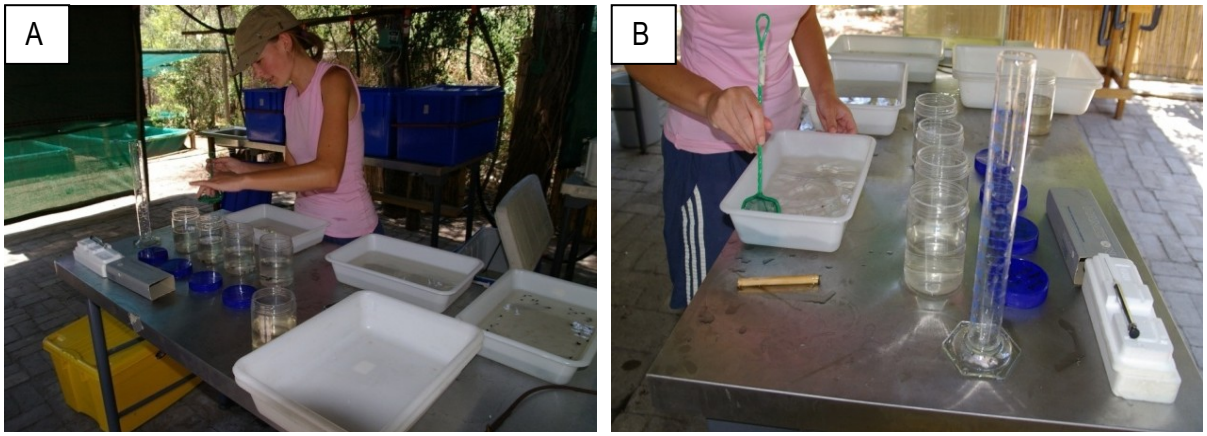

Figure 7.5: Tadpoles were bathed in a 4% formalin solution in order to render them trichodinid free before the second part of the cross-transmission experiment were undertaken.

Hundred uninfested tadpoles and 20 of the fish used in the first part of the experiment (now infested with a trichodinid population) were placed in a clean tank filled with filtered aged water. The degree of trichodinid infestation on both host populations was recorded on a daily basis over a period of six days as proposed by Van As and Basson (1992) (Table 5.2).

2.2.2. Results and discussion

A trichodinid infestation persisted on the fish throughout the experiment. In contrast to the first part of the experiment where only a few trichodinids infested the initially uninfested host group (i.e. *B. barnardi*), a relatively high trichodinid infestation occurred on the initially uninfested tadpoles within 24 hours of the experiment. An interesting observation is that all of the tadpoles examined on Day 1 hosted trichodinids on the gills and that the skin of only one individual was infested on Day 2 (Table 7.7). A moderate trichodinid infestation (Degree of infestation ranging from 2 – 3) was noted on the tadpoles from the second day until the end of the experiment.

It is therefore suggested that the trichodinids that was hosted on *B. barnardi* could form a viable population on *A. poweri* larvae. When the degree of infestation between the two parts of this experiment is compared with one another, it can be seen that the number of trichodinids on both the initially uninfested host groups increased from Day 0 – Day 2. However, the number of trichodinids on these populations decreased during the third day, increased on the fourth day and decreased again until the last day of the experiment (Figure 7.6).

Similar results are found in nature when the degree of trichodinid infestations on various individuals within a single population (fish or tadpoles) is studied. Some of the naturally infested host individuals usually have a high number of trichodinids, while others only host a small number of trichodinids, if any. Kruger (1992) therefore suggested that some hosts within a population may be more resistant to a trichodinid infestation.

Additionally, trichodinids increase in number when the host is stressed (Lom 1961). The hosts utilised during the present study were probably stressed as the hosts were collected from natural environments and placed in small tanks, with a great number of individuals, under experimental conditions. In addition, the hosts had to adapt to the fish food flakes that were provided on a daily basis.

Trichodinids will probably detach from a host in search of a more suitable host. A more suitable host may include a younger and / or a weaker individual of the same species. The skin of a weak host may produce more mucus and more food will thus be available to the trichodinids.

During the experiment, the hosts were kept in a small tank, leading to the hosts being kept in close proximity to one another. This may have also contributed to the translocation of trichodinids to smaller and / or weaker hosts during the experiment, leading to the variance of the degree of infestation noted during the present study.

Table 7.7: Degree of trichodinid infestation on the initial uninfested host population (*Amietophrynus poweri* Hewitt, 1935).

Day of experiment	Degree of infestation on initially uninfested host population										Average	Number of individuals with a skin infestation	Number of individuals with a gill infestation
0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	2	2	2	1	2	2	2	2	1	2	2	0	10
2	3	3	2	2	3	2	2	2	3	3	3	1	10
3	2	2	2	3	2	2	3	1	2	2	2	10	8
4	2	3	3	3	2	2	3	2	3	2	3	8	10
5	2	2	3	1	2	3	2	2	1	2	2	7	10
6	2	2	3	3	3	3	2	2	2	1	2	7	9
Total number of tadpoles infested with a trichodinid species												35	57

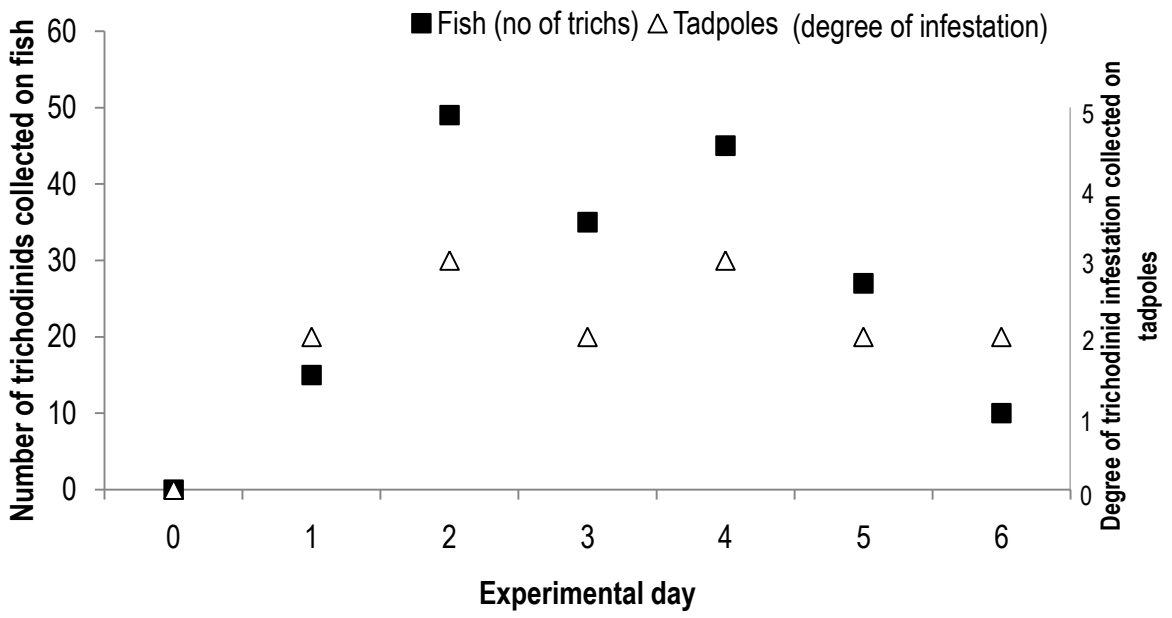

Figure 7.6: Correlation between the trichodinid infestations on both host groups during the experiment.

3. Concluding remarks

Two cross-transmission experiments were conducted to determine if the trichodinids hosted on tadpoles are transmissible to uninfested tadpoles and / or to fishes. It was concluded that the trichodinids could form a viable population on both the naturally uninfested fish population and uninfested tadpoles. It is suggested that the trichodinids encountered on the skin and / or gills of tadpoles under natural conditions may also naturally infest fishes. This may indicate that trichodinids hosted by tadpoles in southern African waters may be fish trichodinids that utilise tadpoles as a facultative host, as was suggested by Lom (1961).

Chapter **8**:

Trichodinid **I**nfestation **I**ntensity on **T**adpoles **U**nder **E**xperimental **C**onditions

'…No amount of experimentation can ever prove me right;
a single experiment can prove me wrong…'

Albert Einstein

A study on the quantitative analysis of the prevalence, abundance and intensity of infestation as well as the distribution pattern of trichodinids on tadpoles was undertaken by Fernandes *et al.* (2011) by examining naturally infested *Rhinella pombali* (Baldissera, Caramaschi and Haddad, 2004) tadpoles from a stream near the city of Juiz de Fora, Brazil. The prevalence, mean intensity and abundance values of the trichodinids on the skin and / or gills of the tadpoles were determined for four tadpole weight classes. Additionally, the diameters of several trichodinids from the same host were measured to determine whether average size of the trichodinids is influenced by the infestation intensity (Fernandes *et al.* 2011). Fernandes *et al.* (2011) found that all tadpoles examined were infested with *Trichodina heterodentata* and that the infestation was uniformly distributed on the tadpoles. It was also determined that heavier tadpoles hosted a higher trichodinid load. Although Churcher *et al.* (2006) mentioned that an increase in parasite intensity can alter parasite characteristics due to food competition, Fernandes *et al.* (2011) could not find a significant variance of the mean trichodinid diameter. These authors concluded that the studied trichodinid populations did not demonstrate density-dependent effects in terms of body size.

In contrast to the finding by Fernandes *et al.* (2011) that larger tadpoles hosted more trichodinids, Lom (1961) studied *T. nigra* and *T. acuta* infestations on various tadpole species in the former Czechoslovakia and came to the conclusion that most of the trichodinids were hosted on the thickened central part of the tail or around the rectum of tadpoles with a length of 1.5 – 2 cm. The author further mentioned that the infestation was gradually lost as soon as the hind legs of the tadpoles began to develop and that trichodinids usually detached themselves before the metamorphosis process of the tadpole host was completed.

A similar observation was made by Kruger (1992) who studied the position of trichodinid infestations on various tadpole species in southern Africa. Kruger (1992) mentioned that fewer trichodinids were generally hosted by tadpoles that already developed hind legs in comparison to younger individuals. In addition, most of the trichodinids hosted by older tadpoles were found on the hind legs and towards the tail of these hosts. In contrast to the observation by Lom (1961), Kruger (1992) noted that older tadpoles hosted lower infestations around the rectum area as well as the webbed area between the toes.

Kruger (1989) conducted an experiment to determine the position of *T. heterodentata* infestations on *Xenopus laevis* tadpoles as these tadpoles undergone various stages of metamorphosis. *Xenopus laevis* can be seen as a suitable host to conduct these experiments as this species has a breeding season of up to six and a half months (Balinsky 1969). Kruger (1989) mentioned that a trichodinid

infestation may occur on the skin of *X. laevis* tadpoles during any of the metamorphosis stages (Figure 8.1), but that the position of the trichodinid infestation may differ at various tadpole developmental stages. As an example, a trichodinid infestation occurred on all parts of the body of the tadpole during the first three metamorphosis stages. Conversely, the position of the infestation became more concentrated near the webbing of the back legs during the following three stages. During Stage 7 – Stage 9, most of the trichodinids were hosted on the webbing between the toes, or on the tip of the tadpole tail. Trichodinids were only observed on the webbing of tadpoles that reached Stages 10 – 12 as well as young frogs. However, no trichodinids were observed on the skin of adult *X. laevis* during the mentioned experiment (Kruger 1989).

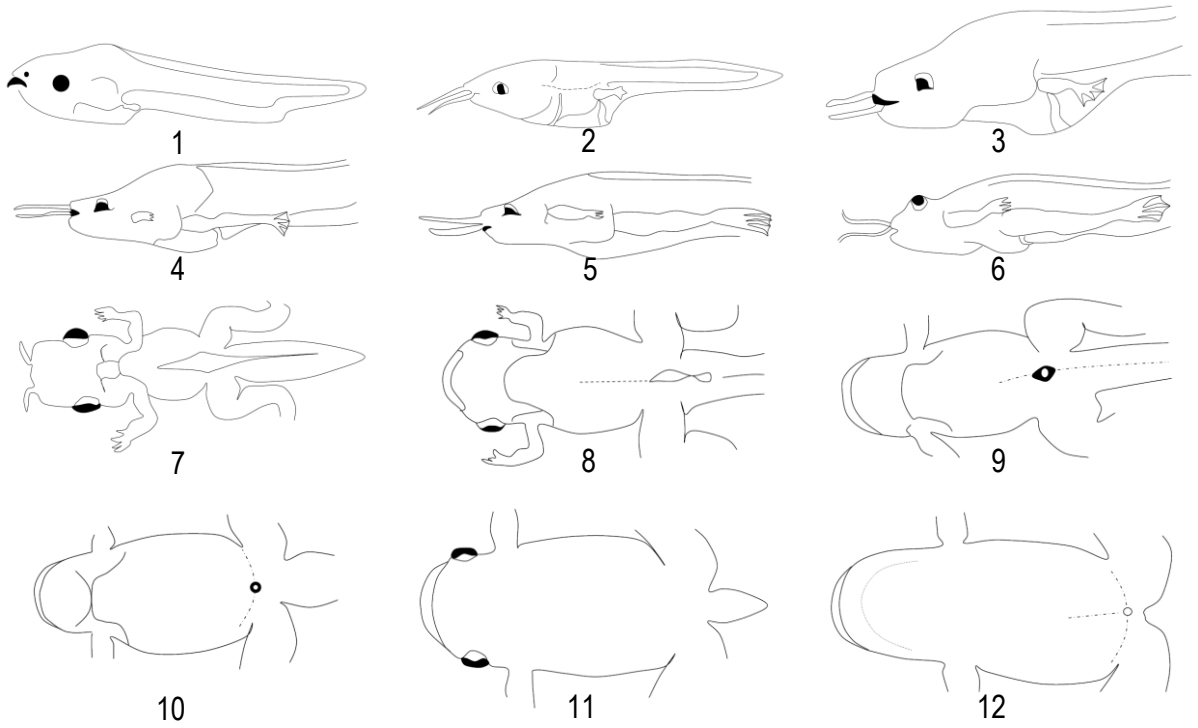

Figure 8.1: Indication of the twelve morphological stages of *Xenopus laevis* Daudin 1802, redrawn from Kruger (1989).

Kruger (1989) mentioned that the position of the trichodinids could be ascribed to the development of adult epidermis of the host as guanophores (indication of adult skin) is present on the abdomen and thigh of tadpoles that reach the fourth stage of metamorphosis. In addition, a large part of the skin of tadpoles that reach Stage 8 is covered with guanophores (Nieuwkoop and Faber, 1967). Thus, it is believed that the composition of adult anuran skin is not a suitable surface for the attachment of trichodinids (Kruger 1989).

Authors such as Thompson *et al*. (1947), Lom (1966) and Kruger (1992) mentioned that external trichodinid infestations cannot be kept on fishes and tadpoles in unnatural environments for a long period of time.

One of the objectives of the present study was to determine if the above statements are indeed the case. Therefore, the degree of trichodinid infestation on different host populations was studied by means of an experiment conducted in October 2007.

1. Material and methods

Possible hosts were collected from various water bodies in October 2007 during a field trip in Botswana. Each host population was kept separate from one another to ensure that no transmission of trichodinids to and from the different possible hosts could occur during the transportation of the hosts to the Leseding Research Camp.

The tadpoles were identified by using either a key by Channing (2001) or by Du Preez and Carruthers (2009) while Skelton (2001) was used to identify the collected fish species. Please refer to Table 5.1 and Figure 5.1 for more information on the host population, including the locality of collection.

Skin and gill smears were made from a number of hosts within each population and a compound microscope was used to determine if the possible hosts were infested with trichodinids. However, if the tadpole was too small, the entire tadpole was compressed between a microscope- and a cover slide. The degree of infestation was noted by means of a similar index (Table 5.2) as proposed by Van As and Basson (1992). In cases where a small number of trichodinids were observed, the number of trichodinids counted was noted, and the degree of infestation was calculated.

Each of the infested (and uninfested) host populations were transferred to separate clean tanks filled with aged water. The degree of trichodinid infestation was recorded on a daily basis by preparing skin and gill smears of ten hosts from each population where possible, as the number of hosts examined during the experiments depended on the number of individuals collected. For example, Population F19HmNA consisted of only two fish samples. Therefore, skin and gill smears were prepared from one fish on Day 1 while the other fish was examined on the second day of the experiment.

In addition, the body length (mm) and tail length (mm) (Figure 8.2) of the hosts used for the preparation of the skin and gill smears were noted.

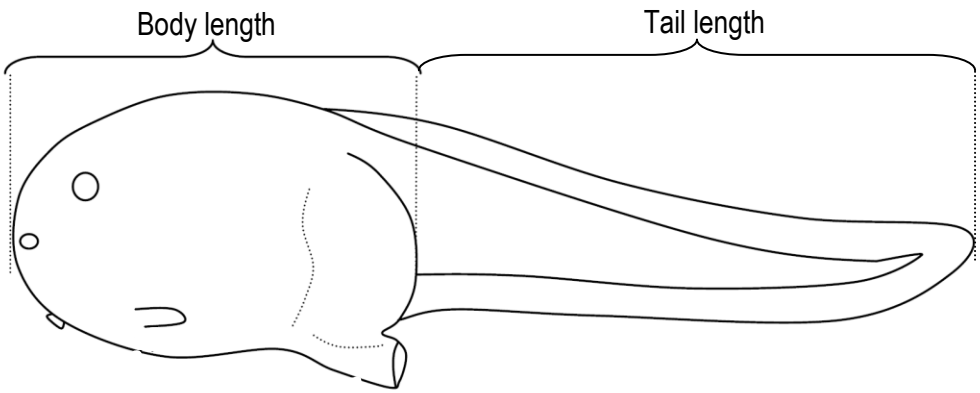

Figure 8.2: The body length and tail length of tadpoles used in the experiment was measured. The leg length was also measured in instances where the back and / or front legs already developed.

The number of hosts dissected within each population was also noted. The intensity of the trichodinid infestation of each population was evaluated / compared with the body length respectively by using 100% stacked columns as these graphs emphasise the proportion of each 'trichodinid infestation degree' within each of the body measurements.

The degree of infestation on the skin versus the degree of infestation on the gills of tadpoles was also examined for a few of the above populations.

2. Results and discussion

2.1. Degree of infestation

Eleven tadpole populations and two fish populations were examined on a daily basis as part of this experiment. Green (2001) noted that the immune system of tadpoles are less effective than that of adult amphibians and it is therefore suggested that the intensity of a trichodinid infestation on juvenile anurans may be related to the developmental stage of the tadpoles. Poynton and Whitaker (2001) mentioned that a heavy trichodinid infestation could be associated with clinical diseases such as skin decolouration, ulcers and reddened gills or skin, where infested (haemorrhage). However, none of the tadpoles showed any side effects due to their trichodinid infestation during the current study.

Fernandes *et al.* (2011) studied the degree of trichodinid infestation on *R. pombali* tadpoles and concluded that heavier tadpoles hosted more trichodinids. A similar conclusion can be made when studying the degree of infestation of *A. gutturalis* tadpoles that were collected from Lake Nxamasere [Populations T10AgNC (a and b), Appendix A] during the current study. These tadpoles were collected

on the same day, in the same pond but placed in different tanks upon arrival at the Leseding Camp. Individuals from Population T10AgNC(b) were generally larger in size and some already developed hind legs before the experiment commenced. Some of these froglets also made use of the floating objects during the experiment (Figure 7.2).

It is a known fact that trichodinids cannot survive in a dry environment and need at least a moistened habitat (Kruger 1992). The skin of a young toad becomes too dry as it leaves the water medium to explore its new terrestrial habitat and Kruger (1992) therefore suggested that the trichodinids have to detach from the toad before it leaves the water medium. However, the trichodinids did not detach from the tadpoles that developed legs or even the froglets that used the floating objects during the current experiment as trichodinids were still observed on the smears prepared from the skin of these individuals. Live froglet specimens were also placed under a light microscope and the trichodinids could be seen moving all over the skin and eye lids of even the froglets that made use of the floating objects.

Although most of the tadpoles from both mentioned populations hosted trichodinids, Population T10AgNC(a) hosted less trichodinids per tadpole (degree of infestation ranging from none to two) than Population T10AgNC(b) (degree of infestation ranging from one to five) (Figure 8.3).

The following should be kept in mind: Although both Populations T10AgNC (a and b) consisted of *A. gutturalis* tadpoles collected from Lake Nxamasere in October 2007, Population T10AgNC(b) consisted of more tadpoles than Population T10AgNC(a). Therefore the tadpole density in the tank that hosted Population T10AgNC(b) was higher than in the tank that hosted Population T10AgNC(a). Anderson and Gordon (1982) mentioned that parasite distribution patterns are determined by the rate of transmission, which is more intense in aggregated hosts. Therefore, hosts living in close proximity to one another (denser populations) could lead to the more rapid and successful translocation of trichodinids to uninfested hosts in comparison to infested individuals living in isolation. Therefore, the phenomenon of Population T10AgNC(a) hosting less trichodinids than Population T10AgNC(b) cannot be ascribed solely by the difference in body size. As a further confirmation of the above, both populations hosted tadpoles with a body size of 7 mm and the tadpoles from Population T10AgNC(b) hosted more trichodinids than tadpoles from Population T10AgNC(a).

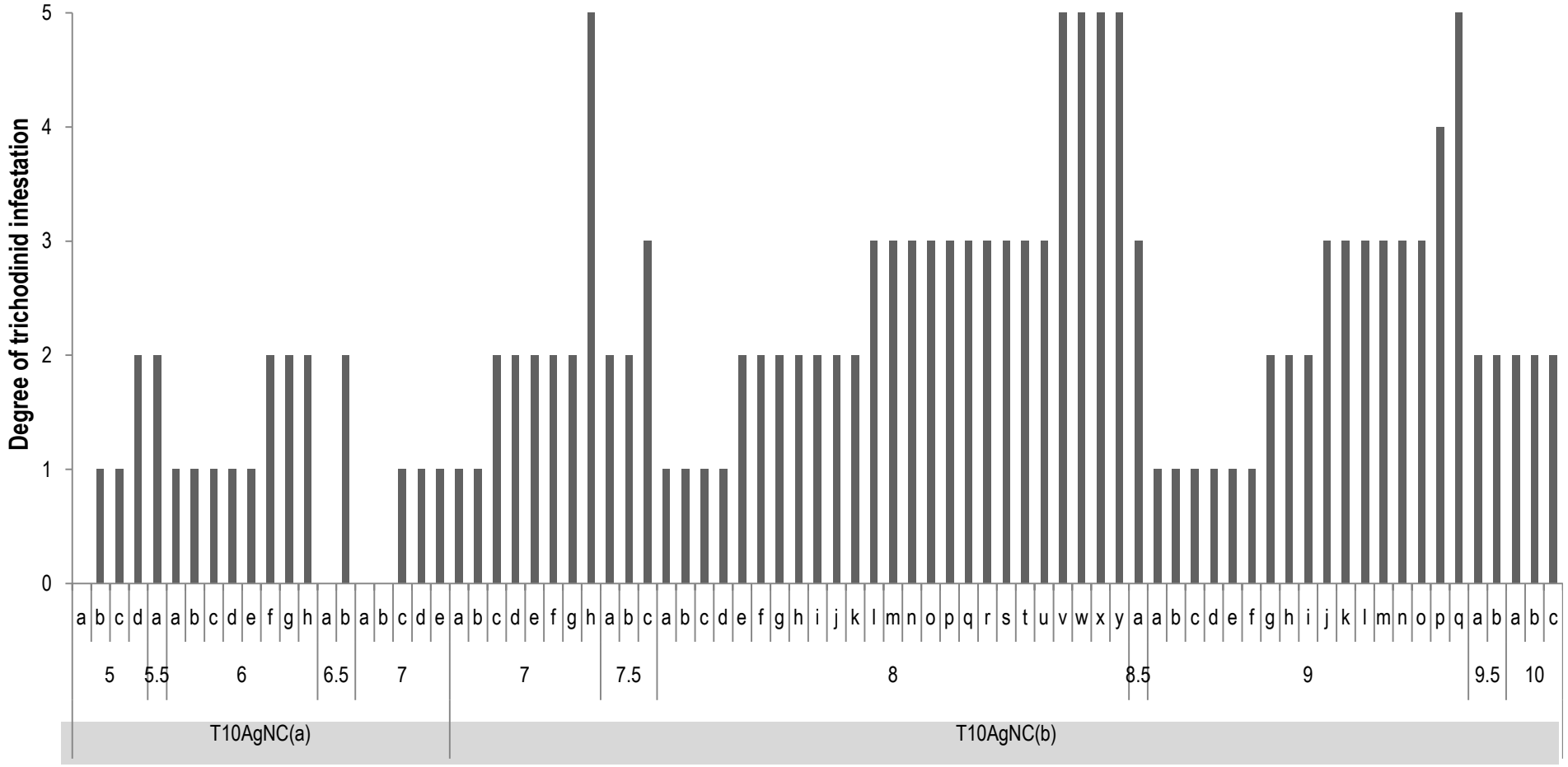

Figure 8.3: Relationship between the degreee of trichodinid infestation and body length (in mm) of *Amietophrynus gutturalis* Poweri, 1927 tadpoles collected from the same pond at Lake Nxamasere, Botswana. Population T10AgNC(a) was kept in a separate tank than Population T10AgNC(b). Please refer to Figure 5.1 and Table 5.1 for more information on the host population.

A better example to use would be Population T5ApNB that consisted of *A. poweri* collected from the Nxamasere Floodplains as these tadpoles were kept in a single tank upon collection thereof (Figure 8.4). The body length of these tadpoles ranged from 4 mm to 8 mm. It was found that a low trichodinid infestation (infestation degree ranging from none to two) occurred on many tadpoles ranging from 4 mm to 7 mm in body length. Some tadpoles with a body length ranging from 5 mm to 8 mm (except the group that consisted of the tadpoles with a body length of 5.5 mm) hosted heavy trichodinid infestations (infestation degree ranging from three to five), with most trichodinids occurring on the skin and gills of tadpoles with a body length of 6 mm.

Population T9ApCF (*A. poweri* tadpoles collected from the crocodile farm, Botswana) was not naturally infested (Figure 8.5). This population was infested during a cross-transmission experiment explained in Chapter 7. The reason why this group is ideal for the comparison between body length and degree of trichodinid infestation is that all the tadpoles were placed simultaneously in a container hosting infested fish. Therefore, all the tadpoles within this population (smaller and larger) were in the same vicinity of the trichodinid infested fish for the same period of time. This means that the occurrence of more trichodinids on the larger (further developed) tadpoles could not be due to a longer accumulation period on the tadpoles.

These tadpoles were divided into two groups according to their body size: small (5 mm – 6.5 mm) and large (7 mm – 8.5 mm). One tadpole from the smaller group was uninfested while four of the larger tadpoles did not host trichodinid infestations. A trichodinid infestation between one and two degrees was hosted by seventeen small tadpoles and twelve larger tadpoles. Only one tadpole (from the smaller group) hosted a trichodinid infestation to a degree of five. Therefore, it does not seem as if the number of trichodinids hosted by the tadpoles depends on the body size of the tadpoles.

This even becomes more evident in Figure 8.6. No pattern of increasing or decreasing of the degree of infestation on each of these populations could be detected when comparing the degree of trichodinid infestation with the body length of the examined host populations.

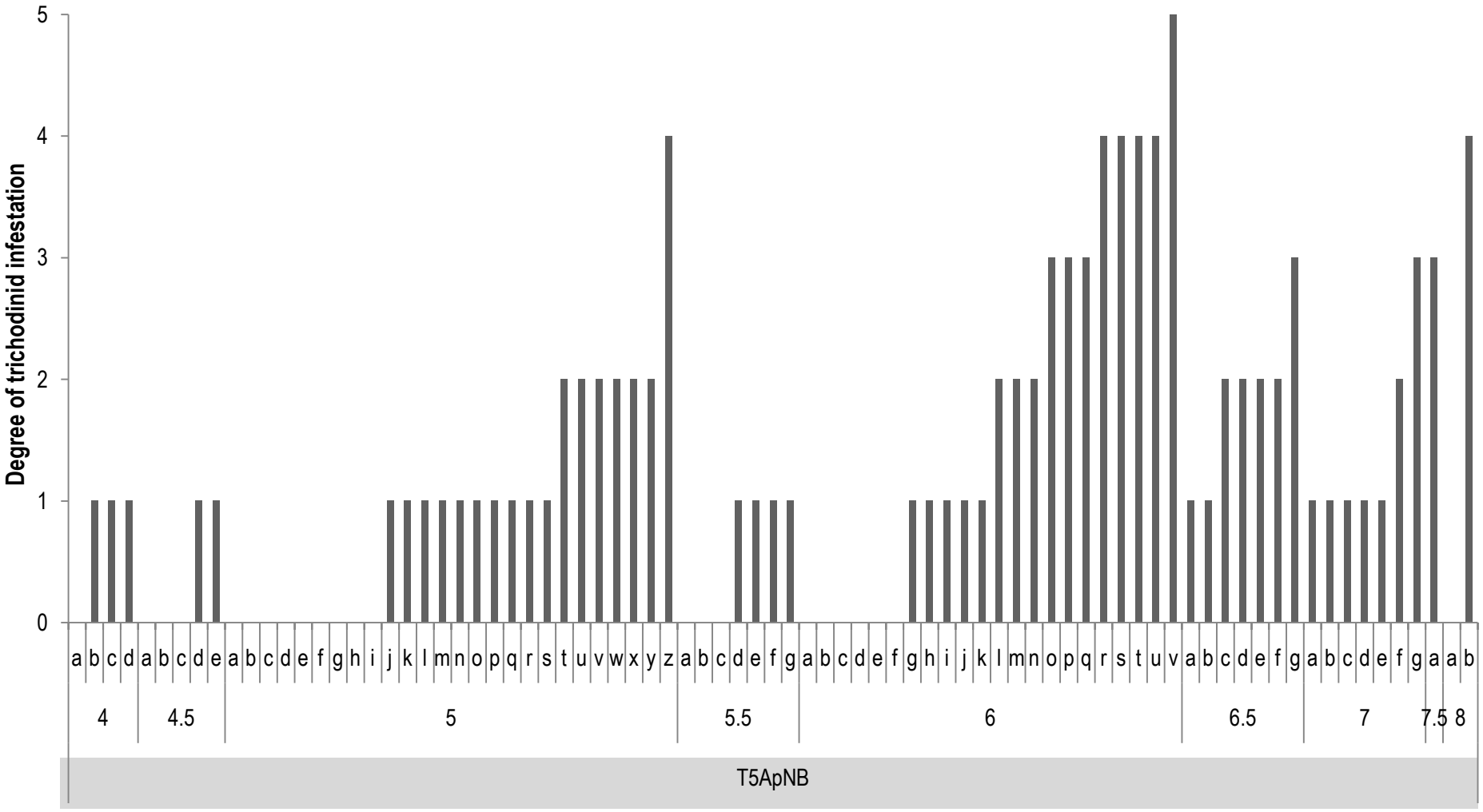

Figure 8.4: Relationship between the degree of trichodinid infestation and the body size of *Amietophrynus poweri* Hewitt, 1935 tadpoles collected from a pond at Nxamasere Floodplains.

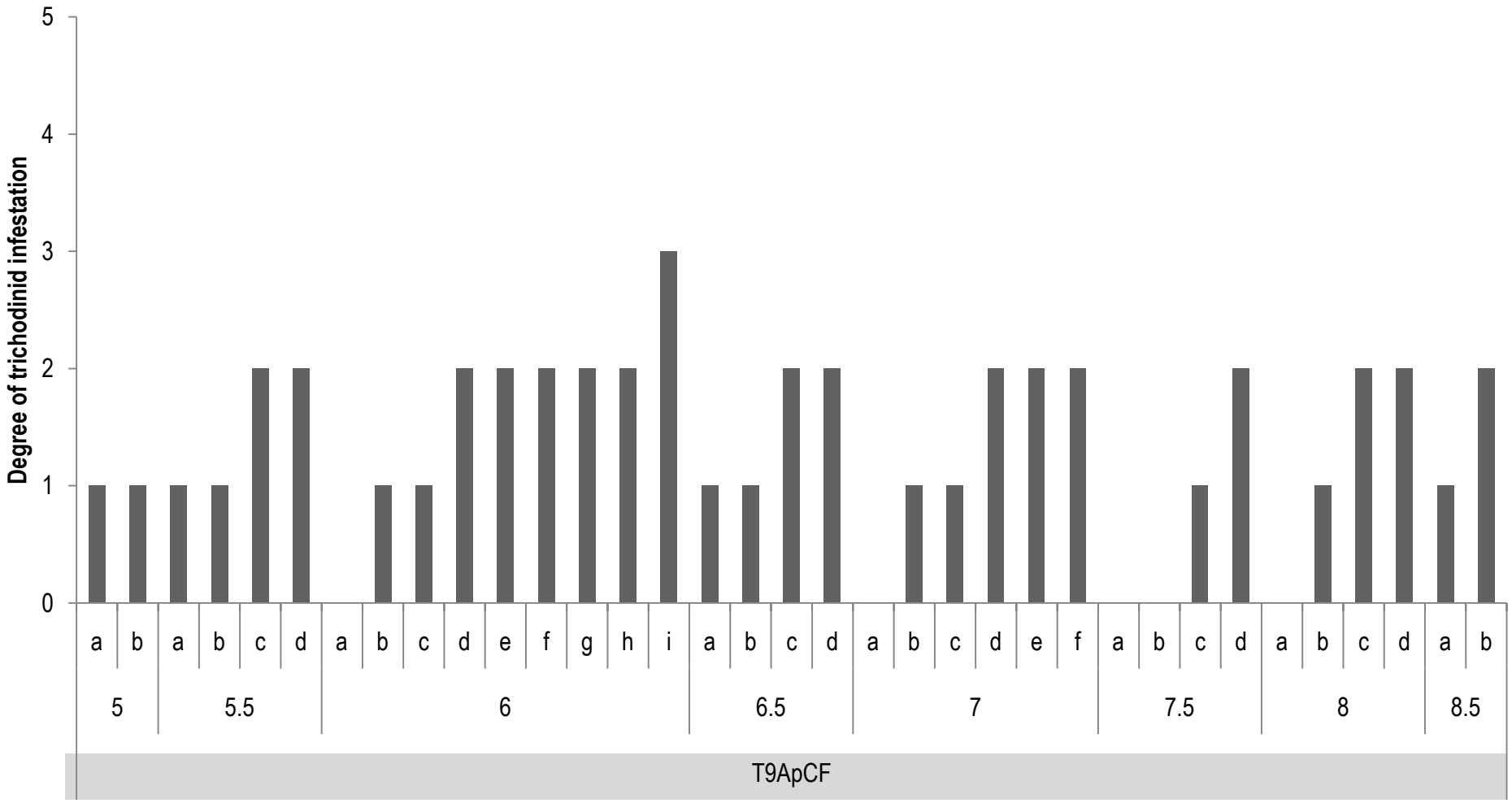

Figure 8.5: Relationship between the degree of trichodinid infestation and the body size of *Amietophrynus poweri* Hewitt, 1935 tadpoles collected from a pond at the crocodile farm, Botswana.

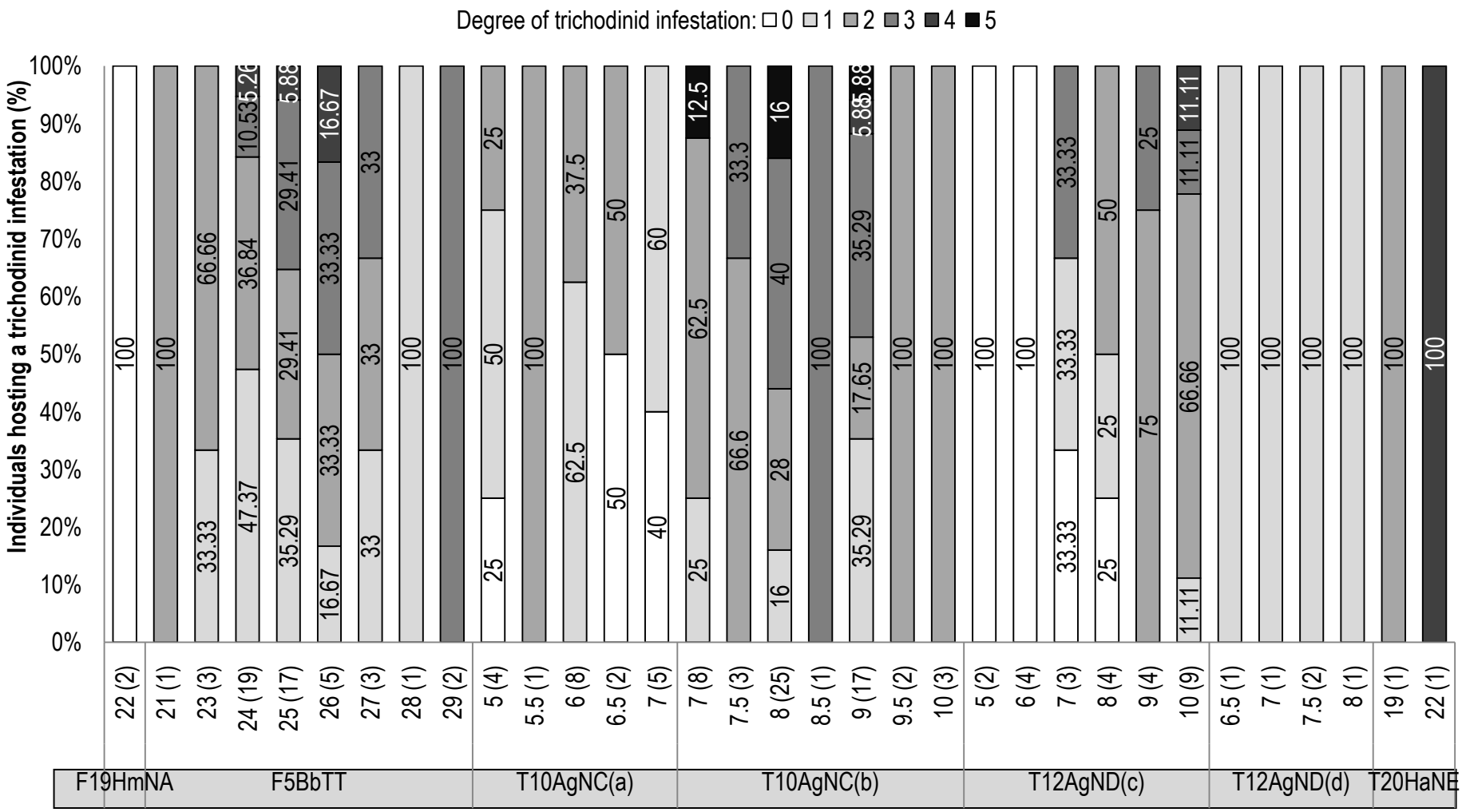

Figure 8.6: Graph indicating the percentage of individual hosts within various populations infested with no trichodinids (degree: 0) to several trichodinids (degree: 5). Please refer to Table 5.1 and Figure 5.1 for a description on the collected hosts.

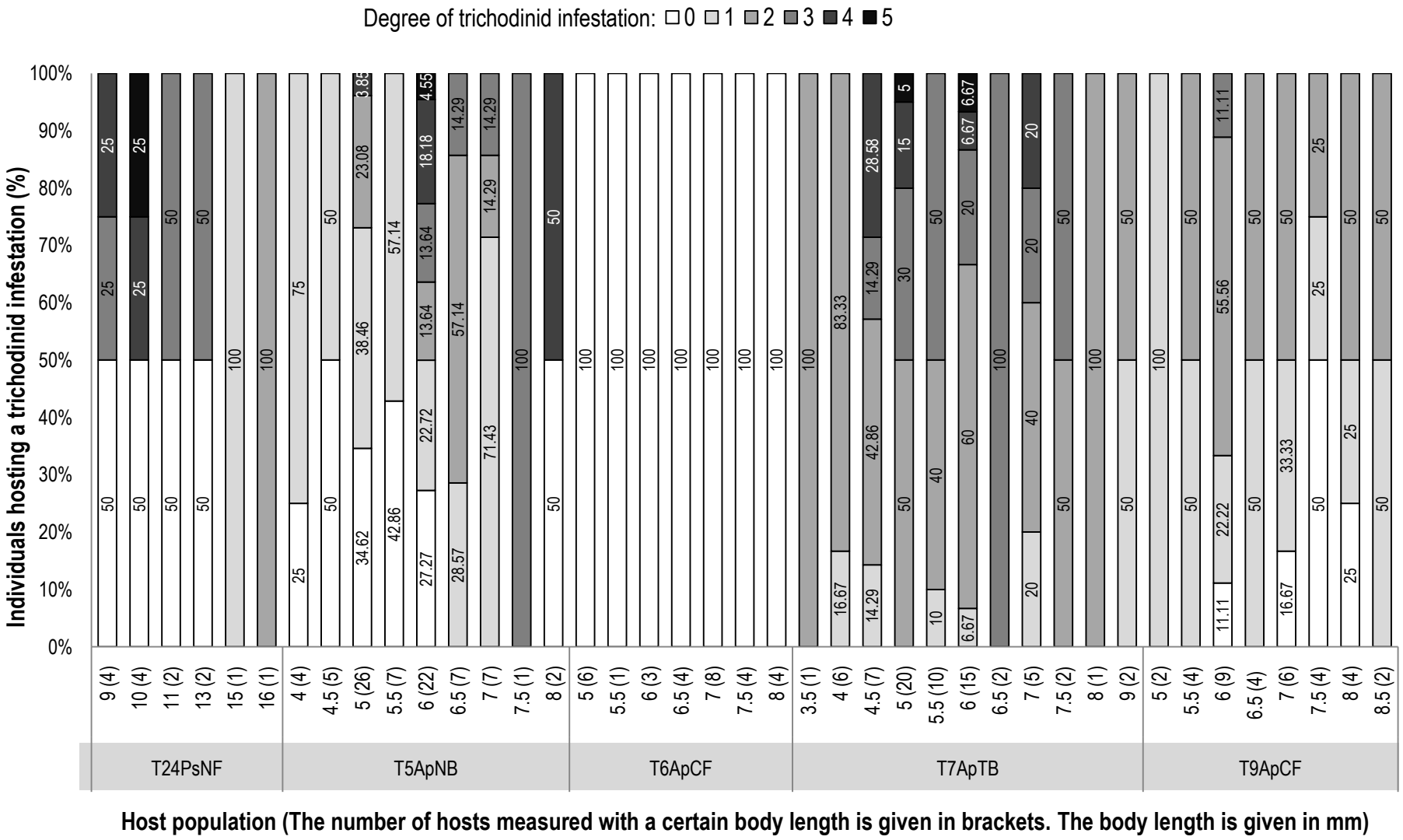

Figure 8.6 (Continue): Graph indicating the percentage of individual hosts within various populations infested with no trichodinids (degree: 0) to several trichodinids (degree: 5). Please refer to Table 5.1 and Figure 5.1 for a description on the collected hosts.

2.2. Position of infestation on tadpoles

Van As and Basson (1987) divided ectotrichodinids into four categories according to their normal position of occurrence on their hosts:

a) Only on skin, never on gills
b) Mostly on skin, sometimes on gills
c) Sometimes on skin, mostly on gills
d) Never on skin, only on gills

Kruger (1992) conducted a study on the occurrence of trichodinids on anuran larvae in southern Africa and concluded that most trichodinids attached to the skin of various tadpole species. It was therefore concluded that these trichodinids should be assigned to the second group as proposed by Van As and Basson (1987), namely the group of trichodinids that occur mostly on the skin of the host.

During the current study, the intensity of the trichodinid infestation on the skin and gill smears that were prepared from some of these tadpole populations was used to determine if the trichodinids favour skin or gills of the host for attachment (Figure 8.7).

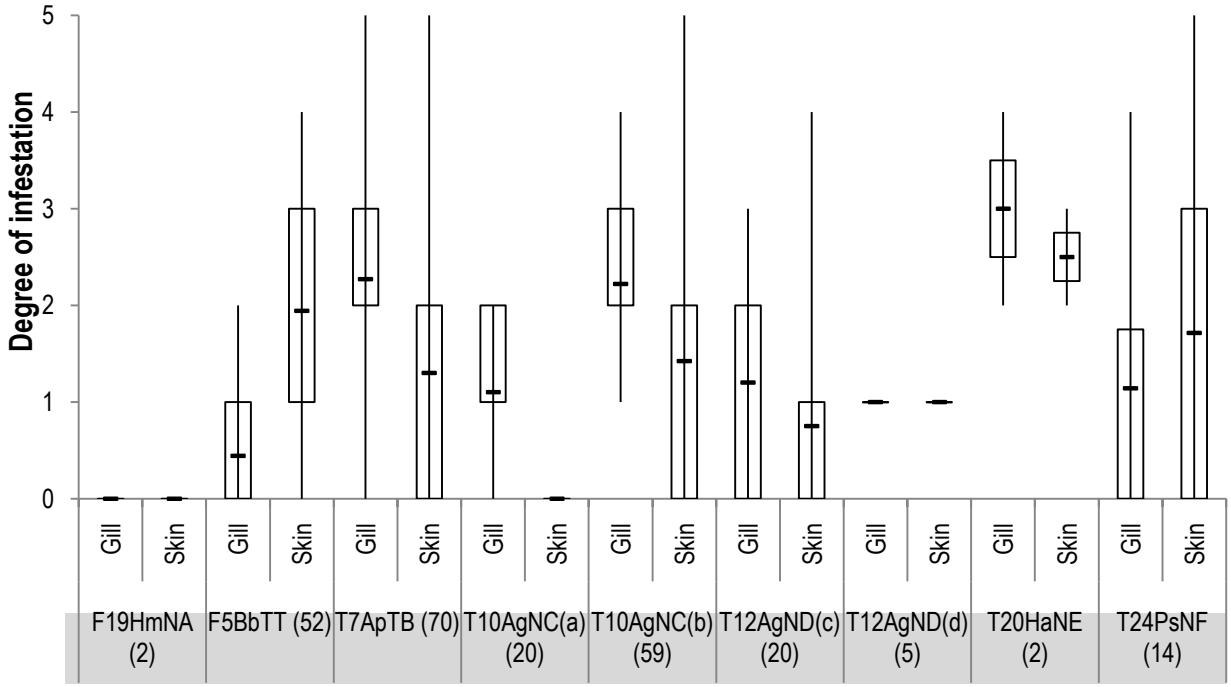

Figure 8.7: Degree of trichodinid infestation on the skin or gills of various host populations examined during the present study. Please refer to Table 5.1 and Figure 5.1 for more information on the host populations.

A higher infestation was noted on the skin smears prepared from the *B. barnadi* fish collected from the Thamalakane Bridge (Population F5BbTT) and the *P. subpunctata* tadpoles collected from Lake Nxamasere (Population T24PsNF) , while more trichodinids were observed on the gills smears prepared from *A. poweri* tadpoles (Population T7ApTB that was collected from the Toteng Bridge), *A. gutturlais* tadpoles (Populations T10AgNC(a), T10AgNC(b), T12AgND(c) that was collected from Lake Nxamasere) as well as Population T20HaNE that consisted of *H. parallelus* that was collected from Lake Nxamasere. It seems as if these trichodinids do not have a preference position for attachment to the host.

3. Concluding remarks

Kruger (1989) mentioned that a trichodinid infestation may occur on the skin of *X. laevis* tadpoles during any of the metamorphosis stages but that the position of the trichodinid infestation may differ as the tadpoles develop into adults. Kruger (1992) noted that trichodinids detach from tadpoles before the metamorphosis process is completed as it seems as if the skin of adult *Xenopus* is not a suitable environment for trichodinids to occur. It is also suggested to be true for other anuran species as the trichodinids are not able to survive on the dry skin of adult frogs.

Lom (1961) mentioned that trichodinids are mostly located on the thickened central part of the tail or around the rectum of young tadpoles and that the infestation is gradually lost as soon as the back legs of the tadpoles develop. Fernandes *et al.* (2011) on the other hand stated that the trichodinid infestation on the examined tadpoles was uniformly distributed over the entire tadpole.

The results of the present study correspond to the finding by Fernandes *et al.* (2011). However, the tadpoles were kept under experimental conditions during the present study, resulting in a closed system, containing a certain number of possible hosts. The increasing number of trichodinids (due to reproduction) had to attach to an available area on these hosts. This may have influenced the finding during the current study.

Chapter **9**:

Body **M**easurements

'…there is one thing even more vital to science than intelligent methods;
and that is, the sincere desire to find out the truth,
what ever it may be…'

Charles Pierce

Since the description of *Trichodina pediculus*, many authors described trichodinids from various hosts. No standard set of morphological characteristics was used by the earlier authors to describe trichodinids and therefore the discovery of trichodinids on a new host or a slight morphological deviation from a known species was seen as enough evidence to describe the collected material as a new species (Lom 1958).

Dogiel (1940) proposed that a few principal characteristics should be used for the determination of a new species or to verify the trichodinid samples to an already described species (Figure 9.1). The principal characteristics proposed by Dogiel (1940) included the following:

- Position of the macronucleus with regard to the macronucleus
- Diameter of the macronucleus vertical to the plane of the bilateral symmetry of the horseshoe
- Length of sector between the terminations of the macronucleus
- Diameter of the adhesive disc and the diameter of the denticle ring measured from the centre of intervening middle parts (from the central part of the denticle)
- Number of denticles in the denticle ring
- Shape of the denticles
- Number of radial pins per denticle
- Diameter of the body width above the adhesive disc
- Position of the contractive vacuole
- The proportion between various measures should be determined (e.g. diameter of the body to the macronucleus diameter)
- Dimensions of individual denticles (blade, central part, ray)

Lom (1958) mentioned that the characteristics proposed by Dogiel (1940) are not sufficient as it does not include the body shape, the course of the adoral spiral, and so forth. Lom (1958) suggested that all trichodinids should be described by using uniform criteria as this would make comparisons of individual species possible.

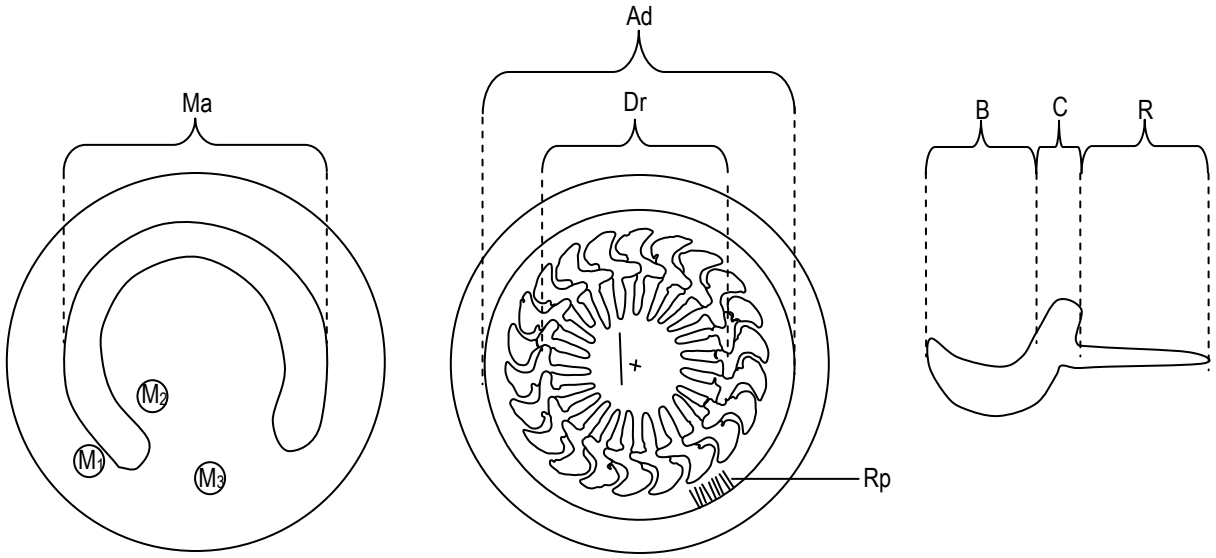

Figure 9.1: Indication of some of the characteristics to be measured according to Dogiel (1940), adapted from Lom (1958).
Abbreviations: **Ad**: Diameter of the adhesive disc (measured without the border membrane); **B**: blade length; **C**: Central part length; **Dr**: Diameter of the denticle ring; **Ma**: Macronucleus; $\mathbf{M_1}$, $\mathbf{M_2}$ and $\mathbf{M_3}$: Possible positions of the micronucleus; **R**: Ray length; **Rp**: Number of radial pins on the striated membrane between two denticles.

The following uniform characteristics for the description of trichodinid species (and / or populations) as suggested by Lom (1958):

- Shape of the body
- Structure of the adhesive disc
- Shape of the denticles
- Dimensions of the denticles
- Number of the denticles in the denticle ring
- Diameter of the denticle ring
- Number of radial pins per denticle
- Width of the border membrane
- Thickness of the velum [the velum was described as a pellicular border or a fold covering the aboral skeletal ring. However, trichodinids do not have velums (Basson – pers. comm.)]
- Distinguishing between the two aboral ciliary rings
- Shape and position of the nuclear apparatus
- Course of the adoral zone

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- Position of the central vacuole
- Host specificity

The trichodinid characteristics proposed by Lom (1958) were reviewed and the characteristics presented in Figure 5.4 were made standard for the description of trichodinid species. This method was used during the current study in order to determine whether one or more trichodinid species was collected from the various host populations.

This method was also used to determine if any differentiation between the different populations should be determined. This was done as Kruger (1992) found that trichodinids hosted on the skin and gills of *X. laevis* tadpoles were generally smaller than trichodinids of the same species that was hosted on fish from the same water medium.

1. Material and methods

The revised standard method for describing trichodinids (Figure 5.4) was used for the description of the trichodinids collected during this study as well as specimens previously collected by the Aquatic Research Group as the tip of the ray diameter as well as the tangent point diameter were also noted. Please refer to Figure 5.4 for an explanation on the additional features measured. This was achieved by using a Zeiss Axiophot compound microscope and a Nikon DXM1200F digital camera to produce digital images of the silwer impregnated specimens. The necessary measurements were made by using the ImageJ software package (ImageJ, version 1.40g, 2008). The measurements were presented as the minimum, first quartile, mean, third quartile and maximum and all measurements are in micrometers (µm). Van As and Basson (1992) suggested that the mode of two of the standard characteristics (number of denticles, number of radial pins per denticle) should be provided rather than the mean value as a trichodinid cannot have, for example 6.25 denticles within the denticle ring. However, the current study noted the mean value of these characteristics due to the nature of the statistical methods used during the present study. The number of specimens measured was also provided. Biometrical data of the trichodinids from the collected host groups were statistically compared with one another by using the values obtained from the above mentioned characteristics.

2. Results and discussion

During the current study, the minimum, first quartile, average, third quartile and maximum value obtained for the various characteristics mentioned above was determined (Figures 9.2 – 9.27, see also Appendix B). A great variation in most of the measurements was observed between the different trichodinid populations. For example, the maximum number of denticles counted from a trichodinid collected on *Oreochromis mossambicus* from the Lowveld Fisheries was 29, with the minimum being 20 (Population F11OmLF, Figure 9.2). However, most trichodinids within this population had between 25 and 27 denticles. In contrast, trichodinids collected from *Cyprinus carpio* that was also collected from the Lowveld Fisheries (Population F8CcLF) had on average 20 (mean value: 20.48) denticles, with a minimum number of denticles counted being 18 and the maximum number of denticles within a trichodinid was 28.

It was found that trichodinids hosted on tadpoles were in general smaller than trichodinids hosted on fish. The only exceptions were the width of the border membrane and the number of radial pins per denticle. With regards to the border membrane, the values measured for trichodinids hosted on tadpoles ranged from 2.4 µm (minimum); 5.05 µm (first quartile); 5.53 µm (average); 6.10 µm (third quartile) and 10.21 µm (maximum) (Figure 9.15) while trichodinids collected from fish had a border membrane of 1.24 µm (minimum); 5.31 µm (first quartile); 6.10 µm (average); 6.64 µm (third quartile) and 10.36 µm (maximum) (Figure 9.14). Therefore, the trichodinids that utilised fish for attachment had in general a thicker border membrane, although the thinnest border membrane was measured from a trichodinid that occurred on fish. It should also be taken into account that the border membrane cannot be measured quite accurately as the thickness of the border membrane may vary within a single trichodinid.

By comparing the number of radial pins per denticle within the fish hosted versus tadpole hosted trichodinids, the minimum, first quartile and average number of radial pins was less for trichodinids that occurred on tadpoles (5, 10 and 10.62 respectively in comparison to 6, 11 and 11.33 for trichodinids hosted on fishes). However, the maximum number of radial pins per denticle that was counted for both groups was 15 (Figures 9.16, 9.17).

The above findings corresponds to the conclusion made by Kruger (1992) that mentioned that trichodinids hosted on *Xenopus* tadpoles were generally smaller than trichodinids of the same species collected on various fish species.

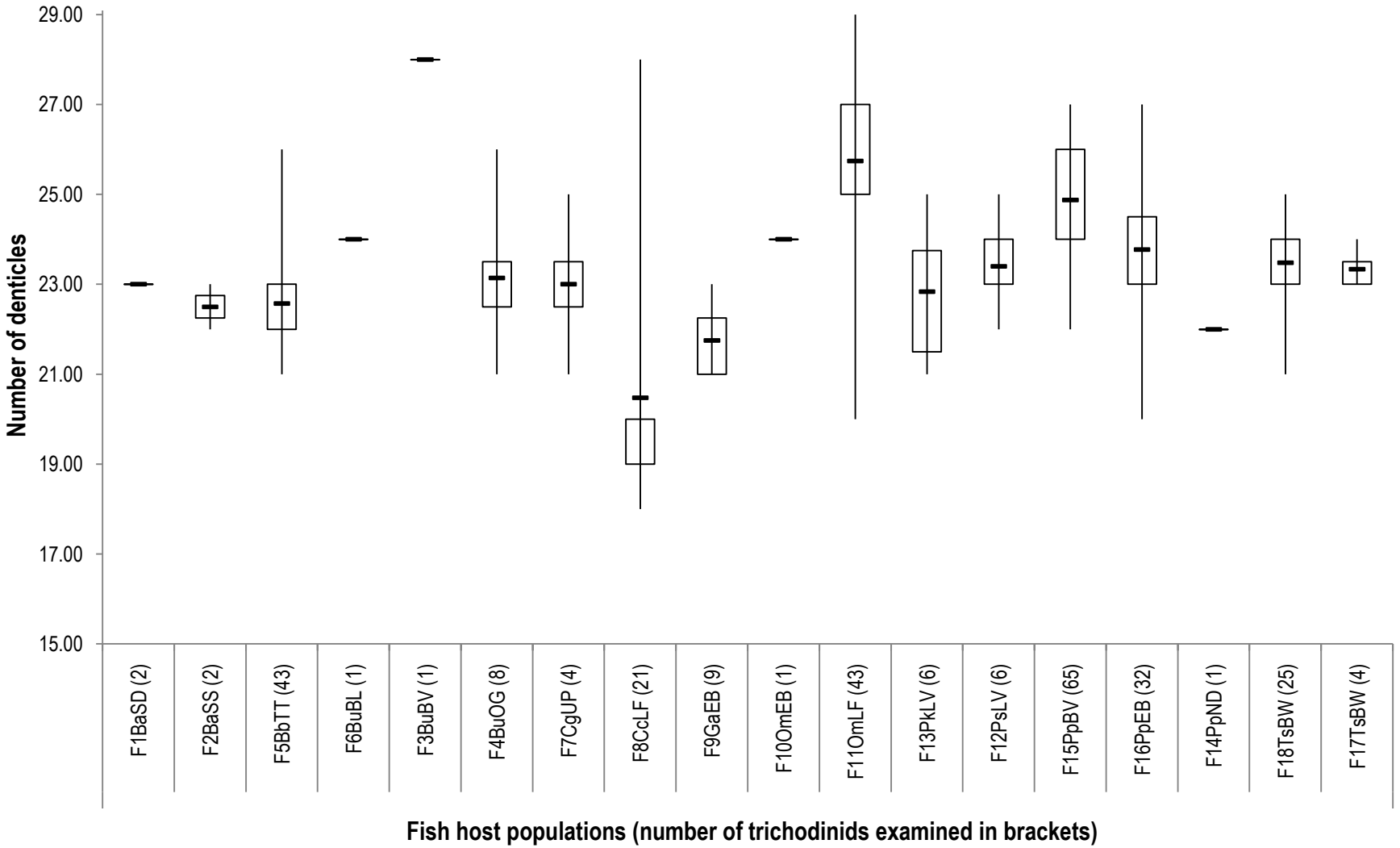


Figure 9.2: The number of denticles within a denticle ring was counted for ectotrichodinids from various fish populations. Please refer to Figure 5.1 and Table 5.1 for more information on the host populations.

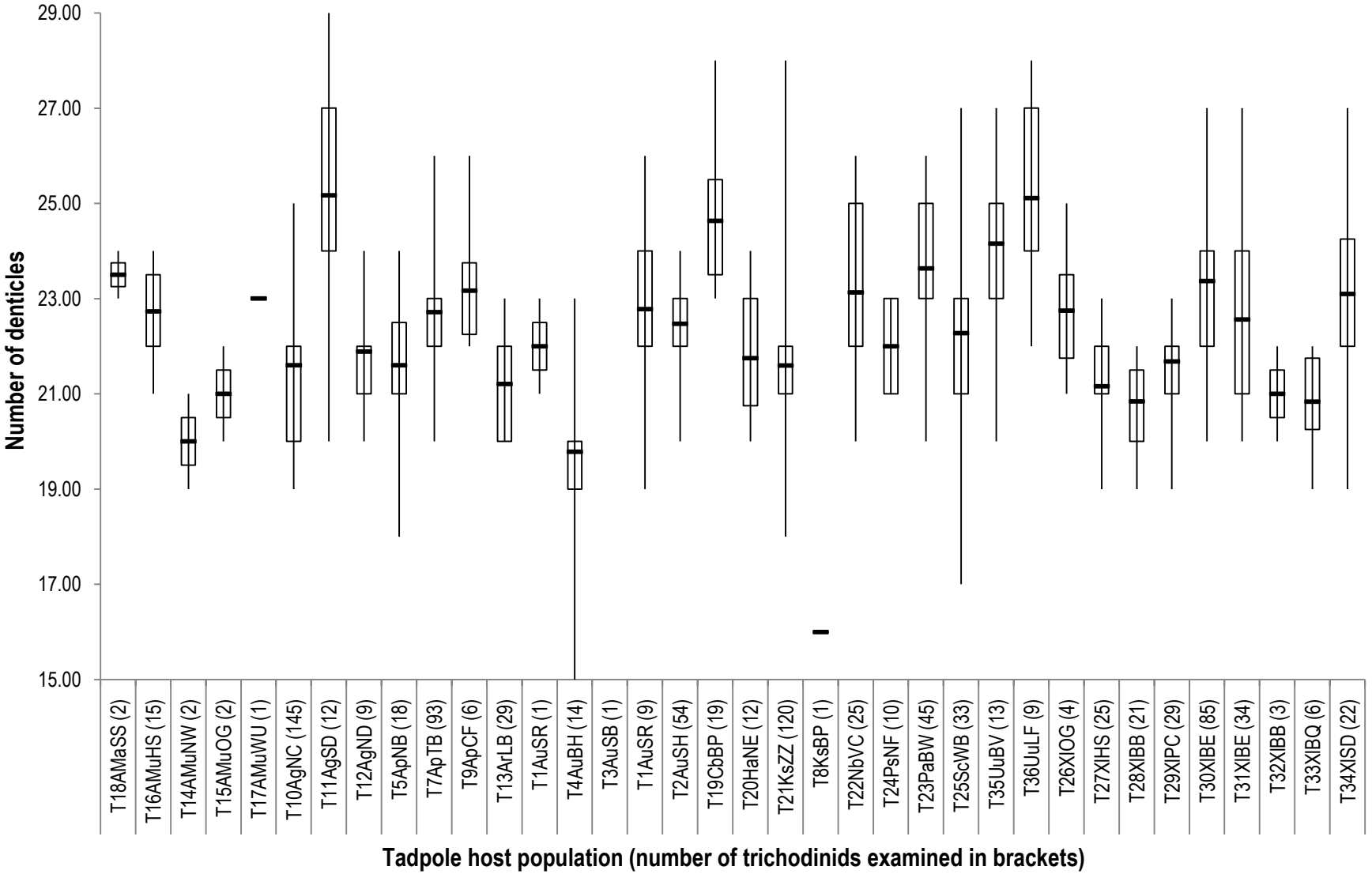


Figure 9.3: The number of denticles within a denticle ring was counted for ectotrichodinids from various tadpole populations. Please refer to Figure 5.1 and Table 5.1 for more information on the host populations.

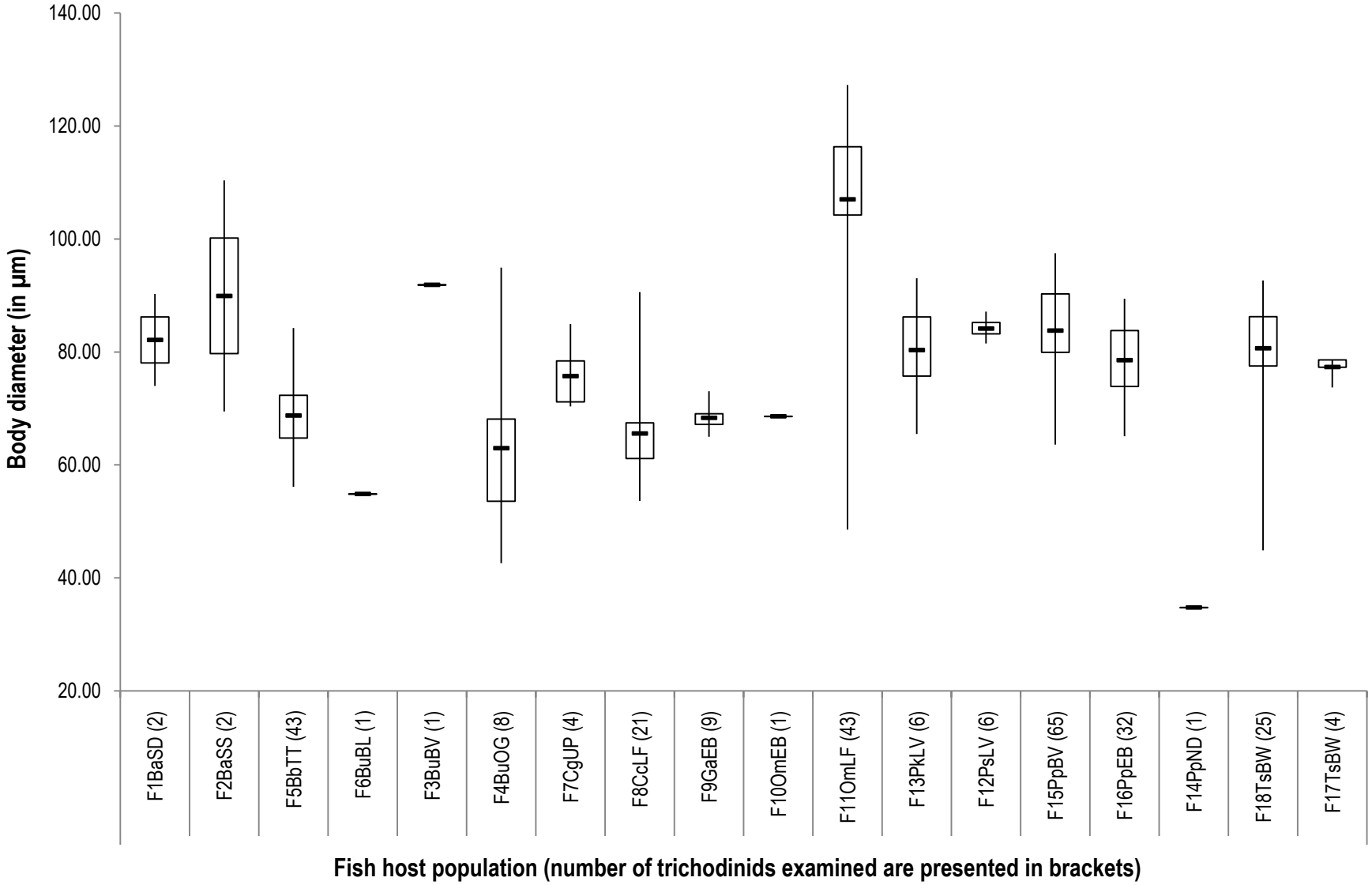


Figure 9.4: Body diameter (in µm) of trichodinids from various fish populations that were studied during the present study. Please refer to Figure 5.1 and Table 5.1 for more information on the host populations.

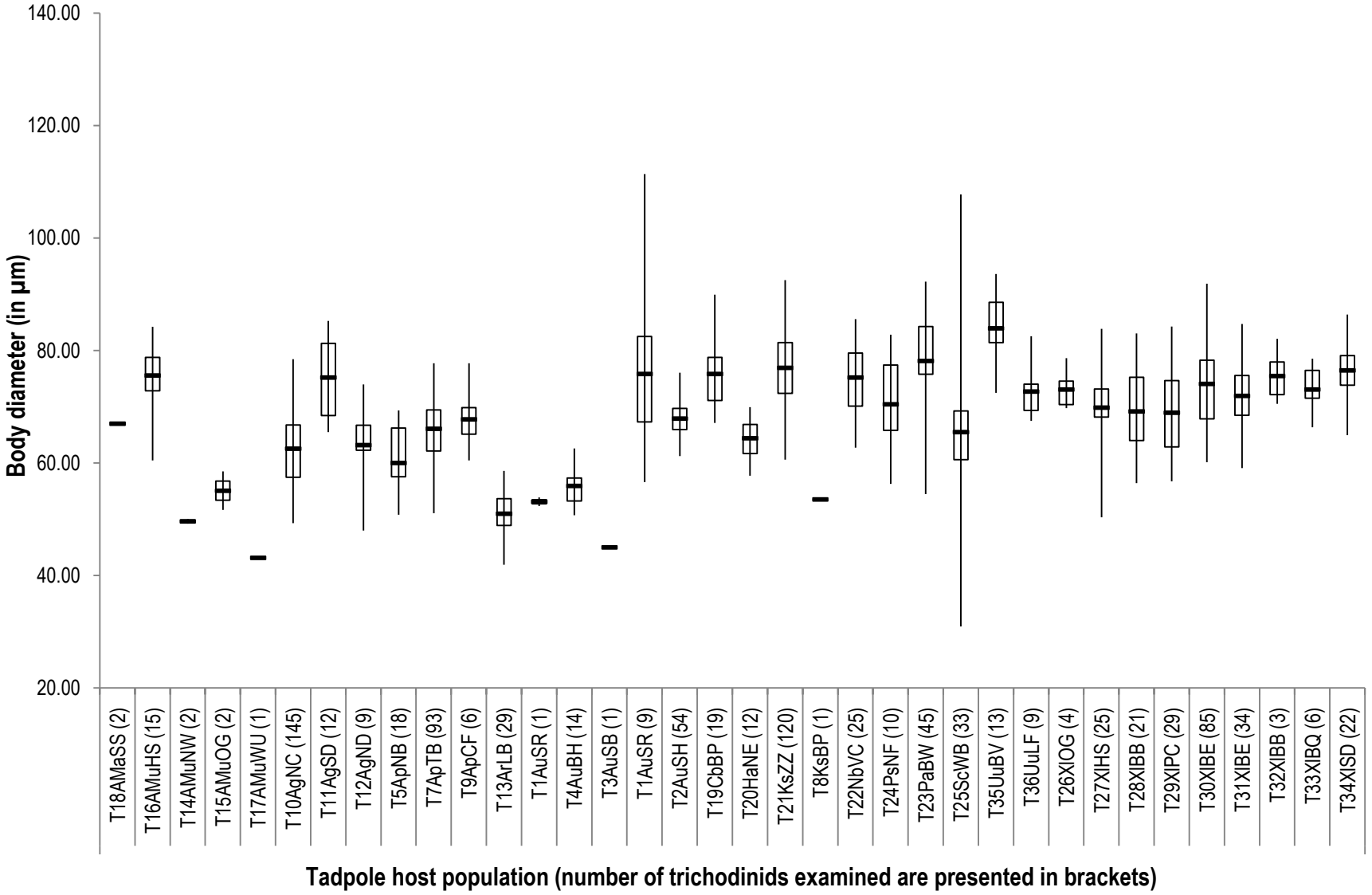


Figure 9.5: Body diameter (in µm) of trichodinids from various tadpole populations that were studied during the present study. Please refer to Figure 5.1 and Table 5.1 for more information on the host populations.

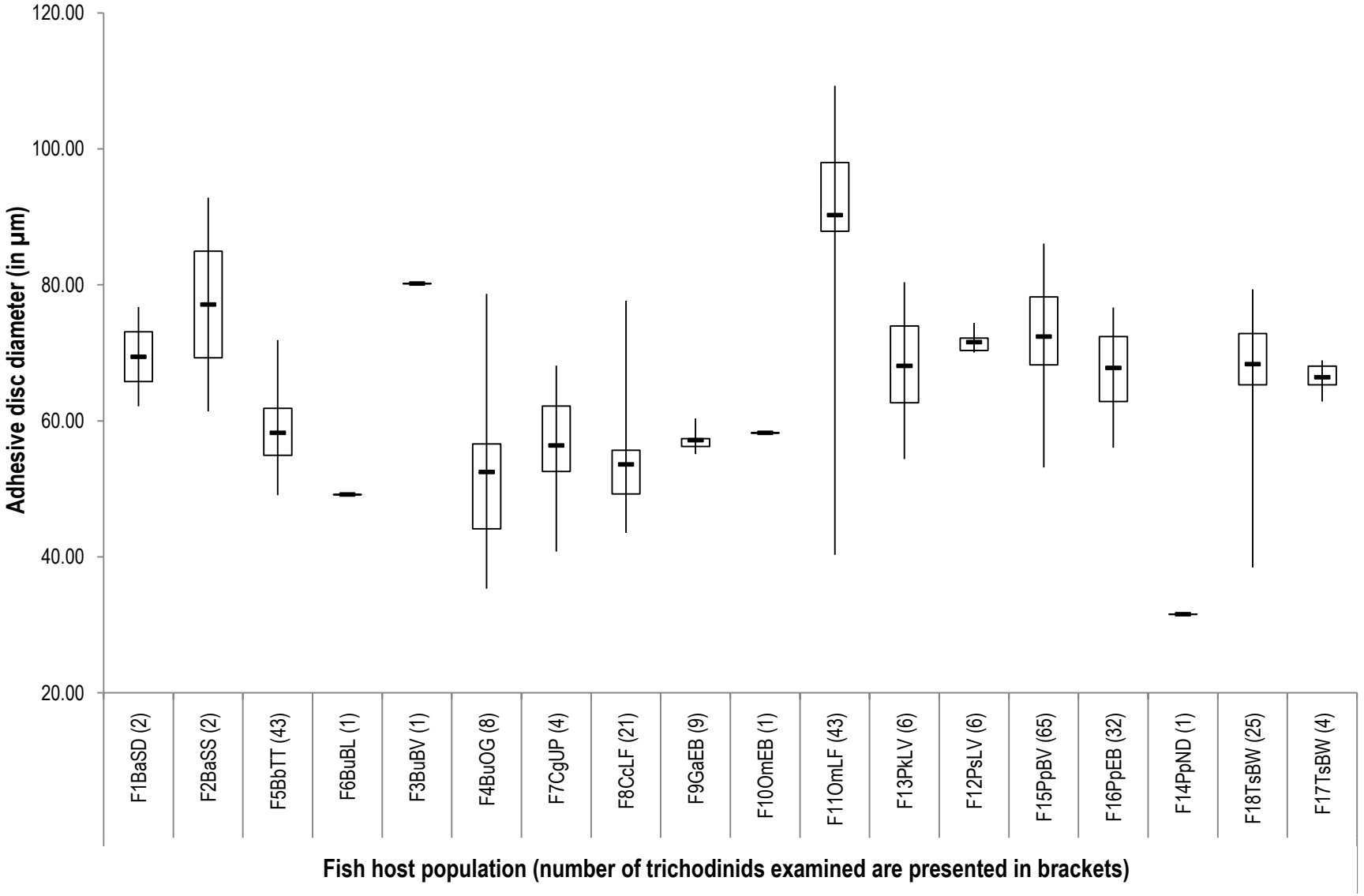


Figure 9.6: Adhesive disc diameter (in µm) of trichodinids from various fish populations that were studied during the present study. Please refer to Figure 5.1 and Table 5.1 for more information on the host populations.

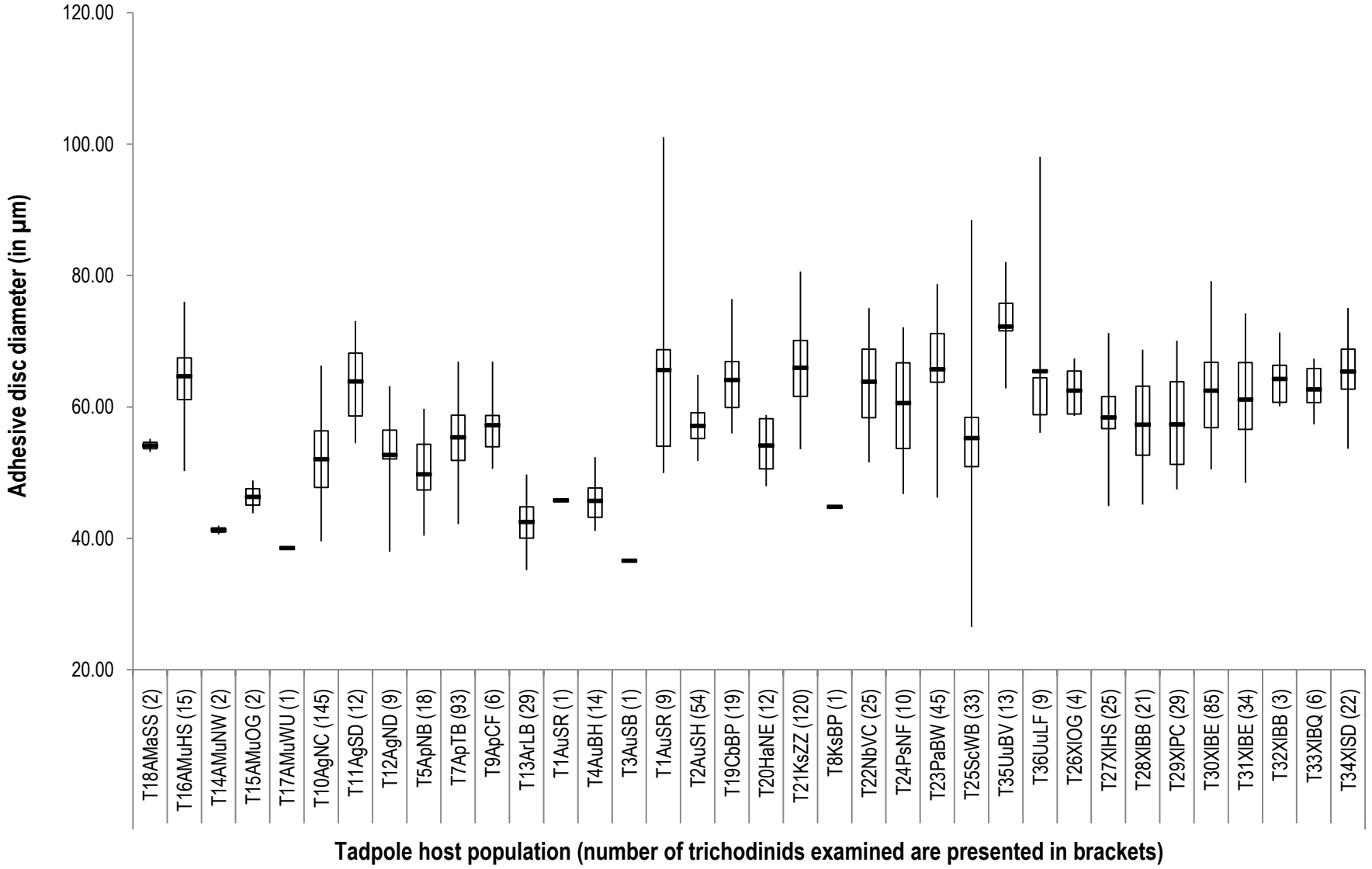


Figure 9.7: Adhesive disc diameter (in µm) of trichodinids from various tadpole populations that were studied during the present study. Please refer to Figure 5.1 and Table 5.1 for more information on the host populations.

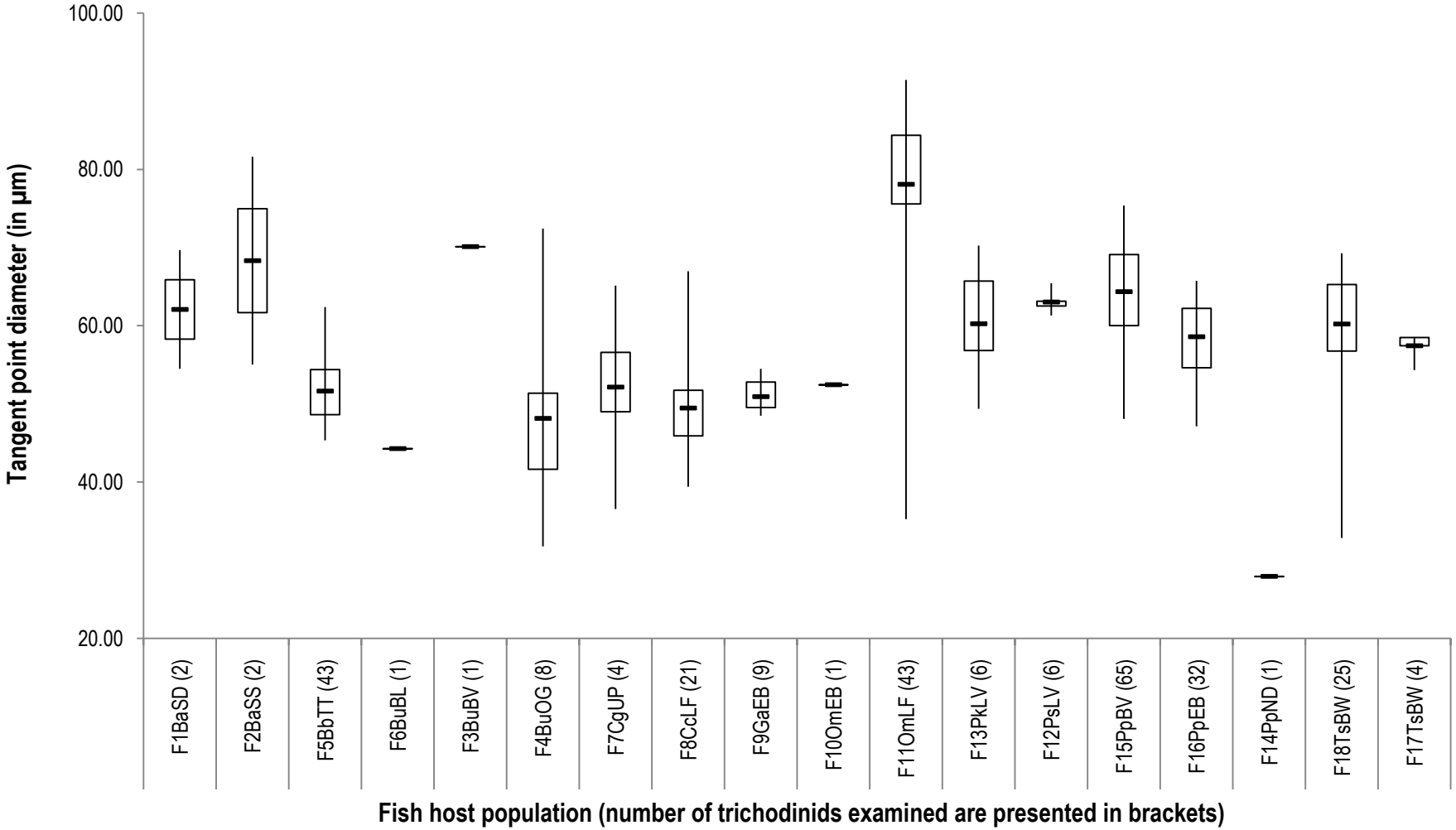


Figure 9.8: Tangent point diameter (in µm) of trichodinids from various fish populations that were studied during the present study. Please refer to Figure 5.1 and Table 5.1 for more information on the host populations.

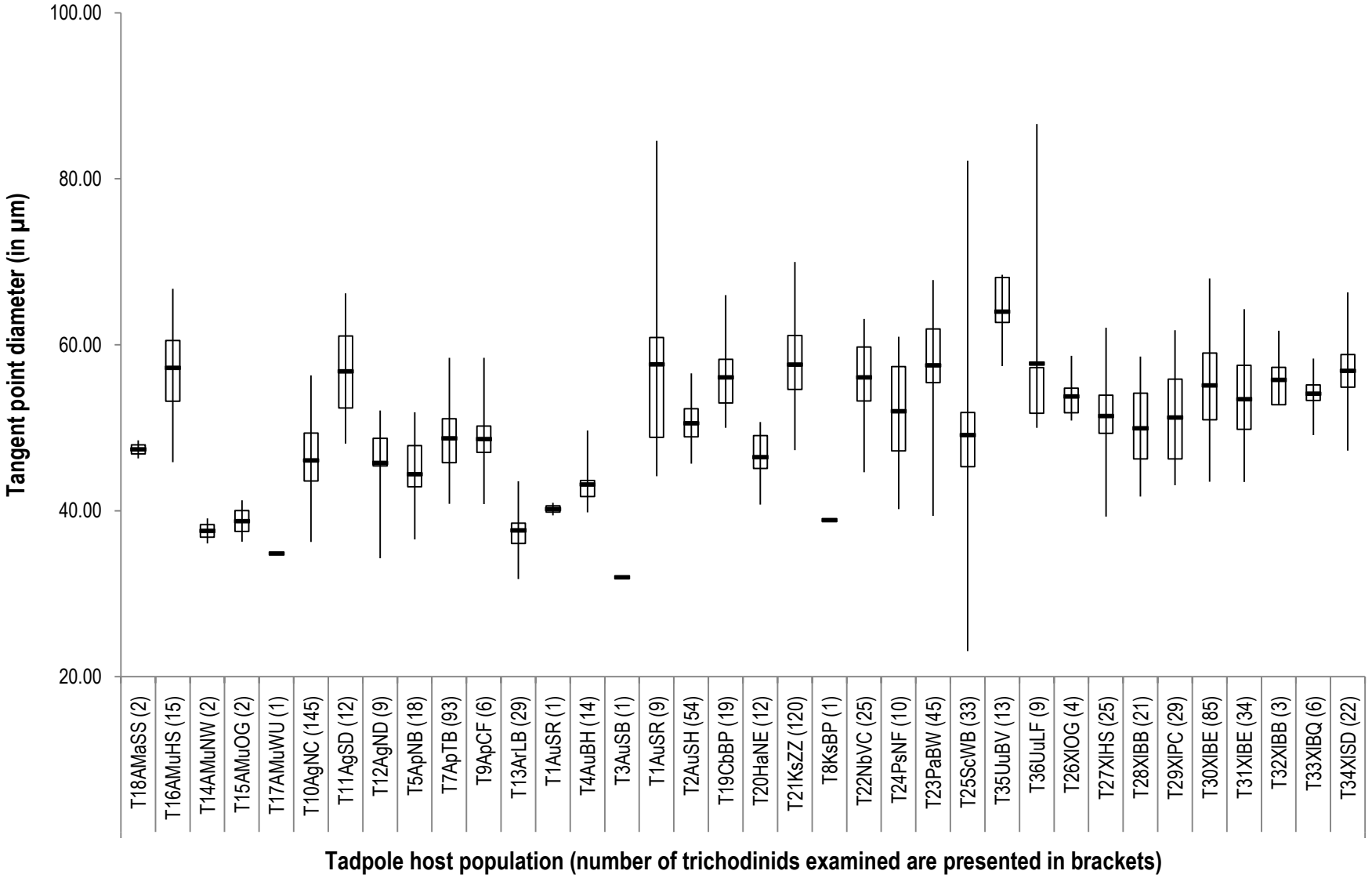


Figure 9.9: Tangent point diameter (in µm) of trichodinids from various tadpole populations that were studied during the present study. Please refer to Figure 5.1 and Table 5.1 for more information on the host populations.

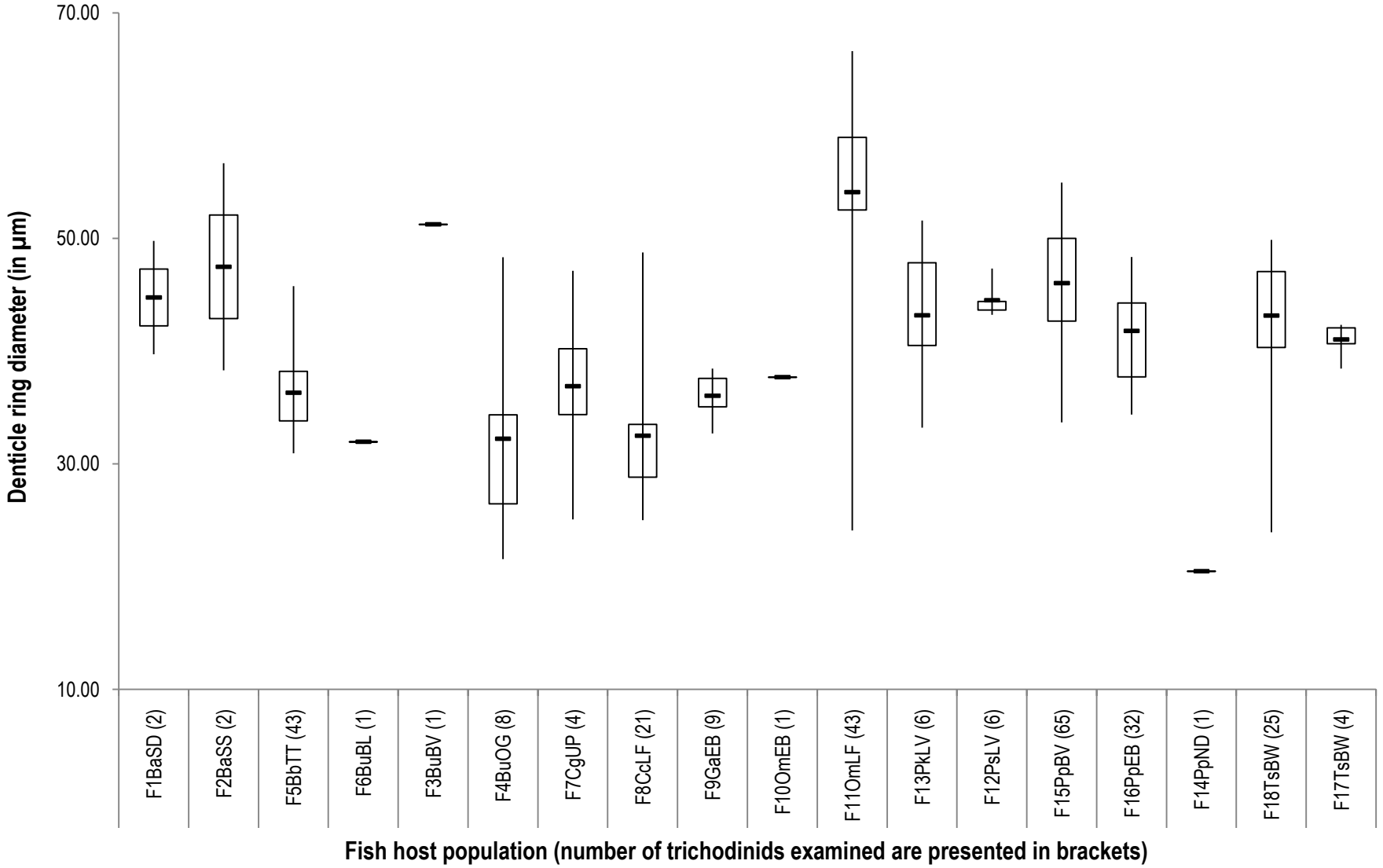


Figure 9.10: Denticle ring diameter (in µm) of trichodinids from various fish populations that were studied during the present study. Please refer to Figure 5.1 and Table 5.1 for more information on the host populations.

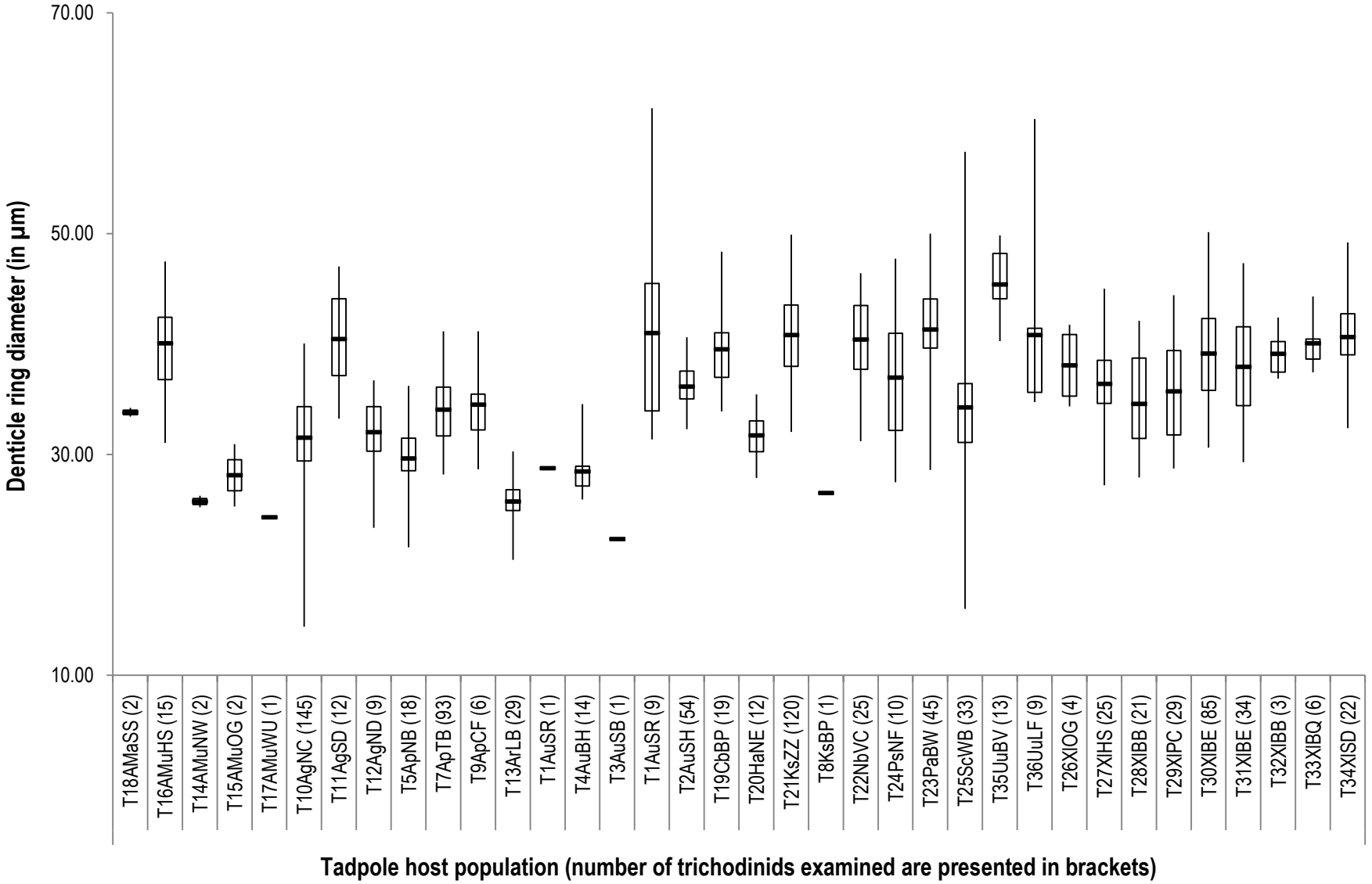


Figure 9.11: Denticle ring diameter (in µm) of trichodinids from various tadpole populations that were studied during the present study. Please refer to Figure 5.1 and Table 5.1 for more information on the host populations.

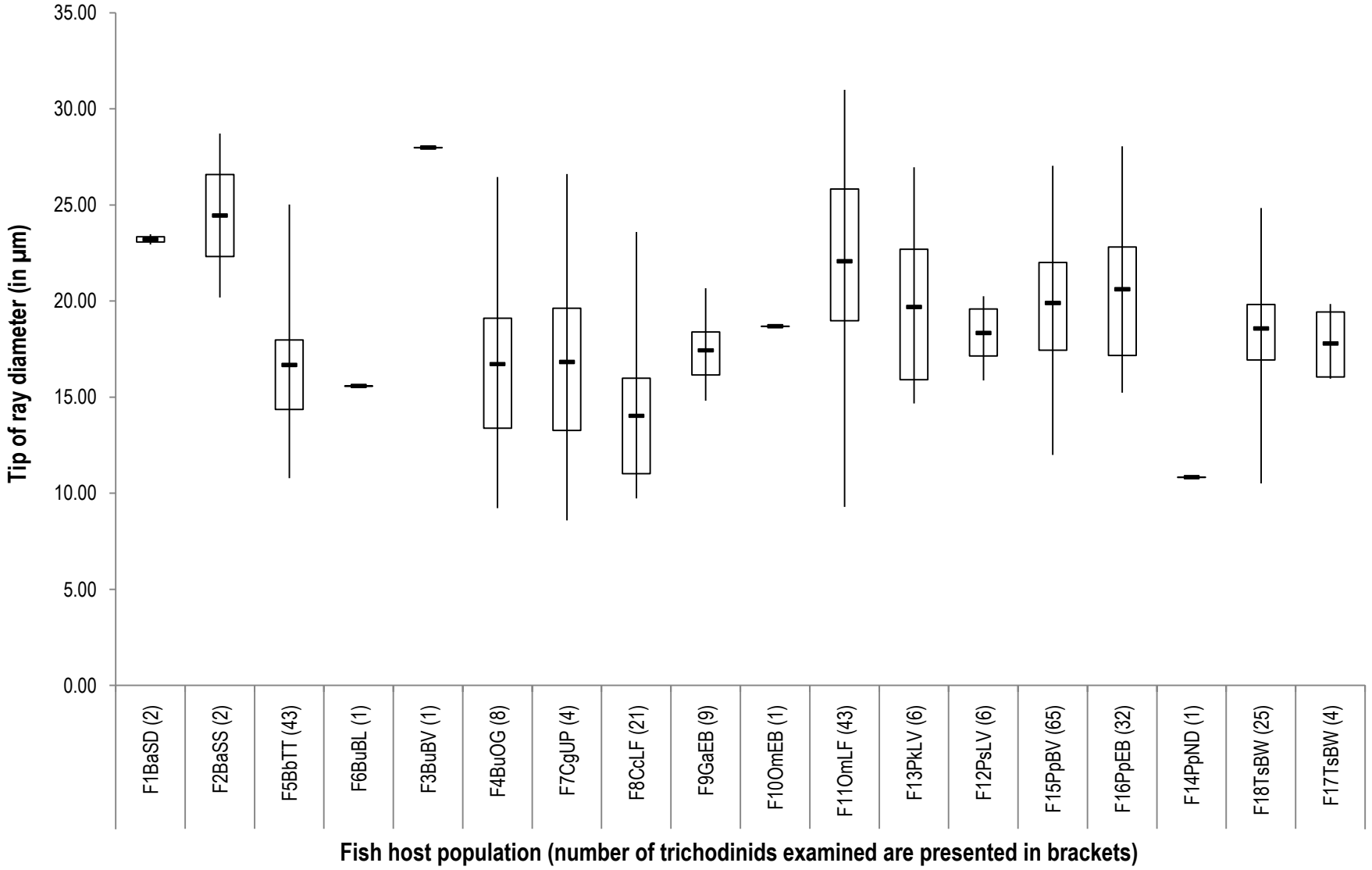


Figure 9.12: Tip of ray diameter (in µm) of trichodinids from various fish populations that were studied during the present study. Please refer to Figure 5.1 and Table 5.1 for more information on the host populations.

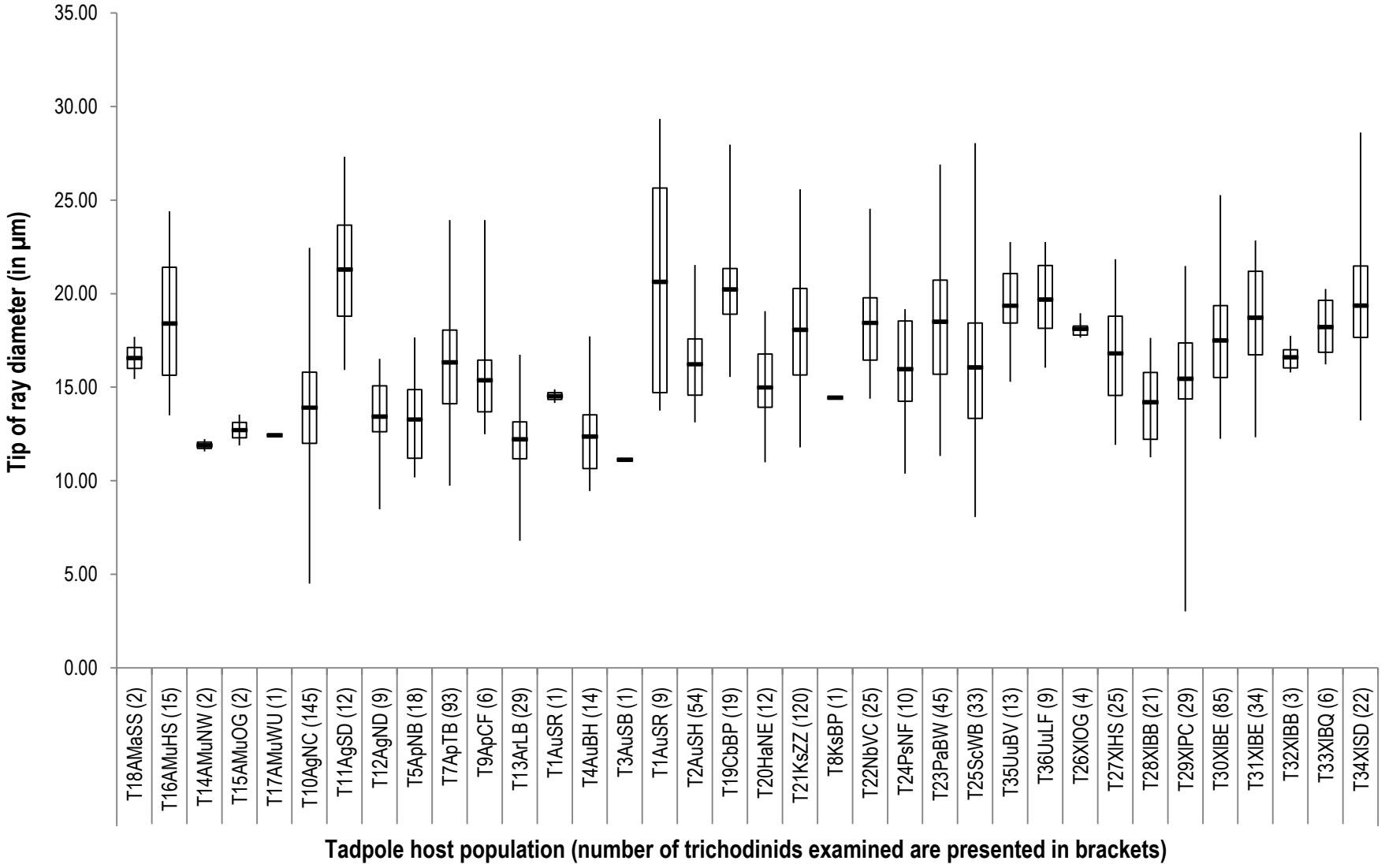


Figure 9.13: Tip of ray diameter (in µm) of trichodinids from various tadpole populations that were studied during the present study. Please refer to Figure 5.1 and Table 5.1 for more information on the host populations.

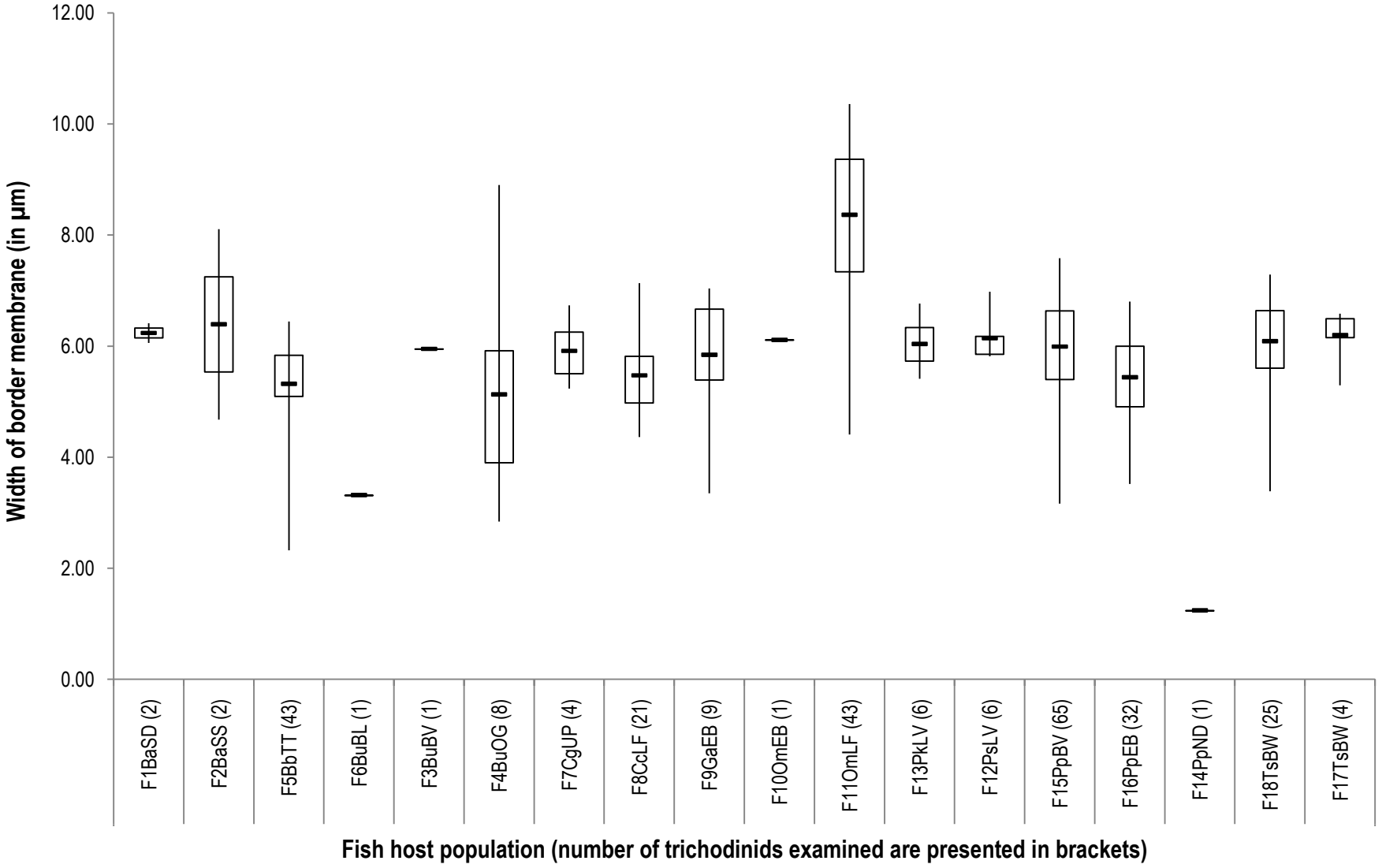


Figure 9.14: Width of border membrane (in µm) of trichodinids from various fish populations that were studied during the present study. Please refer to Figure 5.1 and Table 5.1 for more information on the host populations.

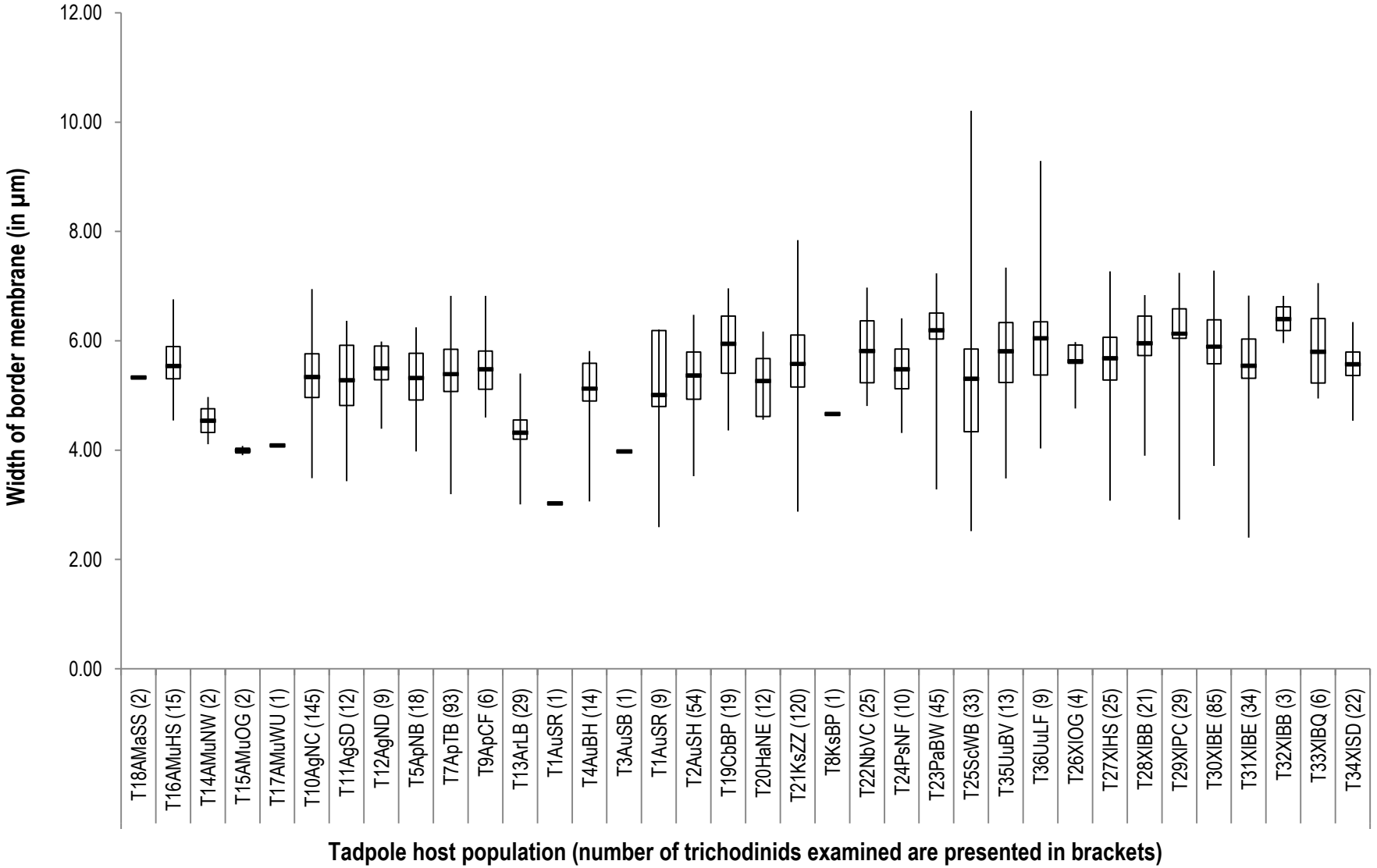


Figure 9.15: Width of border membrane (in µm) of trichodinids from various tadpole populations that were studied during the present study. Please refer to Figure 5.1 and Table 5.1 for more information on the host populations.

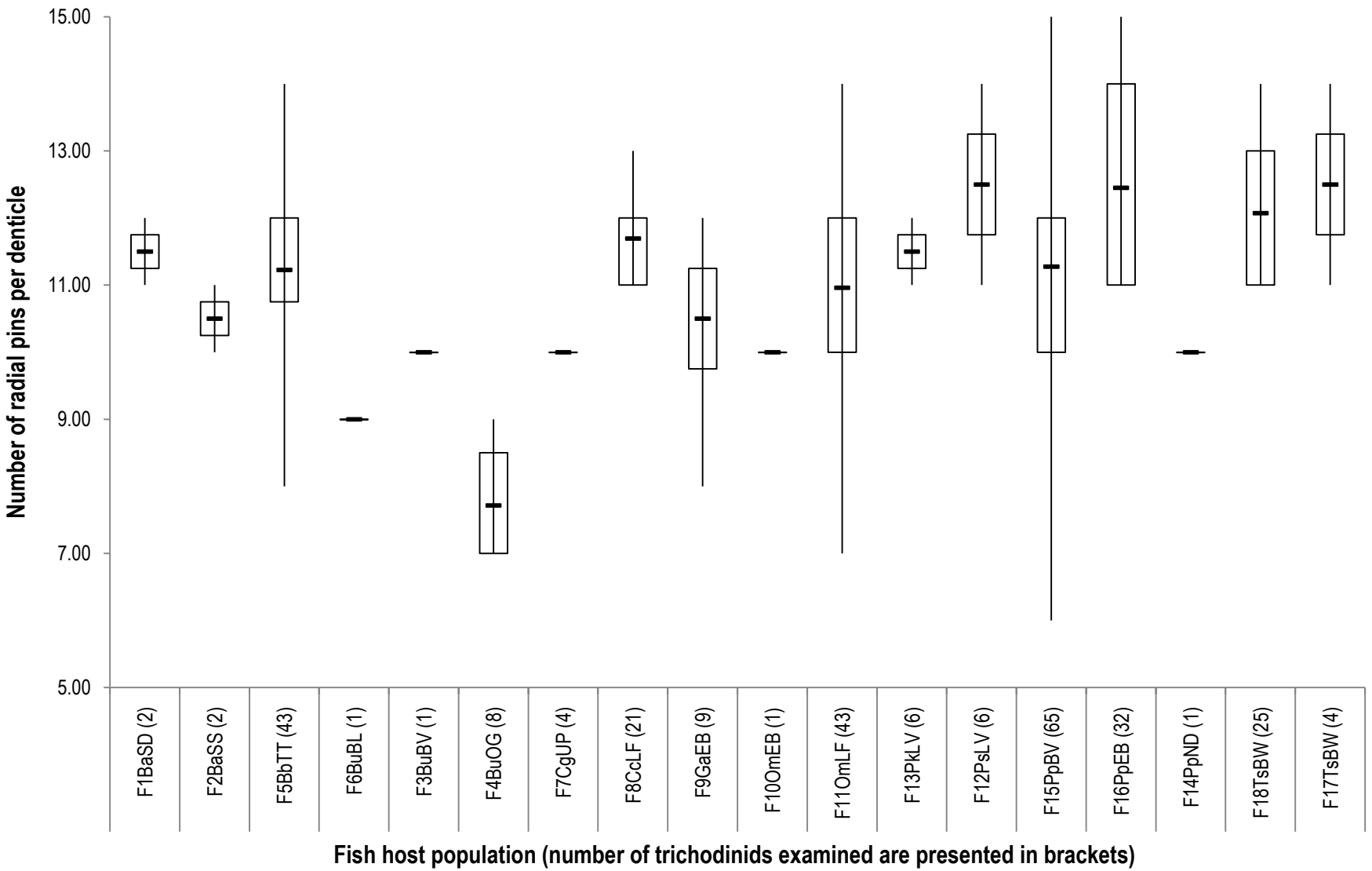


Figure 9.16: Number of radial pins of trichodinids from various fish populations that were studied during the present study. Please refer to Figure 5.1 and Table 5.1 for more information on the host populations.

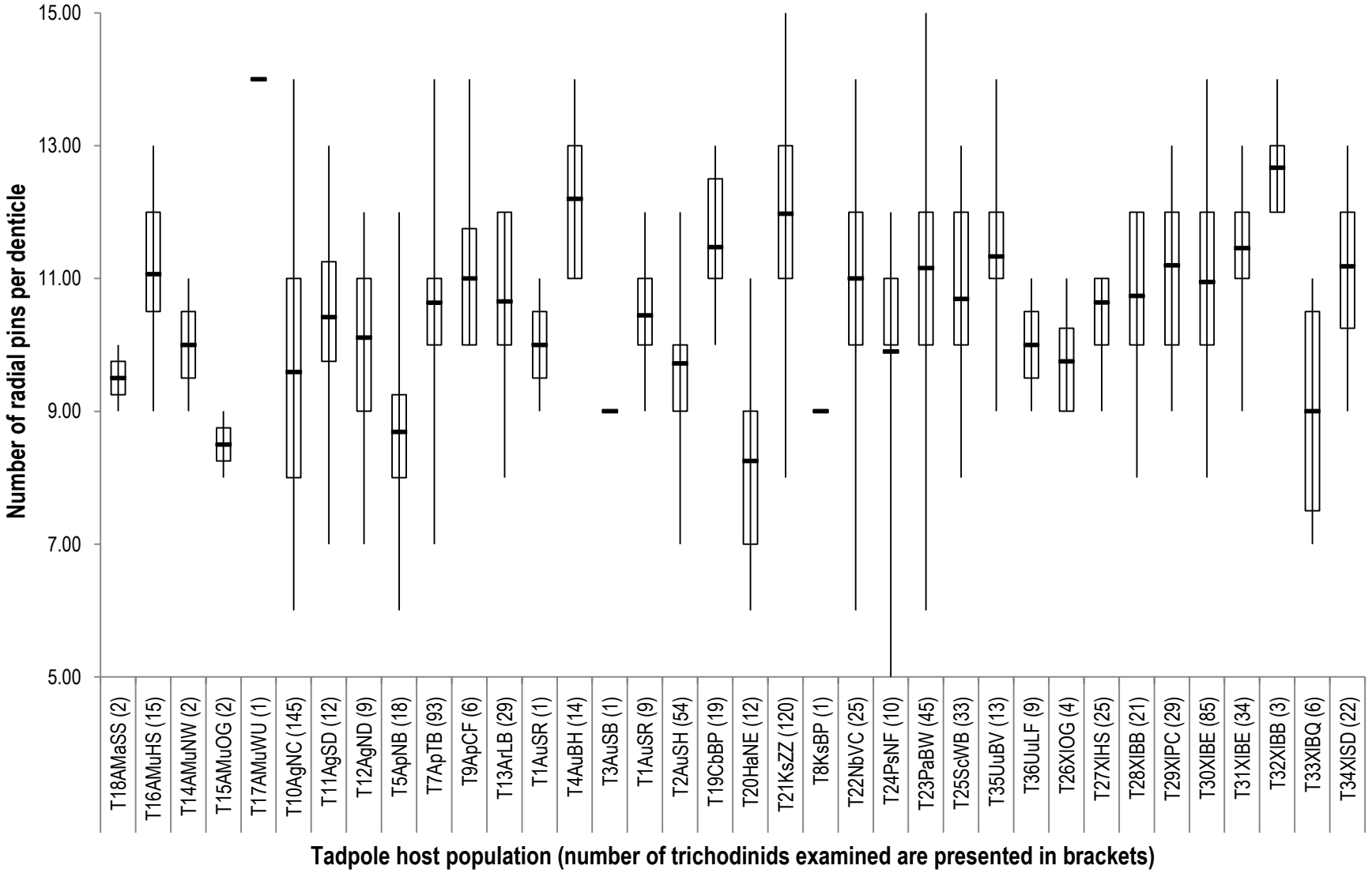


Figure 9.17: Number of radial pins of trichodinids from various tadpole populations that were studied during the present study. Please refer to Figure 5.1 and Table 5.1 for more information on the host populations.

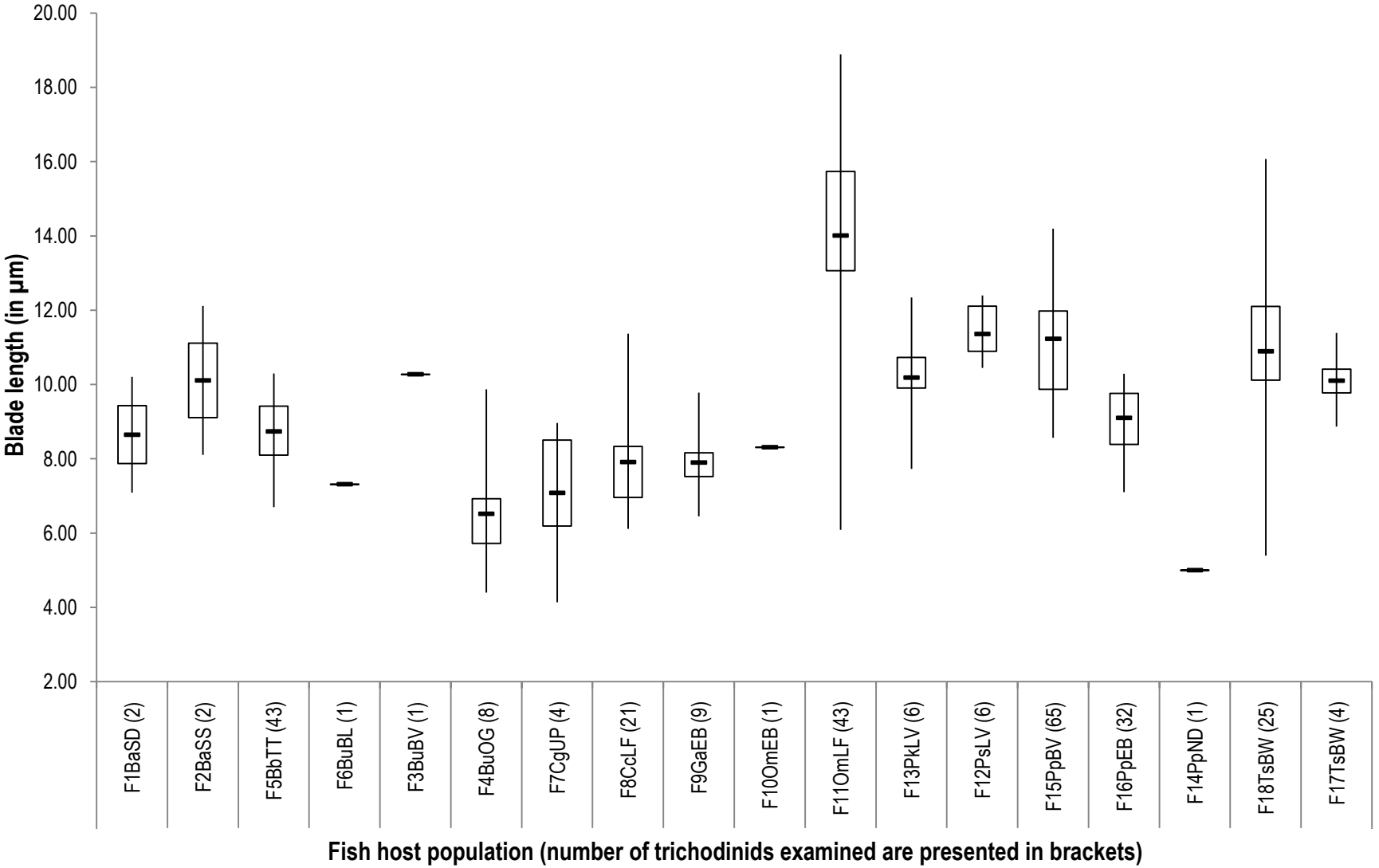


Figure 9.18: Blade length (in µm) of trichodinids from various fish populations that were studied during the present study. Please refer to Figure 5.1 and Table 5.1 for more information on the host populations.

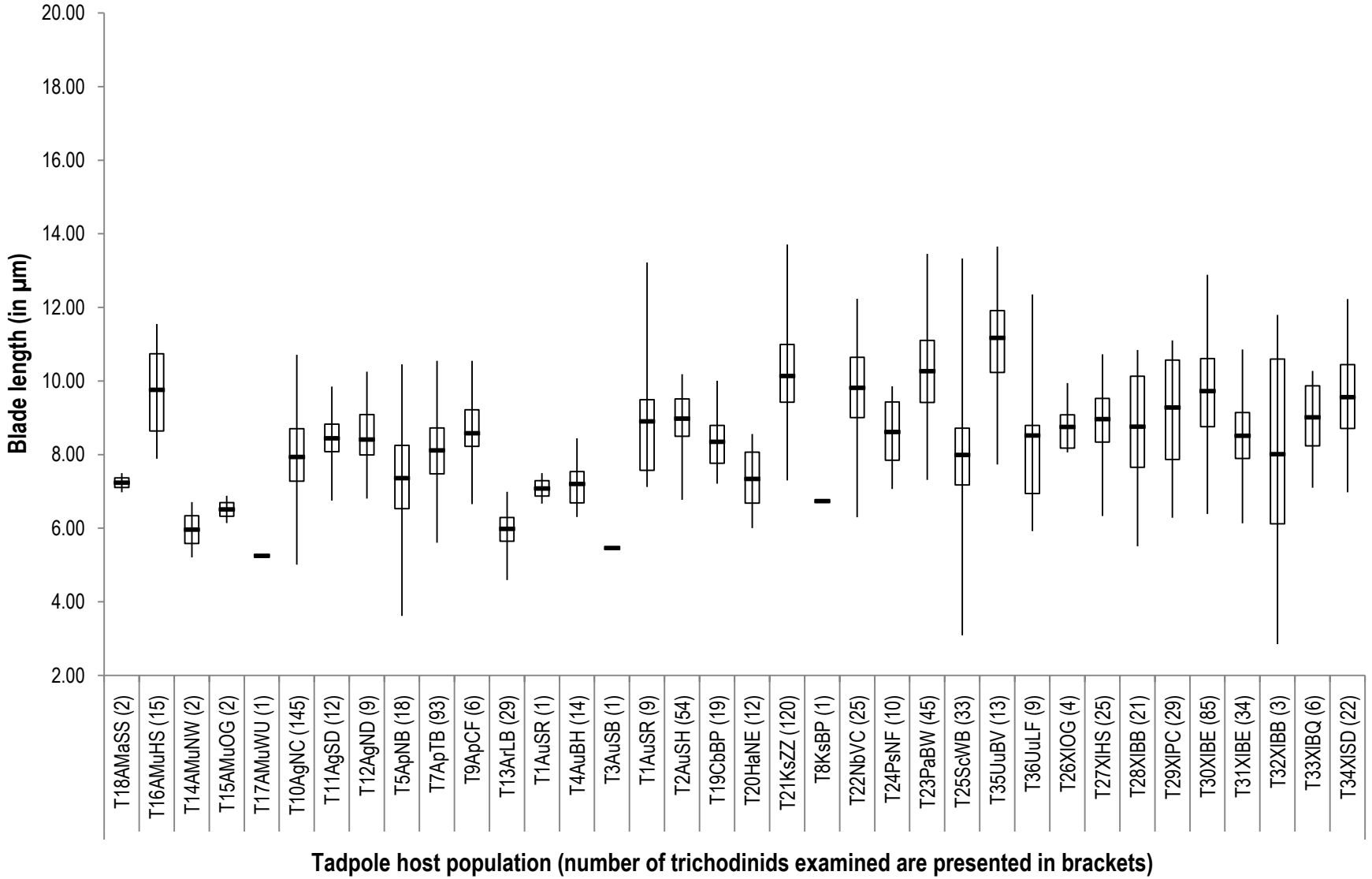


Figure 9.19: Blade length (in µm) of trichodinids from various tadpole populations that were studied during the present study. Please refer to Figure 5.1 and Table 5.1 for more information on the host populations.

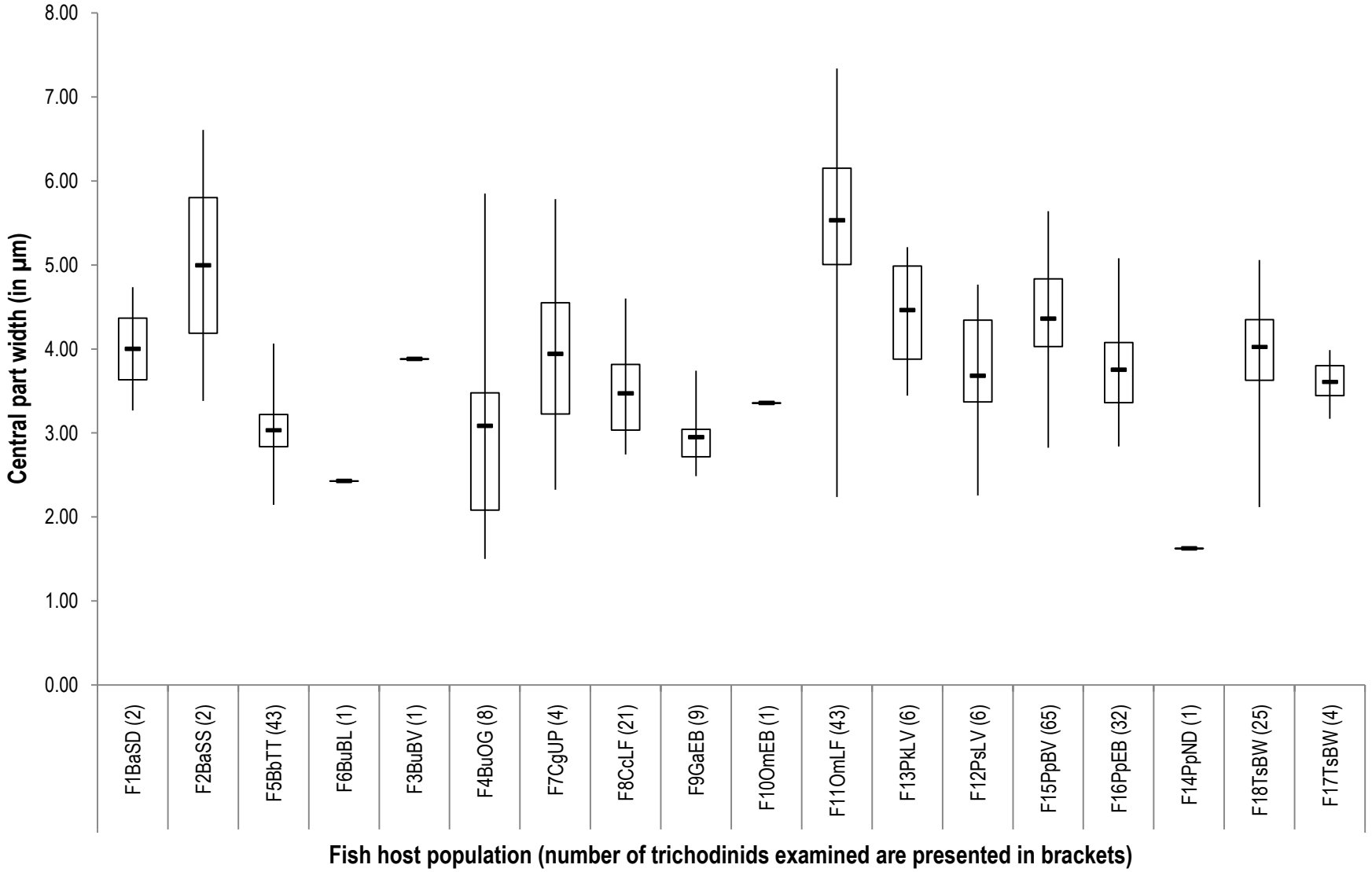


Figure 9.20: Central part width (in µm) of trichodinids from various fish populations that were studied during the present study. Please refer to Figure 5.1 and Table 5.1 for more information on the host populations.

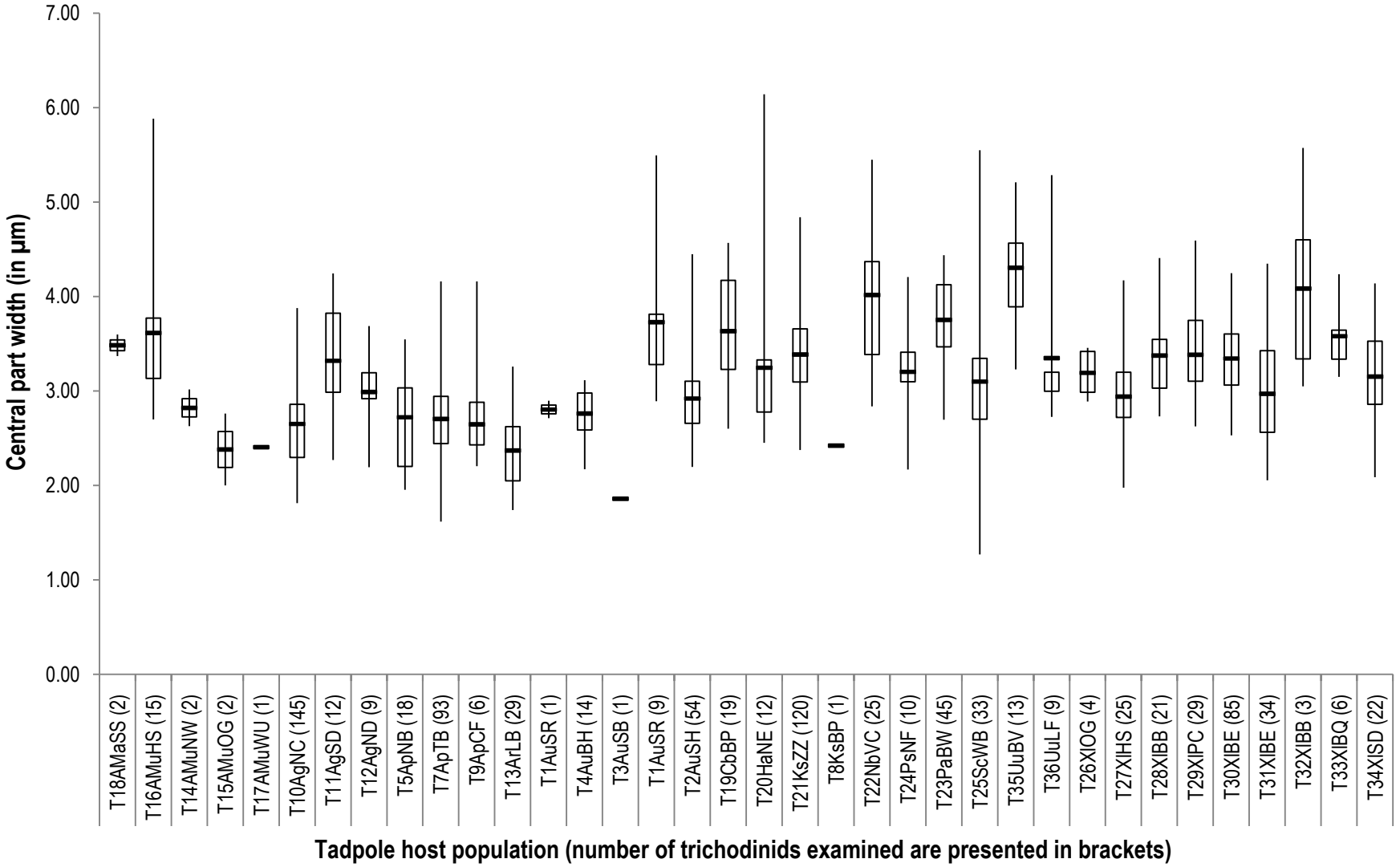


Figure 9.21: Central part width (in µm) of trichodinids from various tadpole populations that were studied during the present study. Please refer to Figure 5.1 and Table 5.1 for more information on the host populations.

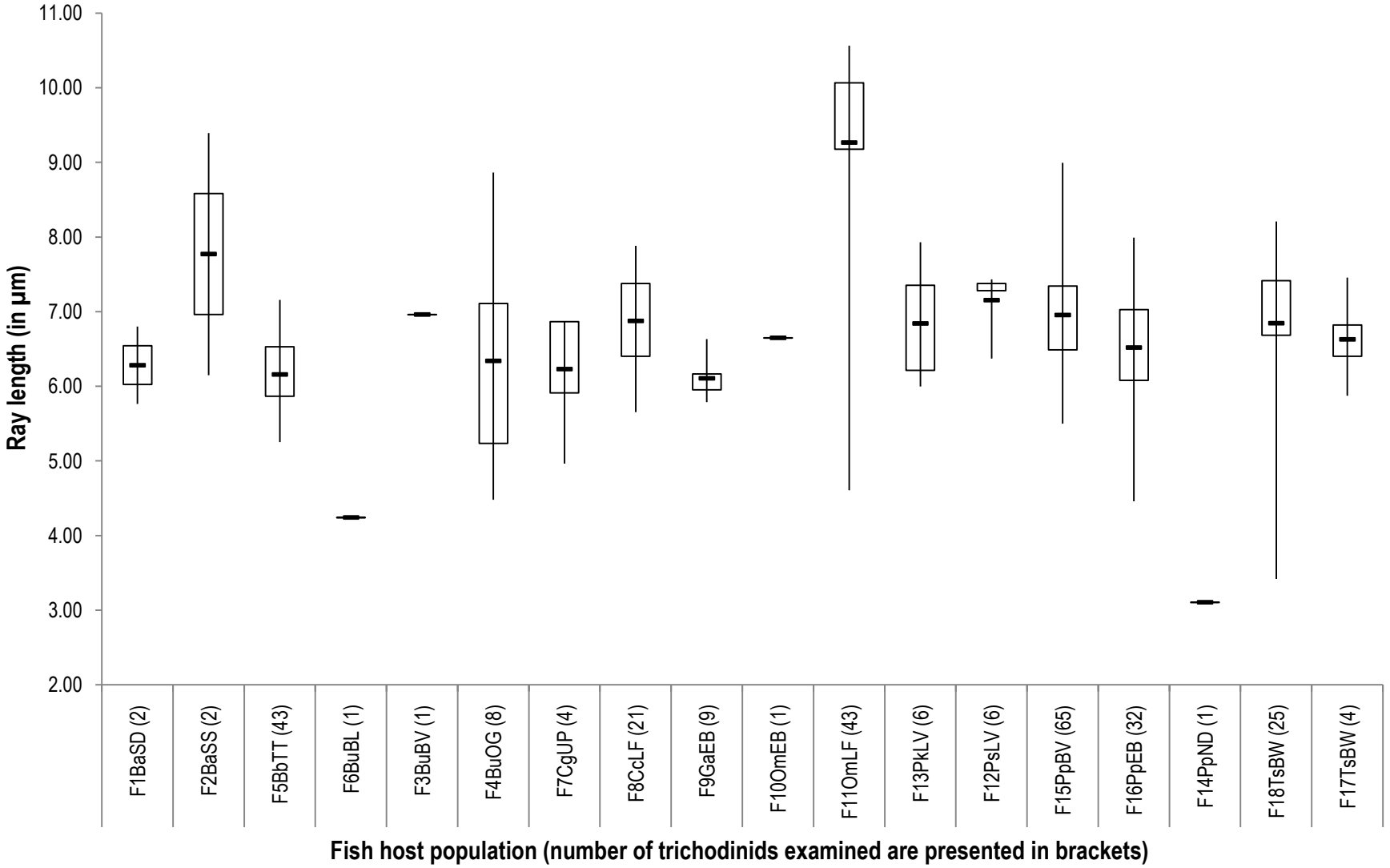


Figure 9.22: Ray length (in µm) of trichodinids from various fish populations that were studied during the present study. Please refer to Figure 5.1 and Table 5.1 for more information on the host populations.

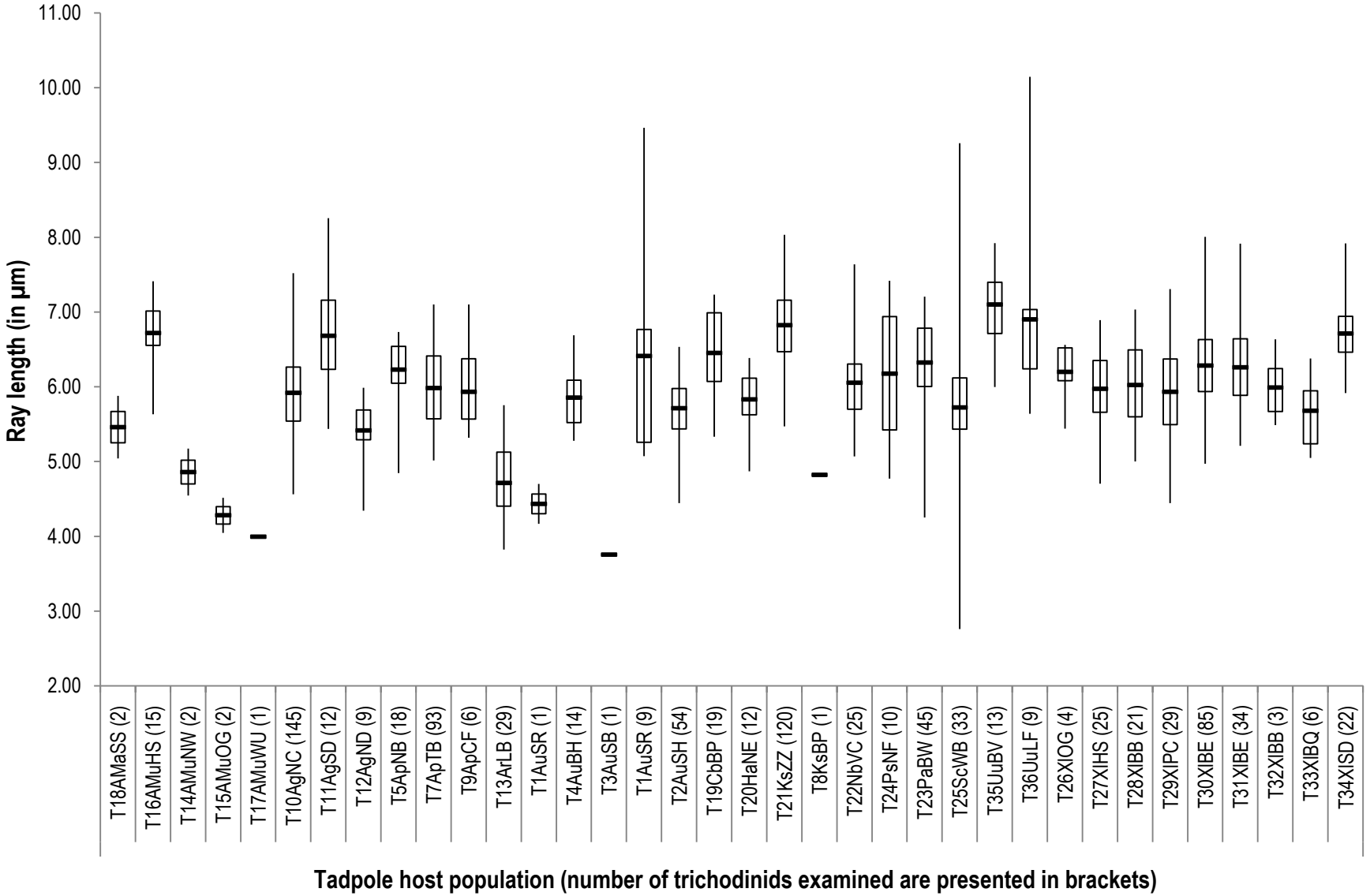


Figure 9.23: Ray length (in µm) of trichodinids from various tadpole populations that were studied during the present study. Please refer to Figure 5.1 and Table 5.1 for more information on the host populations.

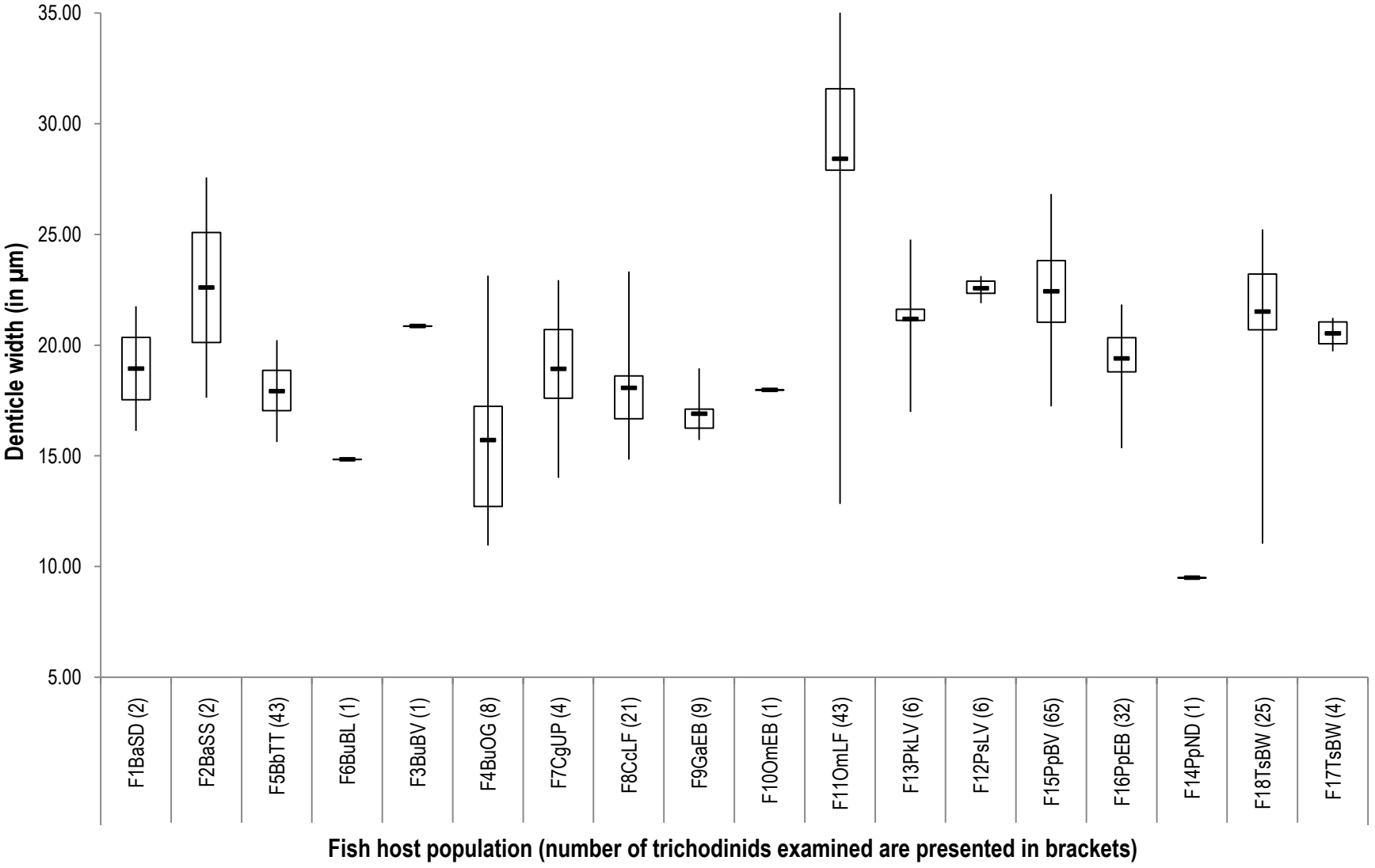


Figure 9.24: Denticle width (in µm) of trichodinids from various fish populations that were studied during the present study. Please refer to Figure 5.1 and Table 5.1 for more information on the host populations.

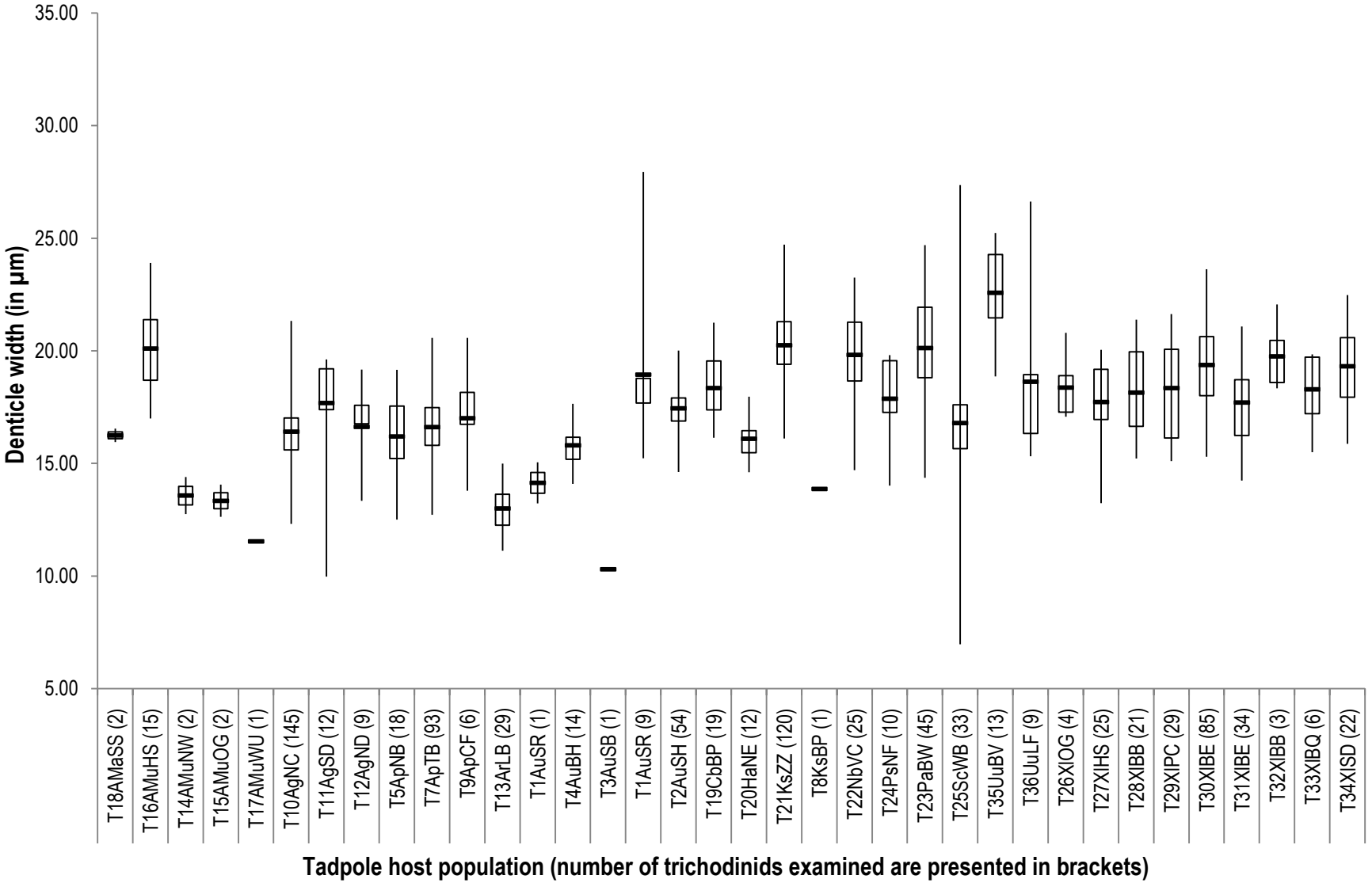


Figure 9.25: Denticle width (in µm) of trichodinids from various tadpole populations that were studied during the present study. Please refer to Figure 5.1 and Table 5.1 for more information on the host populations.

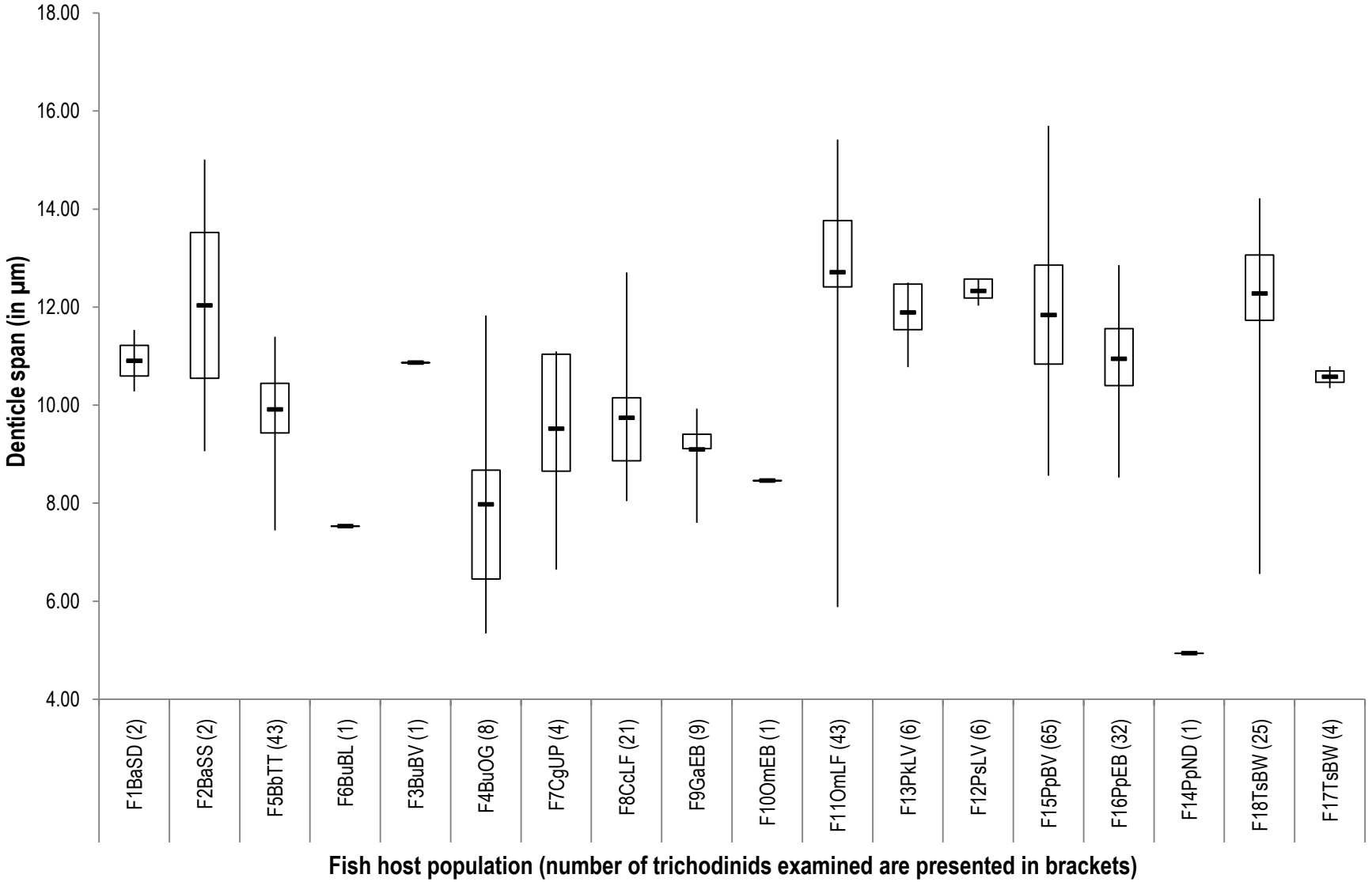


Figure 9.26: Denticle span (in µm) of trichodinids from various fish populations that were studied during the present study. Please refer to Figure 5.1 and Table 5.1 for more information on the host populations.

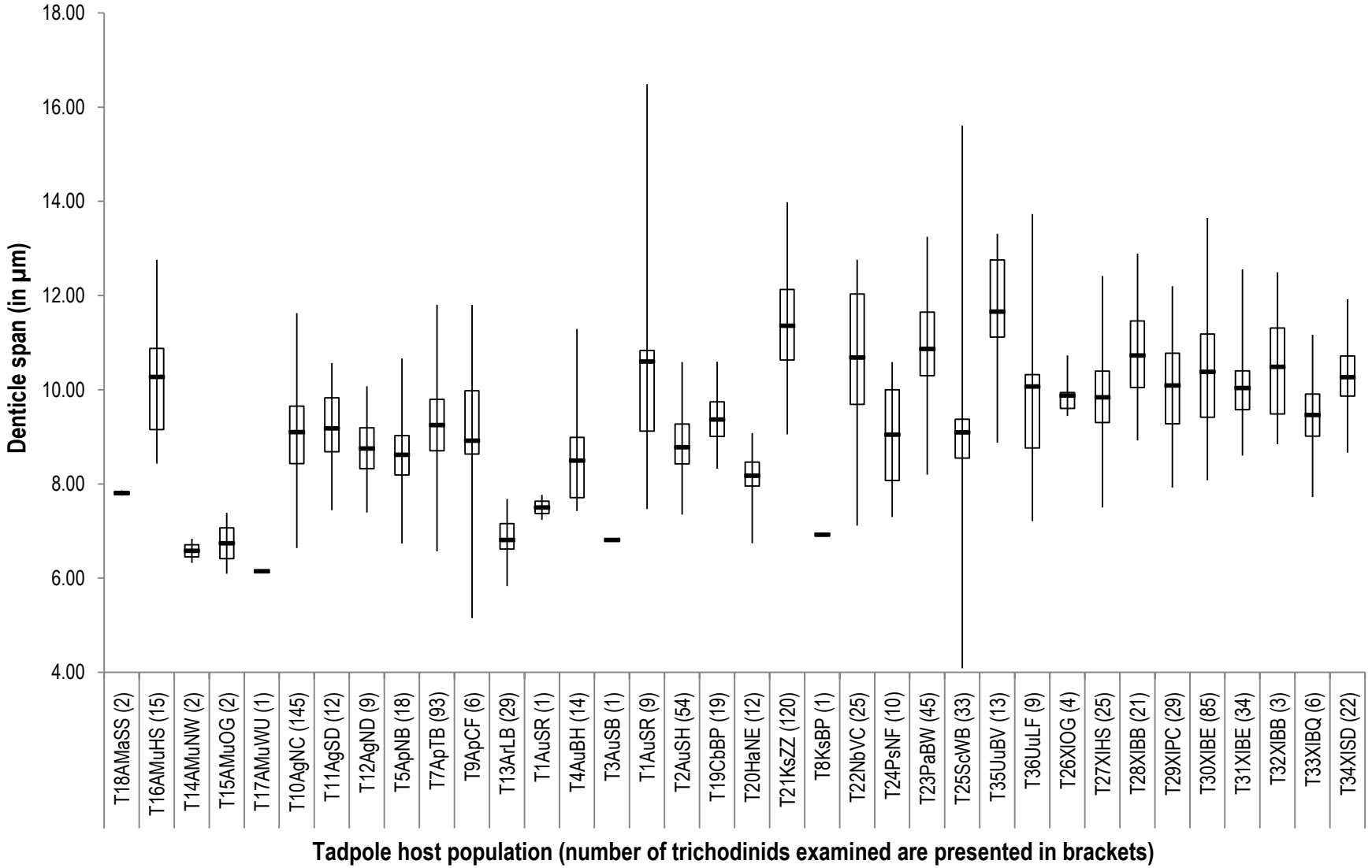


Figure 9.27: Denticle span (in µm) of trichodinids from various tadpole populations that were studied during the present study. Please refer to Figure 5.1 and Table 5.1 for more information on the host populations.

Kazubski (1988) noted that *T. reticulata* on the skin of tadpoles were smaller than trichodinids collected from the skin of fish in the same water bodies at the beginning of summer. However, at the end of summer, only the diameter of the adhesive disc of samples collected on fish versus samples collected on tadpoles was observed. Kazubski (1982) mentioned that different hosts do not play a role in the size of the trichodinids, but that seasonal changes do.

The results obtained for the trichodinids collected from *B. anoplus*, *X. laevis* and the *Amietophrynus* tadpoles collected from Sabella, Wepener, were compared with one another as these populations were collected in the same water medium, in the same season. Twenty two *Xenopus* (Population T34XlSD) tadpoles and two *Barbus* fish (Population F1BaSD) were collected in a gravel dam on the farm Sabella, Wepener in November 2009. Population T18AMaSS consisted of two *Amietophrynus* tadpoles collected at the spruit below the gravel dam. In addition, two fish (Population F2BaSS) were also collected from the spruit in November 2009. In most instances, the measurements for the various characteristics were larger in the case of Population F2BaSS, followed by Populations F1BaSD, T34XlSD and lastly, T19AMaSS (*Amietophrynus* tadpoles collected from the spruit). However, one should take into account that the trichodinids examined in each of the populations were small as 22 trichodinids from *Xenopus* tadpoles were measured, while only two trichodinids for the other three populations were taken into account. Therefore, a conclusion on the trichodinid sizes on various host groups during one season cannot be made by comparing the results obtained from these populations.

A better example would be Population T23PaBW (*Pyxicephalus adspersus* tadpoles) and Population F17TsBW as well as Population F18TsBW (*Tilapia sparrmanii*) that were collected from Loch Logan, Bloemfontein. Population F18TsBW was collected in June 2007, while Population F17TsBW and Population T23PaBW were collected in November 2009.

By comparing the values measured for the two fish populations, it was noted that the average value obtained for most of the characteristics were smaller when Population F17TsBW was examined. Exceptions were the width of the border membrane (Population F18TsBW: 6.09 µm; Population F17TsBW: 6.20 µm) and the number of radial pins per denticle (Population F18TsBW: 12.07; Population F17TsBW: 12.5). In addition, the values obtained for the tadpole trichodinids were generally either similar or smaller than those of Population F17TsBW, with the exceptions being the following:

- Average number of denticles (Population F17TsBW: 23.33; Population T23PaBW: 23.64)
- Body diameter (Population F17TsBW: 77.34 µm; Population T23PaBW: 78.11 µm)
- Adhesive disc diameter (Population F17TsBW: 62.84 µm; Population T23PaBW: 63.70 µm)
- Denticle ring diameter (Population F17TsBW: 38.46 µm; Population T23PaBW: 39.64 µm)
- Tip of ray diameter (Population F17TsBW: 17.79 µm; Population T23PaBW: 18.50 µm) (characteristic is not part of the standard trichodinid characteristics to be measured)
- Blade length (Population F17TsBW: 10.11 µm; Population T23PaBW: 10.26 µm).

Kazubski (1982) believed that seasonal changes play an important role in the size of trichodinids, but this wasn't true in the current study as the two trichodinid populations collected from two different host groups on one day differed more than the trichodinids collected from one host species collected in late winter and late spring.

Lom (1961) mentioned that many trichodinids detach from older fishes during early spring as the general health of the fishes increase during this period. The condition on the skin of the fishes is no longer a preferred habitat for the great number of trichodinids as less food is available for the trichodinids. The trichodinids thus have to search for a new host for their own survival by detaching from the fishes. Trichodinids attach to the skin and / or gills of fry and tadpoles within the same water medium than the older infested fishes. Tadpoles are usually found in shallow, warm water (Du Preez and Channing 2009) that is ideal for the reproduction of trichodinids. Thus, a trichodinid population on fishes persists for long periods, even months or years. However, the tadpoles are only to be found in water bodies for a few months in a year, depending on the species. It is suggested that the age of the trichodinid population on a host may rather influence the average size of the trichodinids.

A high correlation between the fish and tadpole trichodinids examined during the present study and the *T. heterodentata* populations examined by Duncan (1977) as provided by Kruger (1992) were observed for several of the examined characteristics (Figure 9.28). However, the blade length for both groups examined during the present study was longer than in the cases of the populations described by Duncan (1977).

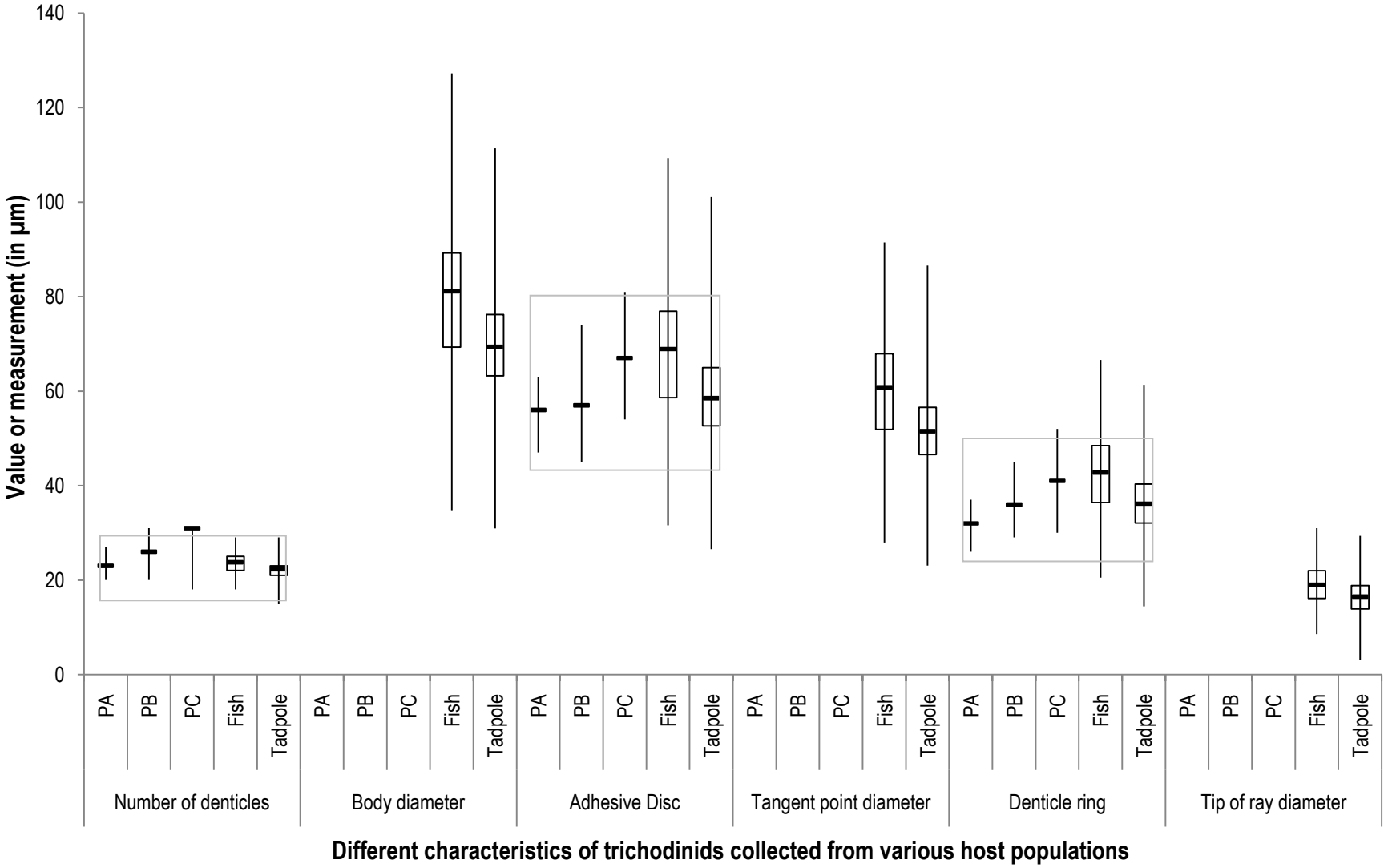


Figure 9.28: Variation of trichodinid measurements obtained from *Trichodina heterodentata* Duncan, 1977 populations collected by Duncan (PA, PB, PC) (information obtained from Kruger 1992) and the trichodinid populations examined during the present study. Note that the Body diameter, Tangent point diameter and Tip of ray diameter for the populations described by Duncan (1977) were not provided by Kruger (1992).

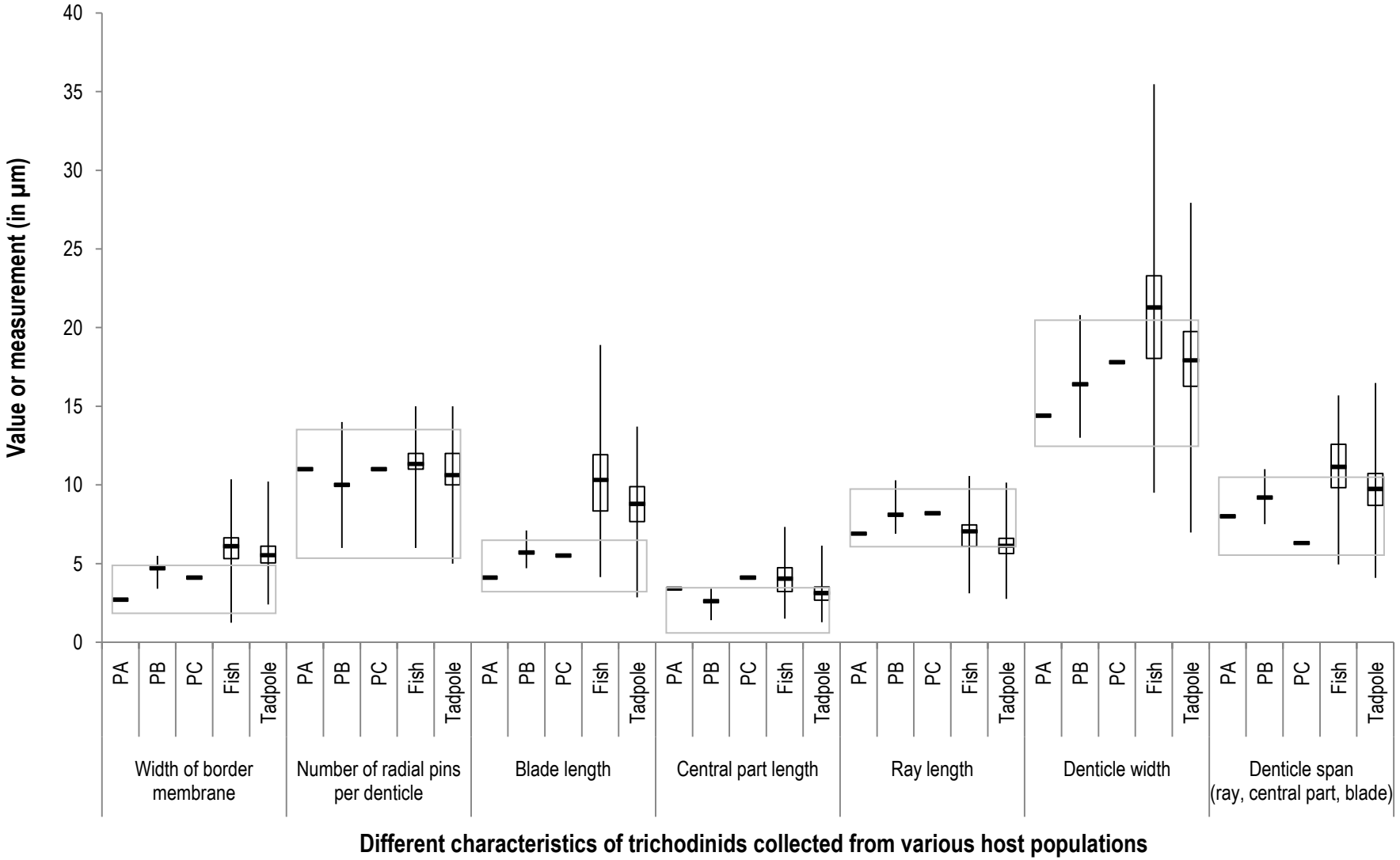


Figure 9.28 (Continue): Variation of trichodinid measurements obtained from *Trichodina heterodentata* Duncan, 1977 populations collected by Duncan (PA, PB, PC) (information obtained from Kruger 1992) and the trichodinid populations examined during the present study.

3. Concluding remarks

Although the measurements obtained from the various populations examined during the present study varied to a great extent, the differences in the measurements were not large enough to assign the trichodinids to more than one species. By comparing the minimum, average and maximum values obtained from the three *T. heterodentata* populations examined by Duncan (1977) with the tadpole trichodinids and fish trichodinids examined during the present study (Figure 9.28), it is suggested that the trichodinid populations examined during the present study should be assigned to *T. heterodentata* although the blade length for both examined groups was longer than in the cases of the populations described by Duncan (1977).

Chapter **10**:

Area of **B**lade, **R**ay and **C**entral **P**art **W**ithin a **D**enticle

'...'tis my intention to inquire into these marvellous structures more narrowly, just for my own amusement...'

Antonie van Leewenhoek

The preceding chapter focussed on the biometric differentiation of the trichodinids collected from the various hosts by using the set of characteristics as proposed by Lom (1958). It was found that the sampled material showed a diverse size range between the populations and even within a single population. As an example, the ray length of trichodinids collected from *Amietophrynus poweri* in Nxamasere, Botswana (Population T5ApNB, see Table 5.1) ranged from 3.62 µm to 10.5 µm with an average length of 7.2 µm. This range is not unusual, as *Trichodina heterodentata* is known to exhibit a considerable variation in the shape of the denticles, especially the shape and length of the ray (Basson and Van As 1994). Further investigation on the morphological differences (if any) of trichodinids hosted on fish and tadpoles respectively, was thus needed.

At present, studies on trichodinids are still mainly concerned with their taxonomy, especially with re-investigating or re-describing the known species as well as describing new species (Özer 2003; Mitra and Haldar 2004). In addition the morphology of the denticles has been widely used in generic discrimination and species identifications of trichodinids (Van As and Basson 1989, 1992). Gong *et al*. (2005) attempted to provide a 'new' way to study the phylogenetic relationship of trichodinids by taking advantage of existing material and technology by quantifying certain denticle characteristics as the denticles have a high systematic value and therefore its changes ought to reflect the evolutionary tendencies of trichodinids. For this experiment, Gong *et al.* (2005) calculated the percentages of the areas of the blade, central part and ray of a single denticle. These percentages were compared within a single denticle. In addition, the percentages of the total area of all the denticles in the adhesive disc was compared to that of the loop of the denticle ring for more than 20 known trichodinid species. The area of the loop of the denticle ring was described in the Chapter 5 (material and methods) as the area of the denticles within the denticle ring as well as the area of the matrix within the denticle ring. Gong *et al.* (2005) used *Urceolaria* as the out-group as this genus is characterised by the absence of rays or blades. Gong *et al.* (2005) used every third or fourth denticle for their calculations and concluded that nearly all studied species had their own fluctuation range within the characteristic values (Table 10.1).

Gong *et al.* (2005) concluded that this may imply that specimens could be assigned to a species by using the newly proposed method. The current study aimed to determine if a similar method as proposed by Gong *et al.* (2005) could be used to distinguish between the trichodinids that naturally infested tadpoles and the trichodinids collected from fish during the current study.

Table 10.1: Comparison of the percentage (%) of the area of the blade, central part and ray within a denticle as well as the denticles within the denticle loop for various trichodinid species, adapted from Gong *et al*. (2005).

Trichodinid species	Blade (%)	Central part (%)	Ray (%)	Denticles within the denticle loop (%)
Trichodina acuta Lom, 1961	53	25	22	70
T. compacta Van As and Basson, 1989	51	27	23	63
T. cottidarum Dogiel, 1940	58	16	26	53
T. domerguei Wallengren, 1987	61	20	19	66
T. heterodentata Duncan, 1977	47	23	30	62
T. jadranica Lom and Laird, 1969	61	23	16	65
T. maritinkae Basson and Van As, 1991	49	24	27	68
T. modesta Lom, 1970	61	22	17	71
T. murmanica Poljansky, 1955	53	24	23	60
T. mutabilis Kazubski and Migala, 1968	58	21	21	61
T. mystusi Asmat and Halder, 1998	55	22	23	57
T. nigra Lom, 1960	54	24	22	65
T. oviducti Poljansky, 1955	57	19	24	54
T. polycirra Lom, 1960	52	20	58	59
T. puytoraci Lom, 1962	60	18	22	53
T. raabei Lom, 1962	51	19	30	65
T. rectuncinata Raabe, 1958	66	17	17	56
T. reticulata Hirschman and Partsch, 1955	55	20	25	58
T. sinonovaculae Xu, Song and Warren, 1999	60	20	20	63
T. urinaria Dogiel, 1940	58	13	29	55

1. Material and methods

Skin and gill smears were prepared from various possible host species and the air dried smears were impregnated by using the silver nitrate impregnation technique as explained in the material and methods chapter. Digital micrographs were produced for the trichodinids on the smears with the aid of a compound microscope. The area of the blade, central part and ray of three (or more) consecutive denticles per specimen was measured by means of the ImageJ software package (ImageJ version 1.40g, 2008). The percentages of the areas of the blade, central part and ray respective to that of a single denticle were determined. In addition, the total area of all the denticles in the adhesive disc was determined by multiplying the average area of the blade, central part and ray of the measured denticles with the number of denticles in the denticle ring. This was done to compare the percentage of the total area of all the denticles in the adhesive disc to that of the loop of the denticle ring (Figure 5.5).

The dimensions are presented as the minimum, mean and maximum values, where after the percentages are given. Please note that all measurements are in micrometers (µm). The number of specimens measured is also given.

2. Results and discussion

When the results obtained from the trichodinids collected from the different fish populations (Figures 10.1, 10.2), it was found that the area occupied by the blades could vary from 41.11% (Population F12PsLV – *Poecilia sphenops* collected from Lake Valencia in 1980) to 51.84% (Population F4BuOG – unknown *Barbus* species collected at the Barrage Vaal in June 1982), giving a fluctuation range of 10.73%. A fluctuation range of 8.8% was observed within the central part of the denticles (21.29% - 30.09%) while the area occupied by the rays had the largest fluctuation range (13.03%) as 18.34% of the denticles of Population F4BuOG was made up of the ray area while 31.37% of the denticles of Population F6BuBL (unknown *Barbus* species collected in Bloemfontein in June 1991) was occupied by the rays. The area occupied by the denticles within the denticle loop varied to a great extent as the denticles of Population F11OmEB (*Oreochromis mossambicus* collected in Bloemfontein during October 1988) occupied only 32% of the denticle loop while 56.6% of the denticle loop of Population F17TsBW (*Tilapia sparrmanii* collected from the Loch Logan Waterfront in Bloemfontein during November 2009) was occupied by the denticles.

By comparing this information with the trichodinids that were collected from tadpole hosts (Figure 10.3, 10.4), it was found that the blade area of tadpole trichodinids occupied a smaller percentage within the denticles (19.78% - 30.07%) while the area occupied by the ray varied between 39.49% and 52.39%. A large percentage of the denticle loop was occupied by the denticles of Population T14AMuNW (unknown *Amietia* species collected near Wurasoord in December 1989) (82.96%) while the said characteristic occupied only 42.6% of the mentioned area in the case of Population T36UuLF (unknown tadpoles collected from the Lowveld Fish Production Facilities in October 1982).

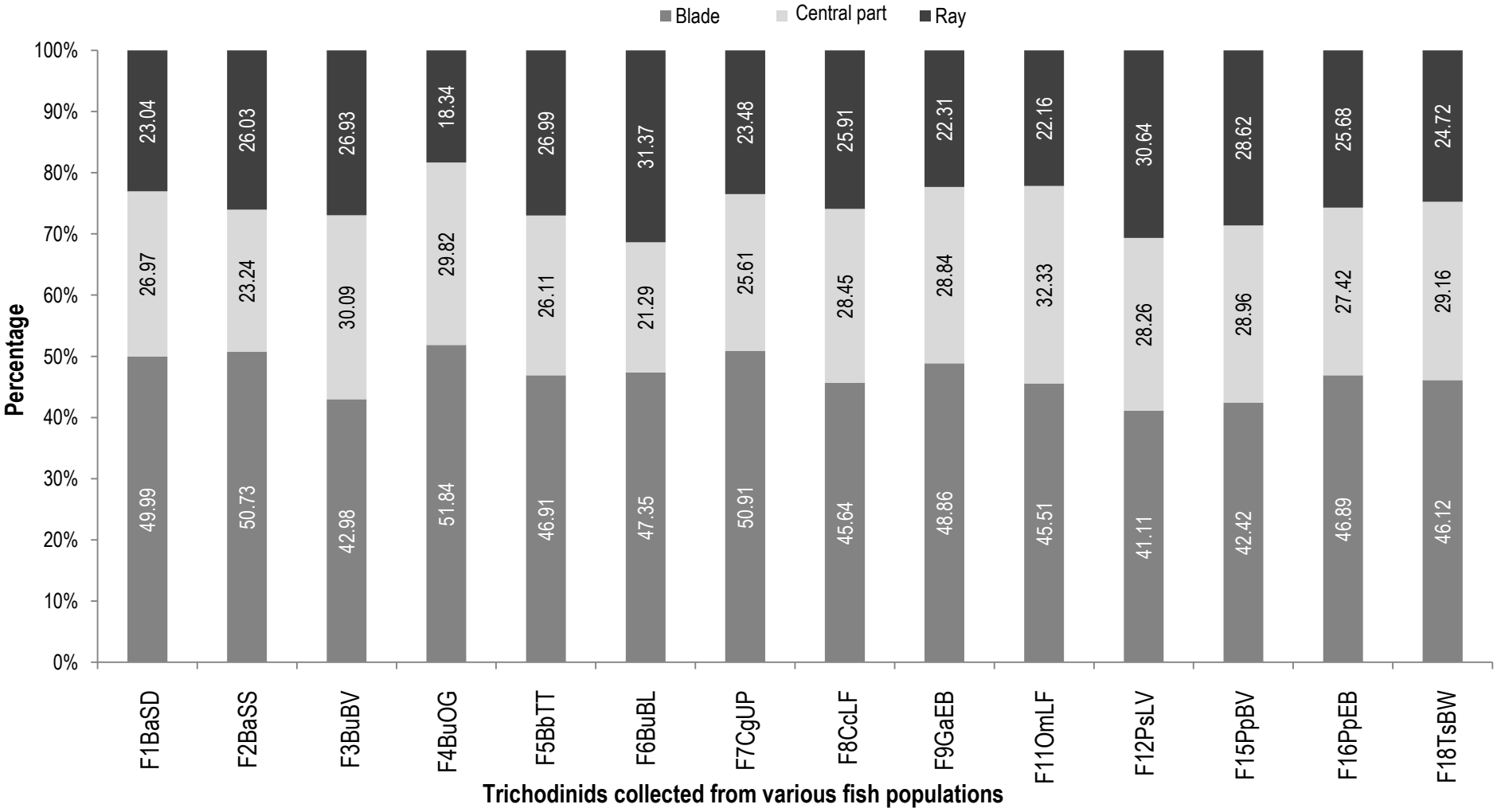


Figure 10.1: Average area of the measured characteristics from trichodinids collected from different fish species (refer to Table 5.1 for an explanation / description on the trichodinid population numbers).

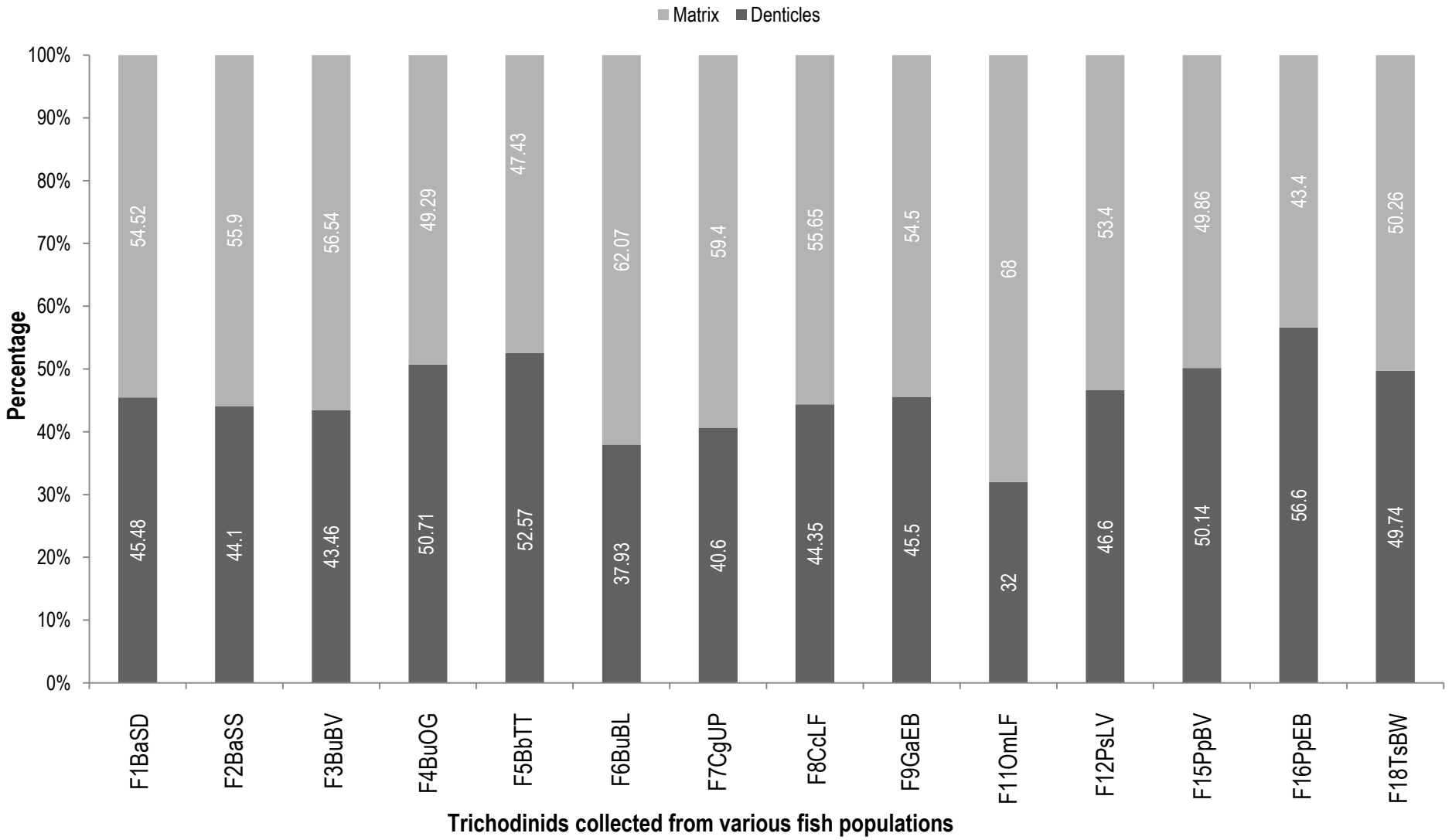


Figure 10.2: Area of the matrix and denticles respectively within the loop of the denticle ring of trichodinids hosted by fish.

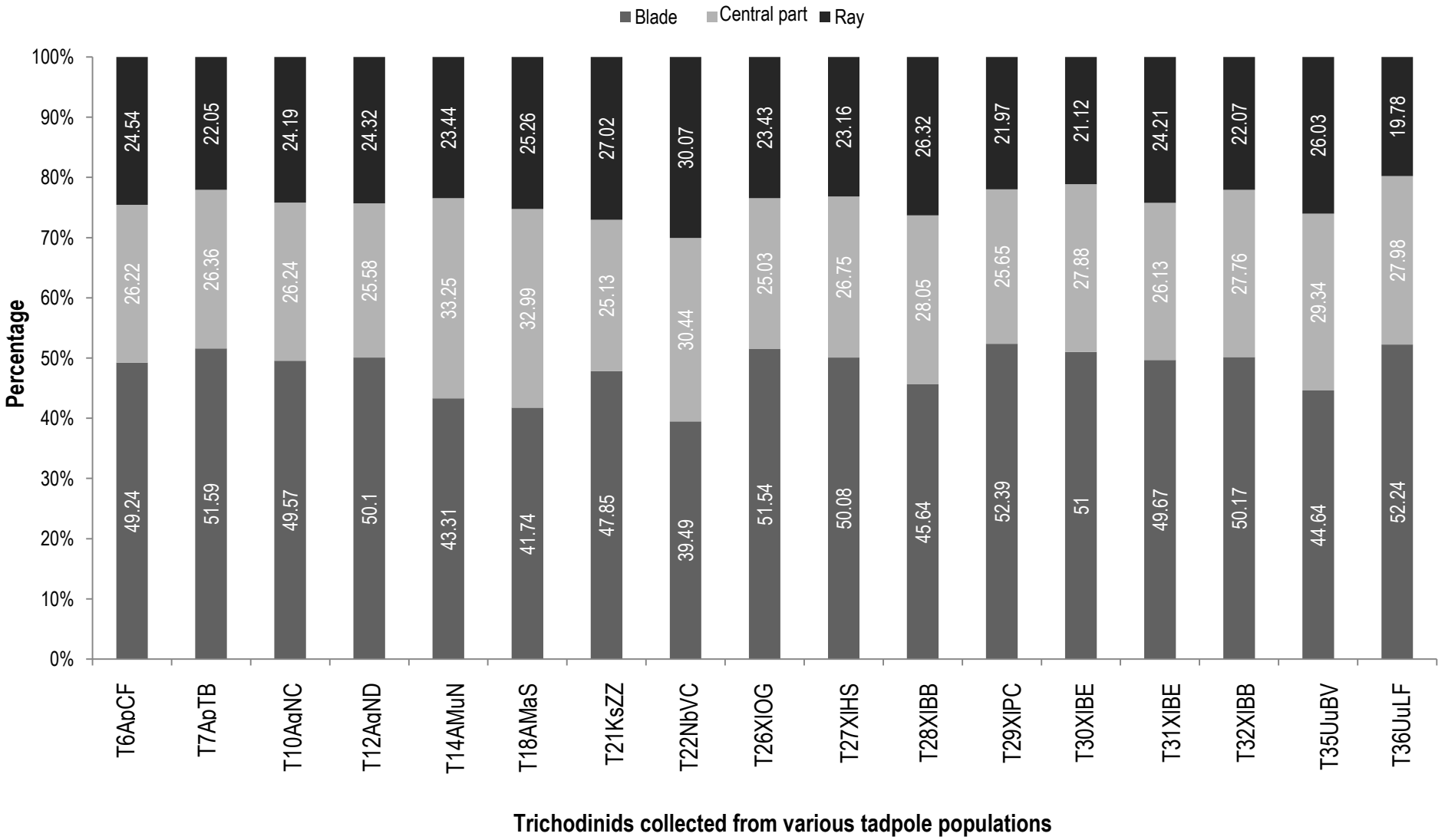


Figure 10.3: Average of the measured characteristics relatively to that of a single denticle (tadpole hosted trichodinids).

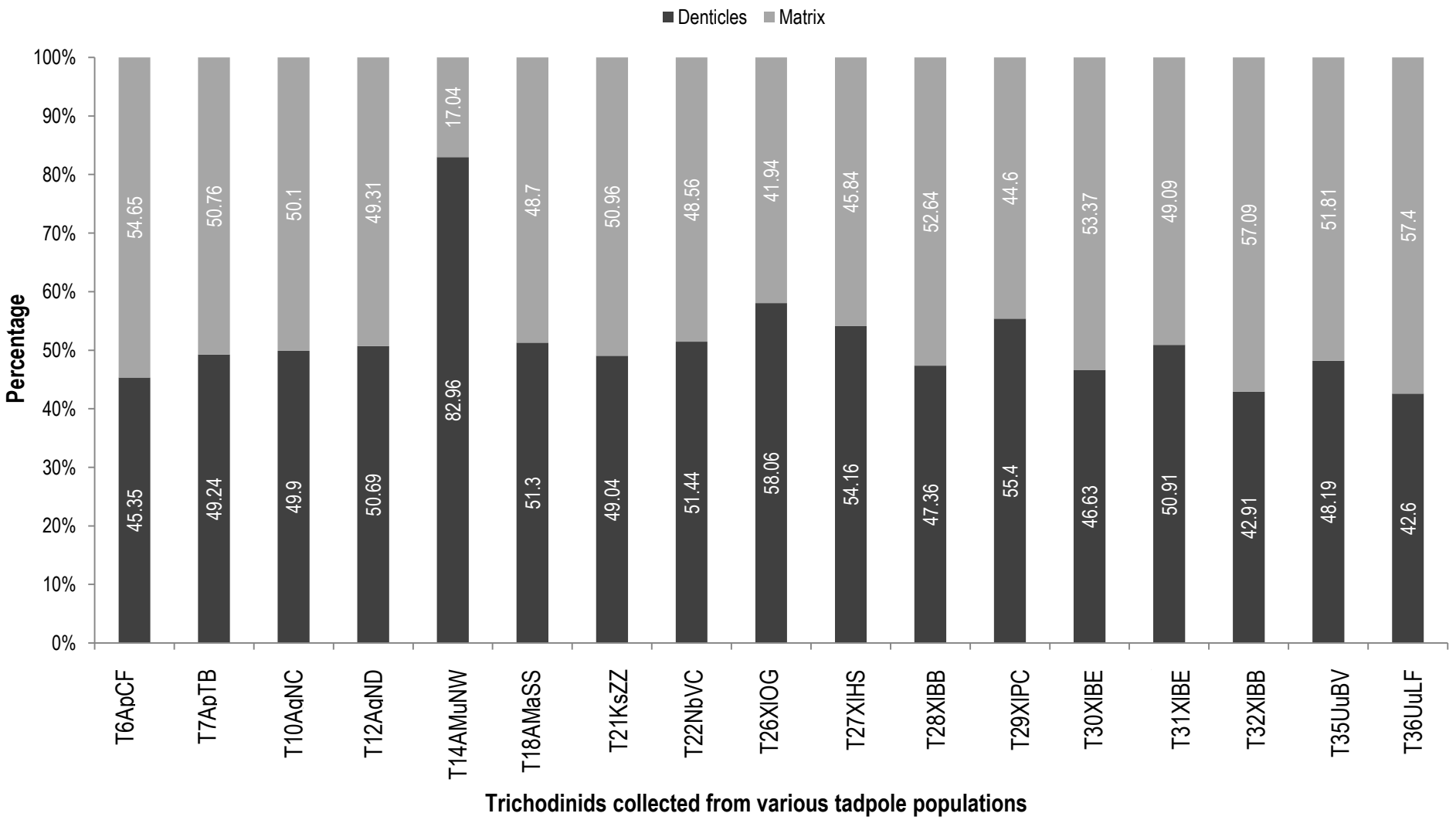


Figure 10.4: Average area of the denticles and matrix respectively to that of the loop of the denticle ring (tadpole hosted trichodinids).

Gong *et al.* (2005) studied only 67 trichodinid samples that represented 20 species in total. Of the 67 samples, only four samples represented *T. heterodentata*. Gong *et al.* (2005) mentioned that the number of samples studied within each trichodinid species were insufficient as variance within each species occur. It can thus be assumed that the variance within each of the measured characteristics for each of the studied species will be larger than determined by Gong *et al.* (2005).

By comparing the *T. heterodentata* values provided by Gong *et al.* (2005) with the values obtained during the present study (Table 10.2), it can be seen that the area occupied by the blade of trichodinids hosted by tadpoles were 1% higher, while the area occupied by the central part of trichodinids collected during this study (fish and tadpole hosted) were 5% higher than the specimens described by Gong and his team. The area occupied by the ray of trichodinids examined during this study and the trichodinids examined by Gong *et al*. (2005) also differ significantly. This said, it should be mentioned that only four *T. heterodentata* samples were taken into account by Gong *et al.* (2005). As previously mentioned *T. heterodentata* is known for its diversity within the group, populations and even within an individual. Therefore, the method by Gong *et al.* (2005) should not be discarded completely, but either more samples per population should be taken into account or more denticles should be measured for every sample.

Table 10.2: Comparison of the percentage (%) of the area of the blade, central part and ray within a denticle as well as the denticles within the denticle loop of trichodinids examined during the present study.

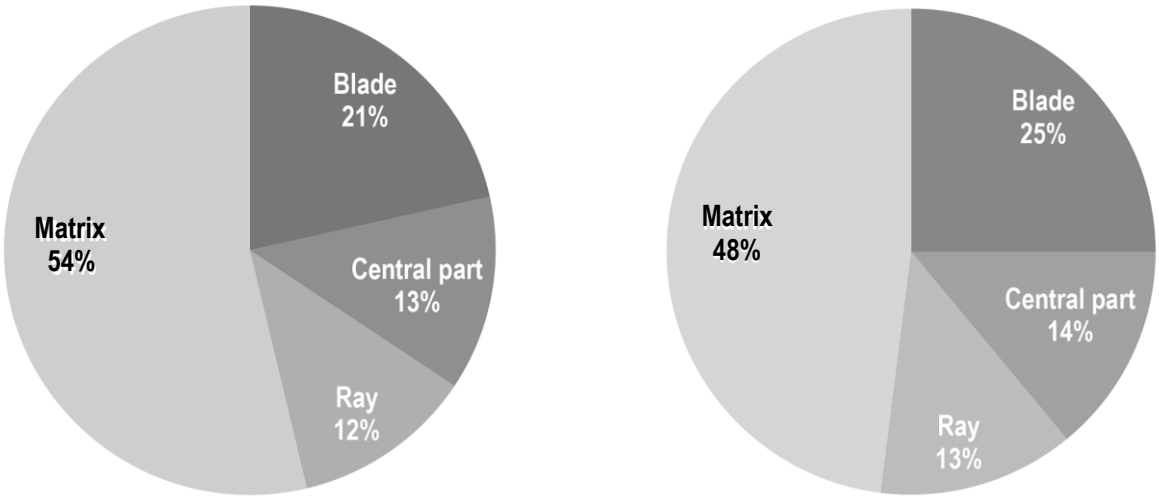
Trichodinids	Blade (%) within the denticles	Central part (%) within the denticles	Ray (%) within the denticles	Denticles (%) within the denticle loop
**Trichodina heterodentata* Duncan, 1977	47	23	30	62
Trichodinids sampled from fish	47	28	25	46
Trichodinids sampled from tadpoles	48	28	24	52

*Values obtained from Gong *et al*. (2005)

Three noteworthy observations were made when studying the area covered by the matrix, blades, central parts and rays within the denticle loop of fish and tadpole trichodinids (Figure 10.5) as it was discovered that:

- The matrix covered 54% of the mentioned area in the case of trichodinids from fish;
- The denticles covered the largest part when trichodinids from tadpoles were examined (52%) and

- The area covered by the blade structures can be identified as a key comparative structure as the area covered by blades in fish trichodinids covered an area of 21% whilst the blade structures of trichodinids from tadpoles covered an area of 25% of the mentioned loop.



Fish trichodinids
(number of populations measured: 18;
number of trichodinid samples measured: 77;
number of denticles measured: 382)

Tadpole trichodinids
(number of populations measured: 14;
number of trichodinid samples measured: 38;
number of denticles measured: 192)

Figure 10.5: Area (%) of the blades, central parts, rays and matrix within *Trichodina heterodentata* Duncan, 1977 on fish and tadpoles respectively.

3. Concluding remarks

A similar method as proposed by Gong *et al.* (2005) was used to describe the ectotrichodinid samples collected from fishes and tadpoles respectively by means of the percentage of the rays, central parts and blades of denticles. It was found that the studied material represents the species *T. heterodentata* if compared to the values obtained for this species by Gong *et al.* (2005). However, a variation between the populations, between fish hosted and tadpole hosted trichodinids and even within a single population was observed. This is not uncommon for *T. heterodentata* as this species is known to exhibit a considerable variation in the shape of the denticles (Basson and Van As 1994). It is therefore suggested that the trichodinids from the fishes and tadpoles examined during the present study belongs to a single species, namely *T. heterodentata*.

Chapter **11**:

Morphological **D**ifferentiation **B**etween **T**he **C**ollected **T**richodinid **P**opulations **(T**hree **C**onsecutive **D**enticles**)**

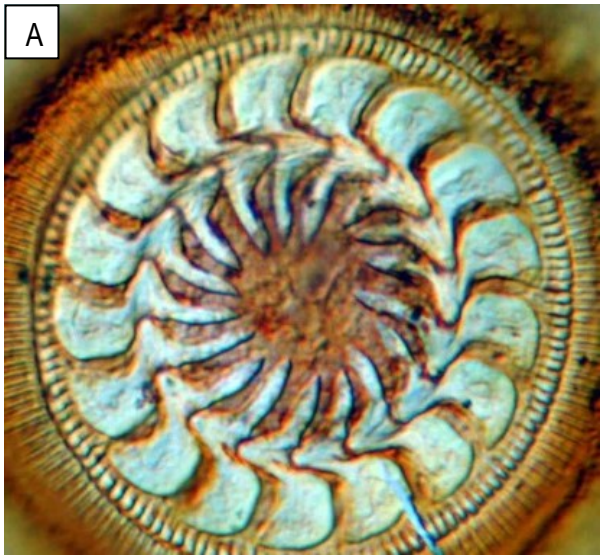
'…the scientist is motivated primarily by curiosity and a desire for truth…'

Irving Langmuir

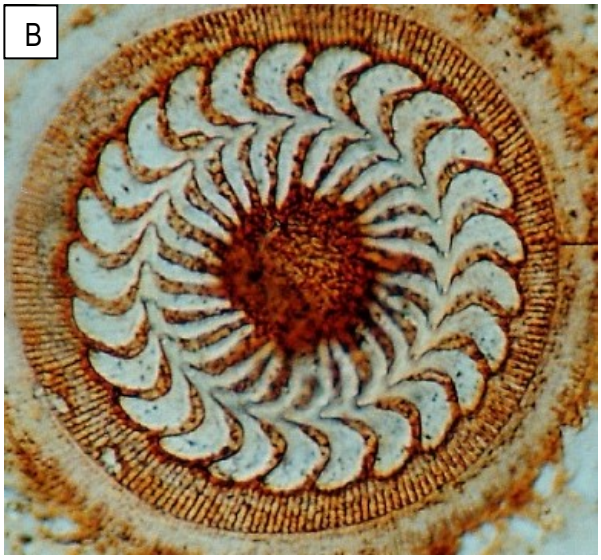
Trichodinid species are differentiated from one another by means of their distribution, host species, position of attachment to host species as well as the morphological characteristics of the denticle ring. For example, ectotrichodinids such as *Trichodina heterodentata* can be found in various countries on various host groups as mentioned before. In addition, *T. heterodentata* attach to the skin and / or gills of their hosts. On a morphological level, the centre of the denticle ring of many trichodinids do not contain a hardened structure (Figure 11.1A and B) while the centre of the denticle ring of certain trichodinids contains a single scleroprotein structure (Figure 11.1C) and other trichodinid species have many small scleroprotein structures in the middle of the denticle ring (Figure 11.1D, E and F). Endotrichodinids that infect the urinary tract of their hosts are usually characterised by slimmer blades as in the case of *T. uretra* Basson, 1989 collected from *Barbus trimaculatus* Peters, 1852 (Figure 11.1F).

Van As and Basson (1989) mentioned that the work of Lom can be described as a milestone in taxonomic terms of trichodinids as the uniform specific characteristics proposed by Lom (1958) aid in the description of trichodinid species. Van As and Basson (1989) further stated that the characteristics proposed by Lom (1958) is sufficient to differentiate between widely differing species, but that the proposed uniform specific characteristics cannot adequately differentiate trichodinid species with relatively small differences in the form of the denticles. Although Lom (1958) acknowledged that the shape of the denticle is a diagnostic feature when describing trichodinids, his method does not include the description and comparison of the shape of the denticles. Van As and Basson (1989) saw the deficiency in the standard method for trichodinid descriptions as proposed by Lom (1958) and introduced additional characteristics that should be taken into consideration when trichodinid populations are to be described. Van As and Basson (1989, 1992) therefore proposed that the morphological characteristics of three consecutive denticles should be studied to support the description of trichodinid species in future.

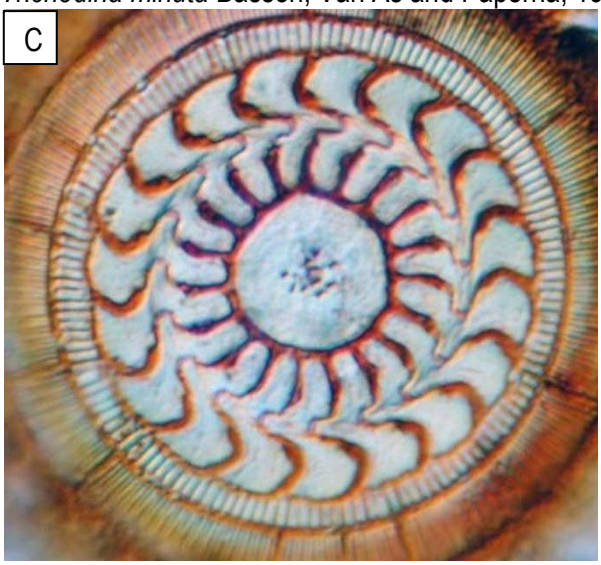
A similar method as proposed by Van As and Basson (1992) was also used during the present study to determine if any morphological differentiation occurred within the trichodinid populations collected from the different host populations as a difference between the trichodinid populations may indicate that the trichodinids adapted to certain conditions on the specific hosts to environmental conditions within the various water mediums and / or to different seasons. Additionally, a significant difference may imply that more than one trichodinid species were hosted on the studied host populations.



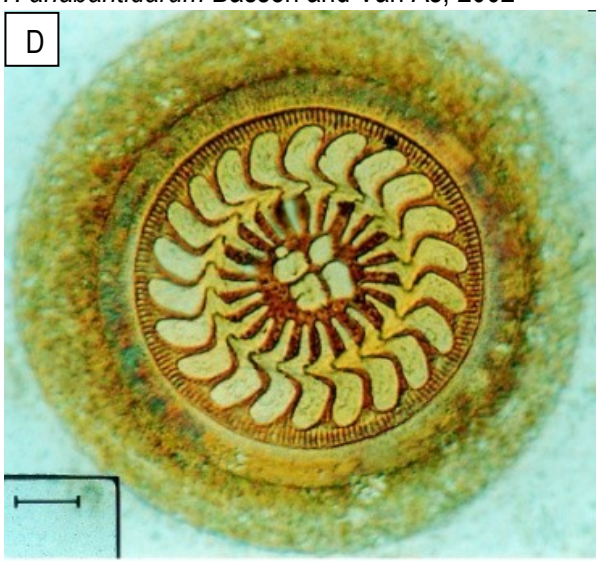
Trichodina minuta Basson, Van As and Paperna, 1983



T. anabantidarum Basson and Van As, 2002

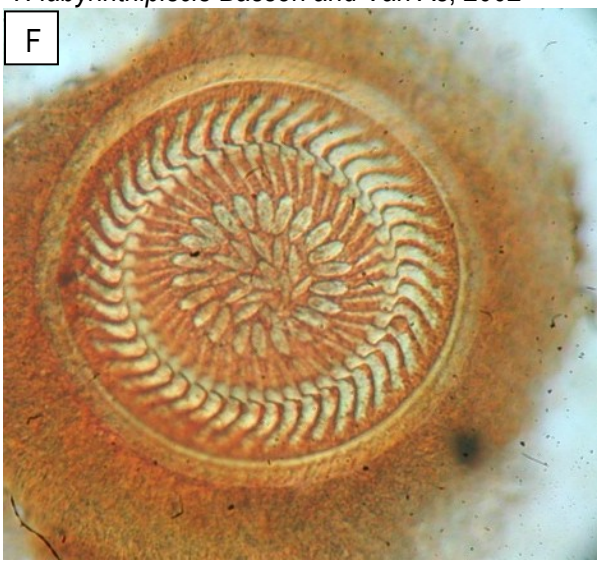


T. compacta Van As and Basson, 1989



T. labyrinthipiscis Basson and Van As, 2002

T. reticulata Hirschmann and Partsch, 1955



T. uretra Basson, 1989

Figure 11.1: Examples of the morphological differentiation between species from the genus *Trichodina* Ehrenberg, 1830 collected from various fish species.

1. Material and methods

Three consecutive denticles were drawn from the images taken from the silver impregnated trichodinids collected from the various hosts as described in Chapter 5 (Material and Methods). A line was drawn from the centre of the adhesive disc towards the tangent point of a denticle and this was repeated for the following two denticles (y-axis, y-1 axis, y+1 axis). A line (x-axis) was also drawn from the middle of the central part of the second denticle, perpendicular to the y-axis (Figure 5.6). Characteristics such as the shape of the tangent point, deepest point of curve of the blade as well as the blade apophysis were used. In addition, the ratio of the denticle above and below the x-axis was also noted.

The results of the morphological characteristics of trichodinids collected previously on tadpoles in southern Africa by Kruger *et al.* (1993) were used to compare to the findings of the current study (Figure 11.2, Table 11.1).

A hierarchical cluster analysis was represented graphically by means of a dendrogram by using Ward's minimum variance method (Ward 1963) to indicate noteworthy similarities and / or differentiation within the studied trichodinid populations. This was achieved by assigning a value to each of the variables of each of the examined denticle characteristics. The dendrogram was produced with the aid a statistical computing language, R (R Development Core Team 2012).

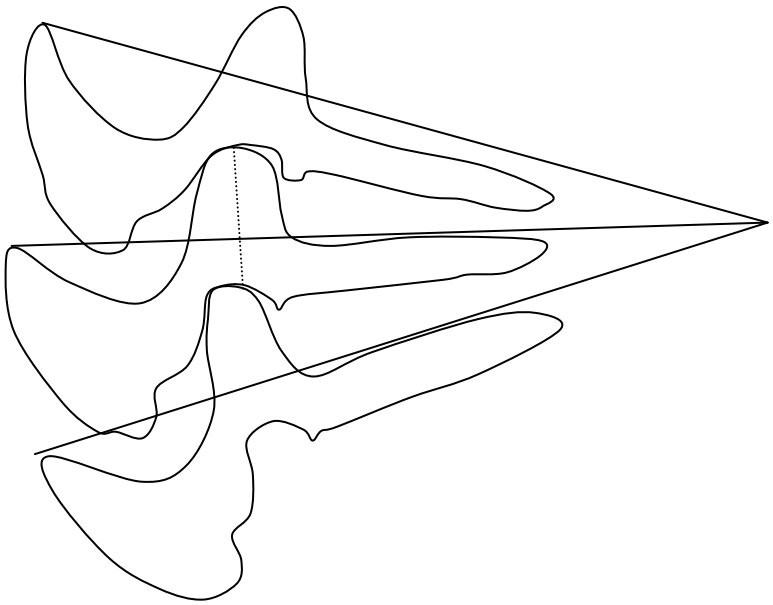


Figure 11.2: A schematic illustration of an example of trichodinids previously collected from *Xenopus laevis* Daudin, 1802 tadpoles in southern Africa, redrawn from Kruger *et al.* (1993).

Table 11.1: A description of the trichodinids previously collected from the skin and gills of tadpoles collected in southern Africa as described by Kruger *et al.* (1993).

Blade form	- Open sickle-shaped
Distal surface	- Rounded
	- Sloping downwards in anterior direction
Tangent point	- Sharp, situated slightly below distal surface
Anterior margin	- Rounded
	- Extending to or slightly beyond y+1 axis
Blade apophysis	- Prominent, forming distinct indentation
Posterior blade surface	- Smooth
	- Deep semi-lunar curve
Blade connection	- Thick (note: Kruger *et al.* (1993) stated that the blade connection is thin, although it is thick in trichodinid terms (Basson, pers. comm.*)
	- Extended
Posterior projection	- Not clearly visible
Central part	- Robust
	- Rounded tip extending more than halfway past y-axis
	- Fitting tightly into preceding denticle
Section above and below x-axis	- Similar
Ray connection	- Short, extends almost directly from central part
Ray apophysis	- Not clearly visible in most specimens
Form of ray	- Straight
	- Some slightly curved in posterior direction
	- Taper slightly to form rounded tip
Length of denticle above x-axis and denticle below x-axis	- 1: 0.7 – 0.8

Professor Linda Basson is a professor at the Zoology and Entomology department, UFS where she specialises in aquatic parasitology, including symbiotic associations between ciliophorans such as Trichodinidae and Urceolariidae and various hosts.

2. Results and discussion

The number of trichodinids drawn and studied as part of this section depended on the number of trichodinids collected from the various host populations as well as the quality of the micrographs taken of the silver impregnated trichodinids.

By studying the drawn denticles, it is evident that the collected trichodinids exhibit a considerable variation in the morphology of the denticles, especially the shape and the thickness of the ray within the individual fish populations (Table 11.2) and tadpole populations (Table 11.3).

Table 11.2: Some of the trichodinids from various fish populations examined during the present study. The denticles were drawn according to the method proposed by Basson and Van As (1989 and 1992). Refer to Table 5.1 and Figure 5.1 for more information on the host populations. Scale = 20µm.

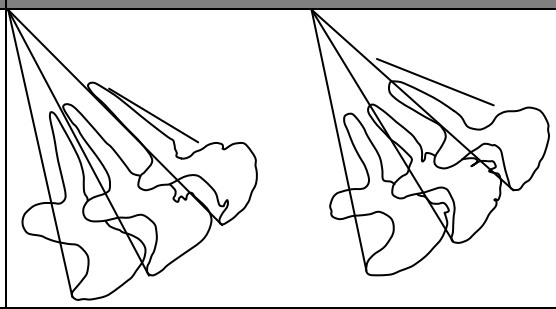
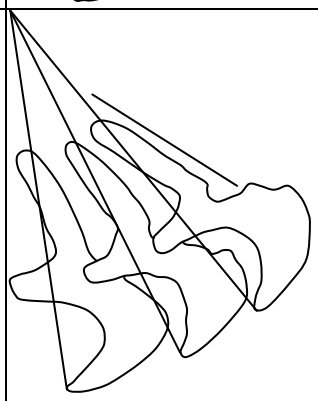
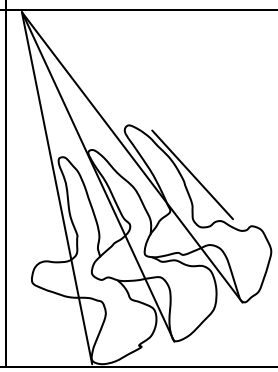
Population description	Examples of trichodinids collected on the various host populations
F1BaSD • *Barbus anoplus* • Sabella Dam, Wepener • November 2009	
F2BaSS • *Barbus anoplus* • Sabella Spruit, Wepener • November 2009	
F3BuBV • *Barbus* species • Barrage Vaal • June 1982	

Table 11.2 (Continue): Some trichodinids from fish populations.

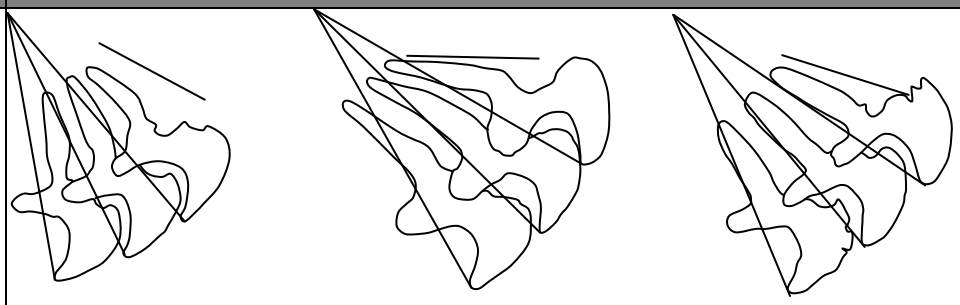
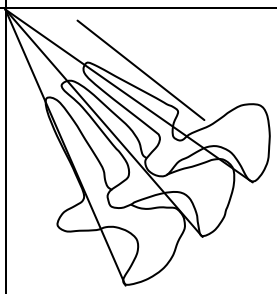
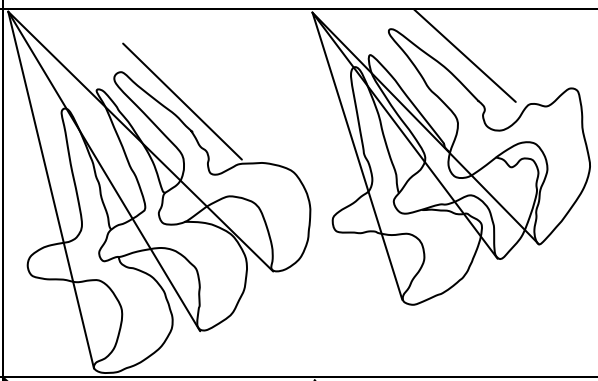
Population description	Examples of trichodinids collected on the various host populations
F5BbTT • *Barbus barnardi* • Thamalakane Bridge, Botswana • October 2007	
F6BsBL • *Barbus* species • Bloemfontein • June 1991	
F7CgUP • *Clarias gariepinus* • W. Uys Production Dam, Blyde River • September 1989	
F8CcLF • *Cyprinus carpio* • Lowveld Fisheries • October 1982	

Table 11.2 (Continue): Some trichodinids from fish populations.

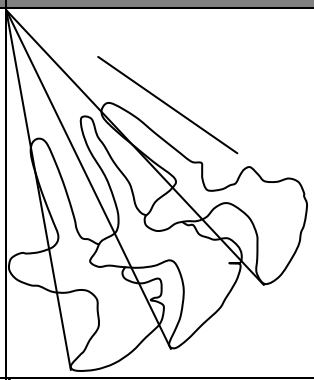
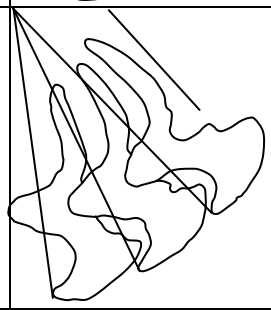
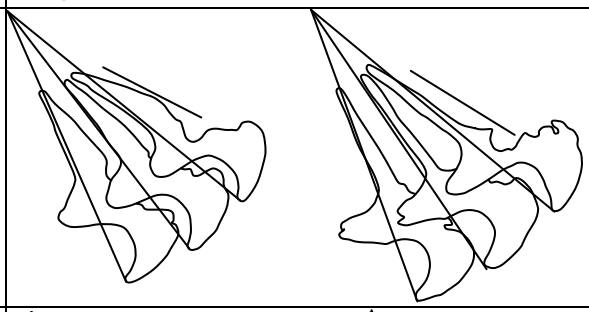
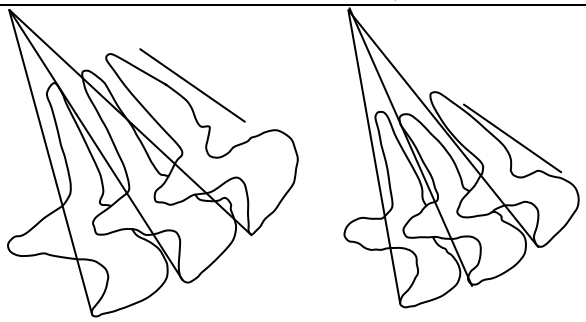
Population description	Examples of trichodinids collected on the various host populations
F9GaEB • *Gambusia affinis* • Bloemfontein • October 1988	
F10OmEB • *Oreochromis mossambicus* • Lowveld Fisheries • October 1982	
F12PsLV • *Poecilia sphenops* • Lake Valencia, South America • 1982	
F14PpND • *Pseudocrenilabrus philander* • Dam near Nelspruit • September 1989	
F15PpBV • *Pseudocrenilabrus philander* • Barrage Vaal • June 1982	

Table 11.2 (Continue): Some trichodinids from fish populations.

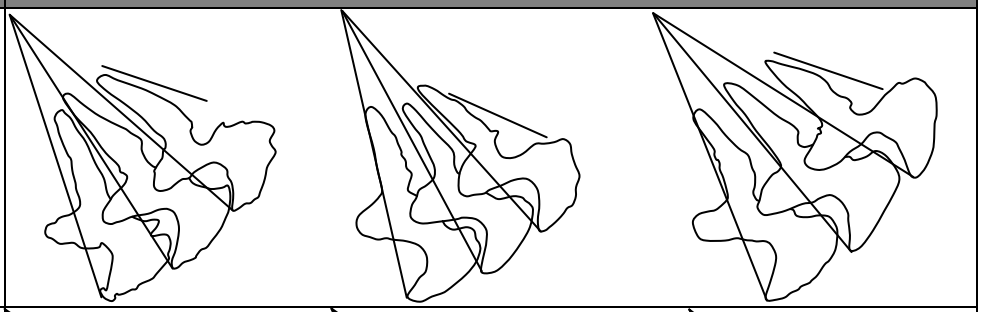
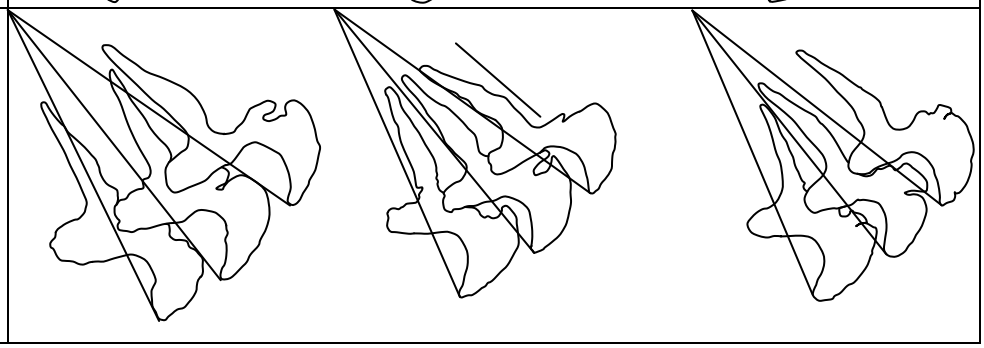
Population description	Examples of trichodinids collected on the various host populations
F16PpEB • *Pseudocrenilabrus philander* • Bloemfontein • March 1992	
F18TsBW • *Tilapia sparrmanii* • Waterfront, Bloemfontein • June 2007	

Table 11.3: Some of the trichodinids from various tadpole populations examined during the present study. Refer to Table 5.1 and Figure 5.1 for more information on the host populations. Scale = 20µm.

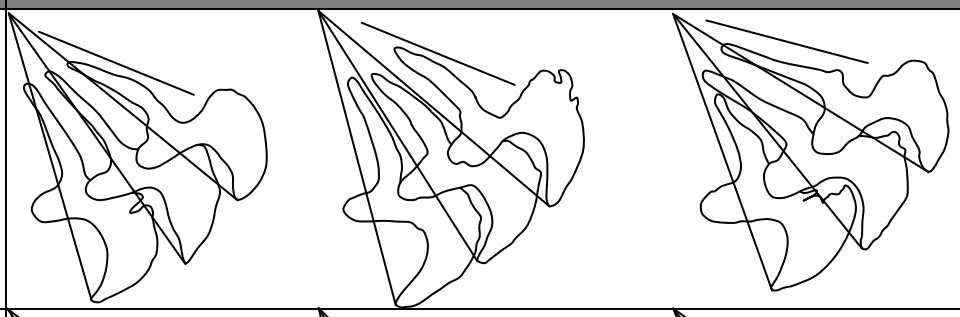
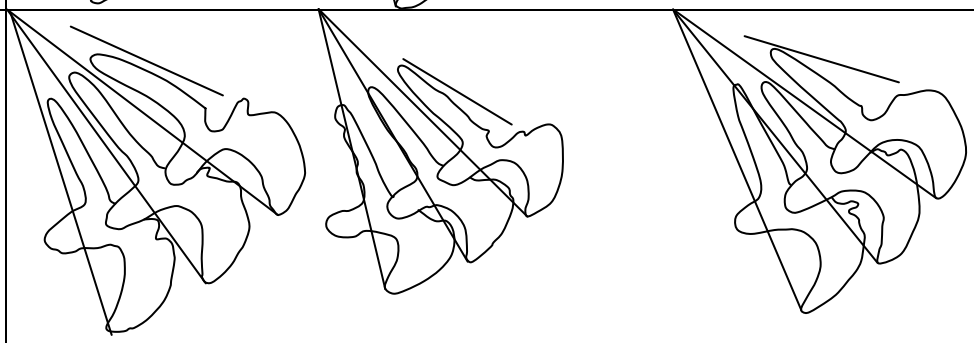
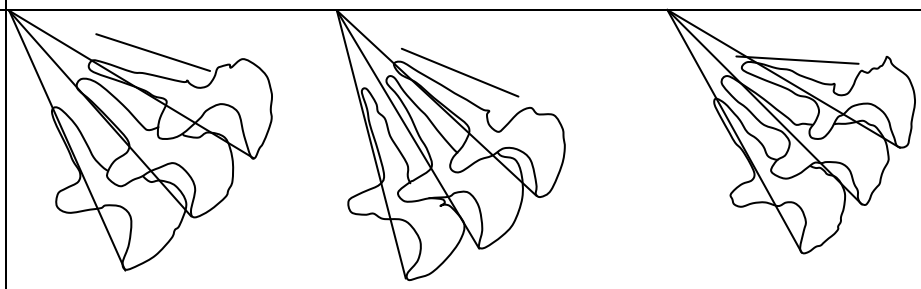
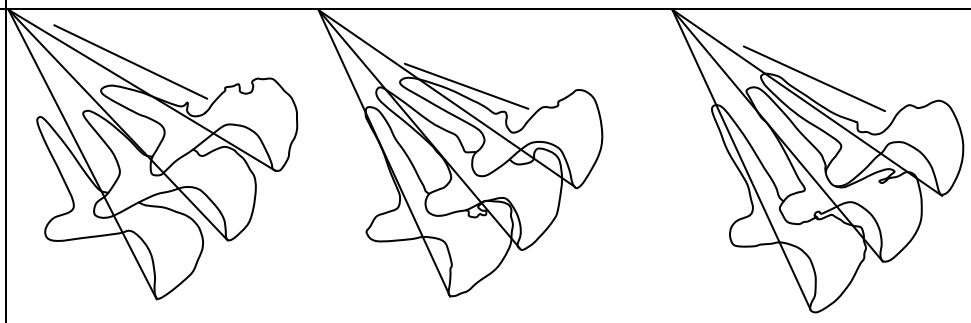
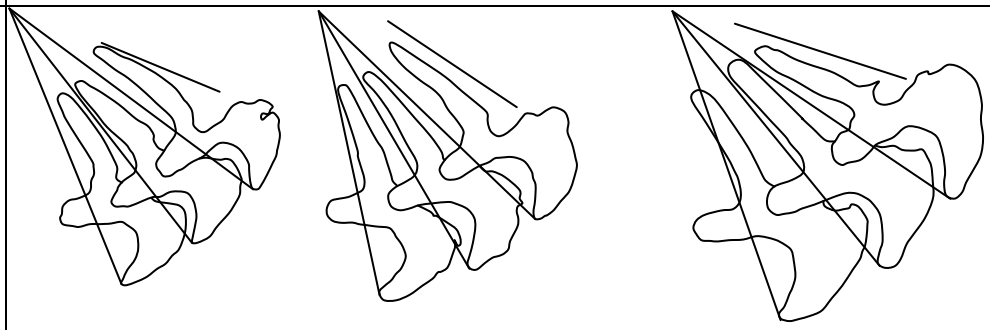
Population description	Examples of trichodinids collected on the various host populations
T4AuBH • Unknown *Amietophrynus* species • Bethlehem • November 1990	
T6ApCF • *Amietophrynus poweri* • Crocodile farm, Leseding Camp, Botswana • October 2007	
T7ApTB • *Amietophrynus poweri* • Toteng Bridge, Botswana • October 2007	
T10AgNC • *Amietophrynus gutturalis* • Nxamasere Floodplains, Botswana • October 2007	
T12AgND • *Amietophrynus gutturalis* • Nxamasere Floodplains, Botswana • October 2007	

Table 11.3 (Continue): Some trichodinids from tadpole populations.

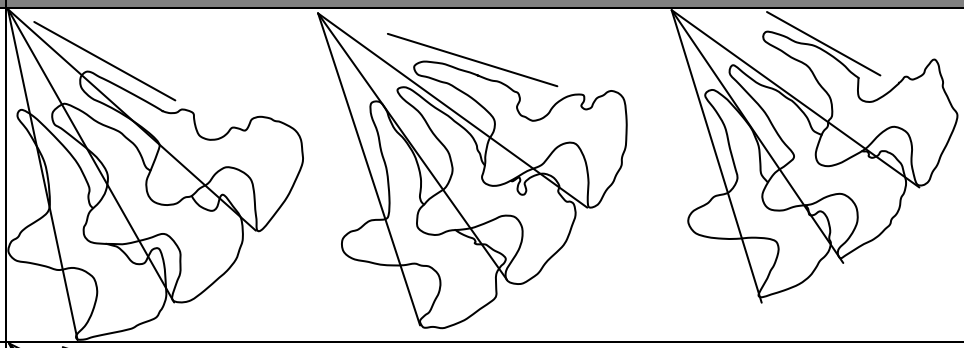
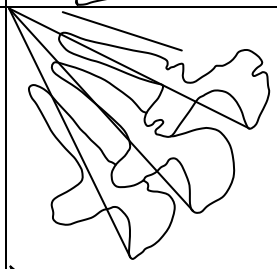
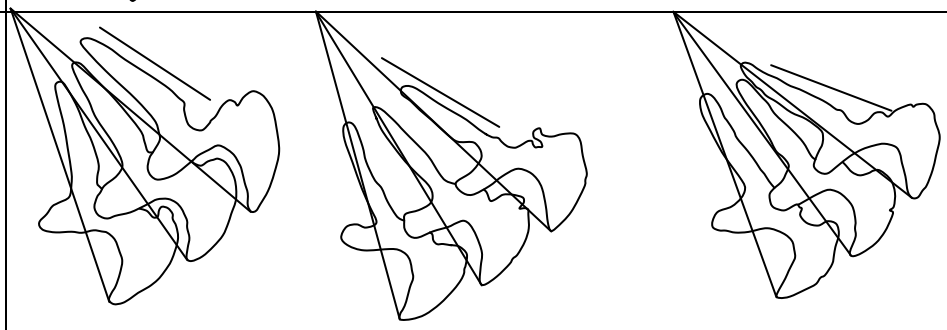
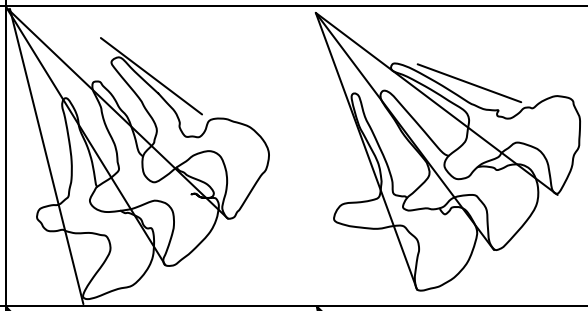
Population description	Examples of trichodinids collected on the various host populations
T14AMsNW • *Amietia* species • Near Wurasoord • December 1989	
T15AMuOG • *Amietia* species • Oribi Gorge • September 1989	
T16AMuHS • *Amietia* species • Hoedspruit • August 1989	
T17AMuWU • *Amietia* species • W. Uys Production Dam, Blyde River • September 1989	
T18AMaSS • *Amietia angolensis* • Sabella Spruit • November 2009	

Table 11.3 (Continue): Some trichodinids from tadpole populations.

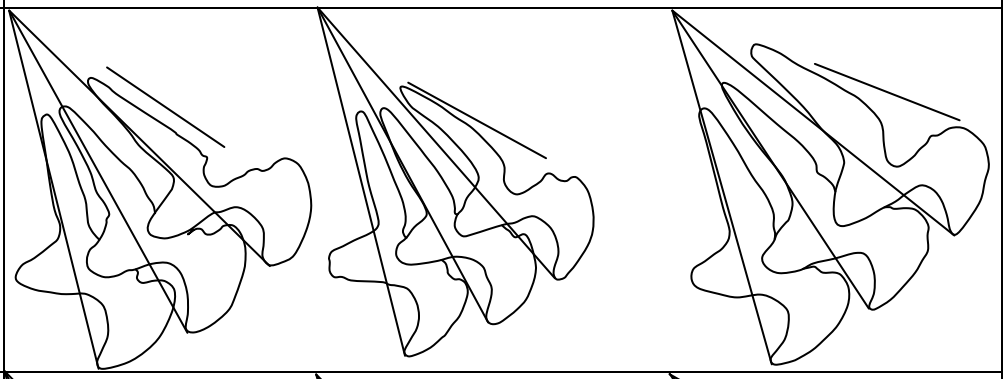
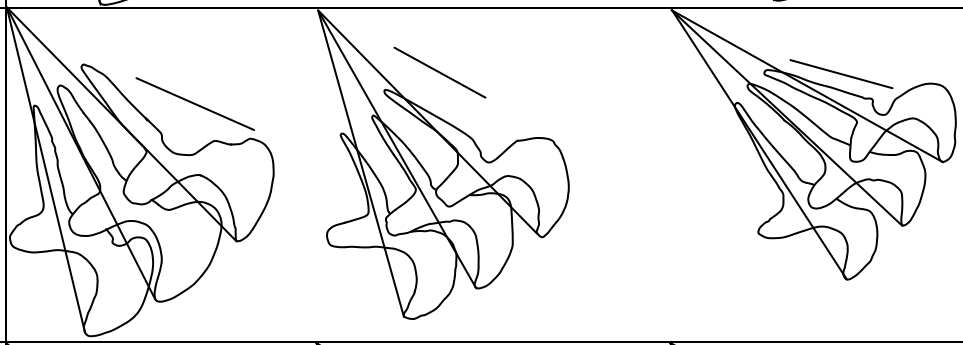
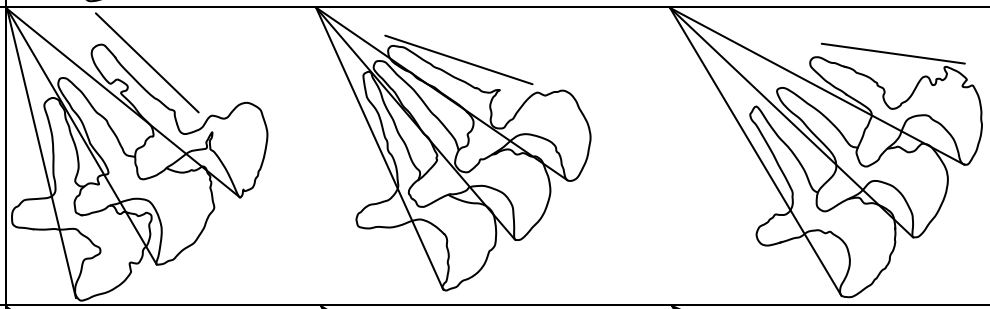
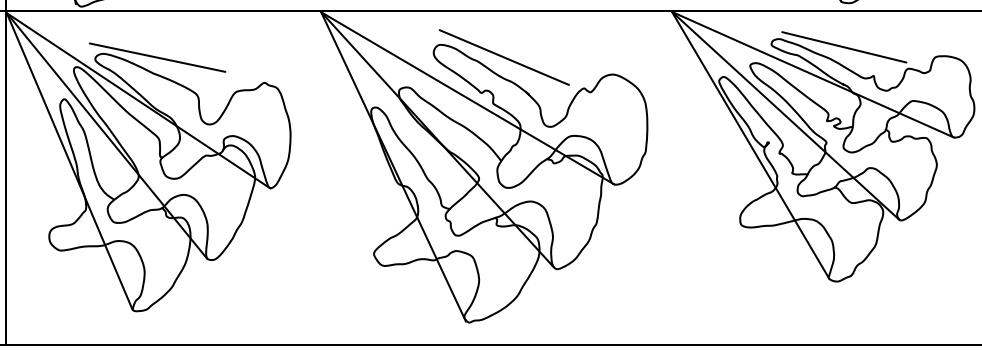
Population description	Examples of trichodinids collected on the various host populations
T21KsZZ • *Kassina senegalensis* • Unknown • May 1990	
T22NbVC • *Natalobatrachus bonebergi* • Vernon Crookes • September 1989	
T26XIOG • *Xenopus laevis* • Oribi Gorge • September 1989	
T27XIHS • *Xenopus laevis* • Hoedspruit • September 1989	
T28XIBB • *Xenopus laevis* • Bloemfontein • April 1989	

Table 11.3 (Continue): Some trichodinids from tadpole populations.

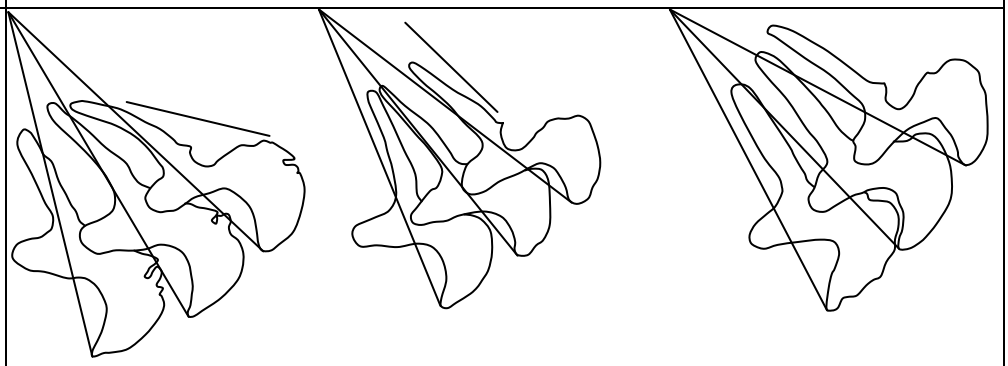
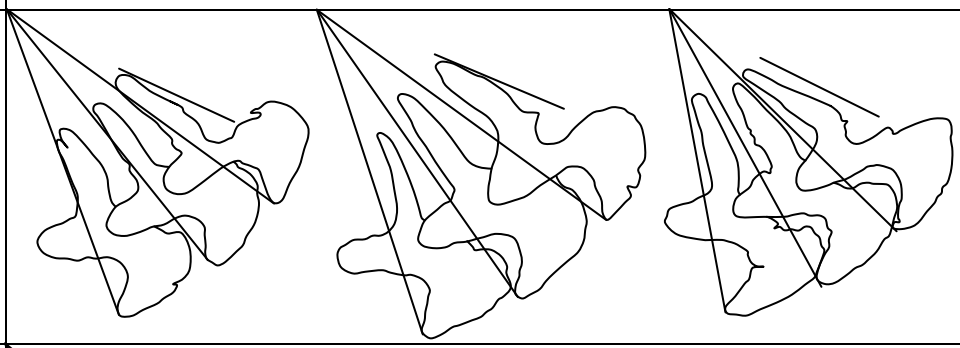
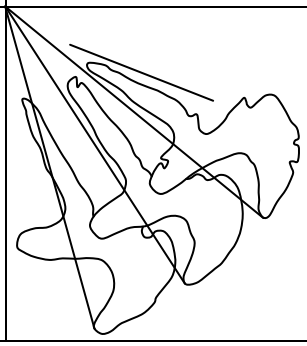
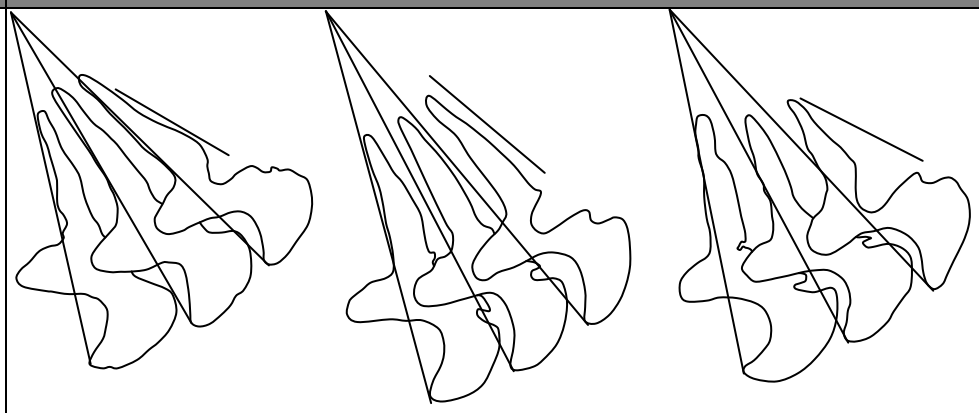
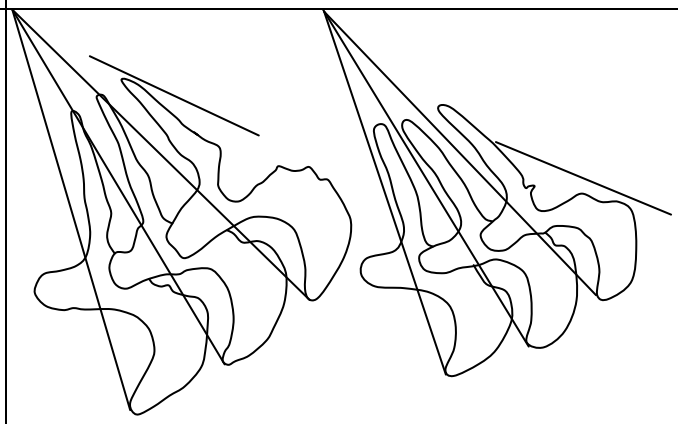
Population description	Examples of trichodinids collected on the various host populations
T29XIPC • *Xenopus laevis* • Pony Club, Bloemfontein • April 1989	
T30XIBE • *Xenopus laevis* • Bloemfontein • October 1988	
T31XIBE • *Xenopus laevis* • Bloemfontein • February 1992	
T32XIBB • *Xenopus laevis* • Bloemfontein • February 1989	

Table 11.3 (Continue): Some trichodinids from tadpole populations.

Population description	Examples of trichodinids collected on the various host populations
T35UuBV • Unknown tadpole • Barrage Vaal • June 1982	
T36UuLF • Unknown tadpole • Lowveld Fisheries • October 1982	

A variation in terms of the form of the denticles within each population was also observed. For example two specimens collected from *Barbus barnardi* differ with regards to the degree in which the rays are curved (Figure 11.3). In addition, the radius measured from the central part of the denticles of one individual is smaller than the other. The individual indicated in blue is smaller than the individual indicated in red and the radius measured from the tangent point toward the centre of the adhesive disc of one this individual is smaller than the other (red). Due to the variation, as many individuals as possible were examined from each of the studied populations.

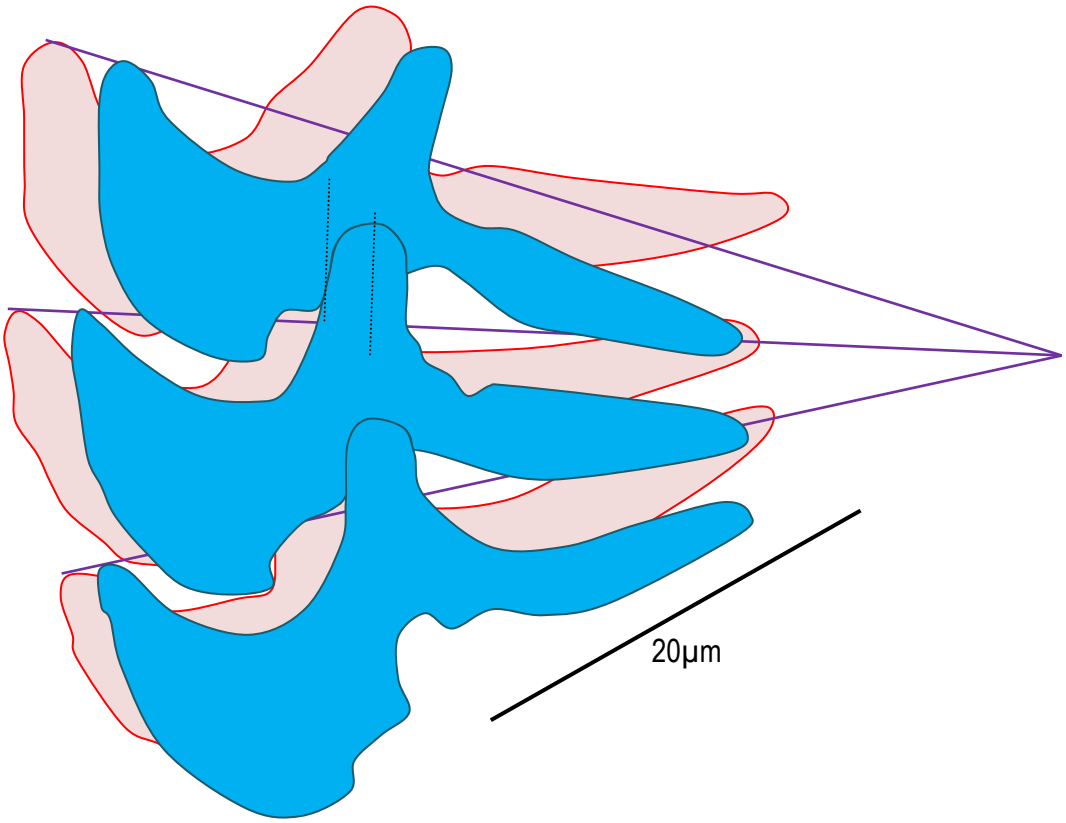
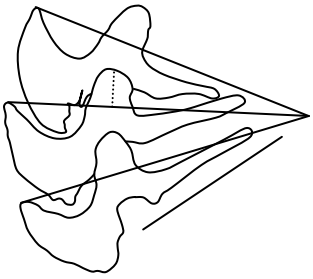
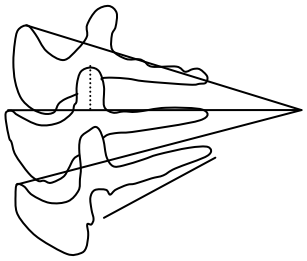


Figure 11.3: Indication of the differentiation between two trichodinid specimens collected from the skin of *Barbus barnardi* Jubb, 1965 used during a cross-transmission experiment.

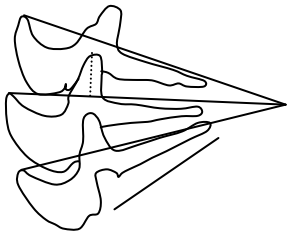
Three consecutive denticles of each the different trichodinid populations examined during the current study are discussed in the following section. Please note that the figure associated with each population group represents a trichodinid from the selected population. As variation within the trichodinid populations was observed such as was illustrated in Figure 11.3, one should keep in mind that the trichodinids shown in the following section may comprise most, but not always all, of the characteristics as discussed within each population.



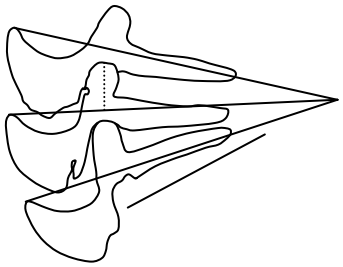
Population Number	- T4AuBH
Host species	- *Amietophrynus* species
Locality	- Bethlehem
Date of collection	- November 1990
Remarks	- Collected and examined for skin and gill trichodinids
Blade form	- Open sickle-shaped
Distal surface	- Rounded
	- Sloping downwards in anterior direction
Tangent point	- Rounded
Anterior margin	- Rounded, not smooth
	- Extending less than halfway past y-axis
Blade apophysis	- Small, rounded
Posterior blade surface	- Smooth
	- Almost L-shaped
Blade connection	- Thick, strong
	- Extended
Posterior projection	- Visible in some instances
Protrusion on lower central part	- Visible in most instances
Central part	- Robust
	- Rounded tip
	- Extending more than halfway past y-axis
	- Fitting tightly into preceding denticle
Section above and below x-axis	- Section above steeper slope
Ray connection	- Short but robust, extends almost directly from central part
Ray apophysis	- Prominent in most cases
Form of ray	- Straight
	- Curved in posterior direction
	- Taper slightly to form rounded tip
Length of denticle above x-axis and denticle below x-axis	- 1 : 1.18



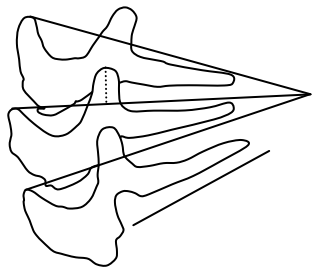
Population Number	- T6ApCF
Host species	- *Amietophrynus poweri*
Locality	- Office, crocodile farm, Botswana
Date of collection	- October 2007
Remarks	- Not naturally infested, part of experiment with T7ApTB, F5BbTT, T9ApCF
Blade form	- Open sickle-shaped
Distal surface	- Rounded - Sloping downwards in anterior direction
Tangent point	- Rounded
Anterior margin	- Rounded, smooth - Extending to or slightly beyond y+1 axis
Blade apophysis	- Sometimes visible
Posterior blade surface	- Smooth - Deep semi-lunar curve in some cases, mostly almost L-shaped
Blade connection	- Thick - Extended
Posterior projection	- Not clearly visible in all cases, small, when present
Protrusion on lower central part	- Visible in some instances
Central part	- Slender - Rounded tip - Extending more than halfway past y-axis - Fitting tightly into preceding denticle
Section above and below x-axis	- Similar
Ray connection	- Short, extends almost directly from central part - Same thickness as most of ray
Ray apophysis	- Small apophysis visible in some instances
Form of ray	- Straight - Equal in thickness throughout - Parallel to y-axis in most instances - Taper slightly to form rounded tip
Length of denticle above x-axis and denticle below x-axis	- 1 : 1.33



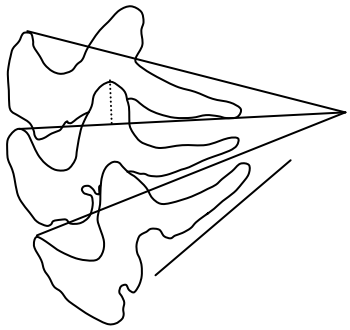
Population Number	- T7ApTB
Host species	- *Amietophrynus poweri*
Locality	- Thamalakane bridge, Botswana
Date of collection	- October 2007
Remarks	- Naturally infested, part of experiment with T6ApCF, F5BbTT, T9ApCF
Blade form	- Open sickle-shaped
Distal surface	- Rounded - Sloping downwards in anterior direction
Tangent point	- Rounded
Anterior margin	- Rounded, not always smooth - Extending to / or slightly beyond y+1 axis
Blade apophysis	- Sometimes visible
Posterior blade surface	- Smooth - Moderately, deep semi-lunar curve
Blade connection	- Thick - Extended
Posterior projection	- Clearly visible in some
Protrusion on lower central part	- Not clearly visible
Central part	- Slender - Rounded tip - Extending more than halfway past y-axis - Fitting tightly into preceding denticle
Section above and below x-axis	- Section below steeper slope
Ray connection	- Short, extends almost directly from central part
Ray apophysis	- Small, when visible
Form of ray	- Straight - Slightly curved in posterior direction - Taper slightly to form rounded tip
Length of denticle above x-axis and denticle below x-axis	- 1 : 1.33



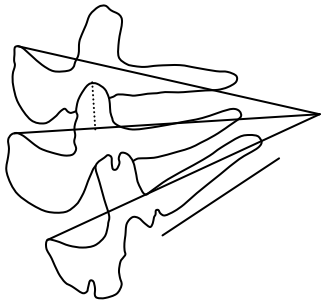
Population Number	- T10AgNC
Host species	- *Amietophrynus gutturalis*
Locality	- Nxamasere, Botswana (Pond 3)
Date of collection	- October 2007
Remarks	- Collected with F19HmNA
Blade form	- Open sickle-shaped
Distal surface	- Rounded, not always smooth
	- Sloping downwards in anterior direction
Tangent point	- Ends sharp mostly, otherwise rounded
Anterior margin	- Rounded
	- Extending to or slightly beyond y+1 axis
Blade apophysis	- Visible in most instances
Posterior blade surface	- Smooth
	- Moderately deep semi-lunar curve
Blade connection	- Thick, robust
	- Extended
Posterior projection	- Visible in some instances
Protrusion on lower central part	- Small protrusion visible in some instances
Central part	- Robust
	- Rounded tip
	- Extending more than halfway past y-axis
	- Fitting tightly into preceding denticle
Section above and below x-axis	- Section below steeper slope, bend in proximal direction
Ray connection	- Short, robust, extends almost directly from central part
Ray apophysis	- Visible in most instances
Form of ray	- Straight
	- Equal in thickness throughout
	- Parallel to y-axis in most instances
	- Taper slightly to form rounded tip
Length of denticle above x-axis and denticle below x-axis	- 1 : 1.23



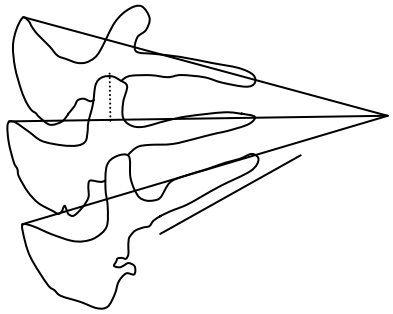
Population Number	- T12AgND
Host species	- *Amietophrynus gutturalis*
Locality	- Nxamasere, Botswana (Pond 4)
Date of collection	- October 2007
Remarks	- Collected and examined for skin and gill trichodinids
Blade form	- Open sickle-shaped
Distal surface	- Rounded
	- Sloping downwards in anterior direction
Tangent point	- Rounded
Anterior margin	- Rounded, not smooth
	- Extending to or slightly beyond y+1 axis
Blade apophysis	- Visible in most instances
Posterior blade surface	- Smooth
	- Deep semi-lunar curve
Blade connection	- Thick
	- Extended
Posterior projection	- Not clearly visible
Protrusion on lower central part	- Not clearly visible
Central part	- Slender
	- Rounded tip
	- Extending more than halfway past y-axis
	- Fitting tightly into preceding denticle
Section above and below x-axis	- Almost similar, section below steeper slope
Ray connection	- Short, robust, extends almost directly from central part
Ray apophysis	- Not clearly visible
Form of ray	- Straight
	- Parallel to y-axis in most instances
	- Taper slightly to form rounded tip
Length of denticle above x-axis and denticle below x-axis	- 1 : 1.33



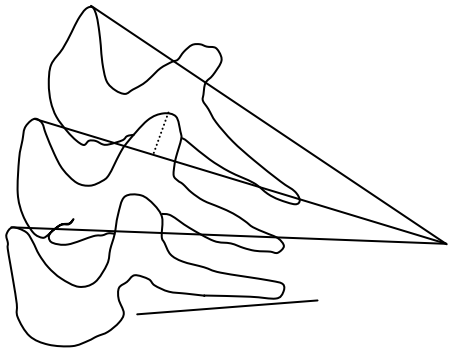
Population Number	- T14AMsNW
Host species	- *Amieta* species
Locality	- Near Wurasoord
Date of collection	- December 1989
Remarks	- Collected and examined for skin and gill trichodinids
Blade form	- Open sickle-shaped
Distal surface	- Rounded
	- Sloping downwards in anterior direction
Tangent point	- Rounded in most cases, may be sharper in some instances
Anterior margin	- Rounded, not smooth
	- Extending slightly beyond y+1 axis
Blade apophysis	- Prominent in some cases
Posterior blade surface	- Smooth
	- Very deep L-shaped curve
Blade connection	- Thick, strong
	- Extended
Posterior projection	- Visible in some
Protrusion on lower central part	- Visible in some
Central part	- Robust
	- Rounded tip
	- Extending less than halfway past y-axis
	- Fitting tightly into preceding denticle
Section above and below x-axis	- Section below steeper slope
Ray connection	- Short, extends almost directly from central part
Ray apophysis	- Not clearly visible
Form of ray	- Straight
	- Slightly curved in posterior direction
	- Taper slightly to form rounded tip
Length of denticle above x-axis and denticle below x-axis	- 1 : 1.23



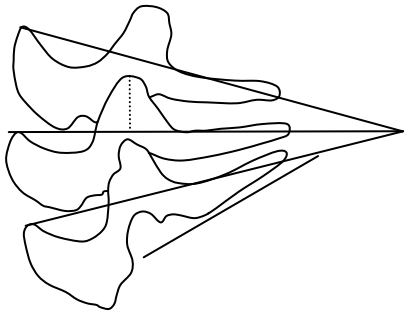
Population Number	- T15AMsOG
Host species	- *Amieta* species
Locality	- Oribi Gorge, Natal
Date of collection	- September 1989
Remarks	- Collected with F4BsOG
Blade form	- Open sickle-shaped
Distal surface	- Rounded
	- Sloping downwards in anterior direction
Tangent point	- Rounded in most cases, sharper in some cases
Anterior margin	- Rounded, not smooth
	- Extending towards, but not beyond y+1 axis
Blade apophysis	- Sometimes visible
Posterior blade surface	- Smooth
	- Moderate to deep L-shaped curve
Blade connection	- Thick
	- Extended
Posterior projection	- Small, when present
Protrusion on lower central part	- Small, when present
Central part	- Robust
	- Rounded tip
	- Extending more than halfway past y-axis
	- Fitting tightly into preceding denticle
Section above and below x-axis	- Almost similar, top of section above steeper slope
Ray connection	- Short, robust, extends almost directly from central part
Ray apophysis	- Not clearly visible
Form of ray	- Straight
	- Equal in thickness throughout
	- Slightly curved in posterior direction
	- Taper slightly to form rounded tip
Length of denticle above x-axis and denticle below x-axis	- 1 : 1.45



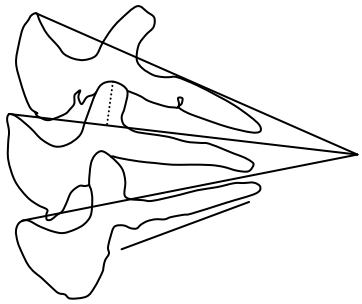
Population Number	- T16AMsHs
Host species	- *Amieta* species
Locality	- Hoedspruit
Date of collection	- August 1989
Remarks	- Collected and examined for skin and gill trichodinids
Blade form	- Open sickle-shaped
Distal surface	- Rounded
	- Sloping downwards in anterior direction
Tangent point	- Rounded in most cases, shaper in some cases
Anterior margin	- Rounded
	- Extending slightly beyond y+1 axis
Blade apophysis	- Not clearly visible
Posterior blade surface	- Smooth
	- Deep L-shaped curve
Blade connection	- Thick, strong
	- Extended
Posterior projection	- Not clearly visible
Protrusion on lower central part	- Not clearly visible
Central part	- Slender
	- Rounded tip
	- Extending more than halfway past y-axis
	- Fitting tightly into preceding denticle
Section above and below x-axis	- Section below steeper slope, bend in proximal direction
Ray connection	- Short, extends almost directly from central part
Ray apophysis	- Not clearly visible
Form of ray	- Straight
	- Parallel to y-axis in most instances
	- Narrows sometimes directly after connection, then thickens
	- Taper slightly to form rounded tip
Length of denticle above x-axis and denticle below x-axis	- 1 : 1.40



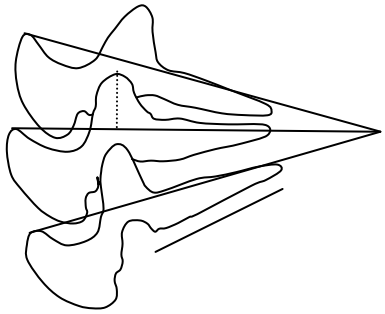
Population Number	- T17AMsWU
Host species	- *Amieta* species
Locality	- Farm Dam, Near Wurasoord, Blyde River
Date of collection	- September 1989
Remarks	- Collected and examined for skin and gill trichodinids
Blade form	- Open sickle-shaped
Distal surface	- Rounded
	- Sloping downwards in anterior direction
Tangent point	- Rounded, sharper in some instances
Anterior margin	- Rounded
	- Extending slightly beyond y+1 axis
Blade apophysis	- Sometimes visible
Posterior blade surface	- Smooth
	- Very deep L-shaped curve
Blade connection	- Thick
	- Extended
Posterior projection	- Not clearly visible
Protrusion on lower central part	- Not clearly visible
Central part	- Robust
	- Rounded tip
	- Extending less than halfway past y-axis
	- Fitting tightly into preceding denticle
	- Small protrusion on lower central part sometimes visible
Section above and below x-axis	- Top of section above smoother, rounder
Ray connection	- Short, robust, extends almost directly from central part
Ray apophysis	- Not clearly visible
Form of ray	- Straight
	- Curved in anterior direction
	- Taper slightly to form rounded tip
Length of denticle above x-axis and denticle below x-axis	- 1 : 1.14



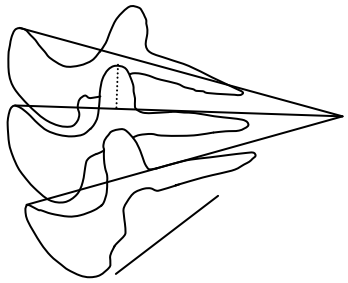
Population Number	- T18AMaSS
Host species	- *Amieta angolensis*
Locality	- Sabella Spruit, Wepener
Date of collection	- November 2009
Remarks	- Collected with F2BaSS
Blade form	- Open sickle-shaped
Distal surface	- Rounded - Sloping downwards in anterior direction
Tangent point	- Rounded
Anterior margin	- Rounded - Extending to y+1 axis, ending beyond in some instances (last denticle)
Blade apophysis	- Small
Posterior blade surface	- Smooth - Deep semi-lunar curve
Blade connection	- Thick - Extended
Posterior projection	- Clearly visible
Protrusion on lower central part	- Not clearly visible
Central part	- Robust - Rounded tip - Extending more than halfway past y-axis, sometimes almost reaching the y-1 axis - Fitting tightly into preceding denticle
Section above and below x-axis	- Section above steeper slope
Ray connection	- Short, robust, extends almost directly from central part
Ray apophysis	- Clearly visible
Form of ray	- Straight - Some slightly curved in posterior direction - Taper slightly to form rounded tip
Length of denticle above x-axis and denticle below x-axis	- 1 : 1.33



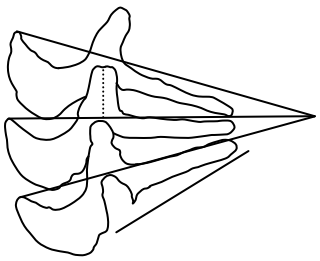
Population Number	- T21KsZZ
Host species	- *Kassina senegalensis*
Locality	- Unknown
Date of collection	- May 1990
Remarks	- Collected and examined for skin and gill trichodinids
Blade form	- Open sickle-shaped
Distal surface	- Rounded
	- Sloping downwards in anterior direction
Tangent point	- Rounded
Anterior margin	- Rounded
	- Extending to y+1 axis
Blade apophysis	- Small
Posterior blade surface	- Smooth
	- Deep L-shaped curve
Blade connection	- Thick, strong
	- Extended
Posterior projection	- Sometimes visible
Protrusion on lower central part	- Not clearly visible
Central part	- Slender
	- Rounded tip
	- Extending more than halfway past y-axis
	- Fitting tightly into preceding denticle
Section above and below x-axis	- Tip of central part of section above rounder
Ray connection	- Short, same thickness as ray, extends almost directly from central part
Ray apophysis	- Clearly visible
Form of ray	- Straight
	- Parallel to y-axis in most instances
	- Tips slightly curved in anterior direction
	- Narrows sometimes directly after connection, then thickens
	- Taper slightly to form rounded tip
Length of denticle above x-axis and denticle below x-axis	- 1 : 1.54



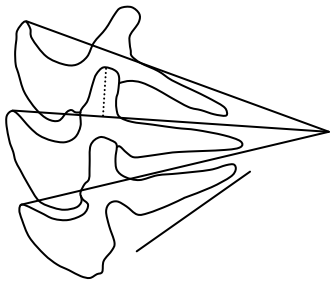
Population Number	- T22NbVC
Host species	- *Natalobatrachus bonebergi*
Locality	- Vernon Crookes
Date of collection	- September 1989
Remarks	- Collected and examined for skin and gill trichodinids
Blade form	- Open sickle-shaped
Distal surface	- Rounded
	- Sloping downwards in anterior direction
Tangent point	- Rounded
Anterior margin	- Rounded
	- Anterior margin extends close to, or just beyond y+1 axis
Blade apophysis	- Sometimes visible, small
Posterior blade surface	- Smooth
	- Moderately deep semi-lunar curve
Blade connection	- Thick
	- Extended
Posterior projection	- Not clearly visible
Protrusion on lower central part	- Not clearly visible
Central part	- Mostly robust
	- Rounded tip
	- Extending more than halfway past y-axis
	- Fitting tightly into preceding denticle
Section above and below x-axis	- Top of central part of section above steeper slope
Ray connection	- Short, robust, extends almost directly from central part
Ray apophysis	- Not clearly visible
Form of ray	- Straight
	- Parallel to y-axis in most instances
	- Taper slightly to form rounded tip
Length of denticle above x-axis and denticle below x-axis	- 1 : 1.33



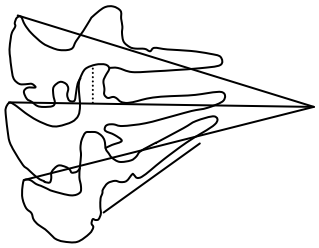
Population Number	- T26XIOG
Host species	- *Xenopus laevis*
Locality	- Oribi Gorge, Natal
Date of collection	- September 1989
Remarks	- Collected with F4BsOG
Blade form	- Open sickle-shaped
Distal surface	- Rounded
	- Sloping downwards in anterior direction
Tangent point	- Rounded, sometimes sharper
Anterior margin	- Rounded
	- Extending slightly beyond y+1 axis
Blade apophysis	- Not clearly visible
Posterior blade surface	- Smooth
	- Deep semi-lunar curve
Blade connection	- Thick
	- Extended
Posterior projection	- Not clearly visible
Protrusion on lower central part	- Not clearly visible
Central part	- Robust
	- Rounded tip
	- Extending more than halfway past y-axis
	- Fitting tightly into preceding denticle
Section above and below x-axis	- Similar
Ray connection	- Short, same thickness as ray, extends almost directly from central part
Ray apophysis	- Small
Form of ray	- Straight
	- Parallel to y-axis in most instances
	- Taper slightly to form rounded tip
Length of denticle above x-axis and denticle below x-axis	- 1 : 1.23



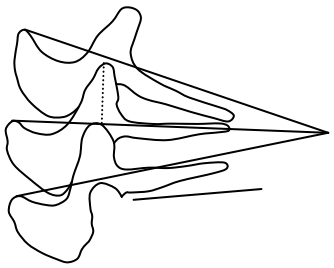
Population Number	- T27XIHS
Host species	- *Xenopus laevis*
Locality	- Hoedspruit
Date of collection	- September 1989
Remarks	- Collected and examined for skin and gill trichodinids
Blade form	- Open sickle-shaped
Distal surface	- Rounded
	- Sloping downwards in anterior direction
Tangent point	- Rounded, sometimes sharper
Anterior margin	- Rounded, not smooth
	- Extending to, but not beyond y+1 axis
Blade apophysis	- Sometimes visible
Posterior blade surface	- Smooth
	- Moderately deep semi-lunar curve
Blade connection	- Thick
	- Extended
Posterior projection	- Clearly visible
Protrusion on lower central part	- Visible in some
Central part	- Slender
	- Rounded tip
	- Extending more than halfway past y-axis
	- Fitting tightly into preceding denticle
Section above and below x-axis	- Section below steeper slope, flattened top
Ray connection	- Short, same thickness as ray, extends almost directly from central part
Ray apophysis	- Prominent in some cases
Form of ray	- Straight
	- Parallel to y-axis in most instances
	- Taper slightly to form rounded tip
Length of denticle above x-axis and denticle below x-axis	- 1 : 1.33



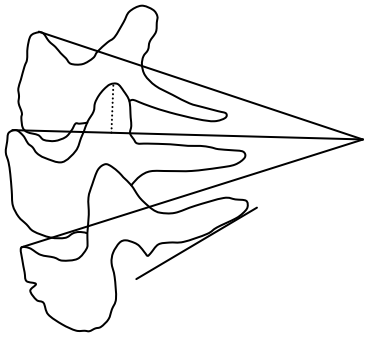
Population Number	- T28XIBB
Host species	- *Xenopus laevis*
Locality	- Bloemfontein
Date of collection	- April 1989
Remarks	- Collected and examined for skin and gill trichodinids
Blade form	- Open sickle-shaped
Distal surface	- Rounded - Sloping downwards in anterior direction
Tangent point	- Rounded
Anterior margin	- Rounded - Extending slightly beyond y+1 axis
Blade apophysis	- Sometimes visible
Posterior blade surface	- Smooth - Deep semi-lunar curve
Blade connection	- Thick - Extended
Posterior projection	- Clearly visible
Protrusion on lower central part	- Not clearly visible
Central part	- Slender - Rounded tip - Extending more than halfway past y-axis - Fitting tightly into preceding denticle
Section above and below x-axis	- Base of section below steeper slope, top of section above steeper slope
Ray connection	- Short, narrower than ray, extends almost directly from central part
Ray apophysis	- Sometimes visible
Form of ray	- Straight - Parallel to y-axis in some instances - Taper slightly to form rounded tip
Length of denticle above x-axis and denticle below x-axis	- 1 : 1.45



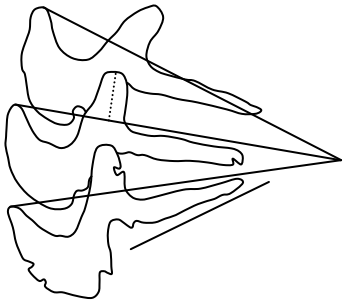
Population Number	- T29XIPC
Host species	- *Xenopus laevis*
Locality	- Pony Club, Bloemfontein
Date of collection	- April 1989
Remarks	- Collected and examined for skin and gill trichodinids
Blade form	- Open sickle-shaped
Distal surface	- Rounded - Sloping downwards in anterior direction
Tangent point	- Rounded
Anterior margin	- Rounded, not smooth - Extending slightly beyond y+1 axis
Blade apophysis	- Sometimes visible
Posterior blade surface	- Smooth - Moderate to deep L-shaped curve
Blade connection	- Thick, strong - Extended
Posterior projection	- Not clearly visible
Protrusion on lower central part	- Not clearly visible
Central part	- Slender to robust - Rounded tip - Extending halfway or more past y-axis - Fitting tightly into preceding denticle
Section above and below x-axis	- Section below steeper slope, bend in proximal direction
Ray connection	- Short, sometimes thicker than ray, extends almost directly from central part
Ray apophysis	- Sometimes visible, small
Form of ray	- Straight - Curved posteriorly - Taper slightly to form rounded tip
Length of denticle above x-axis and denticle below x-axis	- 1 : 1.51



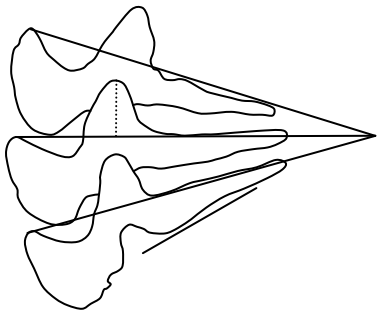
Population Number	- T30XIBE
Host species	- *Xenopus laevis*
Locality	- Bloemfontein Experiment
Date of collection	- April 1989
Remarks	- Cross-transmission experiment with F9GaEB and F10OmE
Blade form	- Open sickle-shaped
Distal surface	- Rounded - Sloping downwards in anterior direction
Tangent point	- Rounded
Anterior margin	- Rounded - Extending to or slightly beyond y+1 axis
Blade apophysis	- Sometimes visible
Posterior blade surface	- Smooth - Shallow semi-lunar curve
Blade connection	- Thick - Extended
Posterior projection	- Not clearly visible
Protrusion on lower central part	- Not clearly visible
Central part	- Robust - Rounded tip - Extending to almost touch y-axis, in some touching y-axis - Fitting tightly into preceding denticle
Section above and below x-axis	- Base of section below steeper slope
Ray connection	- Short, narrower than ray, extends almost directly from central part
Ray apophysis	- Clearly visible in most instances
Form of ray	- Straight - Parallel to y-axis in some instances - Taper slightly to form rounded tip
Length of denticle above x-axis and denticle below x-axis	- 1 : 1.29



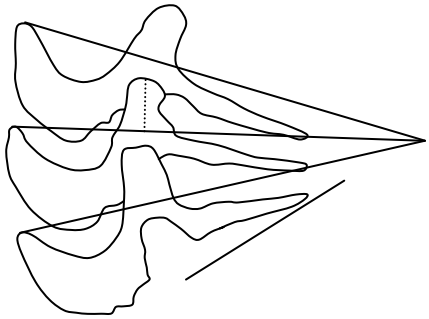
Population Number	- T31XIBE
Host species	- *Xenopus laevis*
Locality	- Experiment, Bloemfontein (Collected from Bloemfontein Zoo)
Date of collection	- February 1992
Remarks	- Cross-transmission experiment with F16PpEB
Blade form	- Open sickle-shaped
Distal surface	- Rounded
	- Sloping downwards in anterior direction
Tangent point	- Rounded
Anterior margin	- Rounded, not smooth
	- Extending to or slightly beyond y+1 axis
Blade apophysis	- Sometimes visible
Posterior blade surface	- Smooth
	- Deep semi-lunar to L-shaped curve
Blade connection	- Thick, strong
	- Extended
Posterior projection	- Visible in some
Protrusion on lower central part	- Not clearly visible
Central part	- Robust
	- Rounded tip
	- Extending more than halfway past y-axis
	- Fitting tightly into preceding denticle
Section above and below x-axis	- Base of section below steeper slope
Ray connection	- Short, robust, extends almost directly from central part
Ray apophysis	- Clearly visible in some denticles
Form of ray	- Straight, strongly developed in most
	- Parallel to y-axis in most instances
	- Taper slightly to form rounded tip
Length of denticle above x-axis and denticle below x-axis	- 1 : 1.23



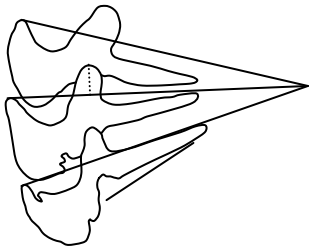
Population Number	- T32XIBB
Host species	- *Xenopus laevis*
Locality	- Bloemfontein
Date of collection	- February 1989
Remarks	- Collected and examined for skin and gill trichodinids
Blade form	- Open sickle-shaped
Distal surface	- Rounded
	- Sloping downwards in anterior direction
Tangent point	- Rounded
Anterior margin	- Rounded, not smooth
	- Extending slightly beyond y+1 axis
Blade apophysis	- Sometimes visible
Posterior blade surface	- Smooth
	- Very deep semi-lunar to L-shaped curve
Blade connection	- Thick, strong
	- Extended
Posterior projection	- Visible in some instances
Protrusion on lower central part	- Not clearly visible
Central part	- Robust
	- Rounded tip
	- Extending more than halfway past y-axis
	- Fitting tightly into preceding denticle
Section above and below x-axis	- Base of section below steeper slope
Ray connection	- Short, same thickness as ray, extends almost directly from central part
Ray apophysis	- Clearly visible in some
Form of ray	- Straight
	- Parallel to y-axis in most instances
	- Taper slightly to form rounded tip, sometimes sharper tip
Length of denticle above x-axis and denticle below x-axis	- 1 : 1.33



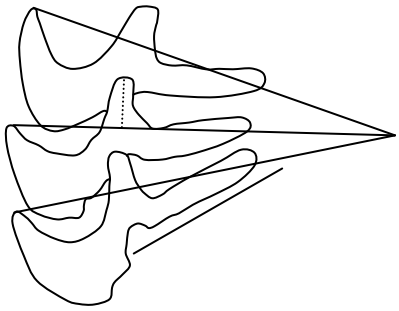
Population Number	- T35UuBV
Host species	- Unidentified tadpole species
Locality	- Barrage Vaal
Date of collection	- June 1982
Remarks	- Collected with F3BaBV and T35UuBV
Blade form	- Open sickle-shaped
Distal surface	- Rounded
	- Sloping downwards in anterior direction
Tangent point	- Rounded
Anterior margin	- Rounded
	- Extending to y+1 axis
Blade apophysis	- Sometimes visible
Posterior blade surface	- Smooth
	- Deep L-shaped curve
Blade connection	- Thick, strong
	- Extended
Posterior projection	- Visible in some
Protrusion on lower central part	- Visible in some
Central part	- Robust
	- Rounded tip
	- Extending more than halfway past y-axis
	- Fitting tightly into preceding denticle
Section above and below x-axis	- Section below steeper slope
Ray connection	- Short, robust, extends almost directly from central part
Ray apophysis	- Small, visible in some instances
Form of ray	- Straight, strongly developed
	- Parallel to y-axis in most instances
	- Taper slightly to form rounded tip
Length of denticle above x-axis and denticle below x-axis	- 1 : 1.45



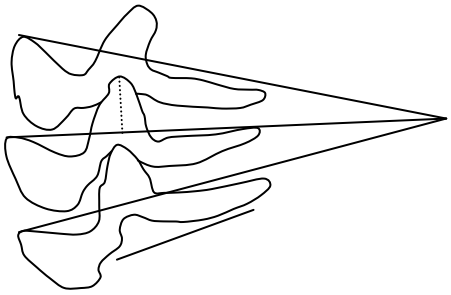
Population Number	- T36UuLF
Host species	- Unidentified tadpole species
Locality	- Lowveld Fish Production Facilities
Date of collection	- October 1982
Remarks	- Collected with F8CcLF and F11OMLF
Blade form	- Open sickle-shaped
Distal surface	- Rounded
	- Sloping downwards in anterior direction
Tangent point	- Rounded
Anterior margin	- Rounded
	- Extending slightly beyond y+1 axis
Blade apophysis	- Sometimes visible
Posterior blade surface	- Smooth
	- Deep semi-lunar curve
Blade connection	- Thick
	- Extended
Posterior projection	- Visible in some
Protrusion on lower central part	- Not clearly visible
Central part	- Robust
	- Rounded tip
	- Extending more than halfway past y-axis
	- Fitting tightly into preceding denticle
Section above and below x-axis	- Highest point at section above longer than section below, rectangular top at some instances
Ray connection	- Short, extends almost directly from central part
Ray apophysis	- Sometimes visible, small
Form of ray	- Straight, delicate
	- Curved in anterior direction
	- Taper slightly to form rounded tip
Length of denticle above x-axis and denticle below x-axis	- 1 : 1.21



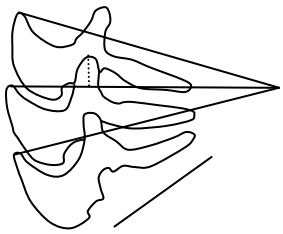
Population Number	- F1BaSD
Host species	- *Barbus anoplus*
Locality	- Sabella Dam, Wepener
Date of collection	- November 2009
Remarks	- Collected with T11AgSD and T34XlSD
Blade form	- Open sickle-shaped
Distal surface	- Rounded
	- Sloping downwards in anterior direction
Tangent point	- Rounded
Anterior margin	- Rounded
	- Extending to or slightly beyond y+1 axis
Blade apophysis	- Sometimes visible
Posterior blade surface	- Smooth
	- Deep semi-lunar curve
Blade connection	- Thick, strong
	- Extended
Posterior projection	- Sometimes visible
Protrusion on lower central part	- Not clearly visible
Central part	- Slender to robust
	- Rounded tip
	- Extending less than halfway past y-axis
	- Fitting tightly into preceding denticle
Section above and below x-axis	- Almost similar; section below steeper slope
Ray connection	- Short, extends almost directly from central part
Ray apophysis	- Sometimes visible, small
Form of ray	- Straight, equal thickness
	- Mostly parallel to y-axis, sometimes curved anteriorly
	- Taper slightly to form rounded tip
Length of denticle above x-axis and denticle below x-axis	- 1 : 1.33



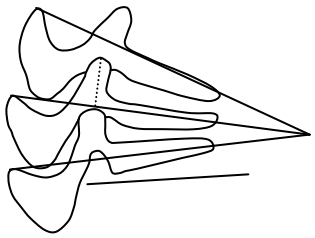
Population Number	- F2BaSS
Host species	- *Barbus anoplus*
Locality	- Sabella Spruit, Wepener
Date of collection	- November 2009
Remarks	- Collected with T18AMaSS
Blade form	- Open sickle-shaped
Distal surface	- Rounded
	- Sloping downwards in anterior direction
Tangent point	- Rounded
Anterior margin	- Rounded
	- Extending beyond y+1 axis
Blade apophysis	- Sometimes visible
Posterior blade surface	- Smooth
	- Deep semi-lunar to L-shaped curve
Blade connection	- Thick, strong
	- Extended
Posterior projection	- Sometimes visible
Protrusion on lower central part	- Not clearly visible
Central part	- Slender
	- Rounded tip
	- Extending halfway or more past y-axis
	- Fitting tightly into preceding denticle
Section above and below x-axis	- Base of section above steeper slope
Ray connection	- Short, extends almost directly from central part
Ray apophysis	- Not always clearly visible
Form of ray	- Straight, equal in thickness throughout
	- Some slightly curved in posterior direction
	- Form strong rounded tip
Length of denticle above x-axis and denticle below x-axis	- 1 : 1.07



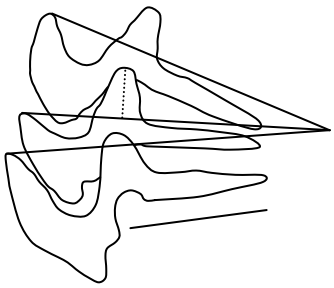
Population Number	- F3BuBV
Host species	- *Barbus* species
Locality	- Barrage Vaal
Date of collection	- June 1982
Remarks	- Collected with T15PpBV and T35UuBV
Blade form	- Open sickle-shaped
Distal surface	- Rounded
	- Sloping downwards in anterior direction
Tangent point	- Rounded
Anterior margin	- Rounded
	- Extending to y+1 axis, but does not reach y+1 axis
Blade apophysis	- Sometimes visible
Posterior blade surface	- Smooth
	- Deep L-shaped curve
Blade connection	- Thick
	- Extended
Posterior projection	- Clearly visible
Protrusion on lower central part	- Not clearly visible
Central part	- Robust
	- Rounded tip
	- Extending more than halfway past y-axis
	- Fitting tightly into preceding denticle
Section above and below x-axis	- Sectin below steeper slope
Ray connection	- Short, same thickness than ray, extends almost directly from central part
Ray apophysis	- Small, when visible
Form of ray	- Straight, thick
	- Mostly parallel to y-axis
	- Taper slightly to form rounded tip
Length of denticle above x-axis and denticle below x-axis	- 1 : 1.14



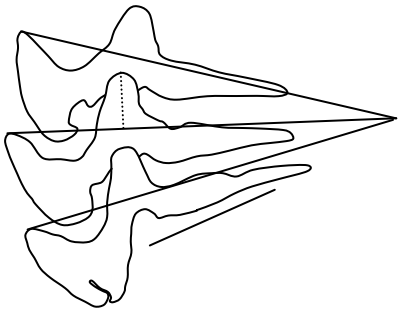
Population Number	- F5BbTT
Host species	- *Barbus barnardi*
Locality	- Toteng Bridge, Botswana
Date of collection	- October 2007
Remarks	- Part of the cross-transmission experiment with T7ApTB, T9ApCF and T6ApCF
Blade form	- Open sickle-shaped
Distal surface	- Rounded - Sloping downwards in anterior direction
Tangent point	- Rounded
Anterior margin	- Rounded - Extending beyond y+1 axis
Blade apophysis	- Visible
Posterior blade surface	- Smooth - Deep semi-lunar curve
Blade connection	- Thick, strong - Extended
Posterior projection	- Not clearly visible
Protrusion on lower central part	- Not clearly visible
Central part	- Slender - Rounded tip - Extending less than, or more than halfway past y-axis - Fitting tightly into preceding denticle
Section above and below x-axis	- Almost similar; tip of section above steeper slope
Ray connection	- Short, same thickness than ray, extends almost directly from central part
Ray apophysis	- Sometimes visible, small
Form of ray	- Straight, narrow, mostly of same thickness - Curved anteriorly - Most of same thickness, some taper slightly to a rounded tip
Length of denticle above x-axis and denticle below x-axis	- 1 : 1.27



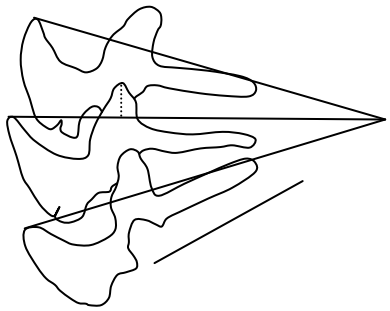
Population Number	- F6BsBL
Host species	- *Barbus* species
Locality	- Bloemfontein (kept in pool at UFS campus)
Date of collection	- June 1991
Remarks	- Kept in pool at UFS campus and dissected for the examination of skin and gill trichodinids
Blade form	- Open sickle-shaped
Distal surface	- Rounded - Sloping downwards in anterior direction
Tangent point	- Rounded
Anterior margin	- Rounded - Extending to reach y+1 axis
Blade apophysis	- Not visible
Posterior blade surface	- Smooth - Deep semi-lunar curve
Blade connection	- Thick - Extended
Posterior projection	- Not clearly visible
Protrusion on lower central part	- Not clearly visible
Central part	- Slender to robust - Rounded tip - Extending more than halfway past y-axis, some cases reaching y-1 axis - Fitting tightly into preceding denticle - Indentation sometimes visible between central part and ray
Section above and below x-axis	- Section below rounder tip, section below steeper slope
Ray connection	- Short, mostly same thickness than ray, others narrower, extends almost directly from central part
Ray apophysis	- Not always clearly visible
Form of ray	- Straight, thick - Mostly parallel to y-axis - Equal thickness, form strong rounded tip
Length of denticle above x-axis and denticle below x-axis	- 1 : 1.33



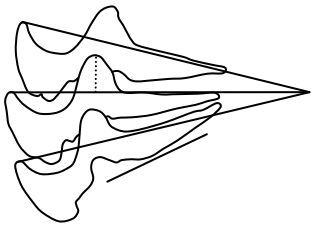
Population Number	- F7CgUP
Host species	- *Clarias gariepinus*
Locality	- Blyde River, Uys Production Dam
Date of collection	- September 1989
Remarks	- Collected and dissected for the examination of skin and gill trichodinids
Blade form	- Open sickle-shaped
Distal surface	- Rounded - Sloping downwards in anterior direction
Tangent point	- Rounded
Anterior margin	- Rounded - Extending beyond y+1 axis, some extending far beyond y+1 axis (see last denticle)
Blade apophysis	- Sometimes visible
Posterior blade surface	- Smooth - Deep L-shaped curve
Blade connection	- Thick - Extended
Posterior projection	- Visible in some
Protrusion on lower central part	- Not clearly visible
Central part	- Robust - Rounded tip - Extending more than halfway past y-axis - Fitting tightly into preceding denticle
Section above and below x-axis	- Almost similar; section below sometimes steeper slope
Ray connection	- Short, same thickness as rays, extends almost directly from central part
Ray apophysis	- Sometimes visible, small
Form of ray	- Straight and thin - Curved in anterior direction - Taper prominently to form sharp tip
Length of denticle above x-axis and denticle below x-axis	- 1 : 1.33



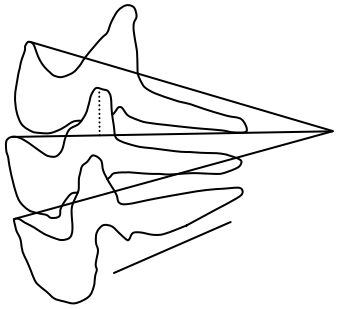
Population Number	- F8CcLF
Host species	- *Cyprinus carpio*
Locality	- Lowveld Fish Production Facilities
Date of collection	- October 1982
Remarks	- Collected with F11OmLF and T36UuLF
Blade form	- Open sickle-shaped
Distal surface	- Rounded
	- Sloping downwards in anterior direction
Tangent point	- Rounded
Anterior margin	- Rounded
	- Extending to or slightly beyond y+1 axis
Blade apophysis	- Not visible
Posterior blade surface	- Smooth
	- Deep L-shaped curve
Blade connection	- Thick, strong
	- Extended
Posterior projection	- Small, when present
Protrusion on lower central part	- Not clearly visible
Central part	- Robust
	- Rounded tip
	- Extending more than halfway past y-axis
	- Fitting tightly into preceding denticle
Section above and below x-axis	- Tip of section above steeper slope
Ray connection	- Short, robust, extends almost directly from central part
Ray apophysis	- Small, when present
Form of ray	- Straight, thick
	- Mostly parallel to y-axis
	- Taper prominently to sharp or rounded tip
Length of denticle above x-axis and denticle below x-axis	- 1 : 1.40



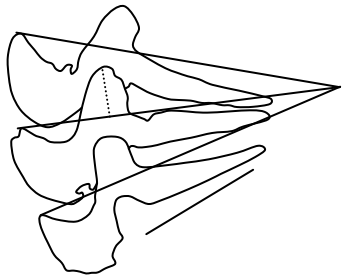
Population Number	- F9GaEB
Host species	- *Gambusia affinis*
Locality	- Bloemfontein
Date of collection	- October 1988
Remarks	- Part of an experiment conducted at the UFS campus
Blade form	- Open sickle-shaped
Distal surface	- Rounded
	- Sloping downwards in anterior direction
Tangent point	- Rounded
Anterior margin	- Rounded
	- Extending slightly beyond y+1 axis
Blade apophysis	- Mostly prominent to very prominent
Posterior blade surface	- Smooth
	- Deep L-shaped curve
Blade connection	- Varies between thin and thick
	- Extended
Posterior projection	- Clearly visible
Protrusion on lower central part	- Visible in most instances
Central part	- Robust
	- Rounded tip
	- Extending less than halfway past y-axis
	- Fitting tightly into preceding denticle
Section above and below x-axis	- Section below steeper slope
Ray connection	- Short, same thickness than ray, extends almost directly from central part
Ray apophysis	- Clearly visible in most instances
Form of ray	- Straight, thick
	- Mostly parallel to y-axis
	- Equal thickness, ending in rounded tip
Length of denticle above x-axis and denticle below x-axis	- 1 : 1.03



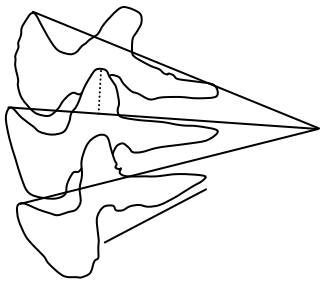
Population Number	- F12PsLV
Host species	- *Poecilia sphenops*
Locality	- Lake Valencia, South America
Date of collection	- 1980
Remarks	- Collected and dissected for the examination of skin and gill trichodinids
Blade form	- Open sickle-shaped
Distal surface	- Rounded - Sloping downwards in anterior direction
Tangent point	- Rounded, sometimes sharper
Anterior margin	- Rounded - Extending slightly beyond y+1 axis
Blade apophysis	- Visible
Posterior blade surface	- Smooth - Deep semi-lunar curve
Blade connection	- Thin - Extended
Posterior projection	- Not clearly visible
Protrusion on lower central part	- Not clearly visible
Central part	- Robust - Rounded tip - Extending more than halfway past y-axis - Fitting tightly into preceding denticle
Section above and below x-axis	- Section above steeper slope
Ray connection	- Short, very robust, extends almost directly from central part
Ray apophysis	- Not always clearly visible
Form of ray	- Straight, very thick at base - Mostly parallel to y-axis, some curved slightly in posterior direction - Taper prominently to narrow tip
Length of denticle above x-axis and denticle below x-axis	- 1 : 1.33



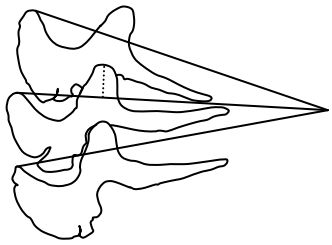
Population Number	- F14PpND
Host species	- *Pseudocrenilabrus philander*
Locality	- Dam near Nelspruit
Date of collection	- September 1989
Remarks	- Collected and dissected for the examination of skin and gill trichodinids
Blade form	- Open sickle-shaped
Distal surface	- Rounded - Sloping downwards in anterior direction
Tangent point	- Rounded, sometimes sharper
Anterior margin	- Rounded - Extending slightly beyond y+1 axis
Blade apophysis	- Sometimes visible
Posterior blade surface	- Smooth - Very deep L-shaped curve
Blade connection	- Thick, strong - Extended
Posterior projection	- Clearly visible
Protrusion on lower central part	- Visible in most instances
Central part	- Robust - Rounded tip - Extending more than halfway past y-axis - Fitting tightly into preceding denticle
Section above and below x-axis	- Section below steeper slope, highest point at section above longer than section below
Ray connection	- Short, extends almost directly from central part - Slightly narrower than ray
Ray apophysis	- Visible
Form of ray	- Straight, mostly of equal thickness throughout ray - Mostly curved in anterior direction - Most cases of equal thickness, ending in blunt rounded tip, other cases taper slightly to form rounded tip
Length of denticle above x-axis and denticle below x-axis	- 1 : 1.40



Population Number	- F15PpBV
Host species	- *Pseudocrenilabrus philander*
Locality	- Barrage Vaal
Date of collection	- June 1982
Remarks	- Collected with F3BuBV and T35UuBV
Blade form	- Open sickle-shaped
Distal surface	- Rounded
	- Sloping downwards in anterior direction
Tangent point	- Rounded
Anterior margin	- Rounded, not smooth
	- Extending to reach y+1 axis
Blade apophysis	- Visible
Posterior blade surface	- Mostly smooth
	- Deep semi-lunar curve
Blade connection	- Thick
	- Extended
Posterior projection	- Not clearly visible
Protrusion on lower central part	- Sometimes visible
Central part	- Robust
	- Rounded tip
	- Extending halfway past y-axis
	- Fitting tightly into preceding denticle
Section above and below x-axis	- Section below steeper slope
Ray connection	- Short, robust, extends almost directly from central part
Ray apophysis	- Clearly visible in some instances
Form of ray	- Straight, thick
	- Curved in anterior direction
	- Taper prominently to form rounded tip
Length of denticle above x-axis and denticle below x-axis	- 1 : 1.45



Population Number	- F16PpEB
Host species	- *Pseudocrenilabrus philander*
Locality	- Barrage Vaal
Date of collection	- March 1992
Remarks	- Cross-transmission experiment with T31XIBE
Blade form	- Open sickle-shaped
Distal surface	- Rounded - Sloping downwards in anterior direction
Tangent point	- Rounded
Anterior margin	- Rounded, not smooth - Extending to or beyond y+1 axis
Blade apophysis	- Visible
Posterior blade surface	- Smooth - Deep semi-lunar to L-shaped curve
Blade connection	- Thick, strong - Extended
Posterior projection	- Not clearly visible
Protrusion on lower central part	- Not clearly visible
Central part	- Robust - Rounded tip - Extending more than halfway past y-axis - Fitting tightly into preceding denticle
Section above and below x-axis	- Section below mostly steeper slope, highest point at section below longer than section above
Ray connection	- Short, same thickness than ray, extends almost directly from central part
Ray apophysis	- Not always clearly visible
Form of ray	- Straight, thick - Mostly parallel to y-axis - Thick at base, taper prominently to form round to sharp tip
Length of denticle above x-axis and denticle below x-axis	- 1 : 1.23



Population Number	- F18TsBW
Host species	- *Tilapia sparrmanii*
Locality	- Waterfront, Bloemfontein
Date of collection	- June 2007
Remarks	- Collected and examined for skin and gill trichodinids
Blade form	- Open sickle-shaped
Distal surface	- Rounded
	- Sloping downwards in anterior direction
Tangent point	- Rounded, sometimes sharper
Anterior margin	- Rounded, not smooth
	- Extending beyond y+1 axis
Blade apophysis	- Visible
Posterior blade surface	- Smooth
	- Deep semi-lunar curve
Blade connection	- Thick, strong
	- Extended
Posterior projection	- Not clearly visible
Protrusion on lower central part	- Not clearly visible
Central part	- Robust
	- Rounded tip
	- Extending more than halfway past y-axis
	- Fitting tightly into preceding denticle
Section above and below x-axis	- Section below steeper slope
Ray connection	- Short, same thickness, extends almost directly from central part
Ray apophysis	- Not always clearly visible
Form of ray	- Straight
	- Some curved in anterior direction, others parallel to y-axis
	- Thick at base, narrows prominently to small, rounded tip
Length of denticle above x-axis and denticle below x-axis	- 1 : 1.27

Stacked columns (100%) were used to emphasise the portion that each of the possible variations contributed for each of the above mentioned characteristics for tadpole trichodinids and fish trichodinids respectively (Figure 11.4 – 11.18). This was done to determine whether the tadpole trichodinids shared the morphological characteristics of the fish hosted trichodinids.

a) Blade form

No variation in the blade form was observed between the trichodinids examined from the two host groups, as well as all the trichodinid populations studied as all the trichodinids had an open sickle-shaped blade (Figure 11.4)

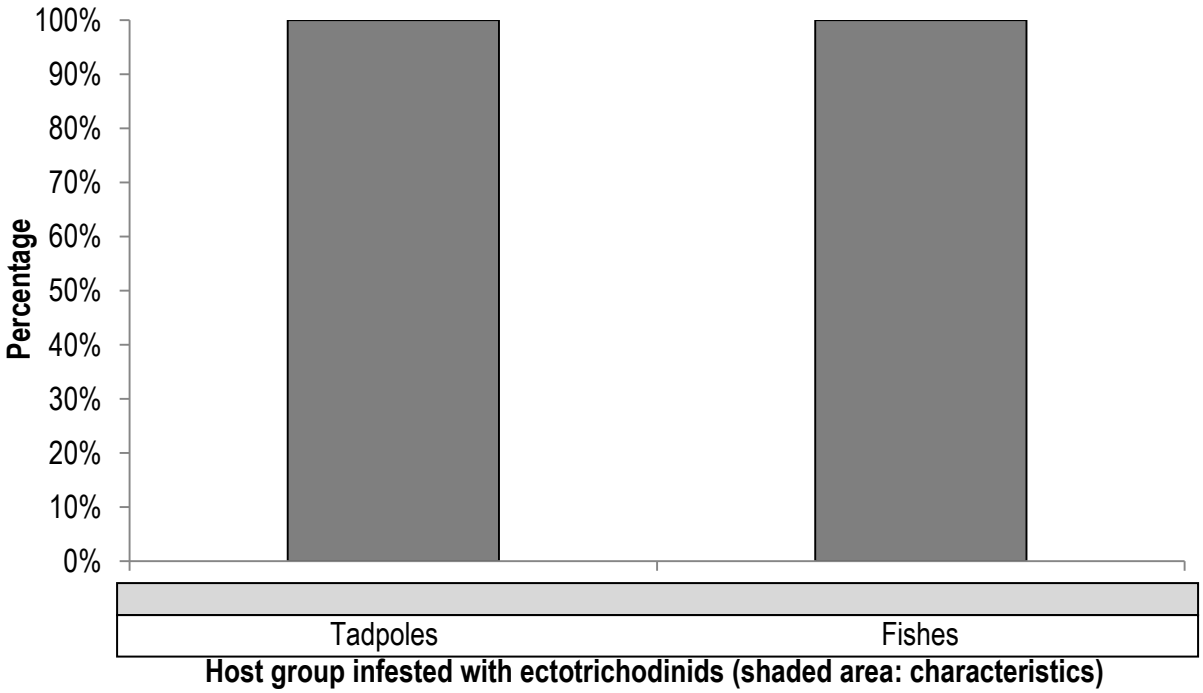


Figure 11.4: Hundred percent stacked diagram indicating the variation in blade form of the tadpole hosted trichodinids versus the trichodinids from fishes: **a**: Open sickle-shaped.

b) Distal surface

All the distal surfaces of the trichodinids examined were rounded and sloped downwards in an anterior direction. Differences were observed regarding the smoothness of the distal surface as most trichodinids had a smooth distal surface. However, almost 5% of the distal surface of tadpole trichodinids was not smooth (Figure 11.5).

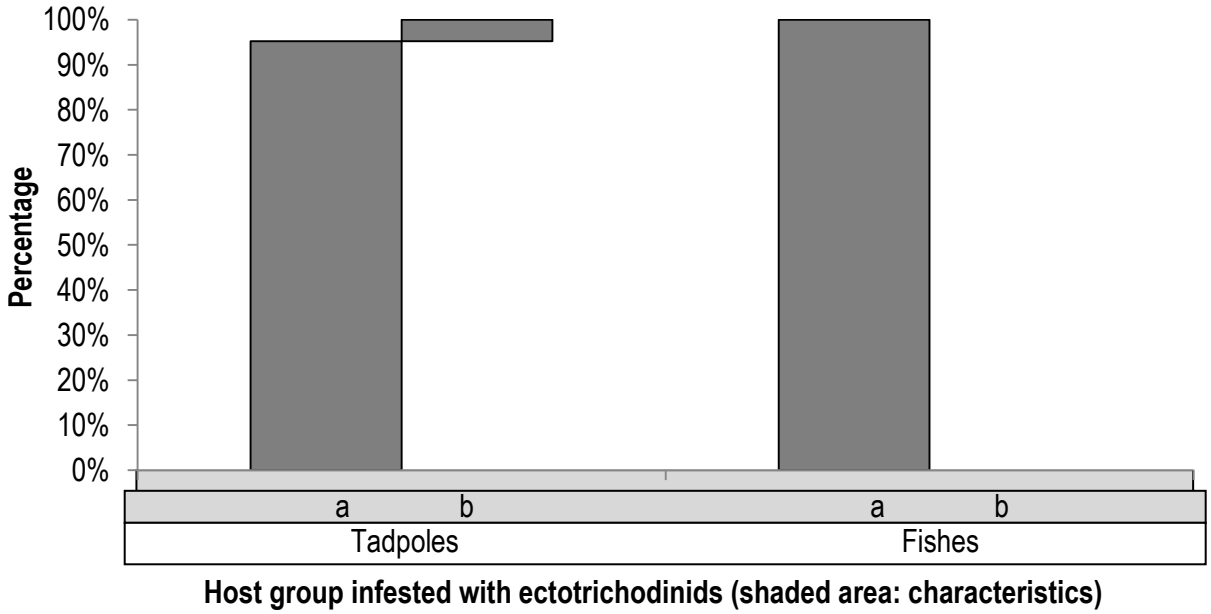


Figure 11.5: Hundred percent stacked diagram indicating the variation in the distal surface form of the tadpole hosted trichodinids versus the trichodinids from fishes: **a**: Smooth; **b**: Not smooth.

c) Tangent point

It was found that the tangent point of most of the tadpole trichodinid populations examined were round in all the examined denticles (67%). In addition, the tangent point of some tadpole trichodinid populations were mostly round (with some sharp) (28%) and about 5% of the tangent points of tadpole trichodinid populations examined ended mostly sharp (with some round). In contrast, the tangent point of fish trichodinids ended round (77%) or mostly round (with some sharp) (23%) (Figure 11.6).

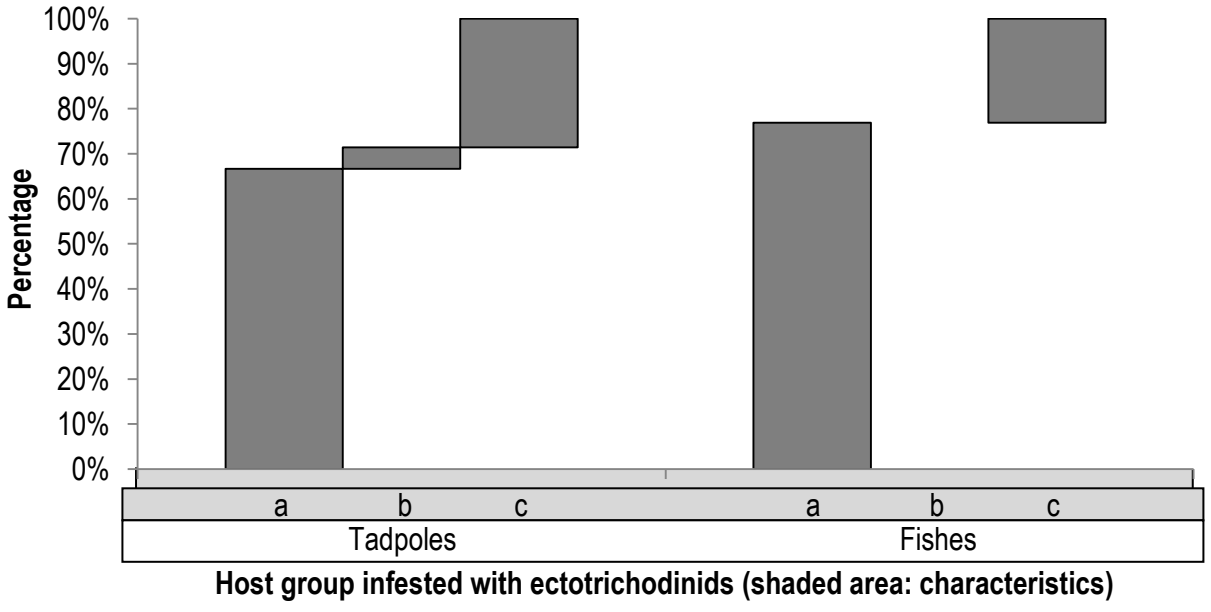


Figure 11.6: Hundred percent stacked diagram indicating the variation in the tangent point of the tadpole hosted trichodinids versus the trichodinids from fishes: **a**: Rounded; **b**: Ended mostly sharp, otherwise rounded; **c**: Rounded in most instances, otherwise ended sharp.

d) Anterior margin

The anterior margin of all the studied material was rounded. However, a great variance was still observed. For example, the anterior margin of 46% of the fish trichodinids extended slightly beyond the y+1 axis while the anterior margin of most of the tadpole trichodinids extended to / or slightly beyond the y+1 axis within a denticle ring (Figure 11.7).

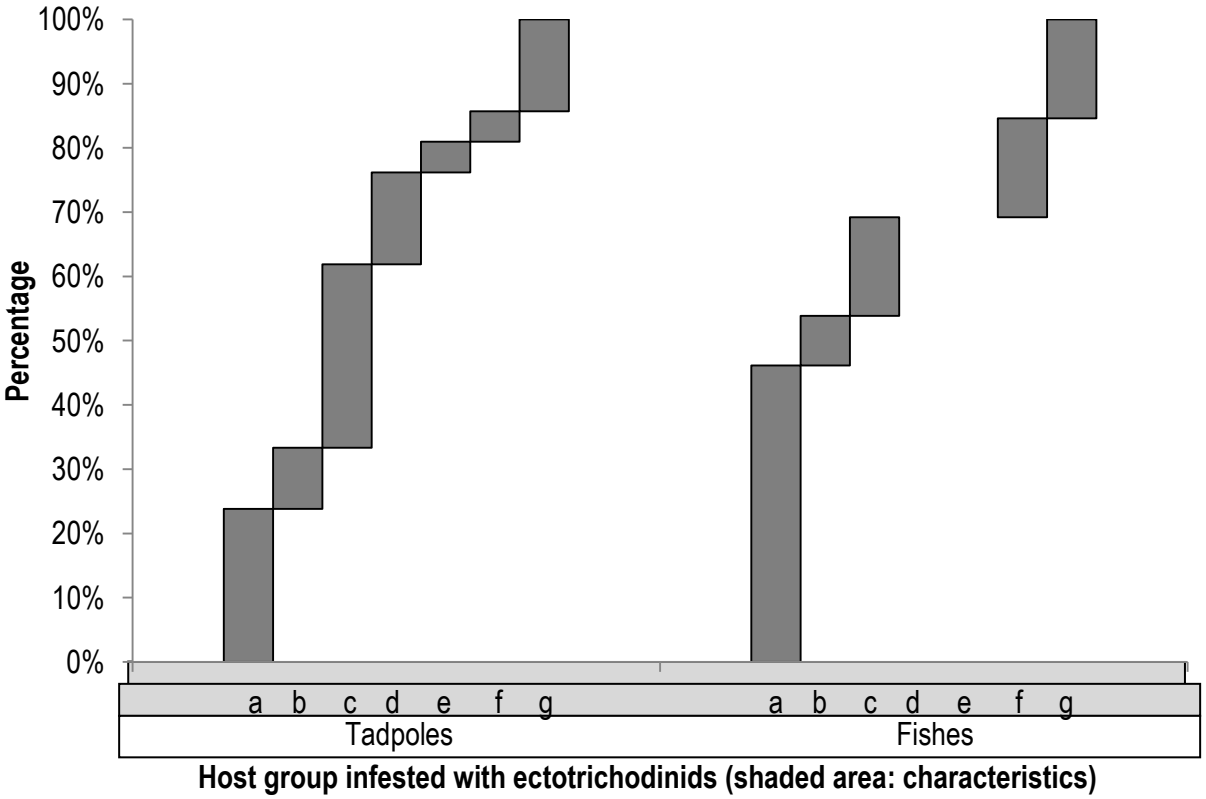


Figure 11.7: Hundred percent stacked diagram indicating the variation in the anterior margin of the tadpole hosted trichodinids versus the trichodinids from fishes: **a**: Smooth, extended slightly beyond y+1 axis; **b**: Not smooth, extended towards but not beyond y+1 axis; **c**: Smooth, extended to or slightly beyond y+1 axis; **d**: Not smooth, extended to or slightly beyond y+1 axis; **e**: Not smooth, extended less than halfway past y-axis; **f**: Smooth, to y+1 axis but not beyond y+1 axis; **g**: Not smooth, extended slightly beyond y+1 axis.

e) Blade apophysis

Four variations with regards to the blade apophysis were noted in tadpole trichodinids:

- Small, when present
- Small, rounded, observed in most instances
- Not observed or not clearly visible
- Visible in most instances

In most cases (61%), the blade apophysis of tadpole trichodinids were small (when observed, in most denticles) while the blade apophysis were not observed in other denticles of the same individual. The

blade apophysis of fish trichodinids were mostly not visible (61%), or not clearly visible (39%) (Figure 11.8).

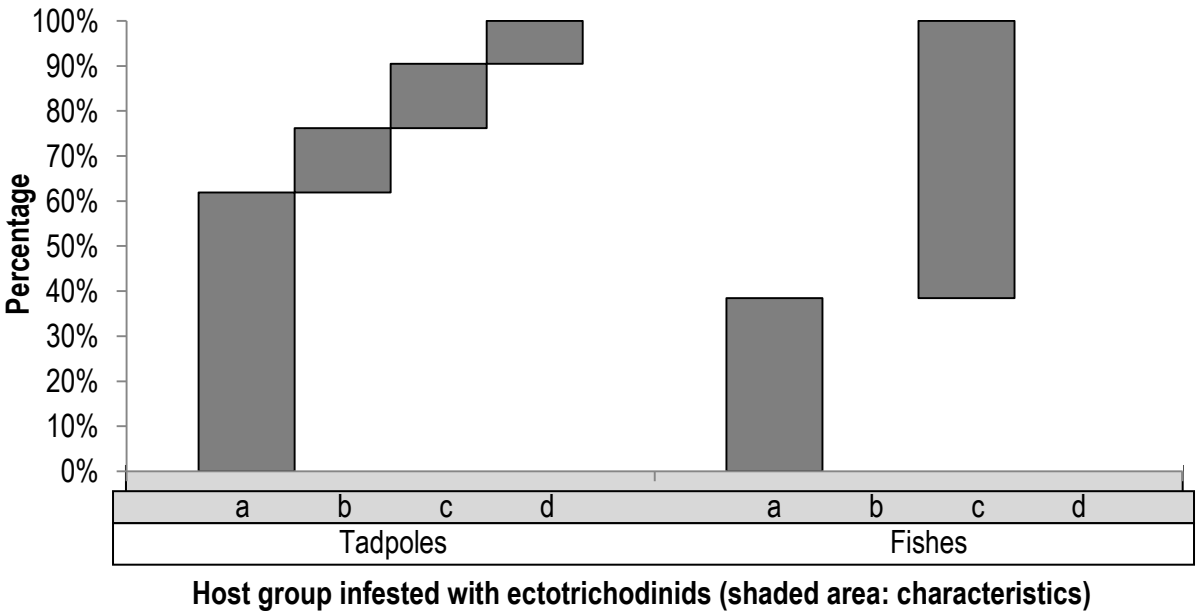


Figure 11.8: Hundred percent stacked diagram indicating the variation in the blade apophysis of the tadpole hosted trichodinids versus the trichodinids from fishes: **a**: Small, when present; **b**: Small, rounded, observed in most instances; **c**: Not observed or not clearly visible; **d**: Visible in most instances or prominent.

f) Posterior blade surface

All the posterior blade surfaces studied had a smooth appearance. Variation in the curvature of the posterior blade was observed (Figure 11.9). The variation can be simplified by dividing the characteristics into three groups:

- All denticles within a trichodinid: semi-lunar
- Semi-lunar and L-shaped forms observed in a single trichodinid
- All denticles within a trichodinid: L-shaped

Although a great variation was observed within the tadpole trichodinids, most of the tadpole trichodinids had a semi-lunar curve (52%). Less variation was observed when the fish trichodinids were studied and 38% of the fish trichodinid populations had an L-shaped posterior blade surface while most of the fish trichodinids also curved in a semi-lunar form (46.15%).

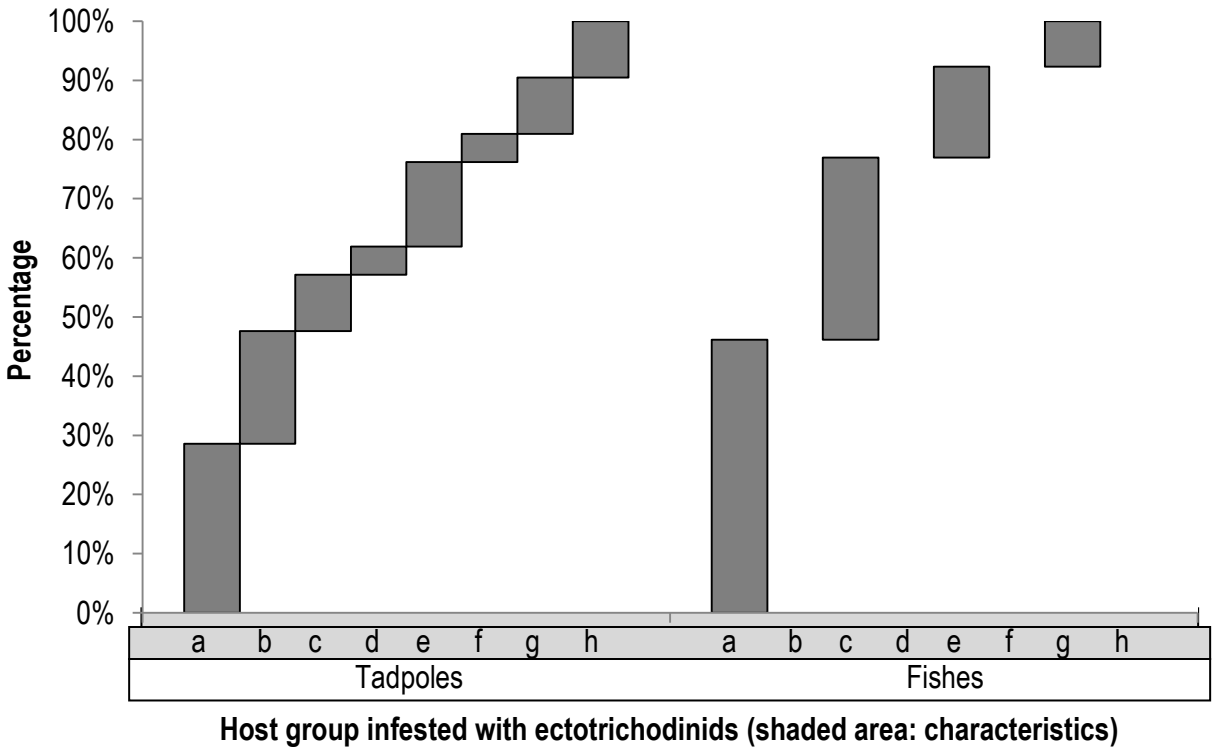


Figure 11.9: Hundred percent stacked diagram indicating the variation in the posterior blade surface of the tadpole hosted trichodinids versus the trichodinids from fishes: **a**: Deep semi-lunar curve; **b**: Moderately deep semi-lunar curve; **c**: Deep L-shaped curve; **d**: Shallow semi-lunar curve; **e**: Deep semi-lunar curve to L-shaped curve; **f**: Almost L-shaped curve; **g**: Very deep L-shaped curve; **h**: Moderate to deep L-shaped curve.

g) Blade connection

The blade connection of most of the fish trichodinids was described as thick and strong while the blade connection of less fish trichodinids were either thick (30.77%), thin to thick (7.69%) or thin (7.69%). Most of the tadpole trichodinids had a thick blade connection while 38.10% of the tadpole trichodinid populations had a thick, strong blade connection (Figure 11.10). In all cases, the blade connections from both fish and tadpole hosted trichodinids were described as extended.

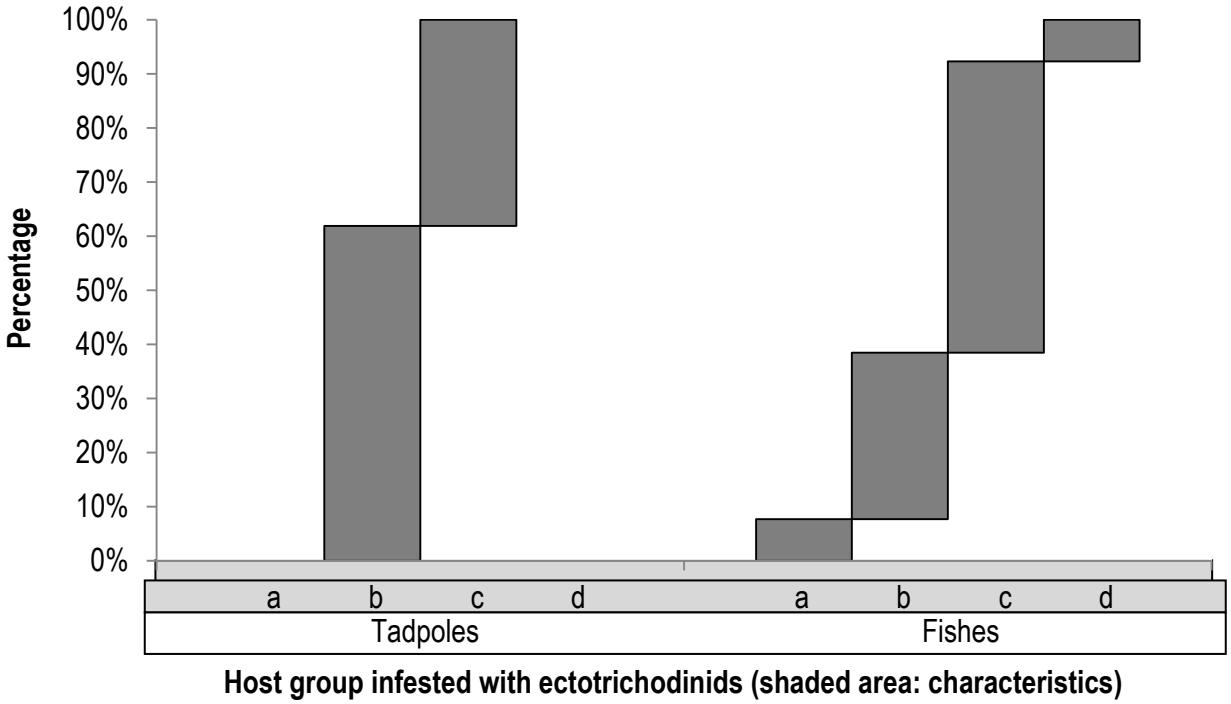


Figure 11.10: Hundred percent stacked diagram indicating the variation in the thickness of the blade connection of the tadpole hosted trichodinids versus the trichodinids from fishes: **a**: Thin; **b**: Thick; **c**: Thick and strong; **d**: Thin to thick.

h) Posterior projection

Variation in both the trichodinid host populations was observed. For example, more than half of the trichodinid populations that were collected from tadpoles had a small posterior projection, when visible (Figure 11.11). The posterior projection of a smaller number of tadpole trichodinid populations were not clearly visible (33.33%) while the posterior projection of only 14.29% of the tadpole trichodinid populations were always clearly visible.

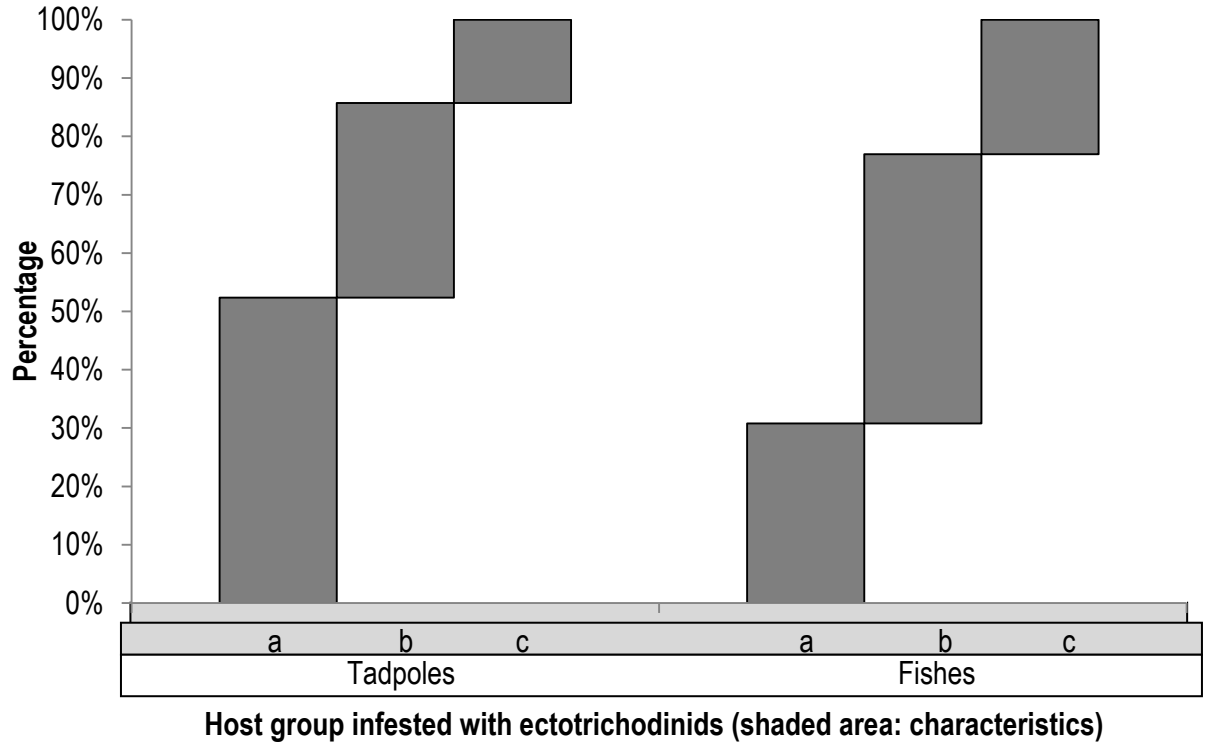


Figure 11.11: Hundred percent stacked diagram indicating the variation in the posterior projection of the tadpole hosted trichodinids versus the trichodinids from fishes: **a** Small, when visible; **b**: Not clearly visible; **c**: Clearly visible.

i) Protrusion on lower central part

The protrusion on the lower central part of most of the trichodinid populations studied (fish- and tadpole trichodinid populations) was not clearly visible (Figure 11.12).

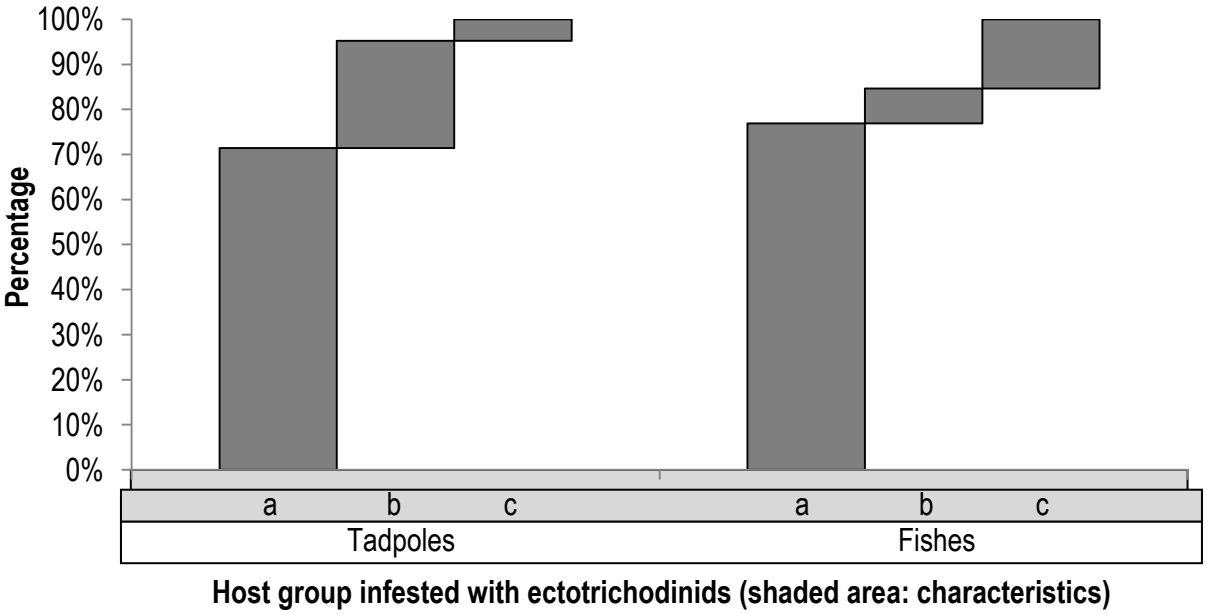


Figure 11.12: Hundred percent stacked diagram indicating the variation in the protrusion on the lower central part of the tadpole hosted trichodinids versus the trichodinids from fishes: **a**: Not clearly visible; **b**: Small when present; **c**: Visible in most instances.

j) Central Part

All the central parts of the studied trichodinids had a rounded tip that fitted tightly into the preceding denticle. In addition, the majority of the trichodinid populations studied had a robust central part that extended more than halfway past the y-axis (tadpole trichodinids: 42.86%, fish trichodinids: 61.54%). It is interesting that the central parts of an additional 33.33% of the tadpole trichodinid populations extended more than halfway past the y-axis. However, these central parts were slender and not robust as the foresaid group (Figure 11.13).

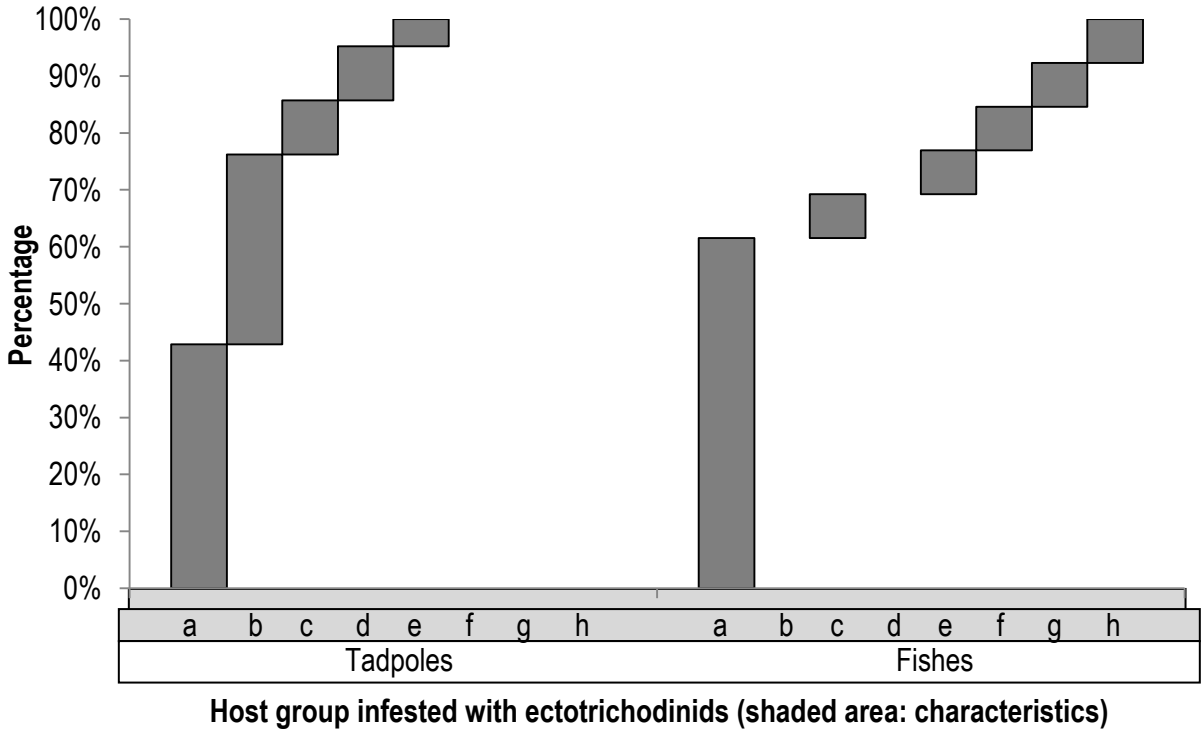


Figure 11.13: Hundred percent stacked diagram indicating the variation of the central part of the tadpole hosted trichodinids versus the trichodinids from fishes: **a**: Robust, extended more than halfway past y-axis; **b**: Slender, extended more than halfway past y-axis, **c**: Robust, extended less than halfway past y-axis; **d**: Robust, extended more than halfway past y-axis sometimes almost reached the y-axis; **e**: Slender to robust, extended halfway or more past y-axis; **f**: Slender to robust, extended less than halfway past y-axis; **g**: Slender, extended halfway or more past y-axis, **h**: Slender, extended less than / or more than halfway past y-axis.

k) Section above and below the x-axis

The trichodinid characteristic with the most variation between the different host groups as well as within the different host groups themselves was the section above the x-axis in comparison to the section below the x-axis (Figure 11.14). Most of the tadpole trichodinid populations were either:

- Steeper sloped at the base of the section below the x-axis
- Steeper sloped for most part of the section below the x-axis, or

- In addition to the steeper sloped section below the x-axis, the denticle also bent in a proximal direction.

In contrast, the section below the x-axis in most fish trichodinid populations were steeper in comparison to the area above the x-axis (30.77%), followed by trichodinids where central parts were almost similar above and below the x-axis, although the area below was still slightly steeper (15.28%).

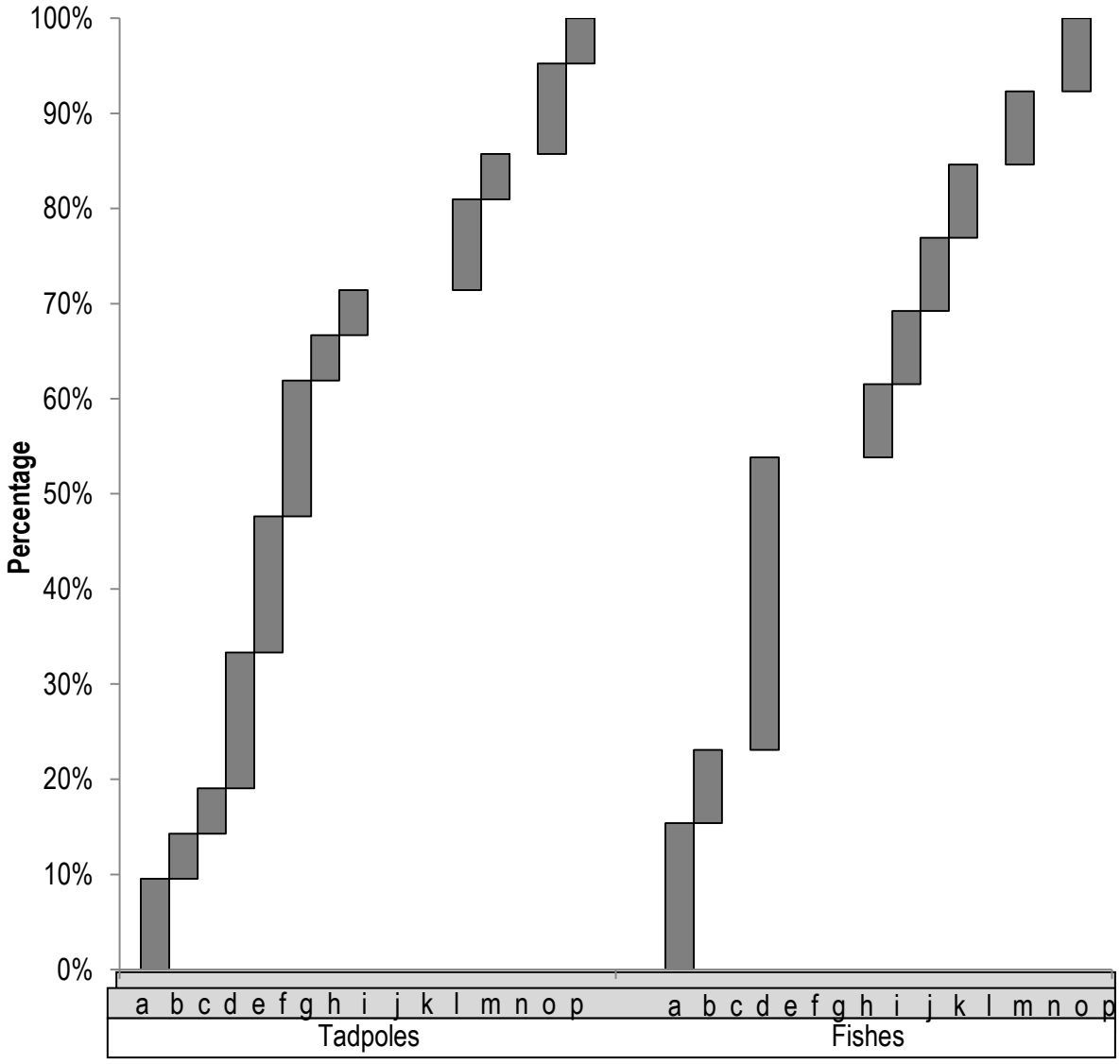


Figure 11.14: Hundred percent stacked diagram indicating the variation of the section above and below the x-axis of the tadpole hosted trichodinids versus the trichodinids from fishes: **a**: Similar; **b**: Almost similar, sb steeper slope; **c**: Almost similar, top of sa steeper slope; **d**: Base of sb steeper slope; **e**: sb steeper slope; **f**: Sb steeper slope, bend in proximal direction; **g**: Sb steeper slope, flattened top; **h**: Base of sb steeper slope, top of sa steeper slope; **i**: Sb steeper slope, sb rounder tip; **j**: Sb steeper slope, highest point at sa longer; **k**: Sb mostly steeper slope, highest point at sb longer; **l**: Sa steeper slope; **m**: Highest point at sa longer, rectangular top at some instances; **n**: Base of sa steeper slope; **o**: Top of sa rounder; **p**: Top of sa steeper slope**; sa**: Section above x-axis, **sb**: Section below x-axis.

l) Ray connection

All the studied denticles had a short ray connection that extended almost directly from the central part. Most of the trichodinid populations hosted on tadpoles had a robust ray connection that was narrower than the ray (33.33%), followed by ray connections that were not robust and had the same thickness than the ray (28.57%). More than half the fish trichodinid populations studied, had a fragile ray connection of the same thickness than the ray (53.85%) (Figure 11.15).

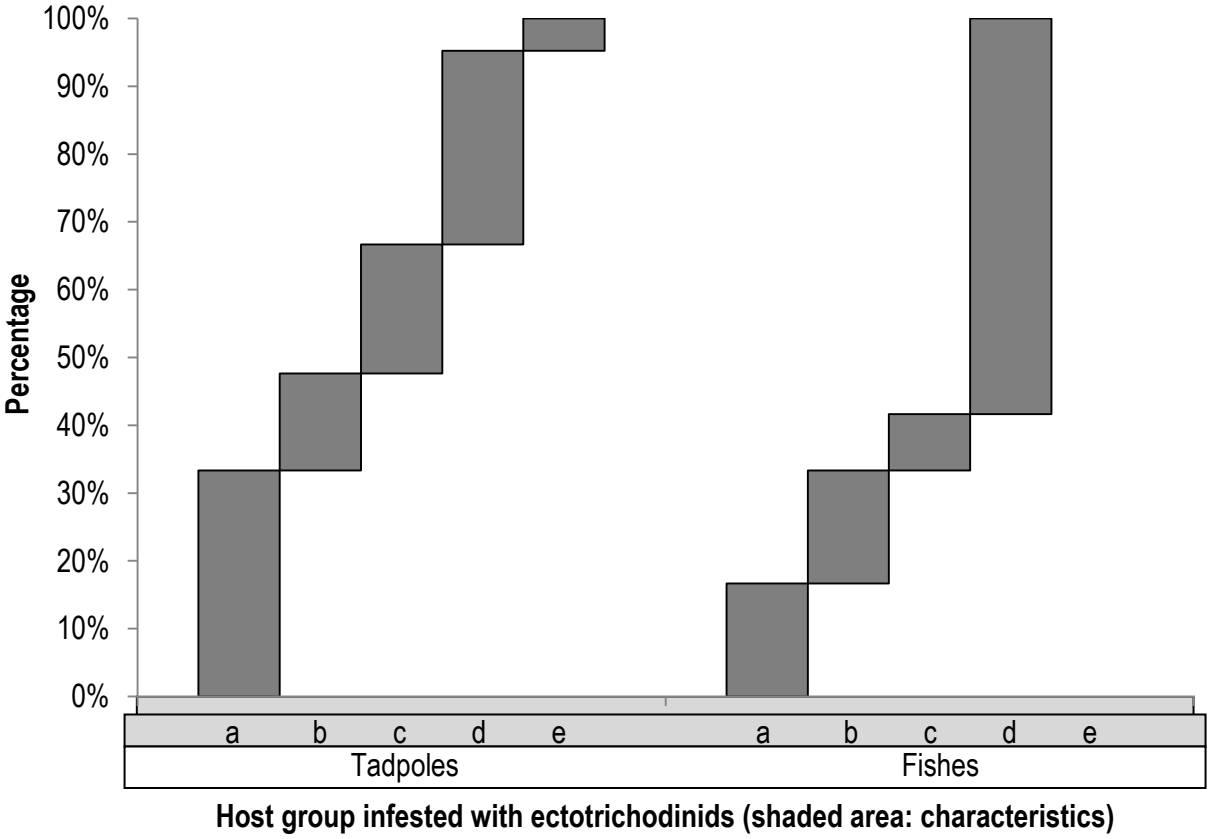


Figure 11.15: Hundred percent stacked diagram indicating the variation of the ray connection of the tadpole hosted trichodinids versus the trichodinids from fishes: **a**: Robust, narrower than ray; **b**: Robust, same thickness as most rays; **c**: Not robust, narrower than rays; **d**: Not robust, same thickness than most of rays; **e**: Not robust, sometimes thicker than ray.

m) Ray apophysis

The ray apophysis of most of the tadpole trichodinid populations were visible (38.10%) although this feature was either absent or not observed in 28.57% of the tadpole trichodinid populations. In contrast, most of the ray apophyses of the fish trichodinid populations were not always clearly visible, or small when present (Figure 11.16).

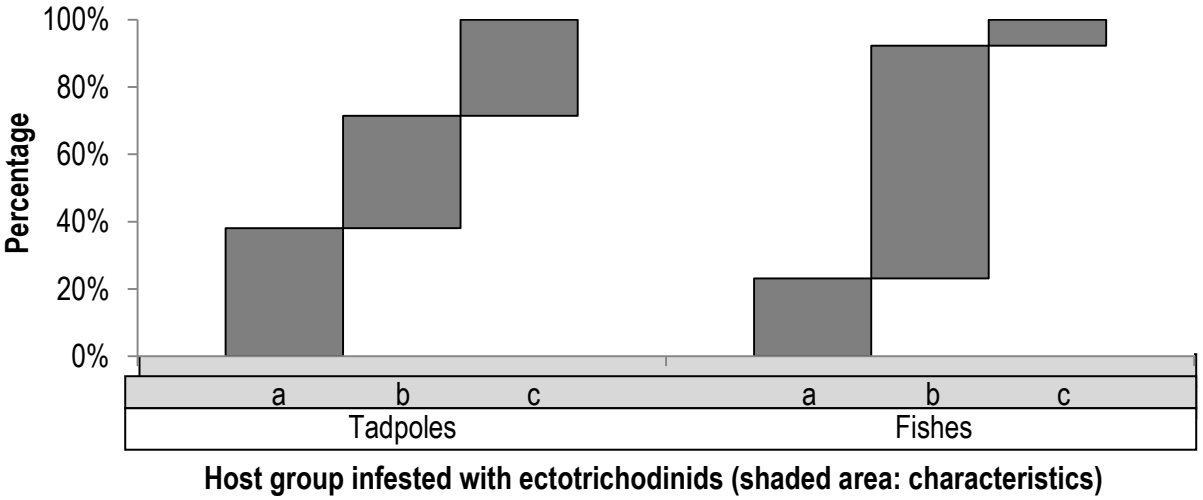


Figure 11.16: Hundred percent stacked diagram indicating the variation of the ray apophyses of the tadpole hosted trichodinids versus the trichodinids from fishes: **a**: Clearly visible to mostly prominent; **b**: Not always clearly visible to small when visible; **c**: Not clearly visible.

n) Form of ray

Most trichodinids from both fish- and tadpole trichodinid populations had a ray of equal thickness throughout that was mostly parallel to the y-axis (Figure 11.17). All the rays studied were straight and tapered slightly to form a rounded tip.

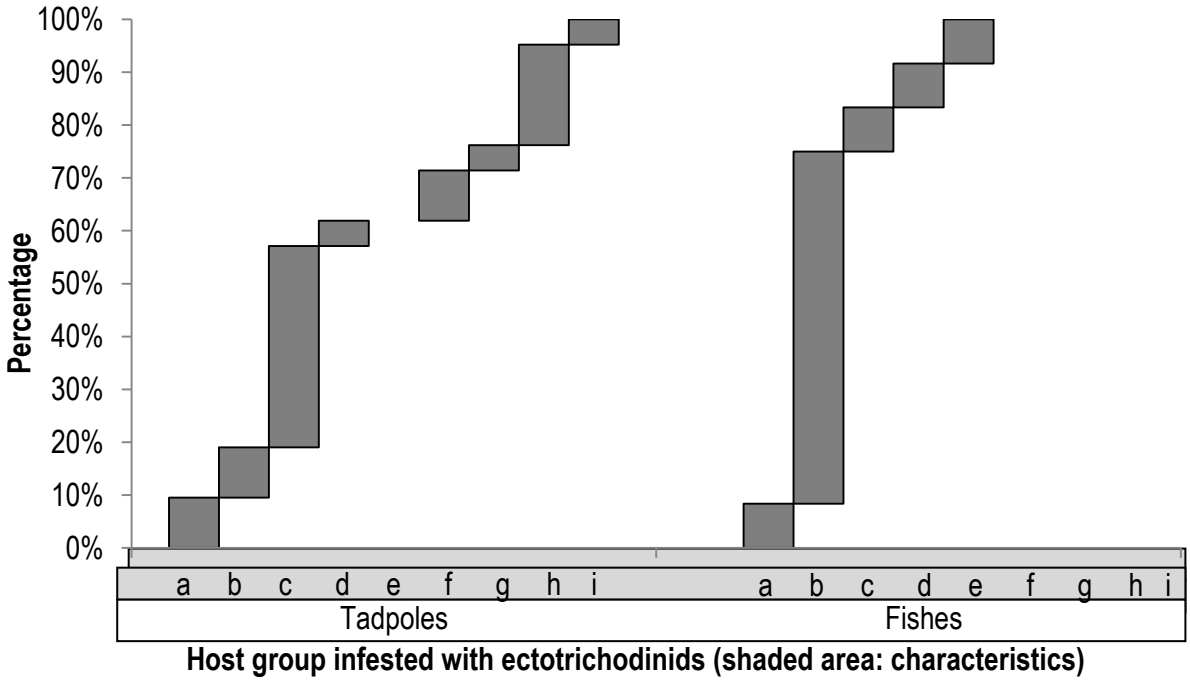


Figure 11.17: Hundred percent stacked diagram indicating the variation in the form of the rays of the tadpole hosted trichodinids versus the trichodinids from fishes: **a**: Thicker base, slightly curved in posterior direction; **b**: Equal thickness throughout, slightly curved in posterior direction; **c**: Equal thickness throughout, mostly parallel to y-axis; **d**: Not of equal thickness, curved in anterior direction; **e**: Delicate, curved in anterior direction; **f**: Equal thickness throughout, curved in anterior direction; **g**: Narrowed sometimes directly after connection before it thickened, parallel to y-axis in most instances; **h**: Become thinner, parallel to y-axis in most instances; **i**: Delicate, curved in posterior direction.

o) Ratio of the length above the x-axis versus the length below the x-axis

Lastly, the ratio of the length of the denticle above the x-axis versus the length of the denticle below the x-axis for each of the trichodinid populations was determined. It was found that both host groups mostly had a ratio between 1 : 1.31 – 1.45. The length below the x-axis in some trichodinids from the tadpole hosts were 1.16 – 1.60 times the length above the x-axis. However, none of the trichodinids from the fish hosts achieved this trait (Figure 11.18).

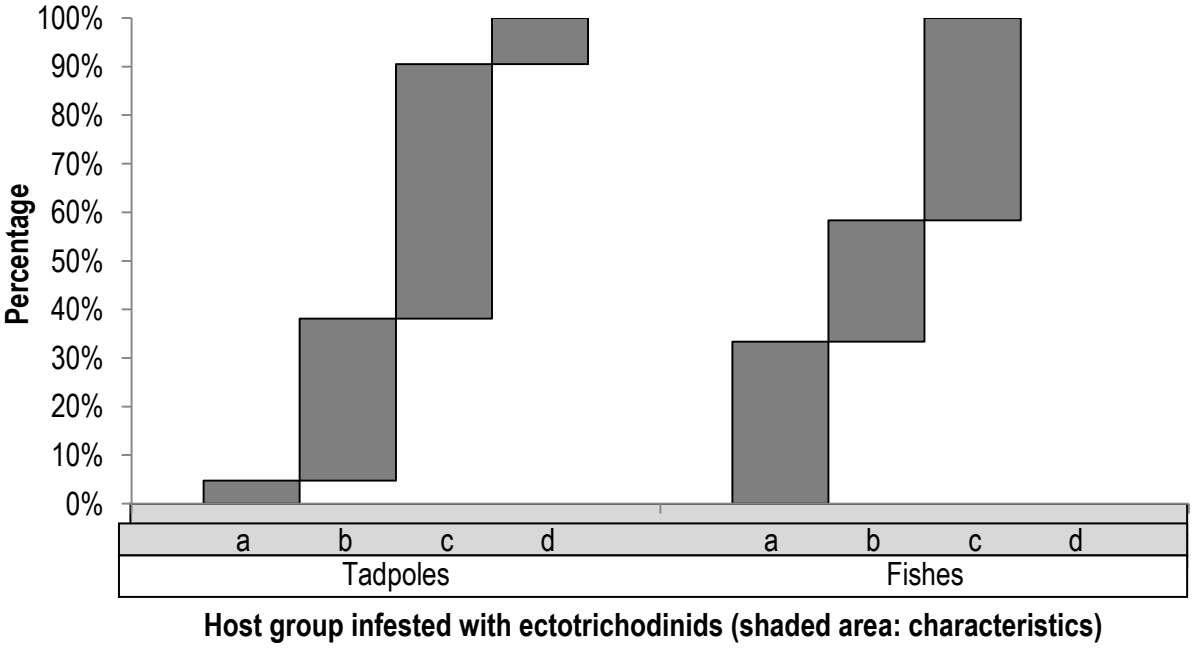


Figure 11.18: Hundred percent stacked diagram indicating the variation of the ratio of the length above the x-axis versus the length below the x-axis for trichodinids collected on tadpoles versus the trichodinids from fishes. **a**: 1 : 1.1-1.15; **b**:1 : 1.16 – 1.30; **c**: 1 : 1.31-1.45; **d**: 1 : 1.46-1.60.

To summarise, it was found that the trichodinids collected from fish and tadpoles during the present study correspond generally with regard to the following characteristics:

- Blade has an open sickle-shaped form (Fish: 100%, Tadpole: 100%, Figure 11.4)
- Distal surface is rounded, sloping downwards in an anterior direction (Fish: 100%, Tadpole: 95.24%, Figure 11.5)
- Tangent point is rounded (Fish 76.92%, Tadpole 66.67%, Figure 11.6)
- Protrusion on lower central part is not clearly visible (Fish: 76.92%, Tadpole: 71.42%, Figure 11.12)
- Central part is robust with a rounded tip fitting tightly into preceding denticle (Fish: 61.53%, Tadpole: 42.85%, Figure 11.13).

Great variations in the following characteristics were observed:

- Anterior margin (Figure 11.7)
- Posterior blade surface (Figure 11.9)
- Blade connection (Figure 11.10)
- Ray connection (Figure 11.15)
- Section above and below x axis (Figure 11.14)
- Ray apophyses (Figure 11.16)
- Form of ray (Figure 11.17)
- Length of denticle above x axis and denticle below x axis (Figure 11.18)

It was also observed that the rays of fish hosted trichodinids were generally thicker and robust in comparison with tadpole hosted trichodinids. Kruger *et al.* (1993) made the same conclusion in their study of fish versus tadpole trichodinids collected in southern Africa. However, this characteristic was not observed throughout all the studied populations and some tadpole trichodinids were also thick and robust in relation to other tadpole trichodinids studied.

Therefore, a list of all the shared characteristics as well as the dissimilarities within the trichodinid host population groups are given in Table 11.4. Although characteristics observed only in one of the two host groups were observed, it is not suggested that these features should be used to distinguish between fish- and tadpole trichodinids. For example, a few tadpole trichodinid populations had an uneven anterior margin that extended less than halfway past the y-axis. Other tadpole trichodinid populations also had an uneven anterior margin, but the anterior margin extended to or slightly beyond the y+1 axis. These characteristics were only observed within the tadpole trichodinid group. However, trichodinids from both host groups had an uneven anterior margin that extended towards, but not beyond the y+1 axis as well as populations with an uneven anterior margin that extended slightly beyond the y+1 axis (Table 11.4). By placing the four characteristics in sequence, it is suggested that, although not observed during the present study, it is possible that fish trichodinids may also have an uneven anterior margin that extend to or slightly beyond the y+1 axis, or even both characteristics that were observed only in tadpole trichodinids during the present study.

Table 11.4: List of the shared characteristics as well as dissimilarities within the trichodinid host population groups.

	Characteristics shared by both trichodinid population groups	Characteristic observed only in the tadpole trichodinid group	Characteristic observed only in the fish trichodinid group
Blade form	• Open sickle-shaped		
Distal surface	• Rounded • Sloping downwards in anterior direction • Smooth	• Not smooth	
Tangent point	• Rounded • Mostly rounded	• Mostly sharp (some round)	
Anterior margin	• Rounded • Not smooth, extending towards but not beyond y+1 axis • Not smooth, extending slightly beyond y+1 axis • Smooth, extending to or slightly beyond y+1 axis • Smooth, extending to y+1 axis but not beyond y+1 axis • Smooth, extending slightly beyond y+1 axis	• Not smooth, extending to or slightly beyond y+1 axis; • Not smooth, extending less than halfway past y-axis	
Blade apophysis	• Small, when present • Not observed or not clearly visible	• Small, rounded, observed in most instances • Visible in most instances	
Posterior blade surface	• Smooth • Deep semi-lunar curve • Deep L-shaped curve • Deep semi-lunar curve to L-shaped curve • Very deep L-shaped curve;	• Moderately deep semi-lunar curve • Shallow semi-lunar curve • Almost L-shaped curve • Moderate to deep L-shaped curve	
Blade connection	• Thick • Thick, strong • Extended		• Thin • Thin to thick
Posterior projection	• Small, when visible • Not clearly visible • Clearly visible		
Protrusion on lower central part	• Not clearly visible • Small when present • Visible in most instances		
Central part	• Rounded tip fitting tightly into preceding denticle • Robust, extending more than halfway past y-axis • Robust, extending less than halfway past y-axis • Slender to robust, extending halfway or more past y-axis	• Slender, extending more than halfway past y-axis • Robust, extending more than halfway past y-axis sometimes almost reached the y-axis	• Slender to robust, extending less than halfway past y-axis • Slender, extending halfway or more past y-axis • Slender, extending less than / or more than halfway past y-axis.

Table 11.4 (Continue): Shared characteristics as well as dissimilarities within the trichodinid host groups. *Abbreviations:* **sa**: Section above; **sb**: Section below.

Section above and below x-axis	• Similar • Almost similar, sb steeper slope • Sb steeper slope • Sa steeper slope • Top of sa steeper slope	• Base of sb steeper slope • Sb steeper slope, bent in proximal direction • Sb steeper slope, flattened top • Base of sb steeper slope, top of sa steeper slope • Highest point at sa longer, rectangular top at some instances • Top of sa rounder	• Sb steeper slope, sb rounder tip • Sb steeper slope, highest point at sa longer • Sb mostly steeper slope, highest point at sb longer • Base of sa steeper slope
Ray connection	• Extended almost directly from the central part • Robust, narrower than ray • Robust, same thickness as most rays • Not robust, narrower than rays • Not robust, same thickness than most of rays	• Not robust, sometimes thicker than ray	
Ray apophysis	• Clearly visible to mostly prominent • Not always clearly visible to small when visible • Not clearly visible		
Form of ray	• Straight • Thicker base, slightly curved in posterior direction • Equal thickness throughout, slightly curved in posterior direction • Equal thickness throughout, mostly parallel to y-axis • Not of equal thickness, curved in anterior direction • Equal thickness throughout, curved in anterior direction	• Sometimes narrowing directly after connection before it thickens, parallel to y-axis in most instances • Become thinner, parallel to y-axis in most instances • Delicate, curving in posterior direction	• Delicate, curving in anterior direction
Length of denticle above x-axis and denticle below x-axis	• 1 : 1.1-1.15 • 1 : 1.16 – 1.30 • 1 : 1.31-1.45	• 1 : 1.46-1.60	

The above mentioned differences cannot be used to distinguish between fish or tadpole trichodinids in general, as many of these characteristics are not exclusive fish or tadpole trichodinid characteristics. For example Kruger *et al*. (1993) stated that the anterior margin of trichodinids collected from *X. laevis* was rounded and extended to or beyond the y+1 axis, while the present study found that trichodinids from both fish and tadpole hosts may have this characteristic. In addition, the anterior margin of a few tadpole as well as fish trichodinid populations a) extended beyond the y+1 axis, b) only reached the y+1 axis, while other populations c) extended less than halfway past the y axis.

Differences between trichodinid populations from each of the studied trichodinid groups (consisting of the tadpole trichodinids or the fish trichodinids) were also observed. For example, the tangent point of most trichodinids collected from various tadpole trichodinid populations ended round. In other tadpole trichodinid populations, the tangent point ended mostly sharp while this characteristic in still other populations ended rounded in most instances although rounded tangent points were also observed from some denticles within these populations (Figure 11.6). The above is also confirmed by examining the dendrograms in Figures 11.19 A and B (see also Appendix D).

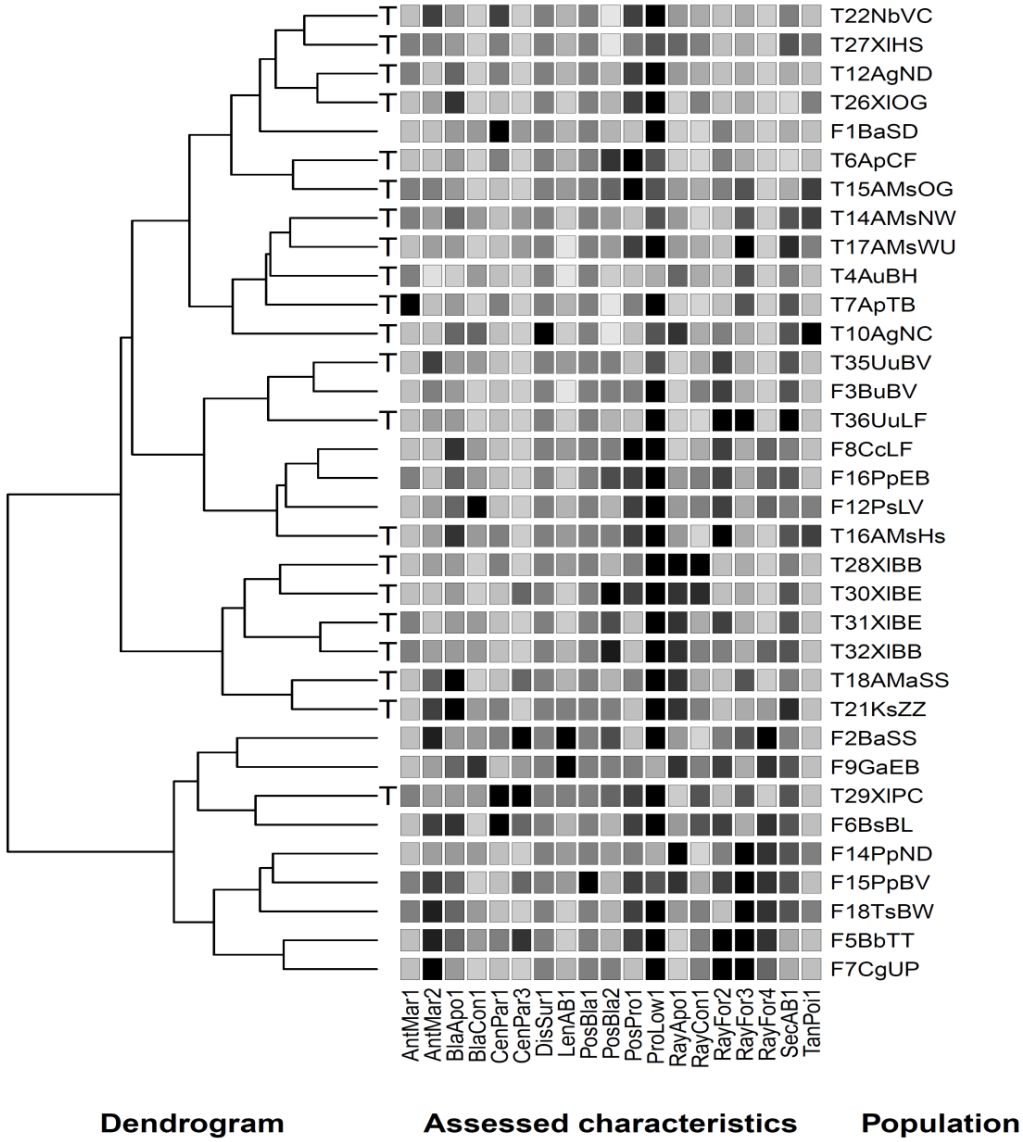


Figure 11.19A: Dendrogram of the assessed trichodinid denticle characteristics. Please refer to Appendix D for more information on the assessed characteristics and to Figure 5.1 and Table 5.2 for more information on the host population. **Note**: **Light colours**: Insignificant correlation; **Dark colours**: Significant correlation.

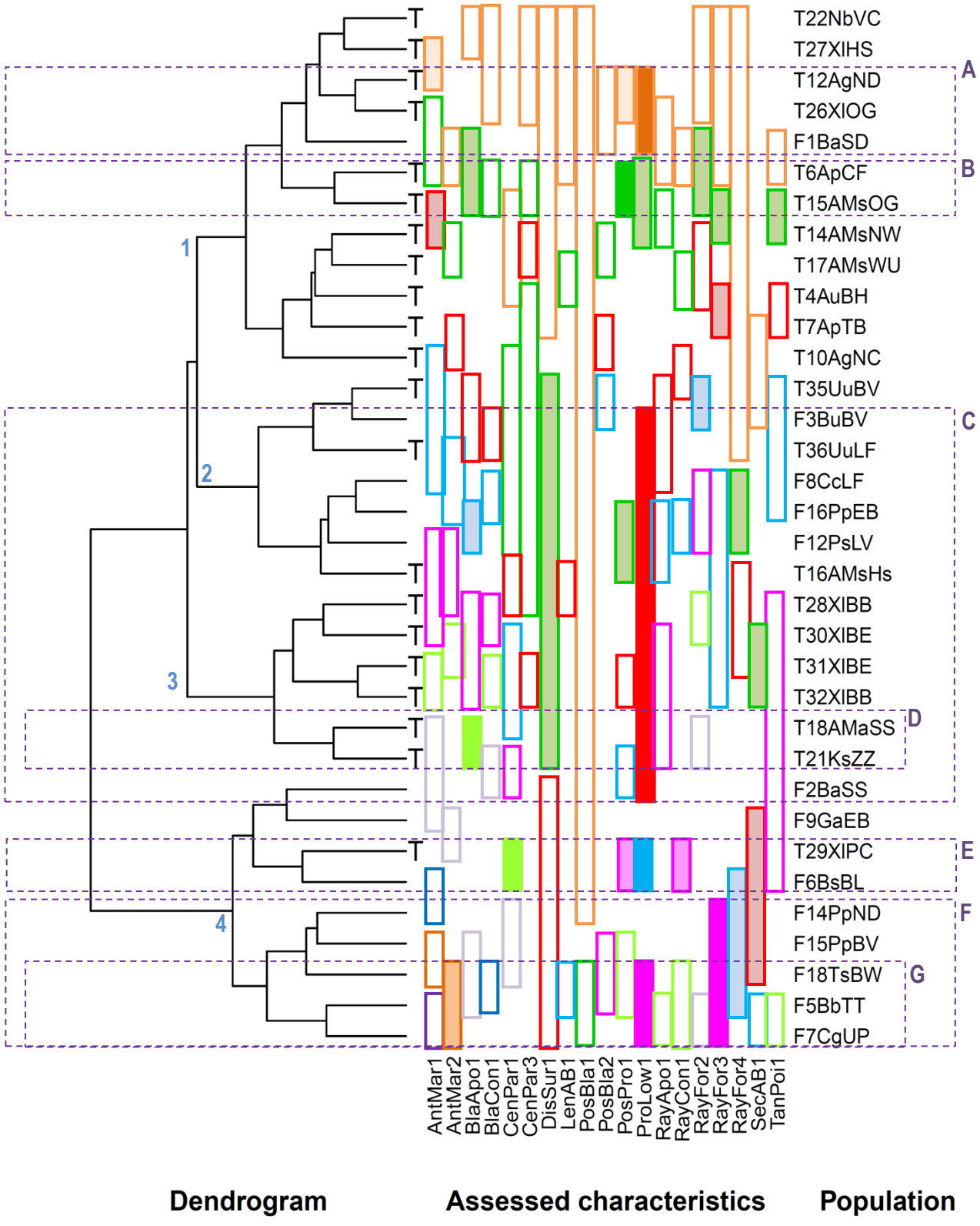


Figure 11.19B: Simplified version of a dendrogram of the assessed trichodinid denticle characteristics. Please refer to Appendix D for more information on the assessed characteristics and to Figure 5.1 and Table 5.2 for more information on the host population. Note: **1-4**: Major groupings, **A-G**: Significant groupings. Similar characteristics with an insignificant correlation are indicated with a border only; features shared to a moderate extend are lightly coloured and features prominently shared within the trichodinid populations are coloured in bright colours.

The results of the simplified dendrogram can be divided into four distinct groupings (Groups 1 – 4):

- Group 1 are represented by 11 tadpole populations and only one fish population (F1BaSD).
- Group 2 are represented by 3 tadpole populations and 4 fish populations.
- Group 3 contains only members from tadpole populations.
- Group 4 are represented by one tadpole population and 8 fish populations.

Although these groupings are observed, one should consider the significance of the shared characteristics. The dotted lines indicate trichodinid populations that significantly shared certain characteristics. By taking these groupings into consideration, Populations F5BbTT, F7CgUP and F18TsBW correlated highly with regard to Characteristic ProLow1. In addition, Characteristic RayFor3 of these populations as well as Populations F14PpND and F15PpBV contribute to a further significant grouping. Five other groupings can be made on the same approach, as can be seen in Figure 11.19B.

The results of the dendrogram did not indicate morphological differences that can be used to distinguish between fish – or tadpole trichodinids, even though the above mentioned groupings (Groups 1-4) mostly consisted of trichodinids of either fishes or tadpoles (except for Group 2 that consisted of three tadpole populations and four fish populations).

In addition, many of the characteristics that were shared between tadpole trichodinids in one of the groups were also noted on trichodinids from fishes in other groups, or even the same group. For example, Populations T12AgND and T26XlOG as well as F1BaSD are grouped as Group 1 as well as Group B as these trichodinids significantly shared Characteristic ProLow1. These three trichodinid populations were collected from different host species, in different seasons, at different localities (Nxamasere, Oribi Gorge and Sabella Dam). In addition, Groups C, E and G also contain trichodinids from fish and tadpole hosts that correlated highly with regards to Characteristic ProLow1.

By taking the morphological characteristics of the trichodinids examined in this part of the study into account, it is suggested that the examined trichodinids should be assigned to one trichodinid species as the above methods as well as the experiments performed in Chapters 6 and 7 could not find clear differentiation characteristics between the different trichodinid populations. These methods aided in describing the trichodinid populations as all belonging to *Trichodina heterodentata*.

This also corresponds with the findings of Kruger *et al.* (1993) that mentioned that all trichodinids previously collected on tadpoles in southern Africa represented *T. heterodentata* (Figure 11.20).

Trichodina heterodentata individuals are known to exhibit a considerable variation in the shape of the denticles, especially the shape of the ray (Basson and Van As 1994). In addition, the denticle blades are described as strongly falcate with an indentation on the anterior edge near its base (Duncan 1977). Duncan (1977) further mentioned that the rays of individuals belonging to the species *T. heterodentata* are usually slender in comparison to other trichodinid species and that younger individuals have smaller rays. Morphological differentiation of this species can also be seen by referring to a trichodinid examined during the current study (Figure 11.20A), a specimen described by Kruger *et al.* (1993) (Figure 11.20B) as well as *T. heterodentata* described by Duncan (1977) (Figure 11.20C).

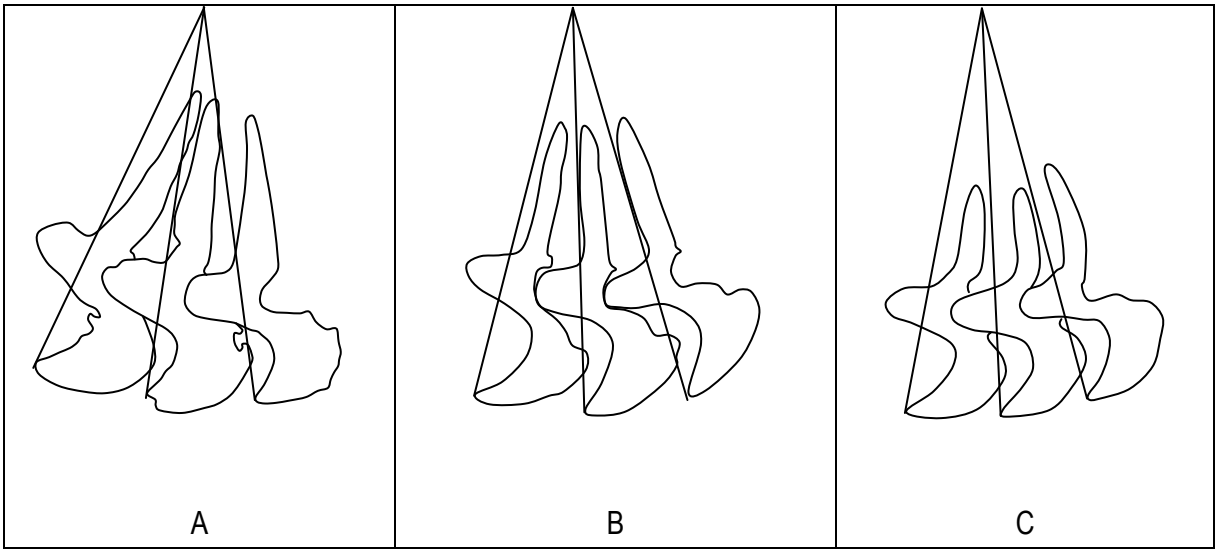


Figure 11.20: Diagrammatic drawings of the denticles of *Trichodina heterodentata* Duncan, 1977 as examples of the trichodinids collected and described during the current study (**A**), Kruger *et al.* (1993) (**B**) and Duncan (1977) (**C**). Note: (**B**) was redrawn from Kruger *et al.* (1993) and (**C**) was redrawn from Duncan (1977).

Although the skin of both fishes and tadpoles has a mucous coating, the skin of fishes and tadpoles differs greatly from one another. It is suggested that the trichodinids have to adapt to these conditions if the trichodinids hosted by one group (e.g. fishes) can form a viable population on the other group (e.g. tadpoles). Therefore the current study also aimed to determine if conditions on different host species have an influence on the morphological adaptations of trichodinids.

With this in mind, the description of trichodinids collected from the same locality on different hosts was compared with one another. Alternatively the morphological characteristics of trichodinids collected from different hosts during cross-transmission experiments were also compared with one another as any morphological differentiation observed from these trichodinid populations may indicate a morphological adaptation of the trichodinids to attach / live on the various host populations.

a) Description of the morphological characteristics of trichodinids hosted by different hosts collected at the same locality

Populations F3BuBV, F15PpBV and T35UuBV

An unknown *Barbus* species (Population F3BuBV), *Pseudocrenilabrus philander* (Population F15PpBV) and unidentified tadpoles (Population T35UuBV) were collected in 1982 from the Barrage Vaal. All these possible host groups hosted a *T. heterodentata* - infestation that shared various morphological characteristics, including an open sickle-shaped blade form and a thick blade connection (Table 11.5).

Table 11.5: Three different *Trichodina heterodentata* Duncan, 1977 populations collected from the Barrage Vaal during 1982 shared the characteristics listed below.

Blade form	• Open sickle-shaped
Distal surface	• Rounded • Sloping downwards in anterior direction
Tangent point	• Rounded
Blade connection	• Extended
Central part	• Robust • Rounded tip • Fitting tightly into preceding denticle
Blade connection	• Thick
Ray connection	• Short, same thickness than ray, extends almost directly from central part
Ray apophysis	• Small, when visible
Form of ray	• Straight, thick • Taper slightly to form rounded tip

Variation within these populations was also observed as the blade connection for both fish populations was delicate while the tadpole trichodinids had a thicker blade connection area (Table 11.6). In addition, the tadpole trichodinids shared features that were observed in only one of the two fish populations. For example the anterior margin of the tadpole trichodinids were smooth (same as Population F3BuBV) and extended to reach the y+1 axis (shared with Population F15PpBV).

Table 11.6: Variation on the morphological characteristics of the trichodinid denticles observed in three different *Trichodina heterodentata* Duncan, 1977 populations collected from the Barrage Vaal during 1982. Refer to Figure 5.1, Table 5.1 for more information on the host populations. Note: Characteristics shared between the fish populations are underlined, characteristics shared between F3BuBV and the tadpole population are indicated in bold and similar characteristics shared between F15PpBV and T35UuBV are indicated in italics.

Trichodinid host	Fishes		Tadpoles
Population number	F3BuBV	F15PpBV	T35UuBV
Anterior margin	• **Smooth** • Extending to y+1 axis, but does not reach y+1 axis	• Not smooth • *Extending to reach y+1 axis*	• **Smooth** • *Extending to reach y+1 axis*
Blade apophysis	• **Sometimes visible**	• Visible	• **Sometimes visible**
Posterior blade surface	• **Smooth** • **Deep L-shaped curve**	• Mostly smooth • Deep semi-lunar curve	• **Smooth** • **Deep L-shaped curve**
Blade connection	• Not strong (delicate)	• Not strong (delicate)	• Strong
Posterior projection	• Clearly visible	• Not clearly visible	• Visible in some
Protrusion on lower central part	• Not clearly visible	• *Sometimes visible*	• *Sometimes visible*
Central part	• **Extending more than halfway past y-axis**	• Extending halfway past y-axis	• **Extending more than halfway past y-axis**
Ray connection	• Not robust	• *Robust*	• *Robust*
Form of ray	• **Mostly parallel to y-axis**	• Curved in anterior direction	• **Mostly parallel to y-axis**
Ratio above and below x-axis	• 1 : 1.14	• *1 : 1.45*	• *1 : 1.45*

Populations F8CcLF and T36UuLF

Unidentified tadpoles (Population T36UuLF) and *Cyprinus carpio* (F8CcLF) were collected during October 1982 from the Lowveld Fish Production Facilities. It was determined that both these populations were infested with *T. heterodentata* and that various morphological characteristics were shared by the respective trichodinid populations (Table 11.7), although differences also occurred between these populations (Table 11.8).

Table 11.7: Two different *Trichodina heterodentata* Duncan, 1977 populations collected from the fish and tadpole populations at the Lowveld Fish Production Facilities during 1982 shared the characteristics listed below.

Blade form	• Open sickle-shaped
Distal surface	• Rounded • Sloping downwards in anterior direction
Tangent point	• Rounded
Anterior margin	• Rounded
Posterior blade surface	• Smooth
Blade connection	• Thick • Extended
Protrusion on lower central part	• Not clearly visible
Central part	• Robust • Rounded tip • Extending more than halfway past y-axis • Fitting tightly into preceding denticle
Ray connection	• Short, extends almost directly from central part
Ray apophysis	• Small, when present
Form of ray	• Straight • Taper prominently to sharp or rounded tip

Table 11.8: Variations in the morphological characteristics of the trichodinid denticles observed in two different *Trichodina heterodentata* Duncan, 1977 populations collected from the Lowveld Fish Production Facilities during 1982. Refer to Figure 5.1, Table 5.1 for more information on the host populations.

Trichodinid host	**Fish**	**Tadpoles**
Population number	**F8CcLF**	**T36UuLF**
Anterior margin	• Extending to or slightly beyond y+1 axis	• Extending slightly beyond y+1 axis
Blade apophysis	• Not visible	• Sometimes visible
Posterior blade surface	• Deep L-shaped curve	• Deep semi-lunar curve
Blade connection	• Thick, strong	• Not strong (delicate)
Posterior projection	• Small, when present	• Visible in some
Protrusion on lower central part	• Sometimes visible	• Visible in some
Central part	• Extending halfway past y-axis	• Extending more than halfway past y-axis
Ray connection	• Robust	• Not robust
Form of ray	• Thick • Mostly parallel to y-axis	• Delicate • Curved in anterior direction
Ratio above and below x-axis	• 1 : 1.40	• 1 : 1.21

b) Description of the morphological characteristics of trichodinids hosted on different host populations during two separate cross-transmission experiments

Populations F5BbTT, T6ApCF and T7ApTB

The morphological differentiation of trichodinids that were collected on various hosts utilised during the cross-transmission experiment (Chapter 7) will be discussed in this section. To recall, *Amietophrynus poweri* tadpoles (Population T7ApTB) were collected from the Thamalakane Bridge and these tadpoles were naturally infested with trichodinids (determined to be *T. heterodentata*). *Barbus barnardi* were collected from the Toteng Bridge and it was determined that these fish did not host a trichodinid infestation, although it is known that *T. heterodentata* may utilise *Barbus* species as a host. For example, Basson *et al.* (1983) collected *T. heterodentata* from *B. paludinosus* Peters 1852 and *B. trimaculatus* Peters 1852. In addition, naturally infested *B. eutaenia* Boulenger 1904 and *B. marequensis* Smith 1841 were also previously collected in southern Africa (Van As and Basson 1989).

The infested tadpoles were placed in the same water medium than uninfested fish. Fish were dissected on a daily basis to determine if a possible trichodinid infestation was present on these individuals. It was found that the fish hosted a trichodinid infestation within 48 hours of the experiment. The now infested fish were placed in the same water medium than uninfested tadpoles collected from a pond near the office area of the crocodile farm (Population T6ApCF). Skin and gill smears were prepared from the latter group on a daily basis to determine the degree of trichodinid infestation (if any) on these tadpoles. Once again, it was found that trichodinids were hosted by the original uninfested group within a short period of time.

Table 11.9: The morphological characteristics of the denticles of trichodinids collected from three different host populations (F5BbTT, T6ApCF, T7ApTB) used during a cross-transmission experiment were determined by means of the method proposed by Van As and Basson (1992). Refer to Figure 5.1, Table 5.1 for more information on the host populations.

Blade form	• Open sickle-shaped
Distal surface	• Rounded • Sloping downwards in anterior direction
Tangent point	• Rounded
Anterior margin	• Rounded
Posterior blade surface	• Smooth
Blade connection	• Thick • Extended
Central part	• Slender • Rounded tip • Fitting tightly into preceding denticle
Ray connection	• Short, extends almost directly from central part
Ray apophysis	• Small, when visible
Form of ray	• Straight • Taper slightly to form rounded tip

Most of the trichodinids examined within the above mentioned populations can be described as having a thick, extended blade connection and a short ray connection that seemed to extend directly from the central part (Table 11.9).

A great variation in the morphological characteristics also occurred between the three populations. For example the posterior blade surface was curve in a moderately to deep semi-lunar position in Population T7ApTB, a deep semi-lunar curved in the fish trichodinids while individuals from Population T6ApCF had a deep semi-lunar to an almost L-shaped posterior blade surface (Table 11.10).

Table 11.10: Variations in the morphological characteristics of the trichodinid denticles from three different host populations that were used during a cross-transmission experiment. Refer to Figure 5.1, Table 5.1 for more information on the host populations. Note: Characteristics shared between the fish population and the naturally uninfested tadpole population are underlined, characteristics shared between the tadpole populations are indicated in italics and similar characteristics of trichodinids from the fish population as well as Population T7ApTB are indicated in bold.

Trichodinid host	Fish	Tadpoles	
Population	F5BbTT naturally uninfested)	T6ApCF naturally uninfested)	T7ApTB (naturally infested)
Anterior margin	• Smooth • Extending beyond y+1 axis	• Smooth • *Extending to or slightly beyond y+1 axis*	• Not always smooth • *Extending to or slightly beyond y+1 axis*
Blade apophysis	• Visible	• *Sometimes visible*	• *Sometimes visible*
Posterior blade surface	• Deep semi-lunar curve	• Deep semi-lunar curve in some cases, mostly almost L-shaped	• Moderately, deep semi-lunar curve
Blade connection	• Strong	• *Not strong*	• *Not strong*
Posterior projection	• Not clearly visible	• Not clearly visible in all cases, small, when present	• Clearly visible in some
Protrusion on lower central part	• **Not clearly visible**	• Visible in some instances	• **Not clearly visible**
Central part	• Extending less than, or more than halfway past y-axis	• *Extending more than halfway past y-axis*	• *Extending more than halfway past y-axis*
Section above and below x-axis	• Almost similar	• *Not similar*	• *Not similar*
Ray connection	• Same thickness than ray	• *Same thickness as most of ray*	• *Same thickness as most of ray*
Form of ray	• Narrow, mostly of same thickness • Curved anteriorly	• Equal in thickness throughout • Parallel to y-axis in most instances	• Some slightly curved in posterior direction
Ratio above and below x-axis	• 1 : 1.27	• *1 : 1.33*	• *1 : 1.33*

Bear in mind that the only population that was naturally infested with a trichodinid species (*T. heterodentata*), was Population T7ApTB and that Populations F5BbTT and T6ApCF were infested during the experiment (directly or indirectly) from the trichodinid infestation found originally on Population T7ApTB. Therefore the trichodinids on all three populations indicate variation within a single trichodinid species, and the differences cannot be seen as an indication of the presence of more than one trichodinid species.

However, the two tadpole hosted trichodinid populations shared more characteristics between these two populations while the fish trichodinids showed less similarities when compared to the two tadpole populations. By only taking the above experiment into consideration, it is possible that one can come to the conclusion that the host group may influence the morphological adaptation of *T. heterodentata*.

Populations F16PpEB and T31XlBE

The morphological characteristics of fish hosted trichodinids (*Pseudocrenilabrus philander* - F16PpEB) and tadpoles (*Xenopus laevis* - T31XlBE) that were used during a cross-transmission experiment in 1992, does not indicate the same morphological variance as between the fish hosted versus the tadpole hosted trichodinids as mentioned in the preceding experiment. Note that most of the trichodinids examined during this experiment shared most morphological characteristics (Table 11.11) although variation also occurred (Table 11.12).

Less variation was observed between these two trichodinid populations in comparison to the trichodinids studied during the first mentioned cross-transmission experiment. By comparing the results of the morphological descriptions of the trichodinid denticles from the two cross-transmission experiments, the blade apophysis of both fish populations were visible in most instances in contrast to the blade apophysis that was only sometimes visible in all the tadpole trichodinids. The posterior projection of the fish trichodinids was not clearly visible although this feature was more readily visible in all three tadpole trichodinid populations.

Although these variations in morphological characteristics were observed between the fish trichodinids versus the tadpole trichodinids, one cannot conclude that *T. heterodentata* hosted on tadpoles will have a specific denticle form in comparison with fish trichodinids of the same species. This is due to the fact that the characteristics that seemed to be more visible in the fish trichodinids during the cross-transmission experiment were also observed in trichodinids that naturally infested tadpoles. For example, the blade apophysis of Populations T4AuBH (*Amietophrynus* species), T18AMaSS (*Amietia angolensis* tadpoles) and T21KsZZ (*Kassina senegalensis* tadpoles) was visible, although small. In

addition, the blade apophysis of Populations T10AgNC and T12AgND (*Amietophrynus gutturalis*) was visible in most instances. It is evident that a different host as per se is not a basis for the morphological differentiation of *T. heterodentata* populations.

Table 11.11: The morphological characteristics of the denticles of trichodinids collected from two host populations (F16PpEB and T31XlBE) used during a cross-transmission experiment were determined by means of the method proposed by Van As and Basson (1992). Refer to Figure 5.1, Table 5.1 for more information on the host populations.

Blade form	• Open sickle-shaped
Distal surface	• Rounded • Sloping downwards in anterior direction
Tangent point	• Rounded
Anterior margin	• Rounded, not smooth • Extending to or slightly beyond y+1 axis
Posterior blade surface	• Smooth • Deep semi-lunar to L-shaped curve
Blade connection	• Thick, strong • Extended
Protrusion on lower central part	• Not clearly visible
Central part	• Robust • Rounded tip • Extending more than halfway past y-axis • Fitting tightly into preceding denticle
Section above and below x-axis	• Not similar
Ray connection	• Short, robust, extends almost directly from central part
Form of ray	• Straight, strongly developed in most • Parallel to y-axis in most instances
Ratio above and below x-axis	• 1 : 1.23

Table 11.12: Variations in the morphological characteristics of the trichodinid denticles from two different host populations (F16PpEB and T31XlBE) that were used during a cross-transmission experiment. Refer to Figure 5.1, Table 5.1 for more information on the host populations.

	T31XlBE	F16PpEB
Blade apophysis	• Sometimes visible	• Visible
Posterior projection	• Visible in some	• Not clearly visible
Ray apophysis	• Clearly visible in some denticles	• Not always clearly visible
Form of ray	• Taper slightly to form rounded tip	• Thick at base, taper prominently to form round to sharp tip

3. Concluding remarks

The method proposed by Van As and Basson (1989, 1992) was used to gather more information on the morphological characteristics of the trichodinid denticles within each population as the principle differentiating characteristics of each genus within the family Trichodinidae are associated with the shape and dimensions of the denticles.

Although differences were observed between the individual trichodinid populations, morphological variation was also observed within a single population and even between individuals. The morphological characteristics of trichodinids collected from different hosts during cross-transmission experiments were used to determine if trichodinids from one source (originally infested host population) adapt morphologically on the skin and / or gills of another host species (be it fish or tadpoles). Additionally, the morphological characteristics of trichodinids collected from the same locality on different host species were also evaluated.

3.1 Trichodinids from different hosts collected at same locality

Populations F3BuBV, F15PpBV and T35UuBV were collected at the Barrage Vaal on the same day. The morphological characteristics of trichodinids examined from all three populations indicated similarities as well as dissimilarities. One should keep in mind that differences within the two fish trichodinid groups were also observed. The same can be said for Populations F8CcLF and T36UuLF that were collected at the Lowveld Fisheries as a few morphological features were observed in both populations while other features were only observed in fish trichodinids or tadpole trichodinids.

With the above in mind, it is suggested that trichodinids do not adapt to fish hosts or tadpole hosts in a specific manner when the above mentioned characteristics are studied.

3.2 Description of the morphological characteristics of trichodinids hosted on different host populations during two separate cross-transmission experiments

Trichodinid populations from two different cross-transmission experiments were also discussed in this section.

Populations F5BbTT, T6ApCF and T7ApTB

To recall, Population T7ApTB was naturally infested with trichodinids and Population F5BbTT was infested with trichodinids originally from Population T7ApTB. Population T6ApCF was naturally uninfested with trichodinids and were infested with trichodinids by means of a cross-transmission experiment between Populations F5BbTT and T6ApCF. Thus, the trichodinids were originally hosted on tadpoles and transmitted to fish, and back to tadpoles.

It was found that many morphological similarities existed between the three trichodinid populations (Table 11.9). Dissimilarities were also observed. For example, the posterior blade surface of:

a) Population T7ApTB were moderately to deep semi-lunar curved

b) The fish trichodinids had a deep semi-lunar blade surface, and

c) The naturally uninfested tadpole population were deep semi-lunar curved in some cases to such an extent that it presented almost an L-shaped curve (Table 11.10).

Populations F16PpEB and T31XIBE

Variation in the morphological characteristics of trichodinids transmitted from the originally infested hosts to the naturally uninfested hosts was also observed. For example, the ray of the tadpole trichodinids tapered slightly to form rounded tips while the fish trichodinids ended either round or sharp (Table 11.12).

By comparing the dissimilarities observed in both mentioned cross-transmission experiments, the blade apophysis of both fish populations were visible in all the denticles examined, while the same feature were only sometimes visible in the three tadpole trichodinid populations (Table 11.13). Although similarities were observed between fish trichodinids from the two fish populations that took part in two different cross-transmission experiments, it cannot be stated that trichodinids morphologically adapt to fish hosts by means of these similar features. This is said as naturally infested tadpoles also contained these features. For example, the blade apophysis of the tadpole trichodinid populations studied during the present study varied to a great extent. The blade apophysis of a small portion of the tadpole trichodinids examined during this study could not be observed, was not clearly visible or only sometimes visible (14.29%) (Figure 11.8). In contrast, most of the blade apophyses of the examined tadpole trichodinids were small, when present (61.90%) or prominent (9.52%). Thus, the differences observed in the fish trichodinids versus the tadpole trichodinids that took part in the two cross-transmission experiments cannot be used to differentiate between fish- or tadpole trichodinids. It is also not suggested that trichodinids adapt to a host group by means of these morphological features.

By evaluating the morphological characteristics of three consecutive denticles within a single trichodinid against the findings from other populations examined during this study, it is suggested that all the studied trichodinids represent one species as no distinct characteristics were observed between different populations.

Table 11.13: Differences observed within trichodinid populations that took part in cross-transmission experiments. Experiment 1 consisted of Populations F5BbTT, T6ApCF and T7ApTB. Experiment 2 consisted of Populations F16PpEB and T31XlBE. Refer to Figure 5.1, Table 5.1 for more information on the host populations. Note: Characteristics shared between Populations F5BbTT and T6ApCF are underlined, Populations T6ApCF and T7ApTB are in italics, Populations F5BbTT and T7ApTB are indicated in bold. Characteristics shared between the same host groups (for example either fishes or tadpoles) are indicated in uppercase. ☐ indicates that the specific characteristic were similar for the trichodinids collected from the various hosts during a specific experiment.

Trichodinid host	Fish		Tadpoles		
Population	F5BbTT	F16PpEB	T31XlBE	T6ApCF	T7ApTB
Experiment	Experiment 1	Experiment 2	Experiment 2	Experiment 1	Experiment 1
Anterior margin	• Smooth • Extending beyond y+1 axis			• Smooth • *Extending to or slightly beyond y+1 axis*	• Not always smooth • *Extending to or slightly beyond y+1 axis*
Blade apophysis	• VISIBLE	• VISIBLE	• SOMETIMES VISIBLE	• *SOMETIMES VISIBLE*	• *SOMETIMES VISIBLE*
Posterior blade surface	• Deep semi-lunar curve			• Deep semi-lunar curve in some cases, mostly almost L-shaped	• Moderately, deep semi-lunar curve
Blade connection	• Strong			• *Not strong*	• *Not strong*
Posterior projection	• NOT CLEARLY VISIBLE	• NOT CLEARLY VISIBLE	• Visible in some	• Not clearly visible in all cases, small, when present	• Clearly visible in some
Protrusion on lower central part	• **Not clearly visible**			• Visible in some instances	• **Not clearly visible**
Central part	• Extending less than, or more than halfway past y-axis			• *Extending more than halfway past y-axis*	• *Extending more than halfway past y-axis*
Section above and below x-axis	• Almost similar			• *Not similar*	• *Not similar*
Ray apophysis		• Not always clearly visible	• Clearly visible in some denticles		
Ray connection	• Same thickness than ray			• *Same thickness as most of ray*	• *Same thickness as most of ray*
Form of ray	• Narrow, mostly of same thickness • Curved anteriorly	• Thick at base, taper prominently to form round to sharp tip	• Taper slightly to form rounded tip	• Equal in thickness throughout • Parallel to y-axis in most instances	• Some slightly curved in posterior direction
Ratio above and below x-axis	• 1 : 1.27			• *1 : 1.33*	• *1 : 1.33*

Chapter **12**:

Conclusion

'…science never solves a problem without creating ten more…'

George Shaw

Amphibians are the most threatened class of vertebrates globally and high mortality rates led to the declining / extinction of many species in the last few decades (Stuart *et al.* 2004, Du Preez and Carruthers 2009). Amphibians and in particular anurans, are used as biological indicators of the general environmental health condition due to their sensitivity to the deterioration of terrestrial and aquatic ecosystems (Du Preez and Carruthers 2009).

Human activities such as over utilisation and habitat loss threaten almost 50% of the rapidly declining species worldwide (Stuart *et al.* 2004). However, the cause of the recent increase in extinctions is still debatable as some of the areas affected by the extinctions are located in undisturbed regions (Du Preez and Carruthers 2009). In addition to the human activities, factors such as predation, climate change, the sporadic outbreaks of chytrid and the depletion of the stratospheric ozone further add to the survival pressures on amphibians (Du Preez and Carruthers 2009). The latter authors also mentioned that anurans are targeted by many parasites such as trematodes, cestodes, mites, leeches and various protozoans as a host.

One such protozoan is the endotrichodinid *Trichodina dampanula* that feeds mainly on the debris within the urinary bladder of adult *Amietophrynus gutturalis*. Although no ectotrichodinids make use of the skin of adult anurans for attachment, representatives from this group are hosted on the skin and / or gills of tadpoles (Kruger 1992). For example, many authors collected various ectotrichodinid species on various tadpoles (*Bufo, Rana* and *Rinella* species) in countries such as China, Brazil, Cuba and Uganda (Table 2.6).

Lom (1961) suggested that the trichodinids hosted by tadpoles are fish trichodinids that utilise tadpoles as a temporary host as tadpoles are not to be found in the water medium throughout the year and no cyst stage for this protozoan group exist. Therefore, infested tadpoles can only be found in a water body containing infested fishes (Lom 1961).

For example, *T. nigra* was hosted by tadpoles in China (Chen 1963) as well as the Czech Republic (Lom 1961), whilst *T. reticulata* was collected from *Rana temporaria* tadpoles in Olsztyn (Poland) by Kazubski (1988). These trichodinids are known fish ectotrichodinids and were also collected on fishes in South Africa. For instance *T. reticulata* was collected from *Oncorhynchus mykiss* while *T. nigra* was collected on *Oreochromis mossambicus*, *Pseudocrenilabrus philander*, *Tilapia sparrmanii* and *Cyprinus carpio* (Basson, pers. comm*).

Thurston (1970) observed trichodinids on *Xenopus laevis* tadpoles in Uganda, but could not identify the trichodinids to species level. Kruger (1992) and Kruger *et al.* (1993) collected trichodinids from various

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tadpole species in southern Africa and concluded that only one trichodinid species was hosted by the examined tadpoles, i.e. *T. heterodentata*. *Trichodina heterodentata* is a known fish trichodinid that exhibit a considerable variation with regards to the shape of the denticles within populations as well as individuals. This trichodinid species was encountered on a wide range of fish hosts (Table 2.7) but seems to favour cichlid fishes when available (Basson and Van As 1987).

1. Present study

Due to the fact that *T. nigra*, *T. reticulata* and *T. heterodentata* are hosted by fishes in many countries and attach to the skin and / or gills to various tadpole species in other countries, the current study was undertaken to determine if trichodinids hosted by tadpoles in southern Africa:

- Represents only one trichodinid species
- Are known fish trichodinids that are temporarily hosted by tadpoles
- Can be morphologically distinguished from fish trichodinids.

In addition, the current study also aimed to determine if trichodinids prefer younger or older tadpoles for attachment and whether certain host species will have a higher degree of trichodinid infestation under experimental conditions.

Trichodinid material from various fish and tadpole populations in southern Africa was collected and examined during the present study, as described in the preceding chapters. A short summary of the discussion of each of the experimental chapters (Chapters 6-8) and morphological chapters (Chapters 9-11) will be presented in the following section. Additionally, suggestions for future studies will also be given for each of the studied sections.

1.1. Degree of trichodinid infestation on different host populations under experimental conditions

The trichodinid infestation on each of the infested populations persisted throughout the experiment and the degree of infestation was either constant, or increased on a daily basis. However, the degree of infestation method as proposed by Van As and Basson (1992) did not give a clear reflection on the increasing number of trichodinids on the hosts as the number of trichodinids on the hosts increased rapidly in comparison to the degree of infestation.

Suggestions for future studies

It is not proposed that the degree of infestation as suggested by Van As and Basson (1992) should be discarded, but rather that the latter method should be used to obtain information on the possible trichodinid infestation on possible hosts under natural conditions. It is proposed that the trichodinids on each of the microscopic smears should be counted to determine the infestation of trichodinids under experimental conditions as this method seems to be more accurate.

Kruger (1992) suggested that some hosts within a population can be more resistant to a trichodinid infestation than other individuals from the same population. Additionally, the manner of trichodinid infestation under natural and experimental conditions should be kept in mind. Trichodinids are transferred from one host to another be means of direct transmission and reproduce rapidly in relative warmer waters. Therefore, hosts that occur in close proximity to each other near the surface of the water medium may host more trichodinids. Younger tadpoles usually occur in high numbers in shallow waters and therefore it is suggested that these tadpoles will host more trichodinids than older tadpoles in natural conditions. Kruger (1992) found that the degree of a trichodinid infestation on older tadpoles were usually less than on the skin of younger tadpoles of the same species. It is therefore suggested that a greater number of tadpoles and or fish within each population for several age groups should be taken into account on a daily basis. It is suggested that at least 30 individuals are used to prepare skin and gill smears on a daily basis.

It is further proposed that the timeframe of similar experiments should be prolonged until the trichodinid infestation decreases or disappears from the host population. This means that more hosts should be collected from each potential host population during possible future studies.

Alternatively, the number of trichodinids could be counted on living tadpoles by using a light microscope. This will aid to determine the infestation rate on each tadpole throughout the experiment as no tadpoles need to be dissected. Each individual tadpole should be marked (e.g. cutting of tail fins / toes in older individuals, or by means of nitrogen) to simplify identification.

1.2. Cross-transmission experiments

The two cross-transmission experiments indicated that the trichodinids that naturally occurred on tadpoles could form a viable population on the naturally uninfested hosts as the trichodinids were transmissible to the uninfested host populations (be it fish or tadpoles) under experimental conditions. This could indicate that the trichodinids naturally infesting tadpoles may also be found naturally on fishes.

Suggestions for future studies

These experiments were undertaken only for a short period of time as the number of hosts within each of the host populations was limited. It is therefore suggested that a similar experiment should be undertaken, but that the following adaptations should be included:

Thirty host individuals from each population should be dissected on a daily basis for the preparation of skin and gills smears. Additionally, the experimental period should be prolonged to at least 14 days.

It is suggested that tadpoles and or fish that are to be used as the uninfested host populations should be bathed in a 15% salt solution for a period of ten minutes in order to render them trichodinid free. The bathed hosts should be transferred to a clean tank filled with aged, filtered water. An infested individual should be euthanized by MS222 (not harmful to trichodinids) in a small volume of water as the trichodinids will detach from the deceased host. Kruger (1989) determined that detached *T. heterodentata* can survive for 20 – 30 minutes in clean water and therefore the euthanized individual should be removed from the water medium as soon as possible and the water containing the free swimming trichodinids should be poured into the tank hosting the bathed individuals. Skin and gill smears should be prepared of 30 bathed individuals on a daily basis for the following consecutive days. The above will aid in determining the multiplication tempo of the trichodinids in a closed system under experimental conditions. This method will also aid in gaining more information on the viability of the trichodinid population (if present) on the specific host under experimental conditions. In addition, the number of days that a trichodinid infestation can be hosted by the specific host under these experimental conditions can be determined.

Alternatively, a gauze / porous obstruction can be positioned in the middle of a tank filled with aged filtered water. The material used for the obstruction should be of such a manner that tadpoles will not be able to move from the one side to the other. However, the openings should be large enough for trichodinids to pass to the other side. A few infested tadpoles (or fish) should be placed on one side of the obstruction while uninfested fish (or tadpoles) are placed on the other side of the obstruction. Once again, the number of trichodinids on the skin and or gill smears prepared of individuals from the original infested as well as original uninfested host groups should be counted on a daily basis. This will aid to determine if trichodinids will leave a current host in search of a new, uninfested host or whether the trichodinids will only utilise hosts in a very close proximity. This will ensure that the trichodinids that are collected on the naturally uninfested hosts have to move towards the naturally infested host population in search of a new suitable host. It will also be interesting to determine if both populations (originally infested and originally uninfested) will host the same number of trichodinids within a given period.

Therefore, it is important to place the same number of tadpoles (or fish) of the same age (size) on each side of the obstruction. It is also preferable that the obstruction should be placed in such a manner that both sides (original infested and original uninfested) are of the same size. This experimental set-up should also aid in determining if the original uninfested host population is a suitable host for the attachment of the trichodinid species being studied.

It is also suggested that the originally infested host population should be removed from the system after a trichodinid infestation (if any) is observed on the naturally uninfested host population.

1.3. Trichodinid infestation intensity on tadpoles under experimental conditions

During these experiments, trichodinids were observed on the skin of froglets that already utilised the floating objects in the tank. This was in contrast to the findings of Lom (1961) and Kruger (1989) that mentioned that trichodinids detach from tadpoles before the metamorphosis process is completed. However, the tadpoles utilised during the present study were kept in experimental conditions, resulting in a closed system containing only a number of possible hosts. The trichodinid infestation on these older froglets may thus be due to the absence of possible attachment areas on younger tadpoles in the water medium, influencing the findings of the current study.

Suggestions for future studies

It is suggested that the degree of infestation on different tadpole sizes should be assessed for individuals collected under natural circumstances. The tadpoles should be measured upon collection and skin and gill smears of the tadpoles should be made in the field or as soon as possible. This will limit the transmission of trichodinids to other tadpoles in captivity.

Living tadpoles should also be examined under a light microscope upon collection to determine the position of trichodinid infestation as soon as the tadpoles are collected to ensure that the noted position of attachment is the natural position of attachment.

1.4. Population description

a) Body measurements (method proposed by Lom 1958)

A great variance in the measurements was obtained during the present study from most of the standard characteristics used to describe trichodinid populations. However, these variances within the populations corresponded to measurements previously obtained for *T. heterodentata* (Duncan 1977, Kruger 1992) and it was therefore suggested that the trichodinids examined during the current study

represented specimens from this species that are known for its great variance (Duncan 1977). It was also found that tadpole trichodinids were usually smaller in size than fish trichodinids.

b) Area of the blade, ray and central part within a denticle (method proposed by Gong *et al.* 2005)

The area of the rays, central parts and blades of trichodinids were determined by means of the ImageJ software and the results were compared to the findings for various trichodinid species by Gong *et al.* (2005). It was found that the studied material corresponded with the results for *T. heterodentata* by Gong *et al.* (2005), although variations between the populations, between fish hosted and tadpole hosted trichodinids and even within a single population were observed.

c) Three consecutive denticles (method proposed by Van As and Basson 1992)

Three consecutive denticles of trichodinids from various host populations were drawn as proposed by Van As and Basson (1992) in order to gather more information on the morphological characteristics of the trichodinid denticles. It was determined that individual trichodinid populations differed from one another and that differences were observed within a single population and even within a single specimen.

In general, the average fish trichodinids differed slightly from the average tadpole trichodinids by having a:

- Anterior margin that extends slightly beyond the y+1 axis,
- Blade apophysis that is usually not observed or not clearly visible,
- Blade connection that is strong,
- Posterior projection that is not clearly visible,
- Section below the x-axis that is steeper sloped,
- Ray connection that is the same thickness than the ray, and
- Ray apophysis that is not clearly visible or small, when visible.

In contrast, most tadpole trichodinids had the following characteristics (Figure 12.1):

- Anterior margin that extends to or slightly beyond the y+1 axis,
- Blade apophysis that is small, when present,
- Blade connection that is thick, but not as strong as the fish trichodinids,
- Posterior projection that is small, when visible,

- Section below the x-axis that is steeper sloped / the base of section below the x-axis that is steeper sloped / section below the x-axis that is steeper sloped and have a flattened top,
- Ray connection that is narrower than ray, and the
- Ray apophysis that is clearly visible, and mostly prominent.

It should be kept in mind that these characteristics were not only seen in fish trichodinids, or only in tadpole trichodinids and therefore one cannot assign a trichodinid to either host group by the above mentioned characteristics.

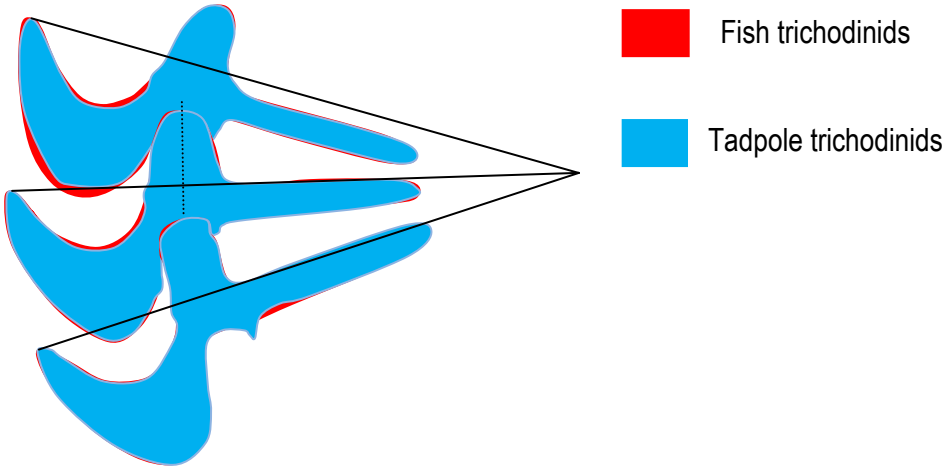

Figure 12.1: Illustration of the general differences observed for *Trichodina heterodentata* Duncan, 1977 collected from various fish and tadpole populations.

Suggestions for future studies

A limitation of the current study was that only a small number of trichodinids were examined for certain populations as only a limited number of material was available. *Trichodina heterodentata* is known to exhibit great variation in the characteristics to be measured as proposed by Lom (1961). The same was observed when the methods proposed by Gong *et al.* (2005) and Van As and Basson (1992) were undertaken. It is therefore it is proposed that future studies should assess as many individual adult trichodinids from each population as possible.

As *T. heterodentata* is known for its considerable variation in the shape of the denticles, it is suggested that more denticles within an individual trichodinid should also be taken into account in future studies using the method proposed by Gong *et al.* (2005).

Alternative characteristics, such as the tangent point diameter and tip of ray diameter that is not included in the standard trichodinid descriptions, should also be measured as this aided the author to

determine the loop of the denticle ring and compare the findings between the tadpole and fish trichodinids by means of a method proposed by Gong *et al.* (2005).

In addition, a comparison between different parts of the denticles, such as the length of the blade versus the length of the ray, length of the central part, width of the blade, etc. should also be investigated, especially in populations with great similarities. The ratio of these additional measurements can also be used to describe differences (if present) between various populations. It is suggested that a line should be drawn perpendicular to the apex of the blade (Figure 12.2). Two additional lines parallel to the above should also be drawn, one at the deepest point of curve, and the other touching the tangent point. The length between each of these lines should be determined as the ratio between adp (length above deepest point of cure) and bdp (length below deepest point of cure) can indicate the deepness of the ray curvature. It is also suggested that one should divide the ray (from area behind ray apophysis to ray tip) into four equal parts as the ratio between these four points (q1, q2, q3, q4) will assist in more accurately indicating the thickening or thinning of the ray. Please refer to Figure 12.2 for an indication of the standard measurements / characteristics as well as proposed additional measures to be taken into account for future trichodinid studies.

2. **Concluding remarks**

To conclude:

- The tadpole trichodinids collected from various populations represented only one trichodinid species.
- The trichodinid species occurring on tadpoles in southern Africa was identified as *T. heterodentata*, a trichodinid species that usually attach to the skin and / or gills of various fish species in southern Africa, as well as globally.
- Although most tadpole trichodinids that were studied shared certain morphological characteristics, such as an anterior margin that extended slightly beyond the y+1 axis, the same characteristics were observed in individuals from the fish trichodinids.

Most tadpole trichodinids were, however, smaller in relation to the fish hosted trichodinids, an observation also made by Kruger (1992).

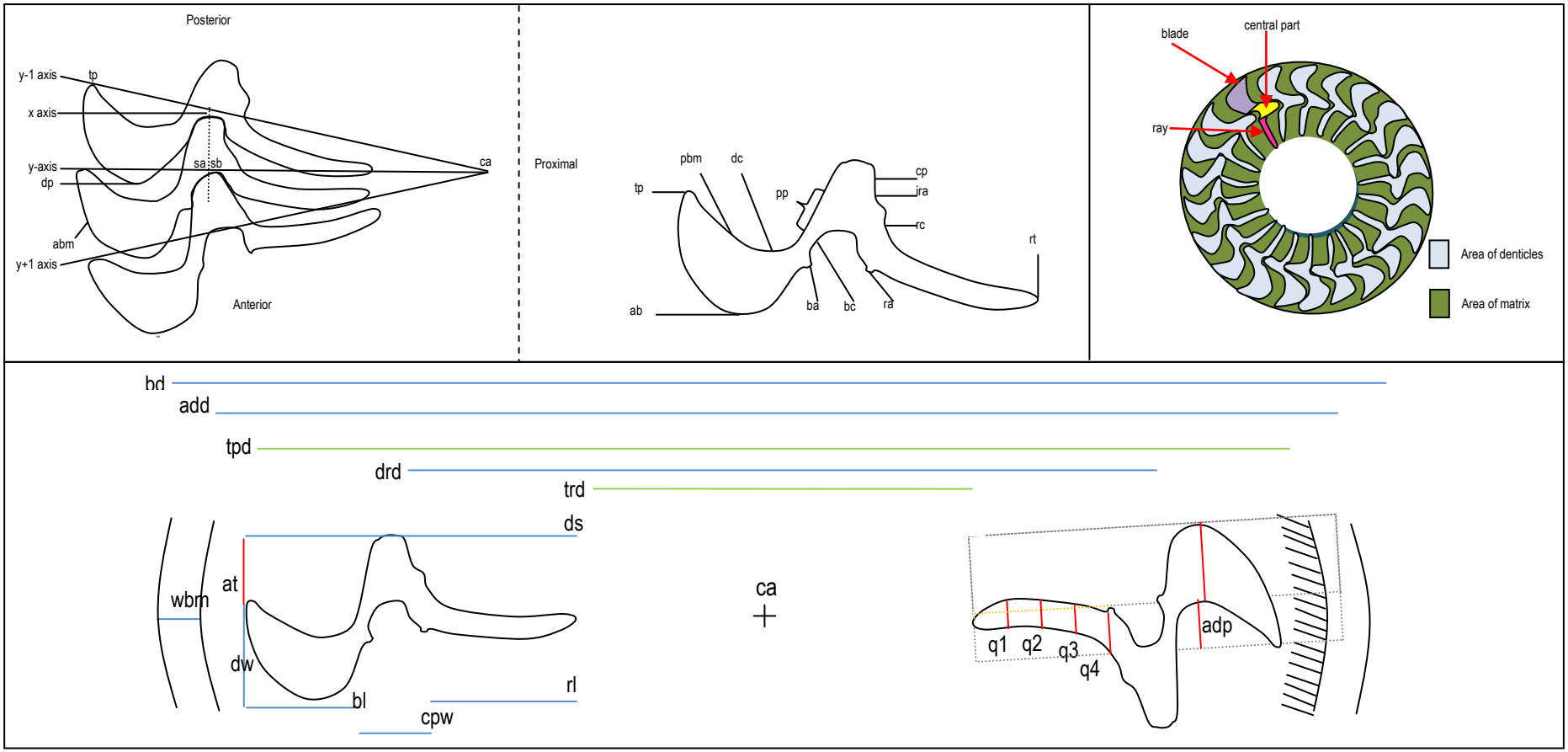

Figure 12.2: Schematic diagram of trichodinid denticles to indicate characteristics measured and / or described in the present study as well as additional characteristics suggested by the present author to be taken into consideration for future studies.

Abbreviations: **ab**: Apex of blade; **abm:** Anterior blade margin; **add:** Adhesive disc diameter; **adp:** length above deepest point of curve; at: length above tangent point; **ba:** Blade apophysis; **bc:** Section connecting blade and central part; **bd:** Body diameter; **bdp:** length below deepest point of curve; **bl:** Blade length; **ca:** Centre of adhesive disc; **ccp:** Section connecting central part and ray; **cp:** Central conical part; **cpw:** Central part width; **dbm:** Distal blade margin; **dp:** Deepest point of curve relative to apex; **drd:** Denticle ring diameter; **ds:** Denticle span; **dw:** Denticle width; **ira:** Lower central part indentation; **pbm:** Posterior blade margin; **pp:** Posterior projection; **q1 – q4:** Area behind the ray apophysis should be divided into four equal parts; **ra:** Ray apophysis; **rc:** Ray connection; **rl:** Ray length; **sa:** Section of central part above x axis; **sb:** Section of central part below x axis; **tp:** Tangent point; **tpd:** Tangent point diameter; **trd:** Tip of ray diameter; **wbm:** Width of border membrane. **Note:** The number of denticles within a denticle ring as well as the number of radial pins per denticle should also be counted. **Blue**: Standard measurements to be noted; **Green**: Additional measurements noted during present study; **Red**: Proposed additional measures to be noted for future studies.

Chapter **13**:

References

'…look deep into nature, and then you will understand everything better…'

Albert Einstein

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Appendixes

'…somewhere, something incredible is waiting to be known…'

Carl Sagan

Information on the body, tail and leg length as well as the degree of trichodinid infestation of various host populations. Please refer to Figure 5.1 and Table 5.1 for more information on the host population. All measurements is = mm.

Abbreviations: **ExpDay**: Experimental day; **HstNo**: individual host examined for the presence of a trichodinid infestation; **BodyL**: Body length; **TailL**: Tail length, **LegL**: Leg length; **SNo**: Number of trichodinids counted on skin smears; **GNo**: Number of trichodinids counted on gill smears; **SGNo**: Number of trichodinids counted on skin and gill smears combined; **SDeg**: Degree of trichodinid infestation on skin smears; **GDeg**: Degree of trichodinid infestation on gill smears; **SGDeg**: Degree of trichodinid infestation on skin and gill smears combined.

Population number	ExpDay	HstNo	BodyL	TailL	LegL	SNo	GNo	SGNo	SDeg	GDeg	SGDeg
T5ApNB	1	1	4.5	6.5	0			0			0
		2	4.5	6.5	0			0			0
		3	5	5	0			0			0
		4	5	6.5	0			0			0
		5	5	6.5	0			0			0
		6	5.5	8	0			1			1
		7	5.5	6.5	0			0			0
		8	6	7.5	0			0			0
		9	6	8	0			0			0
		10	6	8	0			0			0
		11	8	5	0			0			0
	2	1	5	7	0			0			0
		2	5	7	0			2			2
		3	5	7	0			6			6
		4	5	7	0			0			0
		5	5	8	0			0			0
		6	5.5	7.5	0			1			1
		7	5.5	8	0			0			0
		8	6	8	0			7			1
		9	6	9	0			0			0
		10	7	8	0			1			1
	3	1	4	6	0			7			1
		2	4	6	0			0			0
		3	4	7	0			3			1
		4	5	6	0			2			1
		5	5	7	0			40			2
		6	5	7	0			4			1
		7	5	7	0			0			0
		8	5	7	0			1			1
		9	5.5	7.5	0			1			1
		10	6	8	0			3			1
	4	1	4.5	6.5	0			4			1
		2	4.5	6.5	0			1			1

Population number	ExpDay	HstNo	BodyL	TailL	LegL	SNo	GNo	SGNo	SDeg	GDeg	SGDeg
T5ApNB (continue)		3	5	6	0			6			1
		4	6	7	0			1			1
		5	6	7	0			0			0
	5	1	5	6	0			110			4
		2	5	6	0			0			0
		3	5	7	0			31			2
		4	5	7	0			6			1
		5	5	7	0			41			2
		6	5	7	0			3			1
		7	6	8	0			34			2
		8	6	7	0			0			0
	6	1	4	4	0			7			1
		2	4.5	5	0			0			0
		3	5	5	0			1			1
		4	5	5.5	0			0			0
		5	5	6	0			12			2
		6	5.5	6	0			0			0
		7	5.5	6.5	0			8			1
	7	1	5	5	0			3			1
		2	5	5	0			33			2
		3	6	6	0			61			3
		4	6	6	0			10			1
		5	6	6	0			36			2
		6	6	7	0			3			1
		7	6	7	0			32			2
		8	7	7	0			4			1
		9	7	7	0			63			3
		10	7	7.5	0			7			1
	8	1	5	6	0			4			1
		2	6	6	0			133			4
		3	6	6	0			141			4
		4	6	7	0			200			5
		5	6	8	0			52			3
		6	6.5	6.5	0			34			2
		7	6.5	6.5	0			6			1
		8	6.5	7.5	0			17			2
		9	7	8	0			3			1
		10	8	9	0			152			4
	9	1	6	6	0			145			4
		2	6	7	0			105			4
		3	6	7	0			86			3

Population number	ExpDay	HstNo	BodyL	TailL	LegL	SNo	GNo	SGNo	SDeg	GDeg	SGDeg
T5ApNB (continue)		4	6.5	6.5	0			85			3
		5	6.5	6.5	0			21			2
		6	6.5	6.5	0			38			2
		7	6.5	7.5	0			10			1
		8	7	7	0			20			2
		9	7	7	0			7			1
		10	7.5	7.5	0			82			3
T6ApCF	1	1	5	10	0	0	0	0	0	0	0
		2	5.5	11	0	0	0	0	0	0	0
		3	6	14	0	0	0	0	0	0	0
		4	7	15	1	0	0	0	0	0	0
		5	7.5	15	1	0	0	0	0	0	0
	2	1	5	11	0	0	0	0	0	0	0
		2	7	14	0	0	0	0	0	0	0
		3	7	15	0	0	0	0	0	0	0
		4	7.5	15	1	0	0	0	0	0	0
		5	8	16	0	0	0	0	0	0	0
	3	1	5	10.5	0	0	0	0	0	0	0
		2	6	13	0	0	0	0	0	0	0
		3	6.5	13	0	0	0	0	0	0	0
		4	6.5	14	0	0	0	0	0	0	0
		5	7	14.5	1	0	0	0	0	0	0
		6	7	15.5	0	0	0	0	0	0	0
		7	7.5	16	1	0	0	0	0	0	0
		8	8	17	2	0	0	0	0	0	0
		9	8	18	2	0	0	0	0	0	0
		10	8	18	2	0	0	0	0	0	0
	4	1	5	10	0	0	0	0	0	0	0
		2	5	11	0	0	0	0	0	0	0
		3	5	11	0	0	0	0	0	0	0
		4	6	13	0	0	0	0	0	0	0
		5	6.5	13	0	0	0	0	0	0	0
		6	6.5	13	0	0	0	0	0	0	0
		7	7	14	0	0	0	0	0	0	0
		8	7	14	1	0	0	0	0	0	0
		9	7	15	1	0	0	0	0	0	0
		10	7.5	16	1	0	0	0	0	0	0
T7ApTB	1	1	4	4	0				0	2	2
		2	4	5	0				0	2	2
		3	4.5	6.5	0				0	2	2
		4	4.5	5	0				0	1	1

Population number	ExpDay	HstNo	BodyL	TailL	LegL	SNo	GNo	SGNo	SDeg	GDeg	SGDeg
T7ApTB (continue)		5	5	6	0				0	2	2
		6	6	7	0				0	2	2
		7	6	6.5	0				0	2	2
		8	7	10.5	0				0	2	2
		9	7.5	10.5	0				0	2	2
		10	9	9.5	0				0	2	2
	2	1	5	5	0				0	3	3
		2	5	6	0				0	2	2
		3	5	5	0				0	2	2
		4	5.5	5.5	0				0	2	2
		5	5.5	5.5	0				0	3	3
		6	6	6	0				0	1	1
		7	6	6	0				0	2	2
		8	6	6	0				0	2	2
		9	6.5	6.5	0				0	3	3
		10	6.5	6.5	0				0	3	3
	3	1	4	7	0				2	2	2
		2	4	5.5	0				1	1	1
		3	4.5	5.5	0				1	2	2
		4	5	6	0				1	3	3
		5	5	7	0				2	0	2
		6	5	6	0				1	3	3
		7	5.5	5.5	0				1	2	2
		8	5.5	5.5	0				2	3	3
		9	5.5	6.5	0				1	2	2
		10	8	9	0				2	2	2
	4	1	4	4	0				1	2	2
		2	4.5	4.5	0				1	3	3
		3	5	6	0				2	2	2
		4	5	7	0				0	2	2
		5	5	6	0				1	3	3
		6	5	6	0				1	3	3
		7	5.5	6.5	0				2	2	2
		8	6	7	0				0	3	3
		9	6	6	0				2	1	2
		10	7.5	7.5	0				2	3	3
	5	1	3.5	4.5	0				0	2	2
		2	5	7	0				2	1	2
		3	5.5	5.5	0				1	3	3
		4	5.5	5.5	0				0	1	1
		5	6	6	0				1	2	2

Population number	ExpDay	HstNo	BodyL	TailL	LegL	SNo	GNo	SGNo	SDeg	GDeg	SGDeg
T7ApTB (continue)		6	6	6	0				2	1	2
		7	6	8	0				0	3	3
		8	6	6	0				1	2	2
		9	6	7	0				2	2	2
		10	9	10	1.5				1	1	1
	6	0									
	7	1	4.5	3.5	0				0	2	2
		2	5	7	0				0	2	2
		3	5	6	0				3	3	3
		4	5	8	0				2	2	2
		5	5	5	0				2	1	2
		6	5.5	6.5	0				0	3	3
		7	6	6	0				3	3	3
		8	7	6.5	0				2	3	3
		9	7	6	0				2	1	2
		10	7	8	0				1	1	1
	8	1	4.5	5	0				4	3	4
		2	4.5	6.5	0				4	4	4
		3	5	5	0				4	3	4
		4	5	6	0				4	3	4
		5	5	8	0				5	4	5
		6	5	6	0				4	2	4
		7	5.5	7.5	0				3	3	3
		8	6	7	0				4	4	4
		9	6	6.5	1				4	5	5
		10	7	7	0				4	3	4
T9ApCF	1	1	5	10	0			2			1
		2	6	12	0			0			0
		3	7	14	0			0			0
		4	7.5	15	0			0			0
		5	8	15	1			0			0
	2	1	6	12	0			5			1
		2	6	14	1			7			1
		3	6.5	13.5	0			7			1
		4	7	15	0			4			1
		5	7.5	15	1			0			0
	3	1	6	12	0			21			2
		2	6	13	0			64			3
		3	7	15	0			8			1
		4	8	16	1			22			2
		5	8	16	1			9			1

Population number	ExpDay	HstNo	BodyL	TailL	LegL	SNo	GNo	SGNo	SDeg	GDeg	SGDeg
T9ApCF (continue)	4	1	5	10	0			2			1
		2	5.5	11.5	0			19			1
		3	5.5	12	0			37			2
		4	6	12.5	0			43			2
		5	6	13	0			50			2
		6	6.5	13	0			33			2
		7	6.5	14	0			12			2
		8	7	14	0			29			2
		9	7	14	0			13			2
		10	7.5	15	0			47			2
	5	1	5.5	11	0			27			2
		2	5.5	12	0			5			1
		3	6	12	0			23			2
		4	6	13	0			15			2
		5	6.5	13	0			3			1
		6	7	17	0			34			2
		7	7.5	15	0			3			1
		8	8	17	1			26			2
		9	8.5	17	0			10			1
		10	8.5	17	1			11			2
T10AgNC(a)	1	1	5	6	0	0	18	18	0	2	2
		2	5	7	0	0	0	0	0	0	0
		3	5	8	0	0	4	4	0	1	1
		4	5	9	0	0	5	5	0	1	1
		5	5.5	7.5	0	0	14	14	0	2	2
		6	6	7	0	0	2	2	0	1	1
		7	6	8	0	0	1	1	0	1	1
		8	6	8	0	0	48	48	0	2	2
		9	6	9	0	0	6	6	0	1	1
		10	6	9	0	0	26	26	0	2	2
		11	6	9	0	0	8	8	0	1	1
		12	6	10	0	0	1	1	0	1	1
		13	6	10	0	0	16	16	0	2	2
		14	6.5	9.5	0	0	30	30	0	2	2
		15	6.5	9.5	0	0	0	0	0	0	0
		16	7	9	0	0	1	1	0	1	1
		17	7	10	0	0	5	5	0	1	1
		18	7	10	0	0	0	0	0	0	0
		19	7	10	0	0	0	0	0	0	0
		20	7	10	0	0	5	5	0	1	1
T10AgNC(b)	1	1	7	10	0				0	1	1

Population number	ExpDay	HstNo	BodyL	TailL	LegL	SNo	GNo	SGNo	SDeg	GDeg	SGDeg
T10AgNC(b) (continue)		2	7	12	0				0	1	1
		3	7.5	8.5	0				0	2	2
		4	8	11	0				0	1	1
		5	8	10	0				0	2	2
		6	8	13	1.5				0	2	2
		7	9	11	0				0	1	1
		8	9	12.5	2				0	1	1
		9	9	12	2				0	1	1
		10	10	12	3				0	2	2
	2	1	7	11	0				0	2	2
		2	7	11	1.5				0	2	2
		3	7	11	1				0	2	2
		4	7.5	12.5	2				0	2	2
		5	8	4	1				0	1	1
		6	8	12	1				0	1	1
		7	8	13	2				0	1	1
		8	8	13	2				0	2	2
		9	9	13	3				0	1	1
		10	9	14	3				0	2	2
	3	1	8	10	1.5				5	4	5
		2	8	22	2				3	2	3
		3	8	5	1				3	3	3
		4	9	12.5	2				4	4	4
	4	1	7	10	1				5	2	5
		2	8	12	1.5				5	4	5
		3	8	11	1.5				5	3	5
		4	8	9	1				5	3	5
		5	9	13	2				5	3	5
	5	1	7	10	0				2	2	2
		2	8	10	1				2	2	2
		3	8	10	0				2	3	3
		4	9	10	0				2	3	3
		5	9	10	2				3	3	3
	6	1	7.5	9.5	1.5				3	3	3
		2	8	12	2.5				1	3	3
		3	8	11	2				1	3	3
		4	8	11	2				1	3	3
		5	9	12	4				2	3	3
	7	1	8	11	2				1	2	2
		2	8	10	1				2	3	3
		3	8	11	1				2	3	3

Population number	ExpDay	HstNo	BodyL	TailL	LegL	SNo	GNo	SGNo	SDeg	GDeg	SGDeg
T10AgNC(b) (continue)		4	8	11	1				1	3	3
		5	8	10	1				1	3	3
		6	8.5	11.5	2				2	3	3
		7	9	11	2				1	3	3
		8	9	13	3.5				2	3	3
		9	9	13	3.5				1	3	3
		10	10	13.5	4				1	2	2
	8	1	7	10	0				2	1	2
		2	8	9	0				1	2	2
		3	8	13	3.5				1	2	2
		4	9	12	1				1	2	2
		5	9	13	0				1	1	1
		6	9	11	0				1	1	1
		7	9	12	0				1	2	2
		8	9.5	10.5	0				1	2	2
		9	9.5	11	2				1	2	2
		10	10	12	5				1	2	2
T12AgND(c)	1	1	5	6	0				0	0	0
		2	5	6	0				0	0	0
		3	6	6	0				0	0	0
		4	6	7	0				0	0	0
		5	6	7	0				0	0	0
		6	6	7	0				0	0	0
		7	7	8	0				0	0	0
		8	7	9	0				0	3	3
		9	7	9	0				1	0	1
		10	8	10	0				0	0	0
	2	1	10	3	0				2		2
		2	10	5	0				2		2
		3	10	5	0				3		3
		4	10	5	0				2		2
		5	10	6	0				2		2
		6	10	7	0				1		1
		7	9	14	10				1	3	3
		8	8	13	2				1	2	2
		9	8	9	2				2	2	2
		10	8	12	2				1	1	1
		11	9	13	3				1	2	2
		12	9	9	5				1	2	2
		13	9	11	6				1	2	2
		14	10	13	10				4	2	4

Population number	ExpDay	HstNo	BodyL	TailL	LegL	SNo	GNo	SGNo	SDeg	GDeg	SGDeg
T12AgND(c) (continue)		15	10	14	9				1	2	2
		16	10	15	10				1	3	2
T12AgND(d)	1	1	6.5	8	0				0	1	1
		2	7	9	0				0	1	1
		3	7.5	8.5	0				0	1	1
		4	7.5	8.5	0				0	1	1
		5	8	10	0				0	1	1
T20HaNE	2	1	19	29	23	16	15	31	2	2	2
		2	22	44	34	41	200	241	3	4	4
T24PsNF	1	1	15	17	12	1	0	1	1	0	1
	2	1	9	16	0	0	0	0	0	0	0
		2	9	16	0	0	0	0	0	0	0
		3	9	16	0	86	200	286	3	4	4
		4	9	16	0	27	4	31	3	1	3
		5	10	22	0	0	0	0	0	0	0
		6	10	39	20	0	0	0	0	0	0
		7	10	22	0	210	200	410	5	4	5
		8	10	39	20	105	180	285	4	4	4
		9	11	23	0	0	0	0	0	0	0
		10	11	23	0	31	9	40	3	1	3
		11	13	27	5	0	0	0	0	0	0
		12	13	27	5	52	13	65	3	2	3
		13	16	27	22	12	0	12	2	0	2
F5BbTT	1	1	23	29.5		5	50	55	1	2	2
		2	24	30		6	0	6	1	0	1
		3	24	28		3	2	5	1	1	1
		4	24	30		2	0	2	1	0	1
		5	24	30		9	0	9	1	0	1
	2	1	27	34		77	3	80	3	1	3
		2	27	34		33	5	38	2	1	2
		3	24	31		45	31	76	2	2	2
		4	24	31		18	0	18	2	0	2
		5	25	32		26	9	35	2	1	2
	3	1	23	30		34	0	34	2	0	2
		2	24	31		37	1	38	2	1	2
		3	25	31		27	1	28	2	1	2
		4	25	31		33	4	37	2	1	2
		5	21	26		36	0	36	2	0	2
	4	1	26	32		20	0	20	2	0	2
		2	24	30		6	0	6	1	0	1
		3	24	30		150	1	151	4	1	4

Population number	ExpDay	HstNo	BodyL	TailL	LegL	SNo	GNo	SGNo	SDeg	GDeg	SGDeg
F5BbTT (continue)		4	24	30		30	0	30	2	0	2
		5	25	31		17	1	18	2	1	2
	5	1	29	35		64	4	68	3	1	3
		2	24	31		26	0	26	2	0	2
		3	26	30		0	3	3	0	1	1
		4	26	33		11	0	11	2	0	2
		5	24	31		15	13	28	2	1	2
	6	1	23	29		9	0	9	1	0	1
		2	25	31		5	1	6	1	1	1
		3	25	30		11	1	12	2	1	2
		4	24	30		3	1	4	1	1	1
		5	24	29		17	3	20	2	1	2
	7	No hosts examined									
	8	1	24	31		40	4	44	3	1	3
		2	29	37		21	15	36	3	2	3
		3	26	33		111	0	111	4	0	4
		4	24	31		84	0	84	3	0	3
		5	25	32		137	0	137	4	0	4
	9	1	24	31		6	0	6	1	0	1
		2	25	32		4	0	4	1	0	1
		3	25	32		9	0	9	1	0	1
		4	25	32		44	0	44	3	0	3
		5	25	33		38	0	38	3	0	3
	10	1	25	31		31	0	31	3	0	3
		2	25	31		7	0	7	1	0	1
		3	26	32		37	0	37	3	0	3
		4	26	33		48	0	48	3	0	3
		5	24	30		1	0	1	1	0	1
	11	1	25	31		77	0	77	3	0	3
		2	27	33		7	0	7	1	0	1
		3	25	31		67	0	67	3	0	3
		4	24	30		4	0	4	1	0	1
		5	25	31		3	0	3	1	0	1
		6	25	31		1	0	1	1	0	1
		7	28	34		7	0	7	1	0	1
F19HmNA	1	1	22	4	0	0	0	0	0	0	0
		2	22	4	0	0	0	0	0	0	0

Body measurements of the trichodinid populations examined during the present study. Please refer to Figure 5.1 and Table 5.1 for more information on the host population.

Abbreviations: **ab**: Apex of blade; **abm**: Anterior blade margin; **add**: Adhesive disc diameter; **adp**: length above deepest point of curve; **at**: length above tangent point; ba: Blade apophysis; **bc**: Section connecting blade and central part; **bd**: Body diameter; **bdp**: length below deepest point of curve; **bl**: Blade length; **ca**: Centre of adhesive disc; **ccp**: Section connecting central part and ray; cp: Central conical part; **cpw**: Central part width; **dbm**: Distal blade margin; **den**: Number of denticles in denticle ring; **dp**: Deepest point of curve relative to apex; **drd**: Denticle ring diameter; **ds**: Denticle span; **dw**: Denticle width; **ira**: Lower central part indentation; **pbm**: Posterior blade margin; **pp**: Posterior projection; **q1 – q4**: Area behind the ray apophysis should be divided into four equal parts; **ra**: Ray apophysis; **rad**: Number of dadial pins per denticle; **rc**: Ray connection; **rl**: Ray length; sa**:** Section of central part above x axis; **sb**: Section of central part below x axis; **tp**: Tangent point; **tpd**: Tangent point diameter; **trd**: Tip of ray diameter; wbm: Width of border membrane. All measurements in µm.

Host species	den	bd	add	tpd	drd	trd	wbm	rad	bl	cpw	rl	dw	ds
Barbus anoplus	**F1BaSD**												
	23	90.25	76.74	69.70	49.78	23.47	6.41	11	10.21	4.74	6.80	21.76	11.53
	23	73.98	62.13	54.50	39.72	22.95	6.06	12	7.09	3.27	5.76	16.12	10.28
	F2BaSS												
	22	110.38	92.81	81.61	56.66	28.72	8.11	10	12.12	6.61	9.39	27.57	15.01
	23	69.47	61.42	55.04	38.30	20.19	4.68	11	8.11	3.38	6.15	17.63	9.06
Barbus barnardi	**F5BbTT**												
	23	64.85	52.77	47.39	32.29	15.40	5.84	11	8.28	2.91	5.72	16.36	8.62
	22	66.66	55.54	50.27	34.65	13.00	4.90	12	9.44	3.14	5.84	18.30	10.53
	21	61.94	51.96	46.55	32.39	13.37	5.38	12	8.17	2.85	6.04	16.94	10.04
	22	71.57	59.79	55.05	38.43	16.28	5.84	11	10.13	3.27	6.56	19.76	9.59
	22	60.63	49.09	46.31	32.29	10.78	5.73	13	8.32	2.95	5.80	17.15	9.37
	22	65.34	54.83	48.76	33.98	13.89	5.31	-	10.00	3.14	5.91	18.89	10.72
	24	70.86	61.11	53.71	37.37	17.39	5.46	12	9.19	3.08	6.50	18.60	10.47
	23	70.30	59.53	52.56	37.98	18.64	5.86	-	8.56	2.62	5.91	17.40	10.34
	22	72.12	59.84	55.96	39.84	17.70	5.42	11	10.30	3.89	6.09	20.23	10.17
	24	75.74	63.33	55.20	37.84	16.09	5.32	11	9.33	4.07	6.78	19.96	10.45
	23	84.25	71.86	62.39	45.76	25.02	5.98	10	9.01	3.60	7.00	18.85	11.37
	23	72.71	61.84	53.50	37.53	16.55	5.54	12	9.78	3.10	6.58	18.88	10.68

Host species	den	bd	add	tpd	drd	trd	wbm	rad	bl	cpw	rl	dw	ds
Barbus barnardi	23	72.47	61.26	55.12	40.09	22.82	5.31	9	7.93	2.28	6.45	16.72	9.44
F5BbTT (continue)	26	64.70	55.16	50.00	35.16	18.18	4.98	10	6.70	3.45	5.92	15.92	7.45
	22	70.31	64.43	51.93	36.95	17.77	3.44	12	9.11	3.78	5.87	18.43	10.21
	24	68.54	56.89	54.61	39.70	20.73	5.84	10	8.10	2.91	6.26	17.45	8.81
	23	63.95	52.73	47.72	33.47	13.43	5.54	11	8.50	3.24	5.87	17.82	9.21
	24	77.83	66.11	59.35	41.53	21.51	6.41	11	9.32	3.52	7.02	19.73	11.40
	22	70.65	62.52	53.11	37.53	16.65	5.13	12	9.41	2.98	6.98	19.12	10.23
	23	63.35	52.34	45.54	31.69	13.59	4.63	11	7.70	2.65	6.04	17.18	9.46
	22	70.17	58.56	51.87	36.91	15.97	5.74	12	9.69	2.94	5.87	18.20	10.59
	22	70.39	58.62	49.32	34.03	17.01	5.09	11	7.73	3.20	5.72	16.42	9.90
	22	56.13	50.40	46.12	32.68	12.81	4.33	11	9.42	2.46	5.57	17.21	9.16
	22	65.10	55.03	51.61	37.04	17.80	6.15	8	7.37	2.99	5.70	16.45	10.44
		62.78	52.50	45.34	30.94	12.35	6.44	10	8.73	2.35	5.64	16.82	8.75
	23	60.71	52.04	47.96	32.23	11.76	5.20	-	9.53	3.05	5.91	18.38	10.11
	23	64.60	56.17	48.62	33.68	16.27	4.75	12	8.56	2.61	5.93	17.28	9.43
	22	64.56	55.81	47.17	33.85	14.20	4.22	14	8.11	2.63	5.72	16.68	10.15
	22	63.44	53.30	49.40	33.78	14.46	5.11	12	9.49	2.91	5.99	18.82	9.68
	22	67.06	54.29	48.65	34.42	14.26	6.11	12	8.20	3.03	5.27	16.90	10.90
	22	75.20	62.89	53.28	36.41	20.61	6.20	11	9.07	2.73	5.25	17.61	9.46
	24	73.11	62.88	54.87	40.36	17.86	5.58	12	10.02	2.75	6.95	18.85	9.24
	21	67.23	55.68	48.63	33.67	17.52	5.12	10	7.15	2.14	6.30	15.63	9.61
	22	72.47	60.44	54.15	38.48	17.25	5.83	13	9.08	2.82	6.85	19.02	9.52
	23	65.91	60.30	52.35	36.74	17.86	2.32	13	8.02	2.99	6.61	17.84	10.32
	23	72.28	62.03	52.89	37.43	18.61	5.78	12	8.32	3.33	6.03	17.62	9.24
	23	74.93	63.58	57.34	40.88	20.31	5.68	10	8.92	3.47	6.57	18.86	10.33
	24	69.28	59.56	51.44	36.42	17.99	3.65	11	8.09	2.94	6.28	17.77	9.46
	23	67.15	55.41	50.97	34.65	15.45	5.44	12	9.94	3.15	6.00	19.03	10.51

Host species	den	bd	add	tpd	drd	trd	wbm	rad	bl	cpw	rl	dw	ds
Barbus barnardi F5BbTT (continue)	22	72.35	61.91	56.13	39.46	16.17	6.10	10	8.45	3.03	6.19	18.04	11.39
	21	73.21	61.86	55.05	39.10	17.95	6.03	11	7.72	3.27	7.16	18.00	9.92
	21	68.31	58.55	51.58	35.14	14.88	5.31	11	9.30	3.15	6.19	19.22	9.33
	21	70.34	60.52	51.29	36.51	18.97	4.70	10	7.51	3.06	6.00	16.30	10.35
Barbus species	F3BuBV												
	28	91.89	80.19	70.14	51.24	27.99	5.95	10	10.28	3.88	6.96	20.86	10.87
	F4BuOG												
	21	94.92	78.69	72.42	48.33	26.45	7.41	7	7.27	5.85	8.86	21.75	11.83
	24	55.53	46.57	44.15	30.39	20.10	4.30	7	4.77	2.88	5.46	12.58	8.04
	23	87.67	69.64	62.19	41.84	18.78	8.90	-	9.87	3.78	8.58	23.14	10.41
		55.37	45.34	42.67	26.62	13.17	5.42	7	6.50	2.09	6.53	15.01	6.80
	26	58.05	51.54	47.76	31.86	15.91	4.50	7	6.51	3.14	6.62	15.73	8.10
	23	61.58	52.27	45.76	31.35	16.69	3.96	9	6.05	3.38	5.63	13.81	7.88
	23	42.62	35.33	31.78	21.55	9.22	2.84	8	4.40	2.07	4.48	10.96	5.42
	22	48.16	40.49	38.54	25.98	13.45	3.70	9	6.81	1.50	4.54	12.76	5.34
	F6BuBL												
	24	54.90	49.18	44.29	31.97	15.58	3.32	9	7.32	2.43	4.24	14.85	7.53
Clarias gariepinus	F7CgUP												
	23	71.92	60.22	53.73	37.47	17.31	6.73	-	8.36	3.52	6.86	18.81	9.32
	25	84.93	68.11	65.14	47.11	26.60	5.23	10	8.96	4.14	6.87	22.94	11.09
	21	70.38	56.48	53.13	37.93	14.83	5.77	-	4.14	5.78		19.96	11.02
	23	-	40.77	36.55	25.08	8.59	-	-	6.88	2.32	4.96	14.01	6.64
Cyprinus carpio	F8CcLF												
	19	65.46	53.05	48.79	31.24	13.38	6.21	12	8.20	3.29	7.05	18.50	9.86
	18	65.90	53.70	49.53	30.70	13.15	4.98	12	7.22	3.97	7.54	18.50	10.60
	20	64.33	52.67	46.98	29.58	11.05	5.79	11	6.96	3.39	7.14	17.19	9.22
	21	72.62	59.91	53.00	34.12	15.39	6.25	12	8.87	4.13	7.62	20.34	10.46

Host species	den	bd	add	tpd	drd	trd	wbm	rad	bl	cpw	rl	dw	ds
Cyprinus carpio F8CcLF (continue)	21	59.25	47.44	44.21	27.11	11.10	5.24	11	7.65	3.44	6.32	17.46	8.85
	25	85.76	73.09	62.74	45.05	22.10	5.66	12	10.56	3.44	6.99	20.67	11.51
	28	-	-	65.52	47.50	23.59	-	-	9.55	3.95	7.38	20.38	10.15
	20	63.19	51.26	48.28	32.03	13.83	5.54	-	7.78	3.22	7.88	18.06	9.85
	19	73.72	57.48	51.74	32.98	15.99	4.36	-	7.27	3.78	7.40	18.61	11.41
	20	62.05	49.87	45.93	28.81	13.26	7.13	-	6.95	2.91	6.99	16.44	8.58
	20	67.17	55.71	49.67	34.99	17.05	5.47	12	6.62	3.82	5.73	15.81	9.99
	20	68.32	55.68	51.73	33.51	16.45	5.65	13	8.34	4.48	7.21	19.33	9.28
	24	90.57	77.68	66.97	48.74	20.29	6.42	11	11.37	4.60	7.37	23.33	12.71
	19	64.82	52.71	48.22	31.98	10.41	5.88	-	8.78	3.59	6.49	18.39	9.82
	20	64.84	52.79	48.30	31.56	13.59	5.40	-	7.80	3.00	6.77	18.21	9.62
	20	57.44	45.56	43.15	26.43	10.81	5.76	-	7.49	3.04	6.03	16.67	9.23
	19	61.85	49.93	46.30	29.10	9.73	5.09	12	7.63	2.94	7.30	17.68	9.86
	20	61.77	50.91	47.29	29.50	11.07	4.37	-	7.84	2.90	7.43	17.91	8.86
	19	54.00	43.52	39.92	25.92	11.01	4.42	11	6.12	3.10	5.65	14.83	8.04
	18	55.00	45.36	41.12	26.59	10.82	4.96	12	6.34	3.16	6.40	15.78	8.32
	20	53.63	43.89	39.41	25.01	10.64	4.86	11	6.79	2.74	5.72	15.52	8.36
Gambusia affinis	F9GaEB												
	21	69.06	57.40	49.46	35.21	16.29	5.39	12	7.66	2.72	5.95	16.26	9.12
	21	71.66	59.02	54.48	38.45	19.97	6.49	10	7.52	3.04	6.63	17.06	9.80
	21	73.06	60.36	52.78	38.35	16.77	7.04	11	9.78	2.97	6.16	18.95	9.93
	22	67.49	56.23	50.64	35.07	16.15	5.94	9	8.65	2.51	6.33	17.11	9.41
	21	65.01	55.12	49.53	35.41	18.40	6.08	-	7.21	2.49	5.79	15.71	7.60
	23	67.18	56.64	49.72	35.05	14.82	6.71	11	6.45	3.74	6.03	16.35	8.18
	22	68.32	57.11	53.18	37.58	20.67	3.35	11	8.16	2.81	5.84	17.07	9.40
	-	68.42	57.28	48.49	32.71	15.90	6.66	12	7.96	3.29	6.12	16.24	9.32
	23	65.03	55.36	50.22	36.64	17.95	4.92	8	7.69	2.97	6.10	17.36	9.11

Host species	den	bd	add	tpd	drd	trd	wbm	rad	bl	cpw	rl	dw	ds
Oreochromis mossambicus	F10OmEB												
	24	68.63	58.24	52.46	37.69	18.69	6.11	10	8.31	3.36	6.65	17.98	8.46
	F11OmLF												
	26	116.42	98.48	88.42	63.92	26.26	9.23	-	15.60	6.16	9.19	30.77	14.75
	26	106.36	88.87	78.35	54.64	22.49	8.07	11	13.11	6.30	9.52	28.92	13.93
	25	100.95	83.78	71.21	50.17	20.56	8.66	11	12.45	4.04	9.48	25.67	12.18
	29	121.57	104.47	91.44	66.60	30.12	9.40	-	16.38	6.02	10.18	32.61	14.21
	25	116.45	100.06	89.17	63.55	26.67	8.82	13	15.09	6.89	9.75	31.61	14.78
	27	112.90	95.04	79.57	56.38	27.20	9.08	10	12.77	5.21	9.38	27.32	12.29
	26	110.52	94.94	83.15	57.97	22.69	7.34	-	15.70	5.02	10.41	31.35	12.41
	26	116.70	97.83	84.92	61.03	26.93	9.27	-	14.10	5.96	9.80	29.87	13.38
	27	115.80	98.19	87.12	62.48	25.33	8.64	11	13.97	6.75	9.60	15.23	6.60
	25	106.85	88.74	74.81	52.72	20.45	9.36	11	15.67	4.82	9.30	28.76	14.30
	20	65.51	55.43	46.36	32.48	14.57	5.35	-	7.84	3.64	5.07	16.94	9.67
	27	110.27	91.09	82.15	58.36	24.13	9.45	11	15.48	5.62	9.31	30.01	12.58
	28	112.58	94.15	83.54	57.52	22.79	8.72	11	13.80	6.69	9.98	30.52	12.93
	26	105.54	87.05	78.78	54.56	26.05	8.89	-	11.83	5.48	9.56	27.12	12.03
	27	116.23	97.69	80.98	57.62	22.75	10.28	10	15.19	5.18	10.56	30.56	12.73
	28	117.41	98.75	79.94	56.44	21.73	10.36	-	15.89	4.40	9.69	29.43	12.81
	27	110.35	92.34	80.42	56.37	23.65	8.91	-	13.94	5.29	10.35	29.65	12.23
	27	111.71	93.24	84.11	58.29	27.20	9.78	11	13.02	6.08	9.19	28.22	12.94
	27	111.23	92.57	81.30	56.23	23.37	9.44		14.88	4.99	10.15	30.63	13.06
	27	110.68	91.08	80.89	55.93	30.99	10.29	10	11.29	5.06	9.37	24.16	13.14
	27	110.50	91.86	81.68	57.65	26.26	8.86	13	14.19	5.13	10.18	29.39	12.73
	27	118.48	100.60	83.41	58.92	21.39	9.09	-	15.38	6.15	9.16	31.54	12.69
	24	102.88	84.74	73.18	50.65	19.22	-	-	14.03	6.13	8.51	28.77	12.14
	25	100.71	82.51	72.33	49.38	17.43	6.44	-	14.02	4.67	9.32	27.68	12.75

Host species	den	bd	add	tpd	drd	trd	wbm	rad	bl	cpw	rl	dw	ds
Oreochromis mossambicus F11OmLF (continue)	27	111.28	91.88	81.81	57.53	25.92	8.79	-	12.23	5.57	9.63	29.18	12.74
	25	114.42	95.97	84.60	59.00	22.38	8.59	12	15.77	6.57	10.32	32.57	14.40
	27	127.21	109.29	67.49	26.21	9.38	-	-	16.12	6.53	10.38	32.68	15.10
	26	114.32	96.73	83.70	58.90	22.33	8.00	11	14.45	7.00	9.85	31.70	14.13
	25	95.32	81.99	72.67	51.15	23.38	6.16	12	12.91	4.92	8.53	25.64	12.59
	27	105.62	92.57	77.74	53.12	22.16	5.71	11	13.60	5.32	9.13	28.12	12.53
	25	101.83	83.88	76.32	52.31	18.73	8.95	12	14.77	5.15	8.72	29.12	12.92
	26	113.85	95.66	88.90	61.57	23.14	10.22	9	16.69	6.07	10.32	33.51	13.09
	24	102.86	85.66	74.70	50.57	17.54	8.86	-	13.61	4.90	9.97	28.32	13.20
	26	119.28	100.04	87.83	61.81	23.85	9.13	12	15.79	6.52	9.79	32.00	13.65
	25	106.94	96.51	78.27	55.92	26.12	7.30	9	16.11	5.35	8.19	29.10	12.41
	26	109.56	91.54	81.35	55.10	18.29	8.12	11	16.20	5.76	10.26	31.93	12.95
	25	121.98	103.08	88.01	61.71	18.36	9.50	14	18.45	7.07	9.91	35.27	15.42
	25	120.18	102.88	89.04	59.89	17.81	7.24	-	18.89	7.34	9.06	35.47	14.74
	26	101.86	92.40	81.24	57.42	24.42	6.53	7	13.59	5.69	9.42	28.74	13.88
	27	119.54	102.54	89.86	64.26	25.74	9.44	11	17.27	5.42	10.30	33.29	13.20
	20	71.80	59.35	50.93	35.14	14.58	5.39	10	8.21	4.88	5.22	18.19	11.66
	21	66.95	55.02	50.16	31.12	15.29	6.88	-	6.09	3.88	7.76	17.61	8.83
	25	48.59	40.30	35.28	24.10	9.29	4.41	-	6.21	2.24	4.61	12.83	5.88
Petenia krausi	F13PkLV												
	25	87.94	75.64	68.00	49.58	26.95	5.72	-	10.36	3.66	7.44	21.70	12.50
	24	93.04	80.40	70.25	51.58	23.55	5.77	-	12.34	5.21	7.93	24.76	12.40
	21	80.90	68.86	58.80	41.97	17.48	6.17	-	9.94	4.54	6.14	21.39	11.70
	21	80.63	68.63	58.87	42.64	20.14	6.39	12	10.85	4.90	6.45	21.15	12.49
	23	65.48	54.39	49.36	33.21	14.67	5.41	-	7.73	3.45	6.00	16.98	10.78
	23	74.09	60.68	56.16	40.01	15.38	6.76	11	9.90	5.02	7.09	21.10	11.49

Host species	den	bd	add	tpd	drd	trd	wbm	rad	bl	cpw	rl	dw	ds
Poecilia spenops	F12PsLV												
	25	87.13	74.39	65.45	47.32	20.25	5.85	12	10.90	3.37	7.28	22.59	12.57
	23	85.19	72.16	62.74	43.22	17.15	6.98	13	12.11	3.67	7.43	23.12	12.03
	24	81.49	70.06	61.32	44.41	15.87	5.89	11	10.45	4.76	6.37	21.89	12.28
	23	83.21	70.37	63.15	43.64	19.58	5.82	14	10.95	4.34	7.38	22.89	12.18
	22	83.74	70.88	62.52	43.97	18.87	6.18	-	12.40	2.26	7.31	22.34	12.58
	-	81.02	70.73	58.76	42.41	18.48	6.10	12	10.53	3.94	6.61	21.67	11.90
Pseudocrenilabrus philander	F15PpBV												
	26	69.27	59.11	53.00	37.08	15.83	5.28	11	9.68	3.41	6.33	19.27	9.65
	25	63.62	53.14	48.09	33.68	14.97	4.82	10	8.86	3.08	5.92	17.65	8.56
	25	66.27	56.18	49.32	35.10	12.63	4.78	11	10.52	2.82	6.00	19.15	9.64
	27	63.93	57.01	52.27	37.80	18.14	4.40	11	9.11	3.69	5.51	17.83	9.39
	26	80.17	68.66	58.92	42.44	19.43	4.68	12	9.96	4.03	6.03	19.75	9.97
	23	70.26	59.58	51.91	36.37	13.30	5.73	15	9.81	4.10	5.53	19.13	9.42
	26	73.69	63.20	55.34	40.18	16.94	5.07	11	9.57	4.25	5.50	19.73	9.58
	25	70.31	60.84	53.26	37.53	15.87	4.75	13	9.61	3.22	6.49	18.72	9.16
	24	71.32	60.97	52.06	37.06	13.97	5.37	11	9.47	3.33	6.18	18.90	9.72
	27	75.05	64.22	56.60	40.37	20.11	5.24	10	9.01	3.97	6.22	19.42	9.05
	23	63.94	54.90	48.36	33.83	12.00	5.82	12	9.11	3.35	5.70	18.14	9.26
	25	66.78	56.08	50.07	36.21	15.44	4.98	9	8.56	2.96	6.23	17.24	9.72
	26	92.97	79.74	72.05	52.27	22.00	7.10	12	11.88	5.22	7.29	24.62	12.67
	25	91.61	79.51	70.94	51.21	23.28	6.23	11	12.40	4.34	6.95	23.82	12.68
	26	91.11	81.43	72.90	53.25	25.07	6.61	13	13.20	3.79	8.56	25.70	14.19
	23	78.06	65.79	59.91	42.14	17.45	5.81	15	11.32	5.00	6.52	22.69	12.32
	27	80.21	68.22	62.21	44.90	18.29	6.20	10	9.71	4.95	6.64	21.35	10.95
	25	-	-	61.74	46.34	18.04	-	10	11.10	4.93	6.20	21.38	10.83
	25	82.76	70.78	65.66	47.92	21.60	6.08	-	12.23	4.42	6.71	23.01	12.30

Host species	den	bd	add	tpd	drd	trd	wbm	rad	bl	cpw	rl	dw	ds
Pseudocrenilabrus philander F15PpBV (continue)	24	94.36	80.67	70.11	50.49	21.67	6.85	13	11.87	4.75	6.91	23.63	13.58
	25	91.50	78.69	68.56	48.99	24.02	6.36	-	11.56	3.49	7.83	22.61	12.86
	27	90.18	77.33	67.14	49.43	22.55	7.45	11	10.71	4.44	7.25	22.12	11.02
	23	83.43	71.73	66.61	48.31	19.92	4.97	14	12.45	4.43	7.83	24.79	13.41
	23	86.86	74.76	64.82	45.65	16.51	5.62	12	13.20	4.87	6.31	24.54	12.09
	25	83.15	70.91	66.06	47.55	21.02	6.53	10	11.57	3.71	6.66	21.86	11.33
	24	87.88	77.30	67.65	49.27	24.32	5.62	10	9.28	5.22	6.84	21.44	13.32
	24	79.03	68.91	61.10	43.60	14.54	5.15	6	12.24	3.77	6.39	22.83	10.08
	25	90.92	83.79	72.32	52.99	27.04	3.17	12	11.76	4.43	7.06	23.23	13.09
	25	94.40	81.46	70.94	51.79	24.57	5.67	13	11.42	4.12	7.07	23.08	15.01
	24	89.12	76.89	66.74	47.29	26.76	5.44	11	10.96	3.91	7.46	22.31	11.35
	23	89.25	74.92	66.55	45.48	17.48	6.35	11	11.63	5.64	7.12	24.39	13.45
	26	85.50	72.38	64.22	45.84	17.27	7.16	10	11.98	4.79	7.13	23.37	10.86
	24	-	83.54	74.86	54.18	21.77	6.51	13	12.85	5.59	8.99	26.82	12.75
	23	87.70	74.46	65.04	45.24	18.18	5.48	13	11.97	5.03	7.18	24.33	12.44
	26	89.13	76.55	69.11	47.12	25.54	6.14	-	10.83	4.48	6.97	21.75	12.13
	23	81.99	-	68.57	41.91	15.43	6.80	12	9.87	4.41	6.55	21.03	11.83
	25	87.30	76.23	66.52	47.54	19.31	5.91	15	11.11	4.97	7.60	23.41	12.20
	23	92.62	78.51	68.61	51.10	18.82	5.61	10	11.12	4.68	7.27	23.25	12.30
	27	80.37	70.35	64.68	44.55	21.32	6.02	10	9.73	4.14	7.34	20.50	11.78
	25	84.74	71.07	63.98	46.43	19.96	6.56	-	10.85	4.65	7.48	23.08	11.73
	26	85.83	74.05	67.66	49.24	20.97	6.70	12	11.99	4.04	7.10	23.01	12.64
	27	82.43	69.12	63.03	45.85	20.76	6.78	9	11.77	4.22	6.67	22.23	10.44
	26	86.03	75.98	68.82	50.67	20.67	4.84	11	11.92	4.17	7.24	23.10	12.33
	23	87.05	73.52	64.61	45.48	21.22	7.59	11	11.54	4.52	7.06	23.11	12.62

Host species	den	bd	add	tpd	drd	trd	wbm	rad	bl	cpw	rl	dw	ds
Pseudocrenilabrus philander F15PpBV (continue)	26	84.32	68.99	62.72	44.60	19.30	6.20	9	10.71	4.26	6.84	22.03	10.84
	25	-	85.11	75.37	54.94	25.09	6.65	-	13.81	4.22	8.06	25.70	14.62
	25	90.02	78.34	71.27	51.49	20.99	6.70	10	14.01	4.95	7.48	25.88	13.05
	25	91.17	78.09	68.94	48.48	19.06	7.04	12	13.67	4.92	7.12	26.30	14.11
	24	81.33	68.78	64.67	46.87	18.29	6.55	11	11.45	4.43	7.34	23.29	12.52
	27	94.31	81.43	72.68	52.48	26.14	7.23	12	11.24	5.12	8.00	23.97	11.36
	27	89.52	76.91	66.67	46.99	21.47	6.89	10	11.32	4.50	6.49	22.54	9.59
	26	96.89	82.66	72.96	54.21	24.22	6.63	-	13.16	5.52	7.22	25.44	13.62
	24	97.48	85.94	72.61	53.25	23.12	7.01	11	13.99	4.56	7.15	25.40	13.80
	24	86.93	73.47	66.16	46.83	18.04	6.26	12	11.47	4.56	7.01	23.24	11.58
	24	76.39	66.18	59.55	42.66	16.15	5.08	7	11.32	5.23	6.10	23.12	11.51
	25	83.57	70.53	64.01	45.73	19.53	6.49	-	11.13	4.17	7.20	22.99	12.02
	25	88.60	75.54	70.39	49.78	24.41	5.30	9	12.30	4.07	7.89	23.74	12.92
	24	82.84	69.98	61.74	44.39	15.71	6.75	10	10.38	4.83	7.02	21.98	11.78
	22	90.45	77.68	69.99	50.00	19.54	5.41	12	14.20	5.22	7.55	26.42	13.74
	23	96.65	84.57	71.81	52.44	21.69	5.94	14	13.05	4.29	8.64	25.95	15.69
	24	77.07	65.73	60.01	40.72	21.86	6.08	14	9.35	3.75	7.18	19.59	11.43
	27	90.30	77.53	68.96	49.62	23.09	6.38	11	11.59	4.83	7.36	23.42	12.07
	24	79.86	68.28	59.59	42.77	20.36	5.94	11	9.23	3.68	6.44	19.61	11.93
	25	94.73	86.07	71.82	51.01	22.53	5.81	13	11.93	4.70	7.56	24.12	14.15
	26	80.36	67.57	58.96	41.09	17.16	6.80	10	9.49	4.32	6.60	19.91	11.44
	F16PpEB												
	24	89.42	76.03	63.65	46.38	22.53	5.76	14	10.08	3.98	6.95	20.90	12.16
	24	82.93	71.74	61.31	44.09	24.10	5.76	14	8.81	5.08	6.05	19.31	9.97
	27	79.96	71.25	63.86	46.05	27.79	4.16	11	8.08	4.04	6.97	19.19	10.44
	24	83.04	72.30	60.76	44.19	21.48	5.93	11	8.36	4.76	5.93	19.47	10.54
	24	68.67	56.07	47.14	34.57	16.62	6.80	11	7.10	4.00	4.46	15.35	8.82

Host species	den	bd	add	tpd	drd	trd	wbm	rad	bl	cpw	rl	dw	ds
Pseudocrenilabrus philander F16PpEB (continue)	24	85.26	73.97	63.21	46.48	23.65	5.67	11	9.93	3.43	7.02	20.24	10.72
	-	83.74	71.30	61.10	43.03	19.65	6.49	13	9.28	3.07	7.51	20.42	12.09
	24	76.92	65.62	55.85	40.02	16.77	6.58	11	9.58	3.38	6.14	19.02	10.08
	25	76.15	68.99	61.77	44.46	22.37	4.17	12	9.10	3.98	5.89	19.65	10.56
	25	80.07	67.38	58.33	42.44	20.07	6.18	13	10.25	3.88	5.61	19.33	11.22
	24	84.25	70.46	62.57	43.78	18.41	5.36	11	9.85	4.27	7.13	20.98	11.30
	24	82.46	69.96	61.08	43.99	25.13	5.20	14	8.62	3.30	7.23	18.95	11.70
	22	78.30	66.18	56.95	39.78	19.78	5.86	15	8.01	4.50	6.44	18.69	11.87
	24	86.06	73.65	65.75	48.34	24.79	6.11	15	9.74	4.39	6.86	21.00	11.59
	24	87.91	76.68	64.67	48.22	26.44	6.30	11	9.12	4.27	7.18	20.31	11.14
	24	75.86	67.07	57.67	41.89	21.50	4.94	-	8.33	3.78	6.10	18.08	9.88
	25	85.05	73.74	61.10	43.76	21.22	5.96	12	9.73	4.13	7.23	21.84	10.95
	22	65.06	56.25	49.47	34.38	17.18	4.73	-	7.89	3.71	5.70	17.06	10.77
	20	82.04	69.96	60.03	43.19	21.53	6.25	11	9.26	4.04	6.94	20.57	12.85
	26	82.66	75.85	65.28	46.96	28.05	3.52	11	9.07	3.58	7.99	20.30	10.90
	23	84.29	73.10	61.16	42.16	22.24	4.82	12	9.23	2.84	7.04	19.94	11.41
	24	73.91	63.98	56.60	39.12	17.28	5.16	11	9.81	3.04	6.00	19.32	11.20
	22	77.07	69.16	60.53	42.76	20.08	3.67	14	9.08	4.20	7.07	20.20	11.73
	24	83.91	72.65	62.87	46.33	22.00	5.51	15	9.53	4.06	6.15	20.87	11.35
	22	68.40	59.37	52.52	36.85	16.87	5.41	11	8.31	2.92	6.09	17.32	10.28
	23	70.94	60.45	53.15	36.52	15.23	4.36	13	9.47	3.61	6.28	18.83	10.28
	21	70.18	60.15	52.67	37.86	16.64	5.67	13	8.70	3.44	6.57	18.51	11.55
	23	73.81	61.93	54.12	37.29	16.51	6.53	12	9.91	3.48	6.58	19.51	10.91
	22	74.87	63.12	54.78	37.01	15.49	5.59	15	10.25	3.22	6.69	20.46	11.48
	26	69.81	58.77	49.63	34.79	17.29	5.65	-	8.05	3.15	6.12	17.30	8.52
	25	79.93	70.49	62.09	43.80	23.83	5.38	12	10.29	2.94	7.02	20.23	12.01
	26	70.80	62.07	52.98	36.82	17.12	4.54	12	8.40	3.58	5.69	17.68	10.06

Host species	den	bd	add	tpd	drd	trd	wbm	rad	bl	cpw	rl	dw	ds
P. philander	F14PpND												
F16PpEB (continue)	22	34.79	31.56	27.96	20.49	10.84	1.24	10	5.01	1.63	3.11	9.50	4.94
	F18TsBW												
	21	75.05	60.56	52.37	37.50	15.55	6.65	12	10.11	4.44	5.74	20.69	13.03
	24	86.95	73.85	65.26	48.18	20.92	6.66	-	12.41	3.85	7.42	23.27	12.92
	25	82.40	70.18	63.47	46.40	18.95	6.65	-	10.32	4.13	6.88	21.01	11.19
	24	80.36	68.18	63.54	46.27	20.24	5.20	-	12.46	3.70	7.38	22.94	13.14
	24	86.23	72.84	63.33	46.80	19.69	7.29	-	11.89	3.89	7.64	22.96	12.88
	24	84.10	72.04	63.42	45.97	18.79	6.37	-	12.87	3.59	7.24	23.21	13.48
	24	88.06	75.00	66.24	47.89	19.83	6.53	12	11.94	4.03	7.34	23.19	13.06
	22	88.08	75.05	66.18	47.07	19.28	6.07	-	13.04	4.72	8.21	25.23	14.22
	25	92.63	79.34	69.28	-	24.84	6.65	13	11.92	4.65	7.97	24.47	12.82
	22	83.44	69.70	60.55	43.78	19.30	6.64	-	8.50	3.63	7.08	18.65	11.76
	24	90.36	78.01	68.41	49.87	21.67	5.60	13	12.69	4.35	7.58	25.19	13.96
	24	83.64	70.32	60.34	43.69	18.15	6.62	13	10.48	4.11	5.71	22.11	12.92
	24	83.67	71.45	61.61	43.62	19.24	6.42	11	11.47	3.61	5.98	21.79	12.80
	24	78.76	66.01	59.64	42.98	17.70	6.00	-	10.78	4.27	7.38	21.86	11.42
	25	87.41	73.88	66.00	47.95	20.75	6.43	14	11.46	4.26	7.46	22.65	12.85
	23	73.21	63.67	51.98	36.61	15.71	5.21	12	9.93	3.54	6.68	19.70	11.17
	24	76.78	64.88	58.77	42.51	19.43	6.46	12	10.95	4.07	6.75	21.56	12.15
	24	84.22	72.47	64.17	47.38	17.58	5.40	-	16.07	4.88	7.04	23.67	12.96
	23	83.74	72.03	65.71	47.04	21.12	6.66	-	12.10	4.68	7.46	24.12	13.61
	23	80.02	66.57	56.78	39.84	16.76	6.56	11	10.94	3.96	6.71	21.46	11.73
	21	70.56	59.42	51.66	36.03	16.86	5.25	11	7.12	5.06	5.81	18.37	10.27
	23	79.99	68.62	61.54	44.38	16.93	5.37	-	10.91	3.52	6.84	21.67	13.62
	23	77.51	65.29	56.60	39.67	17.71	5.96	11	8.00	3.52	6.90	17.86	11.81
	23	73.67	60.76	55.88	40.49	16.88	6.16	11	8.52	4.03	6.53	19.24	10.68

Host species	den	bd	add	tpd	drd	trd	wbm	rad	bl	cpw	rl	dw	ds
P. philander F18TsBW (continue)	24	44.89	38.45	32.86	23.94	10.51	3.38	13	5.39	2.12	3.42	11.04	6.56
	F17TsBW												
	23	73.70	62.84	54.34	38.46	15.96	6.44	14	10.08	3.74	5.87	19.72	10.67
	23	78.57	67.78	58.49	41.40	16.09	6.47	12	10.09	3.99	6.61	20.99	10.79
	24	78.47	68.90	58.52	42.32	19.29	5.30	11	8.87	3.17	7.46	20.18	10.35
		78.61	66.12	58.48	41.98	19.85	6.58	13	11.39	3.53	6.58	21.24	10.51
Amietia angolensis	T18AMaSS												
	24	66.84	53.12	46.30	33.41	15.43	5.31	9	6.97	3.60	5.04	15.94	7.86
	23	67.13	55.13	48.46	34.20	17.69	5.34	10	7.49	3.37	5.88	16.54	7.75
Amietia species	T16AMuHS												
	23	84.19	76.02	64.89	47.47	21.51	4.54	11	11.54	5.88	6.86	23.90	12.17
	21	78.86	-	66.75	41.60	16.26	5.89	12	11.55	4.02	6.92	22.14	12.76
	24	82.49	73.42	63.26	46.53	24.40	5.65	10	9.84	3.51	7.23	20.58	10.80
	23	76.96	65.12	56.07	39.06	21.33	5.81	9	7.96	3.64	6.58	18.24	8.44
	22	79.21	67.57	58.31	41.33	20.96	5.89	10	8.56	3.82	6.74	19.24	9.49
	23	78.62	65.81	58.95	40.62	15.54	6.76	12	11.09	4.56	6.92	22.53	11.71
	24	78.02	67.15	61.27	43.79	20.42	6.23	12	11.47	3.47	7.41	22.60	10.57
	21	73.46	61.17	53.86	37.19	14.76	5.90	13	10.39	3.44	6.64	20.15	10.65
	22	78.48	68.28	59.74	42.86	21.48	5.31	11	10.14	3.29	7.11	20.47	10.74
	24	74.89	64.48	57.34	41.99	22.72	5.34	11	8.25	2.70	6.04	16.99	8.43
	24	74.94	66.45	55.25	39.86	15.72	4.60	11	9.32	3.57	6.52	20.63	9.13
	23	68.56	58.53	51.67	35.89	17.10	4.75	10	7.88	2.70	6.83	17.60	9.05
	22	60.46	50.23	45.85	31.04	13.50	5.46	11	8.72	2.98	5.63	17.20	9.18
	22	71.96	61.11	52.33	36.37	14.25	5.30	12	9.27	3.72	6.17	19.15	9.98
	23	72.20	59.77	52.50	35.49	16.14	5.65	11	10.36	2.92	7.18	20.10	10.95

Host species	den	bd	add	tpd	drd	trd	wbm	rad	bl	cpw	rl	dw	ds
Amietia species (continue)	T14AMuNW												
	19	49.21	40.58	36.04	25.21	12.22	4.97	9	5.21	3.02	4.54	12.75	6.84
	21	50.02	41.93	39.06	26.26	11.57	4.11	11	6.71	2.63	5.17	14.39	6.32
	T15AMuOG												
	22	58.49	48.80	41.24	30.93	13.53	4.08	8	6.88	2.76	4.05	14.06	7.39
	20	51.63	43.81	36.26	25.29	11.89	3.91	9	6.14	2.00	4.51	12.62	6.09
	T17AMuWU												
	23	43.13	38.51	34.84	24.30	12.44	4.09	14	5.25	2.41	3.99	11.54	6.14
Amietophrynus gutturalis	T10AgNC												
	-	57.14	45.98	44.67	29.81	13.72	6.27	8	6.16	2.74	6.09	15.10	8.63
	-	69.54	58.80	53.59	36.55	17.84	5.56	11	7.86	2.77	6.61	17.35	9.63
	23	65.13	54.84	48.99	33.99	12.26	5.88	12	9.16	3.34	6.02	18.74	10.05
	22	54.65	43.56	41.57	27.87	14.40	5.59	8	6.34	2.74	5.19	14.73	8.58
	22	70.12	59.50	51.85	36.57	16.33	5.47	10	8.80	3.37	6.65	19.18	9.52
	23	60.45	50.60	42.57	28.27	16.03	4.89	8	6.43	2.74	5.47	14.81	8.68
	22	62.08	52.44	45.70	32.27	16.62	5.47	10	7.62	2.78	5.55	15.43	10.36
	22	70.43	58.48	52.81	35.60	18.55	6.15	11	8.10	2.67	6.53	17.01	10.37
	21	53.01	45.42	40.27	27.72	11.24	4.96	10	7.35	2.04	5.29	15.26	8.27
	20	67.59	57.65	48.42	35.52	18.79	3.74	12	6.64	2.55	5.95	15.28	9.06
	24	56.55	44.98	42.00	26.97	13.06	5.54	7	5.73	2.39	5.66	13.62	7.36
	22	49.27	39.53	36.24	23.05	11.96	5.88	7	5.01	2.09	5.46	12.31	7.48
	-	68.28	59.36	52.60	38.13	20.84	5.23	11	8.40	2.40	5.86	16.87	9.30
	21	67.18	56.27	49.50	34.53	17.54	5.54	13	9.17	2.21	6.34	17.21	9.07
	-	63.17	52.21	46.42	31.19	11.57	4.96	8	7.35	3.88	5.22	16.71	8.39
	20	61.94	51.41	45.86	30.40	12.17	5.75	11	7.40	2.85	6.07	16.30	9.13
	20	61.84	51.19	46.16	31.53	13.10	4.32	11	8.23	1.83	6.81	16.59	10.15
	25	73.24	61.73	55.34	39.54	19.11	5.59	14	9.48	3.27	6.98	19.48	9.11

Host species	den	bd	add	tpd	drd	trd	wbm	rad	bl	cpw	rl	dw	ds
Amietophrynus gutturalis T10AgNC (continue)	22	51.53	41.71	38.60	24.95	11.17	4.61	6	7.68	2.22	5.56	15.44	8.25
	23	51.59	41.58	37.33	24.75	11.54	4.83	7	5.57	2.27	5.34	13.83	6.64
	23	58.09	51.38	43.39	29.39	13.86	4.67	8	6.55	2.23	6.00	15.36	8.82
	20	56.41	48.64	42.73	29.07	12.27	3.65	10	7.94	2.98	5.63	15.80	8.87
	21	65.56	54.25	44.93	29.40	11.39	5.40	8	6.81	2.95	5.97	16.21	9.65
	21	66.02	56.70	48.89	34.28	13.90	5.15	12	7.89	3.54	5.54	16.82	9.49
	24	69.22	59.07	52.13	37.26	14.84	5.09	9	10.38	3.76	5.51	18.97	11.62
	-	57.88	51.70	44.16	32.09	13.11	3.74	10	6.99	2.39	4.56	13.76	9.05
	23	68.97	58.91	51.73	34.25	17.22	5.77	9	7.52	3.80	6.91	16.67	9.02
	19	61.05	49.52	41.89	28.80	11.02	5.89	-	8.21	2.37	5.31	15.81	10.37
	22	63.28	52.11	45.04	32.11	12.59	5.40	10	8.39	2.14	5.23	15.70	8.16
	22	69.10	58.59	50.54	33.45	13.73	5.31	13	9.36	3.13	6.46	18.75	9.51
	23	59.53	50.19	45.75	30.23	13.43	5.13	7	8.70	2.72	6.49	17.31	9.11
	20	61.47	52.07	44.03	30.07	13.15	4.94	10	7.55	2.73	5.79	15.60	9.20
	22	59.97	49.96	44.09	30.50	13.67	5.45	7	6.77	2.23	5.43	14.70	8.91
	21	54.41	44.34	39.96	26.80	11.66	5.46	10	6.30	2.64	5.29	13.63	8.05
	-	54.47	44.04	39.70	25.31	11.65	5.54	6	5.89	2.30	5.09	13.86	7.31
	20	56.15	44.04	40.38	26.72	12.23	5.16	10	7.73	2.17	5.25	15.00	9.50
	23	65.15	53.90	48.82	32.35	15.02	5.82	7	7.33	2.91	6.48	16.90	7.79
	22	56.66	46.64	44.16	29.97	12.81	5.05	-	7.28	3.16	5.58	15.62	8.62
	21	64.98	53.42	48.86	34.37	16.58	6.33	10	8.93	3.10	5.83	16.85	10.27
	20	59.29	47.84	43.88	30.03	11.18	5.21	11	8.32	2.82	5.77	16.72	9.06
	20	57.04	47.58	44.17	30.41	14.08	4.07	11	8.34	2.39	5.64	15.79	10.35
	22	56.60	47.57	44.79	31.00	12.00	4.64	-	9.03	2.60	5.37	16.79	9.46
	20	61.60	51.19	44.90	30.67	13.56	5.63	11	7.70	2.32	5.73	16.01	8.77
	20	63.48	54.09	46.61	31.55	12.21	6.39	12	7.73	2.64	6.20	16.48	9.40
	22	57.60	46.13	42.51	27.25	12.29	5.67	7	7.50	2.94	5.52	16.07	8.31

Host species	den	bd	add	tpd	drd	trd	wbm	rad	bl	cpw	rl	dw	ds
Amietophrynus gutturalis T10AgNC (continue)	24	76.16	64.85	56.31	40.05	17.42	5.31	12	9.77	2.77	6.05	18.79	11.09
	20	56.72	44.95	43.58	31.19	11.23	5.83	-	8.68	2.54	5.90	16.35	8.43
	21	67.63	58.21	49.52	35.08	15.94	3.77	11	8.73	3.00	5.54	16.83	10.23
	-	56.12	45.68	39.90	26.64	11.87	5.09	7	6.42	2.57	6.07	14.65	8.70
	23	69.46	57.43	49.50	34.40	15.59	5.72	10	8.36	2.54	5.95	16.49	8.43
	21	66.10	52.95	48.09	33.05	13.85	6.08	10	7.45	2.31	5.84	16.00	9.53
	21	70.33	59.19	52.16	37.35	22.44	5.66	10	8.02	1.81	6.26	16.08	7.85
	20	65.90	53.83	45.54	30.75	12.45	5.76	10	7.19	2.26	6.59	15.80	9.23
	21	66.64	58.28	37.53	14.40	4.50	-	14	10.53	2.26	5.88	19.14	10.14
	23	59.49	48.68	44.15	30.42	11.96	4.96	7	7.17	2.26	6.16	15.70	7.87
	23	73.58	61.40	54.03	38.21	16.95	6.76	10	9.12	2.86	6.39	18.67	9.62
	22	59.18	48.75	43.93	31.12	12.35	5.30	-	7.21	2.63	5.73	15.41	8.65
	22	69.10	58.59	50.54	33.45	13.73	5.31	13	9.36	3.13	6.46	18.75	9.51
	23	59.53	50.19	45.75	30.23	13.43	5.13	7	8.70	2.72	6.49	17.31	9.11
	20	61.47	52.07	44.03	30.07	13.15	4.94	10	7.55	2.73	5.79	15.60	9.20
	22	59.97	49.96	44.09	30.50	13.67	5.45	7	6.77	2.23	5.43	14.70	8.91
	21	54.41	44.34	39.96	26.80	11.66	5.46	10	6.30	2.64	5.29	13.63	8.05
	-	54.47	44.04	39.70	25.31	11.65	5.54	6	5.89	2.30	5.09	13.86	7.31
	20	56.15	44.04	40.38	26.72	12.23	5.16	10	7.73	2.17	5.25	15.00	9.50
	23	65.15	53.90	48.82	32.35	15.02	5.82	7	7.33	2.91	6.48	16.90	7.79
	22	56.66	46.64	44.16	29.97	12.81	5.05	-	7.28	3.16	5.58	15.62	8.62
	21	64.98	53.42	48.86	34.37	16.58	6.33	10	8.93	3.10	5.83	16.85	10.27
	20	59.29	47.84	43.88	30.03	11.18	5.21	11	8.32	2.82	5.77	16.72	9.06
	20	57.04	47.58	44.17	30.41	14.08	4.07	11	8.34	2.39	5.64	15.79	10.35
	22	56.60	47.57	44.79	31.00	12.00	4.64	-	9.03	2.60	5.37	16.79	9.46
	20	61.60	51.19	44.90	30.67	13.56	5.63	11	7.70	2.32	5.73	16.01	8.77
	20	63.48	54.09	46.61	31.55	12.21	6.39	12	7.73	2.64	6.20	16.48	9.40

Host species	den	bd	add	tpd	drd	trd	wbm	rad	bl	cpw	rl	dw	ds
Amietophrynus gutturalis T10AgNC (continue)	22	57.60	46.13	42.51	27.25	12.29	5.67	7	7.50	2.94	5.52	16.07	8.31
	24	76.16	64.85	56.31	40.05	17.42	5.31	12	9.77	2.77	6.05	18.79	11.09
	20	56.72	44.95	43.58	31.19	11.23	5.83	-	8.68	2.54	5.90	16.35	8.43
	21	67.63	58.21	49.52	35.08	15.94	3.77	11	8.73	3.00	5.54	16.83	10.23
	-	56.12	45.68	39.90	26.64	11.87	5.09	7	6.42	2.57	6.07	14.65	8.70
	23	69.46	57.43	49.50	34.40	15.59	5.72	10	8.36	2.54	5.95	16.49	8.43
	21	66.10	52.95	48.09	33.05	13.85	6.08	10	7.45	2.31	5.84	16.00	9.53
	21	70.33	59.19	52.16	37.35	22.44	5.66	10	8.02	1.81	6.26	16.08	7.85
	20	65.90	53.83	45.54	30.75	12.45	5.76	10	7.19	2.26	6.59	15.80	9.23
	21	66.64	58.28	37.53	14.40	4.50	4.00	-	10.53	2.26	5.88	19.14	10.14
	23	59.49	48.68	44.15	30.42	11.96	4.96	7	7.17	2.26	6.16	15.70	7.87
	23	73.58	61.40	54.03	38.21	16.95	6.76	10	9.12	2.86	6.39	18.67	9.62
	22	59.18	48.75	43.93	31.12	12.35	5.30	-	7.21	2.63	5.73	15.41	8.65
	19	61.41	49.91	45.29	31.15	12.84	6.66	12	7.73	2.48	6.19	16.49	7.83
	24	62.46	50.20	49.12	35.26	17.29	5.45	-	8.73	2.92	5.51	16.79	7.97
	24	52.12	42.34	39.53	26.58	11.56	4.80	6	7.69	2.26	5.40	15.39	7.81
	23	63.09	53.37	48.17	33.24	16.90	4.69	7	8.51	2.27	6.38	16.64	8.28
	20	68.09	56.59	49.35	33.98	14.88	5.27	10	8.86	2.27	6.03	17.00	10.99
	-	66.89	55.11	49.42	35.05	16.17	6.06	7	7.44	3.13	6.40	18.22	8.27
	-	62.17	50.57	44.52	29.29	12.40	5.38	10	6.67	2.82	6.20	16.44	9.96
	21	60.80	48.93	43.67	29.48	9.83	6.38	9	8.30	2.62	5.72	17.04	7.96
	22	70.80	59.91	51.26	36.06	16.65	6.19	11	9.76	2.47	6.26	18.90	9.75
	-	62.34	51.67	43.34	30.31	11.83	6.01	12	7.59	2.69	5.29	15.59	7.70
	24	55.78	44.42	40.89	27.11	12.09	5.68	6	6.33	3.25	5.33	14.48	8.44
	22	57.16	46.71	41.48	27.96	12.62	4.99	9	8.27	2.44	5.45	15.62	8.37
	22	65.15	54.23	48.09	33.25	18.61	5.46	8	7.34	2.46	6.61	16.17	9.84
	20	60.38	49.16	43.80	31.01	14.49	5.72	10	9.20	2.85	4.63	16.78	9.28

Host species	den	bd	add	tpd	drd	trd	wbm	rad	bl	cpw	rl	dw	ds
Amietophrynus gutturalis T10AgNC (continue)	20	64.85	53.27	47.20	30.60	13.71	6.77	10	8.23	2.64	6.86	17.12	9.23
	21	65.17	53.83	47.28	32.55	13.21	5.56	11	6.52	3.48	5.91	16.43	10.42
	20	62.93	49.76	44.26	31.33	14.65	4.88	11	8.14	3.01	5.16	15.88	9.42
	22	62.48	51.78	43.76	31.17	14.28	6.18	10	6.52	2.23	5.54	15.13	8.58
	22	57.46	47.97	42.75	28.56	13.78	5.84	7	6.44	2.17	6.11	14.40	7.15
	22	63.73	51.14	44.52	29.41	13.64	4.91	12	7.89	2.53	6.49	16.43	8.64
	20	68.54	59.17	47.43	32.89	13.26	5.47	11	8.11	2.82	6.30	17.29	10.90
	22	69.56	59.98	50.66	36.29	15.64	5.53	11	8.98	2.95	6.22	18.15	9.46
	20	63.81	54.46	46.40	31.33	13.27	5.97	12	8.26	2.72	5.93	16.81	9.29
	22	68.59	58.62	49.68	35.43	14.89	4.78	10	9.61	2.94	6.40	18.76	10.96
	21	62.81	51.14	43.58	32.07	16.15	5.74	10	8.55	1.97	5.54	16.27	9.67
	22	66.81	56.95	47.24	32.24	12.80	3.94	11	8.23	2.27	6.30	16.83	9.68
	20	55.57	47.73	39.26	24.82	11.10	3.94	6	7.05	2.55	5.47	15.39	9.93
	20	55.23	47.79	39.46	26.18	11.79	4.47	11	6.89	2.47	5.95	14.58	9.76
	20	63.17	53.35	47.71	32.91	13.43	4.96	12	8.90	3.13	5.92	17.76	9.06
	19	62.94	53.09	44.00	30.33	12.51	5.05	10	7.35	2.67	6.44	16.31	7.89
	25	63.64	52.89	45.89	31.46	16.03	5.82	11	8.14	2.86	5.85	16.13	9.23
	22	66.73	56.11	47.03	33.45	12.46	4.52	9	8.35	2.73	6.29	17.01	8.36
	19	51.56	44.47	38.76	25.88	8.86	3.95	10	7.53	2.57	4.72	15.13	8.33
	22	60.90	51.02	45.53	31.78	13.66	5.16	10	8.02	2.11	6.35	16.70	8.77
	21	67.95	56.34	51.99	36.90	17.57	6.46	8	8.18	2.28	6.24	16.93	9.43
	24	66.03	55.86	51.16	33.62	11.61	5.68	6	7.04	3.19	6.68	17.13	9.29
	21	62.31	53.56	47.26	33.86	14.33	4.38	-	10.07	1.86	6.08	17.40	8.66
	-	67.35	56.57	50.72	37.93	19.86	4.94	10	6.66	2.44	6.20	15.16	9.20
	21	61.49	50.07	47.54	32.87	13.32	6.33	-	8.35	2.54	6.23	16.92	9.06
	20	66.62	54.92	49.82	35.51	14.07	5.01	11	9.66	2.79	5.91	17.75	10.42
	22	56.47	46.17	44.38	30.82	11.83	5.05	-	7.85	2.82	6.04	16.71	9.10

Host species	den	bd	add	tpd	drd	trd	wbm	rad	bl	cpw	rl	dw	ds
Amietophrynus gutturalis T10AgNC (continue)	21	61.96	51.44	47.84	34.34	18.38	5.82	11	7.91	2.44	6.02	16.42	8.73
	22	53.11	42.24	39.94	26.56	11.78	5.46	-	6.77	2.23	5.67	14.25	8.77
	22	63.49	57.69	51.63	36.37	17.46	3.48	-	7.75	2.27	6.33	17.47	10.07
	24	72.05	63.58	54.68	37.47	18.48	5.51	7	8.01	3.30	6.48	17.72	10.34
	21	53.17	43.42	39.27	27.32	11.98	5.78	6	6.76	2.79	6.68	15.46	7.57
	21	53.78	43.77	39.91	27.78	10.71	5.52	-	7.27	2.55	5.20	14.98	9.39
	23	62.13	51.86	46.52	31.31	12.81	5.01	8	7.93	2.07	5.98	15.76	8.53
	25	55.88	46.39	45.14	31.76	11.84	5.89	-	9.50	2.93	6.56	18.43	8.61
	22	66.45	56.17	49.35	34.79	15.48	5.03	10	7.93	2.45	5.17	15.93	9.25
	22	68.34	56.16	49.57	35.23	16.88	5.98	10	9.42	2.22	5.94	17.73	9.18
	21	58.79	47.28	43.89	28.38	9.57	4.13	8	7.92	2.75	6.22	16.91	8.65
	22	71.78	60.50	53.13	36.24	15.03	5.76	8	10.30	3.44	6.90	20.44	9.74
	23	54.68	45.21	41.43	28.62	13.09	3.95	7	6.09	2.75	4.99	14.13	8.40
	22	73.40	62.39	55.08	39.04	15.80	5.46	12	9.11	3.41	5.77	18.90	11.00
	23	78.44	66.28	54.76	39.14	16.57	6.94	13	10.71	3.57	7.52	21.32	9.67
	21	62.60	51.55	46.43	32.52	16.40	5.40	11	8.02	2.23	5.96	16.43	9.76
	20	61.59	51.85	46.96	30.46	11.85	5.44	9	8.96	3.51	5.99	17.28	9.66
	24	69.42	58.07	50.82	35.76	18.63	6.00	11	8.73	2.54	5.94	17.22	10.60
	22	59.83	49.63	46.51	30.56	14.27	5.07	7	8.19	2.82	6.30	16.26	8.89
	T11AgSD												
	24	76.01	67.19	59.03	42.19	20.02	4.15	12	9.32	3.00	7.36	19.61	10.09
	25	68.99	56.72	49.66	34.73	18.06	6.07	10	7.63	3.31	5.98	16.96	8.62
	24	66.17	59.24	52.95	37.90	19.04	3.43	11	8.62	2.27	6.74	17.53	8.74
	27	76.24	66.10	57.99	43.89	24.62	4.98	7	8.75	3.85	6.31	18.69	9.37
	20	66.67	55.17	50.65	34.87	16.42	5.06	9	9.85	4.24	5.44	19.34	9.81
	22	71.11	60.32	53.48	38.36	20.16	5.62	11	7.55	2.71	6.62	9.97	-
	25	84.14	67.76	62.27	44.71	23.34	6.34	12	8.89	3.28	7.09	18.82	9.85

Host species	den	bd	add	tpd	drd	trd	wbm	rad	bl	cpw	rl	dw	ds
Amietophrynus gutturalis T11AgSD (continue)	27	82.84	70.82	63.59	45.34	26.46	5.51	9	8.50	2.95	8.25	19.43	9.30
	26	65.46	54.45	48.09	33.24	15.92	4.32	10	6.75	3.89	5.52	15.79	7.84
	27	78.47	66.16	56.79	39.87	21.49	5.87	10	8.81	3.82	6.73	19.15	7.44
	26	80.76	69.49	60.65	43.21	22.62	5.61	13	8.41	3.43	6.69	18.23	9.33
	29	85.24	73.06	66.19	47.03	27.32	6.37	11	8.23	3.10	7.42	18.69	10.57
	T12AgND												
	20	52.67	43.61	39.57	27.31	13.55	4.39	12	6.94	2.19	5.14	14.11	8.13
	24	68.34	57.05	48.71	34.17	14.58	5.94	9	7.99	3.23	5.52	16.85	8.32
	22	65.27	54.20	48.90	34.33	15.12	5.90	11	9.11	3.08	5.77	17.75	9.19
	21	62.24	52.08	45.40	32.13	12.62	5.29	9	8.62	2.92	5.39	16.75	8.61
	22	65.55	55.06	48.18	33.93	14.64	5.85	12	9.09	3.17	5.29	17.28	9.42
	24	66.72	56.45	48.48	35.89	15.06	5.42	11	8.33	3.19	5.69	16.82	8.51
	22	73.97	63.16	52.07	36.71	16.51	5.99	11	8.55	3.16	5.99	17.58	10.07
	21	65.57	54.61	46.10	30.28	10.33	5.71	9	10.25	3.69	5.60	19.17	9.11
	21	47.98	37.96	34.27	23.36	8.47	4.97	7	6.80	2.28	4.34	13.34	7.39
Amietophrynus poweri	T5ApNB												
	21	58.67	47.30	42.83	28.47	14.21	5.80	9	6.74	3.02	5.68	15.14	8.79
	20	57.80	49.25	43.47	29.40	13.86	5.19	8	8.11	2.12	6.04	15.74	9.26
	20	50.80	40.98	38.47	26.02	10.17	5.28	9	6.52	2.78	5.44	13.73	8.70
	22	66.22	57.68	51.85	36.21	16.92	6.05	12	10.45	2.15	6.73	19.15	10.66
	18	64.20	53.14	44.67	29.31	11.70	5.69	8	7.55	3.04	6.42	16.84	9.94
	21	57.56	47.45	42.99	28.68	11.13	5.46	6	7.06	2.75	6.29	16.17	8.19
	22	51.72	40.38	37.93	24.29	10.91	4.66	10	5.52	1.95	6.45	14.07	6.73
	21	-	48.53	46.34	30.39	14.14	4.82	9	6.27	2.17	6.39	15.43	8.13
	-	57.80	48.83	45.30	29.64	12.32	3.98	8	8.61	2.54	6.65	17.68	9.12
	22	51.74	41.51	36.53	24.46	12.40	5.49	7	5.52	2.23	4.85	12.87	7.69
	23	69.33	59.71	50.16	35.03	17.66	5.29	10	7.42	3.55	6.50	16.79	9.03

Host species	den	bd	add	tpd	drd	trd	wbm	rad	bl	cpw	rl	dw	ds
Amietophrynus poweri **T5ApNB (continue)**	23	51.69	42.03	37.14	21.58	11.40	4.45	-	3.62	2.19	6.22	12.51	7.02
	23	66.85	55.23	49.14	34.02	15.32	6.25	8	9.49	3.35	6.65	18.72	8.70
	22	59.32	47.52	44.27	29.39	10.67	5.78	8	8.13	2.69	5.95	17.12	9.02
	24	66.57	54.70	48.00	34.41	14.63	5.73	12	8.07	3.09	6.54	16.71	8.43
	22	60.78	53.18	47.36	31.41	15.59	5.23	-	8.50	3.00	6.72	18.74	9.00
	-	69.03	57.86	48.59	31.48	14.95	5.90	8	6.55	3.41	6.54	16.27	8.51
	-	59.79	50.28	44.03	29.29	10.96	4.67	7	8.29	2.97	6.05	17.88	8.20
	T7ApTB												
	25	60.07	50.18	44.86	30.68	15.70	5.42	11	7.90	2.57	5.26	15.91	8.83
	22	68.58	58.87	50.44	36.76	20.36	4.75	12	7.66	2.50	5.39	15.12	9.50
	21	60.08	48.27	40.83	28.77	9.73	5.26	9	6.80	2.43	5.19	14.15	9.12
	-	-	51.32	45.77	30.94	18.05	-	11	7.40	2.27	6.08	15.05	8.93
	23	65.65	53.32	48.80	34.49	16.75	5.83	11	7.88	2.19	6.02	16.86	8.50
	23	63.53	51.29	46.69	33.24	14.12	5.84	12	7.10	1.95	5.61	14.77	8.22
	21	67.08	56.34	50.24	33.74	15.92	5.65	10	8.03	3.66	6.67	18.22	11.41
	22	59.67	48.59	45.30	32.07	12.52	6.38	-	9.61	2.99	5.84	17.98	10.35
	24	73.15	60.84	53.09	38.02	19.47	6.24	10	7.34	3.70	6.17	16.87	8.33
	23	76.85	65.45	58.41	40.46	17.80	5.57	10	9.86	4.16	7.10	20.57	9.84
	22	60.22	50.22	45.82	30.19	13.81	5.49	12	7.88	2.73	6.23	16.88	8.99
	23	63.27	51.77	45.28	32.25	13.79	5.30	12	7.81	2.72	5.01	15.87	8.51
	25	77.72	65.83	55.04	39.64	17.86	6.82	10	8.72	2.79	7.02	18.36	9.57
	21	67.54	56.62	47.34	32.40	16.47	5.20	11	8.08	2.60	6.29	16.83	10.40
	21	61.62	52.62	45.14	30.82	15.40	3.78	10	7.16	2.57	6.21	15.42	8.70
	22	68.98	57.94	49.99	35.22	15.75	5.80	10	8.22	3.54	5.57	17.37	9.79
	22	72.47	61.41	53.16	38.26	18.11	6.21	10	9.27	2.22	6.76	18.68	9.44
	22	75.30	63.03	54.67	39.04	20.56	6.14	11	7.62	2.63	6.77	16.68	10.65
	22	64.47	53.12	46.36	31.50	12.59	5.47	11	8.10	3.05	6.07	16.45	8.02

Host species	den	bd	add	tpd	drd	trd	wbm	rad	bl	cpw	rl	dw	ds
Amietophrynus poweri T7ApTB (continue)	24	64.68	53.92	48.21	34.42	15.88	5.36	10	9.28	1.62	5.71	16.34	9.20
	25	66.73	56.81	50.47	34.72	16.43	5.82	11	8.35	2.65	6.61	17.42	9.72
	23	67.45	57.36	50.13	35.23	17.02	5.08	10	9.52	2.63	5.54	17.83	9.03
	22	68.88	60.82	51.36	36.86	15.61	3.96	14	8.23	3.15	6.52	17.73	9.92
	22	60.36	49.82	43.00	30.41	13.53	6.01	10	7.93	2.25	5.51	15.80	8.06
	26	61.16	52.91	44.80	33.11	15.11	6.07	12	7.85	2.34	5.41	15.80	8.50
	21	68.20	57.29	49.41	34.56	17.14	5.46	12	7.47	1.93	6.41	16.40	9.24
	24	69.39	57.54	48.14	31.99	13.56	5.41	12	8.45	2.92	5.74	17.47	8.97
	20	72.78	60.99	53.58	37.89	18.76	5.58	10	9.29	2.38	6.46	17.74	10.13
	25	62.45	51.06	46.54	32.93	15.35	5.58	12	8.05	2.94	5.62	17.14	6.57
	22	71.17	60.91	51.89	37.21	17.41	5.08	13	8.64	2.54	6.59	17.44	9.27
	23	69.56	58.79	51.75	36.28	19.59	5.10	11	7.57	3.05	6.04	16.71	9.29
	23	62.93	53.39	49.70	36.28	18.34	4.34	11	7.33	3.03	5.86	15.77	8.79
	22	62.23	52.66	48.04	33.50	18.62	4.73	10	6.74	2.32	5.57	14.37	9.16
	25	60.00	50.92	44.76	31.25	18.02	4.38	11	6.71	2.56	5.22	14.13	8.25
	23	62.11	51.93	45.12	32.75	17.95	5.40	10	8.26	2.58	5.92	16.41	9.01
	25	68.20	55.66	50.95	35.89	18.65	5.68	11	9.21	2.54	6.25	17.72	8.15
	23	62.82	52.15	48.29	33.74	13.57	4.92	-	8.85	2.50	5.44	16.85	8.96
	23	72.61	60.78	54.41	38.28	21.89	6.40	10	8.55	2.44	6.77	17.69	9.87
	21	67.29	58.35	54.44	37.71	19.69	3.25	10	8.10	2.76	6.60	17.38	10.51
	23	51.06	42.12	41.21	28.20	15.30	5.03	-	5.60	2.13	5.18	13.03	8.48
	21	68.11	54.90	49.42	34.19	17.70	5.61	11	8.89	2.18	6.25	17.07	9.80
	23	76.88	64.80	57.10	41.15	20.95	5.81	10	8.85	3.87	6.51	18.47	10.63
	23	58.08	48.18	43.07	29.50	10.36	4.51	12	7.91	2.54	5.84	16.14	8.55
	23	62.63	52.24	46.30	32.12	16.36	5.84	7	8.62	2.28	5.95	16.82	9.79
	25	71.65	60.70	54.33	39.28	18.73	5.84	10	7.61	3.24	6.31	17.12	9.15
	22	54.71	45.74	43.03	28.18	11.56	5.09	11	7.43	2.57	5.14	14.75	8.63

Host species	den	bd	add	tpd	drd	trd	wbm	rad	bl	cpw	rl	dw	ds
Amietophrynus poweri T7ApTB (continue)	21	60.15	48.95	47.03	32.46	18.86	5.56	9	6.76	2.89	5.23	14.49	10.09
	25	62.12	55.25	52.10	36.90	19.31	3.87	11	7.57	2.45	6.33	16.77	9.35
	25	63.36	51.48	45.67	32.27	13.97	5.96	11	7.06	2.46	5.19	14.42	8.18
	21	67.67	56.91	47.91	33.37	19.35	5.87	9	6.67	2.32	5.64	14.30	9.71
	24	61.94	52.31	45.66	33.08	16.31	4.81	11	6.94	2.60	5.34	14.85	9.70
	22	58.35	48.94	43.97	29.35	12.96	4.42	11	7.08	3.85	5.22	15.31	7.71
	21	68.70	57.31	51.68	37.31	17.47	6.01	12	9.35	2.66	6.04	17.77	10.19
	24	67.82	57.11	52.09	36.08	18.60	4.92	9	7.96	2.65	7.01	17.14	8.84
	22	58.90	46.59	41.70	28.63	13.39	5.67	9	5.77	2.52	5.09	13.26	9.17
	20	56.71	46.40	42.59	29.87	14.28	5.14	10	8.06	1.79	5.26	15.22	9.26
	23	66.02	53.99	49.23	34.39	17.00	5.72	11	8.35	2.48	5.73	16.44	9.55
	23	65.60	54.50	49.57	34.94	16.58	5.53	11	9.07	2.72	5.93	17.58	10.30
	24	60.90	47.75	42.64	30.14	13.26	5.45	7	6.73	2.25	5.17	13.96	7.46
	24	66.21	53.27	51.38	35.45	18.50	4.79	9	8.11	2.42	6.05	16.05	9.58
	24	54.20	45.56	41.67	28.30	12.80	4.87	10	7.21	2.26	5.11	12.72	10.32
	23	69.99	58.53	50.77	34.70	17.61	5.86	11	8.40	2.01	6.69	16.89	8.45
	22	64.46	56.19	49.86	34.69	17.87	5.81	10	8.94	1.82	6.27	16.91	9.65
	23	70.61	61.13	55.09	39.82	17.68	5.49	11	9.71	3.24	5.93	18.36	9.83
	22	69.98	57.52	48.24	34.44	15.87	5.94	11	8.60	2.54	5.66	17.01	9.34
	23	73.07	61.86	54.28	38.24	17.91	6.26	11	9.40	3.19	6.88	19.26	9.96
	21	68.42	54.25	47.13	31.74	12.44	5.99	11	8.34	2.94	6.46	17.45	9.80
	21	75.34	63.70	55.15	39.29	15.54	6.20	-	10.55	2.75	6.61	19.38	11.80
	24	71.06	61.53	53.90	34.60	15.40	5.29	9	6.57	3.25	6.72	16.81	9.86
	22	70.04	58.29	50.99	35.65	14.63	4.76	11	8.50	2.89	6.72	17.08	8.95
	23	61.99	51.28	43.38	30.83	14.80	6.40	11	8.12	2.68	5.14	15.70	8.81
	23	62.18	51.35	45.41	30.80	14.81	5.61	11	7.69	2.89	6.14	16.17	8.58
	22	64.80	53.36	47.30	32.04	13.17	5.88	12	8.30	2.65	6.14	16.97	8.49

Host species	den	bd	add	tpd	drd	trd	wbm	rad	bl	cpw	rl	dw	ds
Amietophrynus poweri T7ApTB (continue)	23	71.85	60.02	50.87		17.46	6.07	11	8.61	2.69	6.49	17.54	9.76
	22	68.54	57.41	51.08	34.97	13.06	6.77	11	9.04	3.31	5.98	18.64	9.08
	23	77.39	66.90	55.93	39.82	23.93	5.71	12	8.15	2.28	6.49	16.59	10.30
	21	70.00	61.91	49.65	33.15	16.75	3.19	10	7.32	2.72	6.70	16.12	10.71
	23	64.35	53.72	45.05	31.24	13.52	4.59	10	8.73	2.77	5.78	16.88	7.84
	23	60.69	50.50	46.07	31.09	14.10	5.89	-	9.04	3.08	5.80	18.09	8.36
	23	60.08	51.87	42.47	29.99	11.06	4.24	-	7.50	2.52	5.40	15.29	8.46
	21	65.31	53.96	46.78	30.72	13.15	5.65	11	9.30	3.32	6.09	18.65	8.78
	21	67.00	56.63	48.29	34.00	15.12	5.80	10	9.20	2.43	5.87	17.14	9.13
	21	65.52	53.78	45.92	31.38	14.24	5.38	12	8.71	2.89	6.60	18.34	9.79
	24	68.23	57.52	49.66	34.01	17.44	5.37	10	8.01	3.12	5.98	17.27	8.94
	24	66.70	54.96	47.95	34.58	16.85	6.00	11	8.16	2.44	5.20	15.20	9.52
	24	69.58	59.20	51.65	37.28	19.02	5.56	10	7.29	3.31	6.13	16.64	8.88
	23	66.83	55.83	47.84	34.19	13.61	6.13	10	9.39	2.54	6.30	18.05	8.18
	23	68.57	58.19	49.40	35.44	18.54	5.19	-	8.44	2.79	6.25	16.88	9.01
	22	70.02	58.71	50.15	35.56	16.96	3.60	11	8.34	3.02	5.98	16.20	9.80
	23	68.65	58.99	49.36	34.31	17.73	5.83	11	8.16	2.92	6.20	16.96	8.88
	22	68.77	58.69	50.71	36.13	21.71	5.75	-	8.91	3.49	5.88	18.16	10.07
	-	60.53	55.53	47.49	33.83	16.69	4.07	12	7.34	2.45	5.46	15.42	9.03
	23	68.14	58.75	50.13	35.24	18.93	4.48	9	7.40	3.08	5.77	16.33	9.70
	T9ApCF												
	22	66.62	55.49	47.64	33.03	13.60	5.65	10	8.92	2.62	6.47	17.74	8.45
	22	69.80	58.29	51.06	35.77	13.95	6.36	10	9.94	2.83	5.52	18.29	10.08
	23	75.15	66.68	57.75	43.19	20.47	5.86	11	9.31	2.97	6.47	18.70	10.97
	24	64.62	53.40	46.82	31.94	14.69	5.37	13	8.53	2.37	6.09	16.69	9.66
	25	69.84	58.84	47.61	34.48	17.04	4.60	12	8.12	2.90	5.73	16.85	9.18
	23	60.44	50.56	40.79	28.66	12.48	5.03	10	6.65	2.20	5.32	13.78	5.15

Host species	den	bd	add	tpd	drd	trd	wbm	rad	bl	cpw	rl	dw	ds
Amietophrynus rangeri	T13ArLB												
	21	48.90	40.29	37.46	26.46	11.63	4.37	11	6.92	1.85	5.00	13.42	6.66
	22	53.66	43.50	38.70	26.79	12.11	4.59	10	5.95	3.24	4.53	13.58	7.12
	21	48.45	38.05	35.95	25.05	11.84	5.40	10	5.93	2.62	4.15	12.49	7.17
	20	51.19	41.47	37.39	25.41	13.38	4.69	11	5.28	2.16	4.63	12.18	6.62
	20	51.94	42.80	38.47	25.79	10.87	4.49	12	6.49	1.74	5.13	13.44	6.97
	20	48.07	38.39	33.75	22.69	9.61	4.74	10	5.76	1.92	4.29	12.06	5.83
	20	51.13	42.37	38.09	26.96	15.51	3.89	11	5.65	2.00	4.40	11.94	6.85
	20	48.32	38.69	35.34	23.33	11.52	4.56	8	5.25	2.04	4.75	11.97	7.47
	20	51.78	41.81	38.50	26.06	12.57	4.41	11	6.29	2.41	3.82	12.59	7.31
	20	41.91	35.15	31.77	20.44	6.79	3.74	11	4.59	2.48	4.33	11.62	5.99
	23	50.64	44.77	39.94	27.53	10.85	5.27	11	6.91	2.94	5.39	14.99	6.67
	23	55.25	47.99	41.76	28.16	12.90	3.00	12	6.28	3.26	4.51	14.16	7.23
	22	49.50	42.15	37.14	24.16	10.79	4.21	12	6.10	2.80	5.13	13.38	6.56
	22	55.77	48.09	41.00	29.43	15.39	4.20	12	6.35	2.12	5.28	13.77	7.12
	22	54.80	46.40	41.68	28.47	13.11	4.50	11	6.99	2.11	5.29	14.50	7.21
	21	47.54	39.81	35.25	24.38	12.06	4.34	12	5.63	2.25	4.22	12.25	7.20
	20	52.04	43.51	37.31	25.84	13.15	4.33	10	5.74	2.05	5.30	13.22	6.14
	23	53.79	44.78	37.93	25.89	12.54	4.47	10	6.15	2.67	4.69	13.05	6.82
	23	50.57	43.39	36.78	25.33	14.12	4.46	12	5.31	2.36	4.60	12.14	7.16
	21	49.12	40.04	35.79	24.91	11.00	4.55	12	5.91	2.10	4.73	12.66	6.66
	20	46.90	39.07	36.05	24.63	11.02	3.72	11	5.96	1.94	4.69	12.46	7.00
	21	43.20	35.26	33.31	23.11	11.26	3.40	9	5.02	2.35	3.86	11.12	6.40
	23	48.87	41.81	37.92	25.90	12.80	3.67	10	6.20	2.71	4.84	13.65	6.73
	21	54.05	45.39	37.68	25.99	13.63	4.33	11	6.60	2.02	5.16	13.62	6.42
	22	58.58	49.70	43.54	30.28	16.73	4.61	10	5.57	2.50	5.75	13.87	7.68
	22	55.41	46.49	39.19	27.09	13.14	4.54	10	6.28	2.21	4.62	13.01	6.63

Host species	den	bd	add	tpd	drd	trd	wbm	rad	bl	cpw	rl	dw	ds
Amietophrynus rangeri T13ArLB (continue)	21	51.80	44.02	38.40	25.46	11.30	3.99	12	5.66	2.81	4.91	13.46	6.80
	21	51.89	42.69	36.84	25.31	11.43	4.55	9	5.93	2.49	4.16	12.38	6.28
	20	53.51	43.71	37.88	25.56	11.17	4.22	8	6.81	2.59	4.58	13.81	6.68
Amietophrynus species	T1AuSR												
	23	52.34	45.62	39.44	28.82	14.89	3.02	11	6.66	2.71	4.17	13.22	7.24
	21	53.85	45.92	40.96	28.67	14.15	-	9	7.50	2.90	4.70	15.05	7.77
	T4AuBH												
	22	55.63	44.67	42.76	28.54	11.95	5.60	-	6.76	2.23	5.89	15.50	7.74
	20	56.49	44.73	43.79	28.45	12.05	5.81	14	7.35	3.11	5.48	15.85	9.93
	20	51.48	42.32	39.81	25.92	11.36	4.48	12	6.43	2.17	5.72	14.08	7.42
	19	55.51	44.65	42.60	28.62	10.45	5.67	11	8.11	2.91	6.00	17.27	9.18
	19	54.48	42.93	41.71	26.47	9.44	5.81	13	6.66	2.98	6.12	15.67	8.30
	15	62.21	52.35	49.08	28.69	14.01	4.87	-	8.44	2.99	6.69	17.64	11.29
	23	50.70	44.21	41.03	27.73	11.67	3.06	11	6.91	2.63	5.63	15.02	7.70
	19	60.25	49.78	44.41	29.26	10.29	4.51	-	7.99	2.92	5.28	15.99	8.42
	20	56.80	46.01	42.08	28.15	11.27	5.53	-	7.02	3.03	5.69	15.42	7.96
	21	62.56	51.90	49.65	34.55	17.71	4.99	-	7.55	2.89	6.53	17.13	9.72
	19	57.46	48.22	43.13	29.02	16.77	5.28	-	7.50	2.57	6.49	16.22	7.67
	20	54.87	44.00	42.20	29.46	14.48	5.54	-	6.49	2.97	5.32	14.94	7.89
	20	51.55	41.13	40.09	26.62	10.49	5.52	-	7.29	2.49	5.64	15.15	7.64
	20	52.81	42.56	41.72	26.94	11.11	5.08	-	6.30	2.74	5.48	15.28	8.07
	T3AuSB												
	-	44.98	36.58	31.98	22.33	11.12	3.97	9	5.46	1.86	3.76	10.30	6.81
	T1AuSR												
	23	62.72	52.34	47.67	33.95	19.01	5.86	11	7.37	2.89	5.21	16.68	7.95
	21	67.42	54.02	48.82	33.75	13.96	6.20	11	9.24	3.81	5.26	17.91	10.83
	19	67.64	58.88	51.05	35.79	14.71	5.20	9	9.49	3.03	5.85	18.78	10.76

Host species	den	bd	add	tpd	drd	trd	wbm	rad	bl	cpw	rl	dw	ds
Amietophrynus species T1AuSR (continue)	26	82.50	68.71	60.87	45.49	27.97	6.19	10	7.67	3.68	6.84	18.20	9.12
	22	67.28	61.86	53.82	35.92	17.22	2.59	11	7.57	3.66	6.76	17.68	9.86
	24	87.05	76.48	69.31	49.40	25.65	4.80	9	9.78	4.22	6.76	19.31	13.09
	22	111.39	101.06	84.58	61.35	29.34	4.96	12	13.22	5.49	9.46	27.94	16.48
	25	79.79	67.30	58.36	41.90	24.02	6.20	10	8.62	3.49	6.48	18.71	9.84
	23	56.58	49.93	44.15	31.36	13.76	3.06	11	7.12	3.28	5.07	15.22	7.46
	T2AuSH												
	21	64.88	52.95	47.25	32.99	13.11	6.44	11	9.06	3.00	5.44	17.64	8.61
	24	73.21	61.62	55.11	39.13	17.75	4.97	9	9.35	3.66	5.02	17.93	8.51
	22	66.73	56.48	52.42	37.25	19.03	5.09	9	8.30	2.95	5.74	16.82	8.68
	23	66.64	54.84	47.97	34.22	15.59	4.93	12	8.72	2.71	6.53	17.79	7.98
	23	63.51	52.03	45.81	33.06	14.54	5.80	8	8.32	2.86	5.09	16.20	8.53
	23	66.89	54.52	48.03	34.40	14.05	6.02	9	9.11	3.31	5.37	17.51	8.68
	21	71.73	61.64	52.98	38.73	15.72	5.90	8	9.92	3.45	6.23	19.28	9.54
	22	72.42	61.16	54.10	39.07	16.63	5.87	10	9.52	3.49	5.96	18.59	10.59
	23	66.24	53.65	49.19	35.51	15.09	5.77	9	9.67	2.75	5.32	17.50	9.56
	24	69.62	56.06	50.79	37.78	21.53	5.58	10	6.77	3.28	4.72	14.62	9.01
	22	71.56	59.17	51.98	36.77	14.19	6.21	9	9.35	3.69	5.83	19.26	9.72
	22	76.02	64.88	56.55	40.61	17.81	5.91	11	9.99	4.45	6.23	20.01	9.55
	22	65.38	53.66	47.78	33.99	14.74	5.76	9	8.71	3.36	5.60	17.08	8.02
	23	66.75	57.49	50.69	36.03	16.00	4.47	10	9.19	2.37	6.30	17.85	8.22
	23	69.38	55.55	51.96	36.63	13.44	6.20	12	10.18	3.50	5.74	19.16	8.78
	22	67.22	57.12	50.53	35.62	16.32	5.59	10	8.89	2.36	5.77	16.96	8.93
	23	63.75	52.43	47.87	35.65	17.35	5.11	9	7.90	2.46	5.08	15.52	8.03
	23	64.86	55.25	49.47	35.18	14.13	4.35	10	9.61	2.82	5.55	17.74	8.77
	22	69.79	59.93	54.07	37.60	15.28	5.38	10	9.65	3.29	6.44	19.04	9.88
	23	66.18	56.40	49.97	35.30	18.17	4.94	10	8.06	2.68	5.62	15.86	8.25

Host species	den	bd	add	tpd	drd	trd	wbm	rad	bl	cpw	rl	dw	ds
Amietophrynus species T2AuSH (continue)	24	68.55	57.55	50.69	35.87	17.64	6.25	9	8.08	3.12	6.10	16.90	7.49
	24	65.71	55.15	49.57	35.61	17.37	5.70	9	8.99	2.83	5.44	16.71	8.26
	22	67.51	56.27	52.20	37.85	15.80	5.85	8	10.11	3.06	5.53	18.52	9.50
	-	61.20	55.39	48.70	34.78	15.66	3.52	10	9.41	3.03	5.73	17.81	9.36
	24	70.59	60.00	53.18	38.01	17.67	5.50	7	9.31	2.98	6.20	18.10	9.49
	22	67.74	57.16	50.86	36.83	19.02	5.50	11	7.23	3.04	5.92	15.86	8.85
	21	65.99	55.68	50.02	36.04	13.59	4.55	9	9.13	3.62	5.39	17.45	9.21
	23	68.75	58.81	51.54	35.78	16.70	4.72	11	8.68	2.94	5.69	16.94	9.30
	24	71.18	60.64	52.40	36.65	16.44	4.59	11	8.84	2.85	6.31	18.04	8.92
	24	72.62	60.85	52.37	37.27	17.60	4.75	9	8.53	2.76	5.79	17.35	8.45
	22	74.80	63.22	54.88	40.34	17.52	5.88	10	9.57	3.25	5.95	18.79	9.64
	24	72.11	60.00	52.32	38.09	18.20	6.47	9	8.33	2.61	5.99	17.01	8.57
	23	66.34	56.41	49.93	36.37	16.36	4.77	10	8.84	2.57	5.59	16.80	8.05
	23	69.67	60.55	52.56	38.13	18.65	4.16	10	8.84	2.81	6.07	17.57	8.81
	23	70.58	59.69	51.58	37.66	18.96	6.12	11	8.74	2.65	5.98	17.25	8.88
	24	69.55	58.28	52.04	37.37	19.97	5.63	9	8.47	2.69	5.91	16.47	8.54
	21	67.29	58.39	51.58	35.02	14.50	4.88	10	9.15	3.01	6.13	17.96	9.33
	21	64.34	52.68	46.68	32.86	13.70	5.73	10	9.87	2.86	5.41	17.86	8.71
	22	65.90	56.94	49.08	35.80	17.13	4.62	9	9.32	2.57	5.80	17.51	9.29
	21	68.53	58.51	50.40	35.82	15.97	5.61	10	9.21	2.74	5.26	17.25	8.94
	22	69.67	58.86	50.75	35.46	15.60	6.13	11	9.70	2.41	5.95	18.24	8.52
	23	64.33	54.24	47.11	33.27	13.66	5.76	9	8.24	2.84	4.44	15.49	8.26
	22	67.20	56.32	47.47	34.39	15.91	5.58	9	8.34	2.56	5.69	16.80	8.65
	22	66.70	57.87	49.28	35.22	15.57	4.87	9	10.12	2.20	5.79	17.74	8.28
	22	64.38	53.44	48.85	35.10	14.49	5.13	10	9.48	3.30	5.22	17.80	8.50
	23	68.71	57.00	52.67	38.90	20.17	4.98	11	8.48	2.64	5.69	16.97	8.54
	22	71.92	60.60	52.52	38.00	15.83	5.62	9	9.90	2.79	6.51	19.39	9.07

Host species	den	bd	add	tpd	drd	trd	wbm	rad	bl	cpw	rl	dw	ds
Amietophrynus species T2AuSH (continue)	22	67.35	56.93	49.35	35.18	16.80	5.07	9	8.75	2.44	5.68	16.93	7.35
	21	64.89	52.92	48.62	33.51	14.40	4.50	10	8.80	2.84	5.42	16.87	8.34
	22	66.51	55.46	49.51	34.86	14.01	5.43	10	9.17	2.38	6.18	17.77	8.42
	20	64.40	54.98	48.14	34.88	14.70	5.53	11	9.67	2.51	5.14	17.27	9.31
	23	66.78	55.30	50.64	36.19	16.00	5.75	11	9.03	2.87	5.66	17.41	8.45
	22	68.68	58.29	50.53	36.42	15.88	5.02	10	8.42	2.72	5.77	16.78	7.72
	22	62.88	51.78	45.66	32.28	14.30	5.30	9	7.66	2.75	5.52	15.72	9.05
Cacosternum boettgeri	T19CbBP												
	25	70.50	60.26	50.33	36.63	19.64	5.65	10	7.31	3.54	5.33	16.14	8.77
	24	68.57	57.95	50.96	36.14	17.02	5.27	10	8.06	2.60	6.27	16.84	9.04
	24	76.60	65.24	54.65	39.32	20.74	4.36	12	7.82	3.43	6.18	17.26	9.00
	23	68.18	55.96	52.52	36.48	19.41	5.76	11	7.35	3.03	6.69	16.93	9.58
	24	78.85	66.13	57.07	41.13	20.14	6.43	12	8.77	4.24	6.11	19.41	9.01
	28	89.92	76.43	65.28	46.26	24.88	6.31	12	9.42	4.57	7.23	21.24	9.71
	23	76.85	64.94	57.73	40.33	21.94	6.36	11	7.94	3.22	7.10	18.33	9.87
	24	74.84	62.53	54.13	37.87	19.15	6.23	13	8.63	3.42	6.17	18.30	10.01
	27	79.39	67.20	58.43	41.04	20.39	6.89	13	8.90	4.13	7.03	19.68	9.27
	26	84.61	72.52	65.97	48.35	27.95	6.09	10	9.92	4.42	6.03	20.74	9.89
	25	74.07	62.70	54.50	39.32	22.23	5.50	10	7.21	3.07	6.02	16.29	9.51
	27	80.33	67.84	58.27	40.97	22.32	6.73	11	7.71	3.52	7.17	18.26	9.33
	24	67.11	56.56	49.99	33.89	15.54	5.18	13	8.08	3.24	6.40	17.50	8.99
	25	71.71	59.57	51.84	35.34	17.36	5.92	11	7.93	3.15	6.28	17.49	8.32
	24	78.66	66.56	60.46	41.74	20.72	6.52	11	8.64	4.29	6.95	19.96	10.59
	23	68.19	58.09	53.45	37.36	19.16	5.03	13	8.81	3.33	5.96	17.83	9.63
	23	78.01	67.52	58.21	40.97	18.96	5.32	13	10.01	4.21	6.62	20.21	9.77
	26	75.76	64.33	56.61	40.30	18.83	6.47	11	8.75	3.84	5.89	18.23	9.35
	23	78.22	65.52	55.01	37.34	17.79	6.96	11	7.35	3.76	7.14	17.92	8.34

Host species	den	bd	add	tpd	drd	trd	wbm	rad	bl	cpw	rl	dw	ds
Hyperolius parallellus	T20HaNE												
	20	57.72	47.94	40.74	27.87	11.99	4.59	8	6.53	3.38	4.87	15.67	8.32
	22	69.91	58.73	48.98	33.02	13.76	5.30	9	8.56	3.16	6.35	17.96	7.60
	23	65.02	54.18	46.86	32.64	18.11	6.17	7	6.73	2.65	5.93	14.88	8.33
	20	68.51	58.78	49.21	33.03	17.11	4.62	8	6.96	3.36	5.61	16.37	8.40
	21	62.47	53.61	45.72	30.48	13.97	4.55	8	7.26	2.95	5.96	16.38	6.74
	20	65.67	54.94	46.94	33.00	14.50	5.51	11	7.58	2.75	6.06	16.47	9.08
	24	68.47	58.46	50.67	35.43	16.66	4.87	7	7.77	3.22	6.27	16.08	8.08
	24	66.30	58.11	49.53	33.87	19.06	4.59	7	6.00	6.14	5.73	14.72	8.27
	21	60.68	49.79	42.83	28.65	14.47	5.94	9	6.08	2.79	5.48	14.61	7.54
	21	61.98	50.72	45.09	31.29	14.53	5.59	10	8.44	2.45	5.63	16.44	8.07
	23	65.35	53.83	45.07	31.81	14.70	5.63	6	8.07	2.80	5.71	15.85	8.98
	22	60.45	50.18	45.71	29.52	10.98	5.80	9	8.07	3.32	6.39	17.62	8.65
Kassina senegalensis	T21KsZZ												
	22	81.35	69.91	61.43	43.72	18.37	5.84	13	10.99	3.89	7.11	22.20	12.17
	19	83.08	69.75	61.05	42.48	19.85	6.26	13	9.43	3.30	6.91	19.50	12.81
	28	82.06	69.58	61.68	44.99	25.43	5.00	8	8.83	2.64	7.73	18.72	9.80
	28	80.86	68.99	61.62	43.32	22.75	5.46	9	8.24	3.12	7.13	17.98	9.05
	18	70.09	59.48	52.53	36.48	16.62	6.12	13	9.77	3.64	7.24	20.17	11.05
	21	79.01	72.48	62.05	45.03	17.66	3.41	12	10.98	3.38	6.73	20.78	12.79
	23	68.80	59.16	51.16	35.72	15.16	5.80	11	8.67	3.54	6.75	20.13	10.84
	22	69.05	57.03	48.69	35.01	16.27	5.30	12	8.05	3.75	6.45	18.02	10.75
	20	77.26	66.01	58.56	40.97	18.00	5.69	14	10.99	2.99	6.86	21.13	11.69
	19	74.29	65.96	54.95	38.18	18.53	4.25	13	11.64	3.26	6.94	21.52	11.41
	22	76.69	67.76	57.94	41.63	19.61	5.03	-	10.18	3.06	6.61	20.40	11.28
	22	73.11	61.12	53.90	37.78	17.37	5.85	11	9.57	3.58	6.42	19.63	11.00
	22	71.82	60.97	55.54	39.40	16.48	5.58	11	10.72	2.93	6.77	20.64	10.38

Host species	den	bd	add	tpd	drd	trd	wbm	rad	bl	cpw	rl	dw	ds
Kassina senegalensis **T21KsZZ (continue)**	21	75.90	65.82	57.65	41.06	21.31	5.58	12	9.74	3.79	7.04	20.71	12.18
	20	77.50	66.59	58.22	40.81	18.52	5.13	12	9.29	3.13	6.67	19.42	12.20
	19	79.76	67.10	59.46	41.69	16.92	5.20	-	12.68	2.85	7.20	22.64	11.04
	23	72.33	61.80	54.63	38.18	21.11	4.81	14	8.26	3.44	5.80	17.27	10.63
	21	80.98	72.20	62.87	44.46	19.72	5.14	12	-	3.18	7.75	21.39	11.91
	22	67.64	56.57	50.59	33.97	15.27	5.17	14	8.61	3.11	6.05	17.62	10.84
	20	75.72	66.43	60.75	43.12	17.64	5.40	11	11.74	2.75	7.43	21.96	12.71
	21	67.18	55.41	50.16	34.18	15.37	5.73	-	9.24	3.32	6.72	18.93	11.48
	23	73.69	62.70	54.94	39.07	13.16	4.80	13	8.66	4.05	6.50	18.09	10.85
	22	73.74	61.10	55.81	38.47	15.70	6.59	11	10.87	3.73	6.34	20.75	11.13
	21	76.62	64.70	57.71	40.07	15.44	6.13	-	10.11	4.65	5.80	20.01	11.77
	21	75.94	64.13	58.48	40.98	19.18	6.42	-	10.12	3.55	6.99	20.69	11.89
	21	84.92	73.57	62.44	44.68	21.66	5.84	12	11.74	3.11	7.07	21.70	11.66
	21	85.09	72.76	62.87	44.82	20.34	6.13	11	12.14	3.22	6.47	22.11	11.89
	19	82.76	71.35	61.42	42.86	20.26	5.60	13	9.86	4.19	7.29	21.77	12.28
	20	79.75	66.82	62.01	45.40	22.43	7.49	-	11.31	3.20	6.97	21.94	13.19
	23	68.38	56.38	50.74	35.57	19.20	7.00	-	8.33	2.48	5.76	16.38	11.16
	21	75.28	62.73	54.63	38.30	14.78	6.70	12	11.70	3.42	5.47	20.50	12.01
	23	-	71.41	60.95	43.77	19.58	-	14	9.76	3.67	6.60	19.84	11.81
	24	85.71	73.52	64.06	46.37	20.07	5.91	10	11.73	4.08	7.24	22.87	11.98
	20	87.78	74.89	64.02	44.40	21.24	6.70	12	11.34	3.23	7.87	21.67	13.25
	23	90.48	76.66	67.13	48.32	21.94	7.84	-	10.26	3.73	8.03	21.53	12.41
	20	83.32	71.86	62.78	45.94	19.22	5.40	11	11.60	2.89	7.11	21.32	13.24
	21	82.43	71.37	61.04	43.56	21.86	5.28	12	9.92	3.40	6.30	20.14	11.34
	-	78.41	66.76	59.65	42.43	22.01	6.08	11	10.30	3.34	6.62	20.32	10.49
	-	85.85	73.34	63.93	46.90	22.84	6.10	10	11.10	3.90	7.41	21.86	12.20
	21	85.28	72.78	62.33	45.18	19.37	5.85	12	11.19	3.77	7.56	21.89	12.18

Host species	den	bd	add	tpd	drd	trd	wbm	rad	bl	cpw	rl	dw	ds
Kassina senegalensis **T21KsZZ (continue)**	21	70.45	58.58	52.01	35.63	14.14	5.70	14	9.50	3.41	6.01	18.72	10.80
	23	76.96	63.50	55.56	40.14	17.85	5.44	11	11.26	3.07	6.77	20.86	10.98
	21	82.55	70.60	60.61	43.12	18.01	6.83	13	11.10	3.57	7.05	21.56	12.60
	22	76.75	65.07	58.00	41.23	16.79	6.38	12	9.79	3.02	7.55	19.69	11.18
	21	77.62	66.74	57.16	40.73	15.72	5.83	-	11.24	2.68	7.69	21.82	11.52
	23	79.76	67.80	59.07	41.69	19.52	5.97	12	10.86	2.67	7.16	20.07	10.84
	21	86.44	74.47	63.95	44.90	23.49	6.37	12	10.07	4.07	7.27	21.17	12.82
	23	75.22	62.11	53.12	36.81	13.43	6.10	-	9.51	3.56	5.82	19.26	9.21
	22	73.56	66.05	56.79	40.50	18.31	4.03	11	8.35	3.05	7.09	18.38	10.89
	20	73.86	61.64	54.94	36.35	15.70	6.44	-	8.93	4.13	6.95	19.79	11.95
	19	68.98	62.67	52.93	37.14	13.72	2.87	11	9.99	3.58	6.48	20.02	10.58
	21	77.75	65.57	56.73	39.92	14.96	6.87	-	11.12	3.46	7.60	22.12	10.65
	21	86.89	73.87	63.43	45.03	25.57	6.34	12	11.10	3.51	7.65	21.94	13.85
	19	78.95	69.46	59.68	41.05	20.38	4.50	13	10.49	3.70	7.42	21.16	13.98
	21	69.83	57.04	50.59	35.80	16.10	4.41	-	9.78	3.06	6.13	18.79	10.63
	22	72.38	60.54	53.84	36.87	13.82	5.97	11	9.16	3.55	6.95	19.48	10.37
	22	79.48	68.51	59.42	41.15	16.04	5.44	13	10.20	3.78	6.95	21.21	11.16
	24	79.08	67.73	57.26	41.28	17.91	6.20	11	9.73	3.90	6.87	20.65	10.69
	22	73.89	62.84	55.53	39.18	16.92	6.26	11	10.18	3.51	6.42	20.08	10.61
	22	84.40	71.15	62.05	43.71	20.42	7.16	11	11.25	3.58	7.07	21.65	11.81
	24	65.50	58.07	51.76	36.13	12.56	3.68	12	10.57	3.13	5.87	19.40	9.64
	22	72.95	61.51	54.21	37.84	14.08	5.95	12	10.58	2.73	6.85	19.94	10.39
	21	71.12	59.67	54.53	38.77	19.69	5.62	13	7.63	2.84	6.27	16.11	10.55
	21	77.55	66.47	61.94	43.16	20.87	5.64	12	9.65	3.43	6.99	20.01	12.25
	21	65.42	54.68	47.30	32.04	14.45	6.01	11	7.88	3.08	5.89	16.59	9.23
	20	79.39	67.76	57.29	40.45	13.61	5.28	15	10.60	3.51	6.69	20.60	11.17
	21	70.42	59.18	54.04	37.94	20.26	5.84	13	8.31	3.10	6.59	17.78	10.32

Host species	den	bd	add	tpd	drd	trd	wbm	rad	bl	cpw	rl	dw	ds
Kassina senegalensis **T21KsZZ (continue)**	21	77.67	66.40	62.19	43.95	22.88	5.06	12	9.37	3.74	6.84	19.85	12.09
	23	67.08	56.49	48.81	34.42	11.78	5.00	11	10.13	3.13	6.47	19.34	9.62
	20	79.51	67.52	58.06	40.32	13.50	5.21	12	12.13	3.49	6.51	21.93	12.27
	23	67.76	57.03	50.04	-	12.00	5.69	12	9.75	3.31	6.12	19.17	9.90
	22	78.71	66.50	59.26	42.73	18.13	5.98	12	12.21	2.50	6.85	21.89	10.79
	21	60.57	53.55	48.58	33.24	12.93	3.40	12	8.47	3.09	5.88	17.74	10.13
	22	81.45	69.97	59.74	42.62	20.71	5.46	-	10.14	3.26	6.49	19.86	11.19
	20	83.88	72.70	64.15	45.56	20.32	5.21	11	9.25	3.05	7.72	20.08	12.34
	21	72.96	65.45	55.53	39.39	18.39	3.99	12	10.96	2.63	6.97	20.15	12.00
	22	86.65	74.10	64.98	47.34	21.93	6.19	12	11.02	3.50	7.57	22.31	13.15
	23	81.24	69.21	58.71	40.54	17.06	5.00	-	11.06	3.12	6.65	20.30	11.15
	24	71.48	62.65	55.76	40.00	22.02	5.03	10	7.81	2.85	6.09	16.44	9.62
	21	66.59	56.87	51.00	35.72	13.92	5.62	12	7.31	4.27	6.24	17.74	10.20
	22	70.22	59.90	52.96	36.78	12.94	6.44	11	9.03	3.97	6.49	19.24	9.26
	24	73.58	62.82	54.98	39.57	17.87	5.50	14	9.52	3.15	6.25	19.39	9.97
	22	71.86	60.55	53.61	37.71	16.12	6.19	-	9.96	3.34	6.23	19.59	10.87
	24	70.50	63.50	56.27	39.87	14.48	3.98	13	9.47	3.44	6.28	19.76	10.13
	24	76.36	65.20	58.17	41.41	17.26	6.05	11	11.29	2.88	6.56	20.81	10.30
	21	84.48	72.61	55.66	45.14	19.74	6.24	13	10.77	3.43	7.27	21.23	13.15
	20	87.59	76.70	61.07	43.13	23.38	5.07	11	9.22	3.06	7.66	19.54	12.13
	22	69.57	57.88	51.17	37.82	14.74	6.30	-	10.42	3.57	6.08	19.84	10.24
	23	82.70	72.67	62.90	44.46	20.77	5.45	-	9.46	4.06	7.50	21.00	11.91
	20	83.49	72.04	63.40	43.83	18.86	5.81	-	9.87	4.24	6.77	21.69	12.21
	22	72.71	60.57	53.71	38.03	17.12	6.28	-	7.32	3.52	6.85	18.12	9.08
	20	80.04	68.15	57.89	41.91	18.25	5.92	12	8.80	3.42	7.17	19.48	13.43
	22	72.36	62.17	55.05	37.92	19.65	5.26	-	7.30	3.33	6.44	17.70	11.00
	21	74.71	62.72	54.64	38.93	17.27	5.95	-	9.96	3.40	7.12	20.26	10.62

Host species	den	bd	add	tpd	drd	trd	wbm	rad	bl	cpw	rl	dw	ds
Kassina senegalensis T21KsZZ (continue)	21	72.92	61.32	54.45	37.68	11.93	6.06	-	10.64	3.22	6.62	20.11	11.85
	22	77.47	68.52	59.04	43.03	18.23	3.36	-	11.57	2.92	7.09	21.27	11.75
	23	88.01	76.04	66.03	49.20	23.64	6.28	-	12.52	2.38	7.94	23.04	12.33
	21	82.70	72.26	62.59	44.72	18.56	5.95	12	10.90	4.15	6.67	22.13	12.84
	22	76.80	65.98	56.58	39.44	17.62	5.48	12	8.14	3.56	7.14	18.78	12.15
	23	71.65	62.85	57.16	39.91	16.34	4.79	12	9.51	2.88	7.10	18.68	10.32
	22	80.81	69.01	61.06	43.69	20.75	6.23	-	10.93	2.90	7.68	21.28	11.83
	23	77.27	66.78	59.27	41.03	18.44	6.11	12	10.70	3.10	6.60	20.66	11.25
	21	86.07	73.32	62.01	45.37	17.22	6.24	12	13.70	3.80	7.25	24.72	12.00
	22	92.48	80.60	69.97	49.91	24.60	5.95	12	12.91	3.71	7.51	24.28	13.67
	22	65.73	57.34	51.32	35.93	15.15	4.16	11	10.89	3.17	6.39	20.35	9.68
	21	67.65	58.61	50.48	36.24	12.80	3.20	-	10.41	2.82	7.00	19.63	11.06
	21	78.22	68.38	60.66	43.52	20.18	4.72	14	10.17	3.86	6.78	20.84	11.31
	21	72.57	63.23	57.42	38.88	15.33	5.89	-	9.49	3.84	6.29	19.78	11.01
	21	76.48	66.89	56.63	41.07	20.31	5.70	-	10.38	3.31	6.30	20.43	11.17
	21	79.59	69.07	57.40	40.92	18.39	5.81	11	9.76	3.18	6.88	19.89	11.02
	21	78.36	68.21	59.32	42.08	19.64	4.35	13	10.54	3.65	7.09	20.88	11.19
	21	75.74	65.16	55.72	37.87	16.05	5.72	-	9.42	2.88	7.46	19.29	10.41
	20	78.18	67.35	58.01	41.19	20.76	4.53	-	9.10	3.83	6.73	19.31	11.82
	21	68.06	58.82	52.22	35.28	15.29	5.35	11	10.34	3.22	6.24	20.01	10.32
	22	81.56	70.52	60.25	43.04	18.81	5.97	13	10.27	3.29	6.72	20.26	12.13
	22	68.29	58.06	51.08	35.90	14.79	4.93	-	8.27	3.23	6.47	17.55	11.12
	19	82.21	69.82	61.22	42.50	18.24	6.09	13	12.56	3.59	7.21	22.95	12.68
	25	80.62	73.31	60.80	43.29	19.30	5.00	14	10.13	4.84	6.69	21.19	11.23
	22	74.28	63.41	56.07	39.13	15.54	5.95	-	9.95	3.20	6.26	19.39	10.76
	21	84.23	72.59	63.14	45.15	23.87	5.89	12	12.20	4.07	7.85	22.84	11.71

Host species	den	bd	add	tpd	drd	trd	wbm	rad	bl	cpw	rl	dw	ds
Kassina senegalensis	T8KsBP												
	16	53.53	44.77	38.85	26.51	14.44	4.66	9	6.73	2.42	4.82	13.87	6.92
Natalobatrachus bonebergi	T22NbVC												
	-	64.03	51.55	50.61	36.47	-	5.22	11	-	2.84	5.70	-	8.87
	25	79.27	68.85	60.85	45.41	23.17	5.86	8	10.48	4.04	6.21	20.09	10.91
	22	81.07	70.00	61.72	43.63	21.02	6.57	11	10.08	5.45	6.27	22.24	12.12
	24	83.10	70.34	59.72	43.13	21.98	6.06	10	8.68	4.09	6.42	18.92	10.63
	23	70.11	57.22	51.18	35.17	19.64	6.97	-	7.24	3.25	6.42	16.42	10.09
	23	80.98	68.95	59.72	43.79	17.62	5.73	12	11.63	4.29	6.11	21.89	12.47
	23	74.72	62.77	55.01	-	-	6.48	10	8.94	4.10	5.84	18.81	9.95
	25	75.47	63.63	54.55	39.50	17.22	5.81	12	9.70	3.76	6.35	19.03	9.39
	26	62.70	51.80	44.64	31.20	15.68	4.81	6	6.30	3.35	5.46	14.70	7.12
	22	75.78	66.07	57.35	39.97	17.86	5.56	12	10.49	4.11	5.97	20.25	12.28
	26	69.52	58.35	51.28	36.88	21.09	5.28	12	6.46	3.69	5.68	15.87	8.58
	25	77.60	65.93	54.90	39.89	18.83	6.26	-	9.02	3.28	6.21	18.21	9.69
	25	74.41	61.30	55.59	40.13	19.91	6.71	10	8.96	3.21	6.02	18.01	9.68
	21	78.54	66.15	55.72	39.26	16.85	5.39	14	10.88	5.14	6.53	22.79	12.03
	21	73.87	62.84	56.60	41.52	17.52	5.82	12	11.30	4.48	6.04	21.48	11.36
	22	85.55	75.01	63.10	46.40	17.62	6.77	12	12.07	4.95	5.55	23.25	12.38
	25	79.51	67.34	57.70	41.82	19.20	6.42	11	9.73	3.84	5.78	19.27	9.77
	23	79.91	68.77	62.09	44.43	24.54	5.46	11	9.71	4.37	7.64	21.08	12.76
	22	71.42	57.96	53.22	37.01	14.61	6.27	13	10.54	3.38	6.13	20.43	11.00
	22	80.72	71.58	61.28	44.48	16.05	4.93	12	12.23	4.24	6.59	23.03	12.13
	22	78.06	65.73	56.71	42.25	16.03	5.23	10	10.36	4.60	5.07	20.12	10.90
	23	68.12	56.85	56.13	39.53	15.27	5.08	13	10.97	4.21	5.92	21.19	11.70
	22	78.12	68.53	57.76	43.45	19.35	5.15	12	9.93	4.66	5.46	19.78	11.46
	20	69.71	59.80	51.58	36.14	14.38	5.09	11	10.56	4.18	6.30	20.50	10.44

Host species	den	bd	add	tpd	drd	trd	wbm	rad	bl	cpw	rl	dw	ds
N. bonebergi T22NbVC (continue)	-	67.28	57.96	52.80	37.92	18.56	6.36	8	9.26	2.86	5.70	18.13	9.33
Ptychadena subpunctata	T24PsNF												
	23	68.16	59.67	50.89	35.51	18.57	5.65	7	8.67	2.17	6.76	17.57	8.35
	-	67.05	55.73	49.80	32.26	14.21	5.61	5	8.71	3.21	7.42	19.64	7.50
	-	74.55	65.41	57.33	39.67	19.16	4.97	11	9.51	3.15	7.10	19.30	10.00
	21	78.38	67.04	54.24	38.16	18.06	6.02	11	7.16	3.69	5.77	17.79	9.99
	23	79.35	69.13	57.51	41.42	18.39	5.56	12	9.66	3.47	7.00	19.81	10.21
	23	71.23	65.75	57.39	47.72	15.77	4.31	11	7.57	3.13	5.88	16.15	10.59
	22	82.82	72.09	60.97	43.91	19.03	6.41	11	9.85	4.20	6.67	19.77	9.82
	21	61.12	51.17	46.34	32.15	11.69	5.75	10	8.76	3.18	5.30	17.21	7.98
	21	65.39	52.97	45.05	31.24	10.38	5.88	10	9.19	3.08	5.08	17.40	8.70
	22	56.26	46.73	40.18	27.47	14.40	4.63	11	7.06	2.73	4.77	14.01	7.29
Pyxicephalus adspersus	T23PaBW												
	25	68.32	58.50	48.47	36.47	15.65	5.26	15	8.59	3.19	5.24	17.02	9.10
	23	57.76	47.48	42.34	29.65	14.47	5.28	12	7.95	3.21	5.20	16.30	8.59
	23	54.45	46.17	39.36	28.58	11.32	3.28	10	7.31	2.69	4.25	14.36	8.20
	23	80.01	66.76	59.77	41.07	13.26	6.51	12	11.55	4.30	6.79	22.22	11.68
	23	76.99	63.74	55.43	39.64	14.42	6.42	13	10.04	4.21	5.42	19.89	11.08
	22	70.85	57.26	50.44	35.34	15.10	6.63	9	9.42	3.35	6.05	18.61	10.10
	24	83.36	70.37	59.61	41.98	22.85	6.17	12	11.10	3.52	6.54	21.08	11.45
	24	75.77	63.78	56.15	39.72	18.14	5.19	14	9.45	3.88	6.06	19.21	10.42
	21	68.47	56.29	49.81	34.05	13.50	6.32	10	9.48	3.03	6.18	18.34	10.29
	22	70.02	58.33	50.07	34.42	13.08	6.81	10	10.34	3.47	6.02	19.45	10.35
	24	85.05	73.01	64.12	46.69	20.71	7.23	12	11.26	4.31	6.92	22.15	12.61
	25	80.77	68.47	60.83	44.68	21.68	6.50	12	10.27	3.58	6.19	19.91	11.03
	23	76.97	65.16	58.00	41.21	17.92	5.55	11	8.75	3.52	6.76	18.65	11.48

Host species	den	bd	add	tpd	drd	trd	wbm	rad	bl	cpw	rl	dw	ds
Pyxicephalus adspersus T23PaBW (continue)	23	84.98	72.44	64.16	46.77	22.61	5.63	11	12.15	4.32	6.82	23.11	13.03
	23	78.51	68.38	60.23	42.93	17.15	5.72	12	11.55	4.32	6.59	22.23	12.18
	23	79.75	68.20	60.18	42.56	18.08	6.43	13	11.48	4.19	6.36	21.93	11.67
	24	79.86	66.89	59.25	42.88	15.69	6.26	12	10.95	3.82	6.52	21.04	10.84
	24	83.19	71.18	63.03	45.12	18.18	5.94	13	13.45	4.44	6.99	24.69	13.25
	23	80.21	68.22	60.30	42.95	18.98	6.04	12	10.99	3.58	5.92	20.52	11.74
	23	84.23	73.20	62.97	47.21	19.47	5.74	13	11.66	3.77	6.56	21.45	12.47
	24	79.53	66.57	59.06	41.36	19.15	6.05	11	9.35	3.93	6.93	20.47	10.64
	23	85.44	70.80	61.91	43.97	18.13	6.54	10	11.30	4.30	6.75	22.26	12.10
	23	85.73	71.14	62.06	44.08	18.11	6.82	12	11.84	4.08	7.20	22.98	11.80
	24	82.82	70.42	61.64	43.78	18.68	6.47	12	10.74	4.38	6.74	22.05	11.65
	25	75.88	64.60	57.33	41.20	19.86	6.18	11	8.96	3.79	6.00	18.81	10.31
	23	71.99	60.21	54.76	39.51	15.77	6.47	11	10.63	4.17	5.34	19.89	10.81
	25	85.40	72.80	63.70	46.44	21.13	6.14	12	12.13	3.76	6.84	22.07	10.68
	23	71.15	57.74	51.27	36.42	13.70	6.78	12	10.35	3.10	6.39	19.60	10.12
	25	78.97	66.13	56.88	40.86	25.35	6.10	10	7.83	3.63	6.90	17.59	10.61
	25	77.14	64.29	57.84	40.44	15.88	6.02	12	10.99	3.75	7.18	21.97	10.73
	20	77.36	63.80	55.04	38.31	16.40	6.28	12	9.88	3.97	6.50	20.61	11.43
	24	76.73	64.08	56.38	40.45	18.60	7.05	10	10.07	4.03	5.90	19.90	10.43
	25	76.91	64.20	55.83	39.94	16.61	6.58	11	10.46	3.83	6.56	20.23	9.94
	-	84.49	72.64	62.85	44.89	20.45	6.03	9	10.50	3.96	6.78	21.03	10.27
	24	81.44	67.89	59.71	43.50	20.44	6.22	11	10.45	4.12	6.37	20.43	10.30
	25	92.23	78.68	67.80	50.00	21.87	6.16	11	12.35	3.64	7.20	22.66	11.88
	22	75.42	62.25	56.42	41.02	19.38	6.60	8	9.39	4.24	5.85	18.69	11.38
	23	79.44	66.25	56.23	40.92	18.84	6.47	9	10.65	3.24	6.00	19.51	10.60
	24	79.72	65.80	58.23	42.70	22.06	6.48	10	7.99	3.99	5.99	17.79	9.59
	25	86.93	73.53	63.69	48.25	25.51	6.30	9	10.41	3.20	6.62	19.72	10.75

Host species	den	bd	add	tpd	drd	trd	wbm	rad	bl	cpw	rl	dw	ds
Pyxicephalus adspersus T23PaBW (continue)	24	67.60	55.65	50.28	35.46	15.15	6.26	11	8.84	3.36	6.13	18.15	9.71
	25	86.16	72.98	60.71	43.96	21.16	6.83	11	10.18	3.71	6.62	20.71	11.28
	25	92.04	78.27	65.59	49.07	26.89	6.36	11	9.83	3.21	6.20	19.06	11.17
	26	84.89	73.15	64.69	47.82	26.60	7.10	12	10.13	3.60	7.07	20.53	10.43
	23	60.12	49.26	43.97	30.93	14.36	5.48	6	8.90	3.18	5.09	16.53	8.66
Schismaderma carens	T25ScWB												
	-	65.05	52.66	45.83	31.74	11.14	5.90	12	8.97	3.31	5.49	17.96	8.90
	17	62.52	55.06	47.95	32.35	9.93	4.38	13	8.03	3.61	5.80	17.85	9.17
	27	80.35	69.99	58.45	40.76	21.46	5.74	12	8.16	3.78	6.77	18.70	9.94
	-	72.02	59.38	51.53	36.81	17.98	5.83	11	6.42	2.73	6.34	15.50	8.77
	22	63.97	55.83	48.39	33.62	13.83	5.28	-	8.44	2.87	5.40	16.34	8.58
	23	65.41	55.71	49.24	34.07	11.53	4.37	8	10.04	2.68	5.82	18.66	7.47
	19	56.99	45.70	40.53	27.10	11.24	5.40	9	7.41	2.87	4.86	15.71	9.06
	23	107.73	88.43	79.52	57.21	24.71	10.21	11	13.33	5.46	8.88	26.65	15.61
	23	66.02	53.50	48.88	35.40	20.59	6.55	12	8.21	2.72	6.11	16.42	9.48
	25	70.19	60.87	53.70	37.84	20.71	4.18	12	8.69	2.85	6.13	17.51	9.07
	-	66.73	56.79	49.87	33.34	18.96	5.49	9	7.67	3.38	5.44	16.01	8.29
	25	69.43	61.56	52.74	37.42	18.39	5.25	10	7.64	3.31	6.28	17.50	9.43
	17	55.77	44.82	42.24	29.33	14.44	6.09	-	7.89	3.22	4.83	16.66	10.06
	25	66.47	56.77	52.88	36.03	16.54	6.07	11	7.27	3.88	6.67	16.91	9.18
	20	52.05	42.84	37.39	24.74	10.19	4.78	11	6.97	2.58	4.95	14.30	8.47
	21	103.16	87.94	82.17	57.40	28.04	9.40	12	12.43	5.55	9.26	27.36	14.88
	18	63.62	52.73	48.48	35.33	15.78	6.09	-	8.91	3.33	5.53	17.35	11.82
	22	64.55	52.39	47.70	31.77	15.19	6.41	8	7.21	3.27	5.63	15.91	8.88
	23	70.75	59.10	50.29	35.18	18.54	5.61	11	8.78	3.00	5.87	17.45	9.12
	24	68.48	56.78	50.84	36.29	18.78	4.10	10	8.04	2.43	6.41	16.70	8.74
	23	71.06	60.80	55.23	38.78	17.79	5.44	10	9.01	3.42	6.04	18.89	9.42

Host species	den	bd	add	tpd	drd	trd	wbm	rad	bl	cpw	rl	dw	ds
Schismaderma carens T25ScWB (continue)	25	69.08	57.99	52.69	38.41	16.95	5.81	10	9.59	2.67	5.58	17.93	8.60
	23	61.31	51.06	47.67	33.25	17.64	5.59	-	6.69	2.68	5.90	15.08	8.58
	22	62.75	55.69	49.69	34.54	16.96	3.44	10	6.38	3.18	5.51	15.30	8.59
	22	59.09	50.40	43.74	29.62	11.83	5.01	13	7.90	2.96	5.73	16.17	8.78
	23	59.88	48.15	42.91	30.60	14.72	5.06	12	7.54	2.71	4.59	15.13	8.47
	23	61.37	53.05	46.60	31.24	15.79	3.50	-	7.06	3.47	5.55	16.82	8.69
	23	-	55.82	49.20	35.19	15.35	4.23	-	8.35	2.40	5.68	16.98	9.36
	25	68.20	58.16	51.23	35.97	18.28	5.55	9	7.45	2.84	5.61	15.71	8.24
	21	54.77	46.83	40.82	28.86	14.06	3.54	9	6.08	2.78	4.10	13.16	7.02
	22	30.92	26.53	23.07	16.00	8.06	2.52	11	3.09	1.27	2.76	6.97	4.09
	20	39.90	34.44	29.77	19.96	8.37	2.94	12	5.94	1.96	3.56	11.76	6.29
	21	46.73	39.10	33.40	23.29	9.97	4.19	13	5.88	1.98	4.33	12.07	6.50
Unknown tadpole species	T35UuBV												
	25	77.43	65.61	59.08	40.27	15.65	5.10	12	10.34	4.24	7.26	21.22	11.61
	23	88.94	76.35	68.08	49.36	19.43	5.86	11	11.39	5.16	7.40	23.76	11.33
	25	83.18	71.77	64.31	45.07	16.36	6.69	11	12.93	4.52	7.59	24.52	11.12
	23	89.92	75.78	68.44	48.37	19.20	5.23	11	11.39	5.21	7.47	24.27	11.82
	25	85.32	73.43	63.94	45.72	20.32	7.34	12	11.91	4.38	6.71	23.05	11.93
	27	72.44	63.72	57.43	41.54	22.14	4.81	9	9.96	3.23	6.00	19.16	8.88
	27	82.73	71.58	64.15	46.91	18.91	5.76	12	9.75	4.29	7.24	21.46	10.20
	25	93.58	82.06	68.33	48.20	22.21	5.74	14	13.65	3.78	7.92	25.23	13.20
	23	75.17	62.83	57.53	40.43	19.83	6.33	10	7.73	4.57	6.50	18.86	12.75
	27	88.41	75.89	68.15	49.84	22.76	6.67	11	11.09	3.97	6.70	22.25	11.09
	23	-	72.40	65.35	46.18	21.07	3.48	-	11.17	3.89	7.28	22.10	11.20
	20	82.74	72.73	62.68	44.19	15.29	6.23	11	13.63	3.82	7.32	24.65	13.12
	21	87.26	74.45	64.13	44.11	18.43	6.25	12	10.23	4.91	6.90	22.95	13.31

Host species	den	bd	add	tpd	drd	trd	wbm	rad	bl	cpw	rl	dw	ds
Unknown tadpole species	T36UuLF												
	27	-	98.09	86.59	60.38	22.04	9.29	9	11.44	5.28	10.15	26.63	13.73
	23	71.98	60.49	53.48	35.61	18.14	5.76	11	8.79	3.20	7.45	18.94	10.10
	22	82.52	70.87	60.91	43.46	16.85	6.61	-	12.35	3.86	7.03	23.12	12.30
	25	68.30	56.56	50.20	34.74	16.04	5.74	10	5.92	2.73	6.24	15.31	7.21
	24	67.45	56.06	49.99	35.51	20.35	5.37	-	6.94	3.00	6.45	16.33	8.76
	25	73.05	63.26	57.24	40.83	22.75	4.03	-	7.99	3.06	5.97	17.29	9.45
	28	69.69	58.81	51.73	37.39	20.44	6.12	-	7.62	3.06	5.64	16.25	10.07
	27	71.51	60.08	53.17	38.03	21.51	6.34	-	6.82	2.94	6.60	16.38	8.69
	25	76.86	64.45	56.34	41.42	19.06	5.13	-	8.78	3.02	6.60	17.40	10.32
Xenopus laevis	T26XlOG												
	22	69.76	59.00	50.85	34.36	17.65	5.86	9	8.06	2.89	6.56	17.34	9.68
	23	70.55	58.63	52.10	35.60	18.03	5.90	11	8.79	3.02	6.30	18.26	9.65
	21	78.62	67.39	58.65	41.74	17.82	5.98	10	9.94	3.46	6.51	20.79	10.73
	25	73.19	64.79	53.45	40.55	18.96	4.76	9	8.21	3.41	5.44	17.07	9.44
	T27XlHS												
	20	72.83	61.56	55.55	39.77	19.97	5.03	11	9.78	3.41	6.35	19.08	11.30
	21	66.06	53.98	48.83	33.28	15.62	6.06	10	7.51	3.03	5.75	16.04	9.23
	20	63.60	51.37	45.59	31.02	13.55	6.11	10	8.94	1.98	6.05	16.95	9.42
	22	77.01	64.46	57.03	40.30	19.78	5.89	11	9.48	3.21	6.59	19.17	10.91
	22	69.95	57.76	51.93	36.65	14.42	5.91	11	9.33	3.34	6.33	19.21	10.25
	21	73.14	61.86	53.02	37.99	17.13	5.11	11	9.36	2.82	6.14	18.26	9.90
	20	70.62	59.39	49.71	34.62	17.12	5.72	11	8.36	2.79	6.27	17.24	10.06
	21	68.25	57.59	49.31	35.41	16.31	5.10	10	9.27	2.81	5.32	17.19	8.54
	22	71.02	59.35	51.18	37.04	18.30	5.90	11	8.34	2.97	5.26	16.66	9.33
	22	68.16	56.70	49.68	34.79	18.79	5.28	10	7.88	2.66	5.66	15.87	9.81
	22	74.35	63.09	55.40	39.58	21.83	6.17	10	8.04	2.74	6.41	17.05	10.01

Host species	den	bd	add	tpd	drd	trd	wbm	rad	bl	cpw	rl	dw	ds
Xenopus laevis T27XIHS (continue)	21	74.11	61.26	53.92	38.70	18.49	7.27	10	9.60	2.72	6.56	18.76	10.39
	20	68.82	56.97	51.63	36.03	14.56	6.07	11	10.07	3.06	6.14	19.46	10.20
	23	72.30	60.13	51.67	37.14	19.21	5.81	11	8.66	2.75	5.99	16.98	9.58
	21	71.51	60.07	52.13	37.99	17.85	5.83	11	9.36	2.82	5.88	18.01	10.18
	22	70.67	58.88	52.95	38.52	17.45	5.78	11	9.84	3.06	5.56	17.95	9.30
	22	63.78	52.19	46.22	31.65	13.11	5.89	11	8.22	3.06	5.81	16.75	8.21
	19	74.05	61.84	52.87	38.07	15.78	6.46	11	10.46	3.20	5.95	19.33	10.60
	21	63.97	51.87	47.12	32.90	11.92	6.10	10	8.66	3.37	5.46	17.31	8.56
	21	83.83	71.25	62.04	45.01	21.03	5.83	9	10.72	2.62	6.57	19.89	11.53
	21	78.49	66.96	58.41	41.75	19.50	4.88	11	9.53	4.17	6.27	20.05	12.41
	22	71.96	60.20	54.97	38.28	14.72	5.70	11	9.48	3.33	6.89	19.70	9.60
	20	72.17	60.94	53.61	37.41	18.77	5.77	11	9.10	2.71	6.51	17.97	10.77
	21	50.32	44.89	39.29	27.21	12.46	3.08	11	6.33	2.22	4.70	13.24	7.50
	22	54.75	45.23	40.80	28.85	12.51	5.24	11	7.68	2.68	4.91	15.01	8.40
	T28XIBB												
	22	60.16	49.12	44.34	29.70	12.21	5.94	10	7.32	2.95	5.34	15.63	8.92
	20	56.41	45.11	41.70	27.90	11.91	5.80	-	6.19	3.34	5.60	15.31	9.92
	-	66.12	54.70	48.17	32.31	16.43	6.22	11	5.51	4.41	6.77	16.65	9.24
	22	63.66	52.72	46.68	32.25	14.95	4.53	12	8.50	2.73	6.40	17.06	10.05
	19	65.96	54.06	46.65	32.35	12.89	5.04	10	6.71	3.03	5.50	15.21	10.42
	21	64.38	51.57	44.11	30.33	11.45	6.45	10	8.78	3.25	5.12	17.48	10.40
	20	65.76	52.65	46.24	31.45	11.91	6.84	12	8.62	2.77	6.07	17.09	10.45
	-	59.64	47.22	44.15	31.59	11.25	6.23	-	9.77	3.31	5.00	18.82	10.13
	20	72.72	60.75	53.16	37.03	12.87	6.45	11	10.13	3.27	6.52	19.94	11.79
	20	63.99	54.79	47.04	32.80	14.32	3.90	10	7.65	2.98	6.71	16.52	8.98
	20	70.08	59.27	52.58	36.54	14.95	5.36	9	8.82	2.91	5.84	18.51	11.46
	21	75.21	64.00	53.76	37.04	14.83	6.61	10	9.13	3.19	5.85	18.23	10.45

Host species	den	bd	add	tpd	drd	trd	wbm	rad	bl	cpw	rl	dw	ds
Xenopus laevis	21	83.04	68.72	58.59	42.11	17.31	6.52	10	10.52	4.40	7.03	21.38	11.68
T28XIBB (continue)	21	60.22	51.06	43.55	28.47	11.72	5.73	10	8.68	3.09	5.62	17.19	9.63
	22	81.11	67.32	57.00	40.39	14.71	6.40	12	10.84	3.32	6.49	20.10	11.39
	20	74.31	62.88	54.16	38.72	13.35	5.86	12	10.36	3.94	6.04	19.95	11.98
	21	77.23	65.92	55.28	39.00	15.79	6.33	11	10.75	3.25	6.37	20.39	11.38
	21	77.90	65.79	56.02	40.26	17.63	5.88	12	9.63	3.53	6.87	20.08	12.89
	22	72.38	59.27	52.18	36.78	15.71	5.67	12	8.52	3.76	5.22	18.77	11.44
	22	65.60	53.07	47.31	30.33	15.87	6.72	8	7.29	3.89	5.62	15.83	11.50
	21	76.12	63.13	55.81	38.87	16.08	6.54	12	10.13	3.54	6.49	20.88	11.07
	T29XIPC												
	19	56.71	50.80	43.58	30.10	12.91	2.73	12	7.94	2.91	5.36	15.94	10.85
	21	59.64	47.44	43.06	28.77	12.90	6.52	9	7.79	3.28	5.15	16.32	9.30
	22	62.84	50.73	46.64	32.08	16.32	6.40	-	7.55	2.62	5.89	15.60	8.57
	23	61.37	51.01	45.52	31.31	16.25	5.94	10	7.56	3.18	6.37	16.31	9.67
	22	62.09	51.24	45.73	30.77	14.61	6.56	9	7.01	2.78	5.74	15.55	10.69
	-	64.02	52.02	49.07	34.00	17.44	6.58	11	6.70	3.33	5.21	15.36	7.92
	21	58.83	48.05	43.14	28.72	-	6.19	10	-	2.98	5.85	-	9.19
	-	63.71	51.57	45.00	29.05	-	6.45	12	-	3.94	5.72	-	-
	22	66.71	54.63	50.33	35.69	3.02	4.90	-	6.76	4.24	4.44	15.11	8.21
	23	81.50	67.83	61.73	44.41	21.48	7.24	10	6.28	3.64	5.49	15.57	8.53
	21	71.02	59.43	52.66	37.87	14.80	6.04	-	10.50	3.90	6.16	18.97	10.13
	22	70.62	58.08	52.33	37.37	15.99	6.01	13	10.57	3.10	5.49	19.23	10.43
	22	66.81	54.47	49.48	35.18	14.49	5.80	10	9.49	3.19	5.58	19.05	9.75
	22	72.62	61.09	55.89	38.23	17.78	6.77	13	8.81	3.85	6.12	18.77	11.78
	21	76.29	63.86	55.84	39.41	16.85	6.37	11	10.51	2.89	7.31	19.94	11.26
	23	77.10	63.82	58.31	42.15	15.65	6.54	13	10.82	3.75	6.37	21.63	10.69
	22	63.23	52.15	47.96	32.52	14.24	6.38	12	9.80	3.18	5.72	18.48	9.87

Host species	den	bd	add	tpd	drd	trd	wbm	rad	bl	cpw	rl	dw	ds
Xenopus laevis T29XIPC (continue)	23	78.48	65.33	57.83	41.18	19.21	6.94	12	10.94	3.29	7.08	21.04	11.79
	22	77.95	67.09	58.36	41.73	17.29	6.38	11	10.21	4.59	5.88	20.33	11.39
	22	74.65	61.09	54.93	38.09	16.24	6.75	11	11.10	3.06	6.45	20.52	10.08
	-	84.25	70.08	60.44	43.10	19.61	6.96	12	10.56	3.85	6.80	21.01	10.75
	-	62.65	52.68	46.23	31.77	12.97	6.60	11	8.77	3.47	5.64	15.86	8.77
	22	71.32	59.25	51.62	35.80	15.70	5.25	12	10.92	3.79	6.01	19.99	9.96
	21	77.68	65.37	58.25	39.75	18.57	6.11	12	9.74	3.31	6.58	20.51	12.20
	22	60.15	48.55	47.24	31.98	13.03	6.51	-	9.24	3.50	5.26	17.61	9.06
	19	63.10	50.44	45.21	31.74	9.21	6.37	11	10.26	2.69	5.42	18.64	9.46
	22	73.91	62.68	53.94	38.31	16.07	6.25	10	11.10	3.20	6.68	19.79	10.72
	21	69.94	57.61	49.78	35.08	16.61	6.96	13	8.88	3.25	5.96	18.09	10.07
	22	69.44	64.43	55.68	39.71	17.83	3.31	10	10.74	3.35	6.35	20.15	11.47
	T30XIBE												
	23	71.72	60.20	53.55	36.12	14.60	6.07	9	8.66	3.53	6.40	19.05	9.95
	24	74.65	63.13	56.31	39.89	17.70	5.66	10	9.44	3.52	6.40	19.31	10.97
	24	75.15	63.24	57.31	40.47	17.37	6.14	10	11.55	3.44	6.32	21.62	10.96
	22	81.64	68.27	59.96	42.94	17.55	6.56	13	10.15	3.41	8.01	21.69	12.55
	22	74.47	62.27	55.36	39.48	16.42	6.75	13	6.90	3.11	6.55	17.07	10.83
	23	79.98	66.36	57.95	42.11	19.43	6.19	10	8.65	3.61	6.32	18.76	10.08
	27	72.64	61.41	55.53	41.30	21.64	5.63	10	8.64	2.53	6.51	17.39	9.45
	23	76.61	65.82	56.79	40.71	15.52	7.28	11	10.91	3.59	6.37	20.98	11.25
	25	82.41	70.28	60.74	44.87	20.89	6.71	9	11.79	3.32	7.00	21.57	10.88
	23	87.29	75.22	65.17	47.78	23.41	6.54	11	10.60	4.25	7.23	21.68	11.45
	24	88.83	75.49	63.29	45.20	23.55	7.13	11	10.51	3.73	7.05	20.63	11.34
	21	81.45	72.13	64.89	44.58	21.12	5.58	14	12.88	3.41	7.63	23.62	13.64
	25	74.97	61.93	53.45	39.07	15.69	6.71	10	10.90	3.43	6.15	20.09	9.79
	23	84.02	70.13	61.36	44.98	21.35	7.02	9	11.24	3.41	6.92	21.33	11.39

Host species	den	bd	add	tpd	drd	trd	wbm	rad	bl	cpw	rl	dw	ds
Xenopus laevis T30XIBE (continue)	20	78.27	67.01	58.82	42.59	20.86	6.19	11	10.58	3.62	6.12	20.24	12.12
	24	77.82	65.74	57.47	39.95	18.40	5.58	13	8.36	3.15	7.30	18.38	11.28
	21	70.77	59.12	53.58	37.34	15.05	6.66	11	10.58	3.37	6.01	19.87	11.19
	22	82.51	70.70	63.01	44.40	22.62	6.14	12	10.41	3.72	7.23	21.13	12.92
	23	73.47	61.27	54.46	38.11	15.40	5.95	10	10.57	3.07	6.40	19.68	11.08
	22	75.46	63.44	55.95	39.39	16.51	6.56	-	9.61	3.66	6.77	19.21	10.47
	24	83.03	69.64	60.53	43.57	19.31	6.28	-	10.01	3.68	6.78	19.88	11.86
	21	81.27	70.08	60.04	43.14	20.34	6.16	11	11.21	3.25	6.03	21.15	11.41
	24	86.58	74.16	64.29	46.62	21.27	6.56	14	11.08	3.17	7.03	22.69	10.39
	25	81.63	68.70	60.81	44.75	19.75	5.83	10	10.08	3.72	6.64	20.55	11.07
	25	75.17	61.81	53.31	37.06	19.99	5.89	-	8.07	3.18	5.99	16.68	10.15
	20	81.15	68.88	58.98	41.41	15.87	5.19	13	12.42	3.90	6.86	22.78	12.14
	23	89.14	75.35	64.97	47.14	20.66	6.15	12	11.83	3.93	6.90	23.57	11.86
	22	78.27	65.54	58.68	41.27	19.36	7.21	12	10.62	3.36	6.67	20.98	11.63
	25	91.86	79.12	67.98	50.13	25.27	6.14	11	10.42	3.28	6.99	20.66	12.72
	23	75.65	63.24	56.08	39.17	17.49	5.66	10	10.70	2.98	6.12	20.06	11.11
	23	80.31	67.33	59.75	42.39	20.89	6.26	11	10.15	3.04	7.07	20.23	11.18
	25	80.89	68.66	59.49	42.84	17.85	6.70	13	11.50	3.33	6.93	21.94	10.49
	21	76.39	63.64	55.30	38.59	16.46	6.55	12	10.14	3.38	6.68	20.35	10.67
	27	63.95	54.54	49.59	35.45	20.84	4.35	-	6.74	3.19	6.32	16.02	8.54
	23	74.90	65.30	59.14	41.57	17.02	5.04	12	10.69	3.43	6.06	20.50	11.40
	23	77.86	66.77	57.06	42.39	21.22	5.66	11	9.86	2.90	5.29	18.49	10.49
	23	72.44	59.68	50.90	35.83	19.03	6.54	11	6.94	3.81	5.57	16.25	10.20
	23	76.15	63.68	55.95	37.39	15.53	6.26	11	10.64	3.73	6.17	20.87	10.54
	24	82.11	71.14	60.30	42.36	19.26	5.79	10	9.66	3.60	6.63	20.20	11.41
	22	64.93	54.17	49.10	34.45	14.50	5.95	11	8.96	3.34	5.73	17.81	8.98
	24	71.81	58.57	53.45	36.91	16.39	6.38	11	10.35	3.05	5.72	19.63	9.16

Host species	den	bd	add	tpd	drd	trd	wbm	rad	bl	cpw	rl	dw	ds
Xenopus laevis	20	62.09	50.66	45.15	31.13	13.35	6.53	-	8.24	3.25	5.21	16.07	9.56
T30XIBE (continue)	-	87.10	73.18	61.40	43.20	18.38	6.26	13	10.71	3.98	7.45	21.97	11.86
	23	63.02	54.99	48.12	-	13.34	4.64	12	9.01	3.04	5.30	17.02	9.12
	22	66.00	53.75	48.27	33.91	14.60	6.37	12	8.76	2.82	5.98	17.90	9.62
	22	63.47	54.32	48.31	34.37	13.75	4.93	11	8.20	2.99	5.46	16.74	9.23
	24	67.42	56.03	49.65	35.58	14.59	5.60	10	9.97	2.93	5.54	18.37	8.43
	23	68.90	55.36	50.94	36.10	18.53	5.85	10	8.20	3.28	6.28	17.88	9.79
	27	77.10	64.74	57.78	41.57	17.97	5.63	9	10.17	3.78	6.33	20.83	9.42
	22	73.62	61.08	54.00	37.28	15.43	6.14	10	9.93	3.56	6.49	20.07	10.41
	24	75.68	63.18	57.04	42.35	18.71	6.21	10	10.16	3.75	6.01	20.01	10.75
	25	70.49	61.28	55.21	40.51	21.95	6.04	10	9.65	3.86	5.86	19.14	9.99
	21	68.83	57.03	50.14	34.55	13.12	6.75	10	9.64	3.15	5.80	18.26	10.27
	23	67.08	55.29	48.72	34.21	17.92	4.71	10	7.05	2.97	5.78	15.62	8.96
	25	79.07	68.72	59.54	41.09	19.14	5.63	11	11.14	3.20	6.48	21.15	11.17
	25	78.22	68.18	59.09	42.31	19.71	6.38	12	11.14	3.47	6.33	20.76	10.88
	25	74.48	62.82	54.45	39.48	16.02	5.95	9	9.98	3.75	6.11	19.57	8.95
	22	60.12	50.48	43.49	30.61	14.24	5.27	9	7.13	2.92	4.97	15.65	9.11
	24	72.00	61.04	55.06	38.44	16.96	4.84	13	9.12	3.86	5.76	18.45	10.66
	24	63.63	52.65	48.21	32.80	14.75	5.38	-	8.65	2.94	5.79	17.95	8.27
	25	74.07	62.41	56.61	41.98	18.88	5.07	8	11.21	3.14	6.25	20.57	9.32
	24	69.39	58.17	51.40	36.58	15.79	6.21	10	8.92	3.75	5.60	18.19	8.94
	23	65.81	56.81	50.26	35.12	16.50	3.71	9	8.64	3.40	5.36	17.28	8.08
	24	64.61	55.43	50.99	36.56	15.30	4.69	-	9.86	3.00	6.05	18.56	9.50
	24	66.38	55.68	49.86	35.35	15.67	4.35	11	8.73	3.02	5.76	17.99	9.35
	23	65.76	54.72	48.12	34.24	12.24	5.68	12	9.95	2.67	5.58	18.01	9.04
	25	75.37	63.70	55.43	39.74	17.43	6.17	11	10.03	3.39	6.11	19.94	10.92
	23	73.94	62.37	55.74	39.36	15.85	5.81	11	10.89	3.33	6.26	20.05	10.47

Host species	den	bd	add	tpd	drd	trd	wbm	rad	bl	cpw	rl	dw	ds
Xenopus laevis T30XIBE (continue)	23	67.86	56.05	49.43	35.15	14.49	5.04	12	8.91	3.00	5.93	17.49	10.41
	24	69.24	59.22	52.61	36.48	15.69	6.10	9	9.35	3.11	6.08	18.86	9.26
	27	74.36	61.68	55.95	39.50	18.87	6.17	9	9.57	3.20	5.94	18.69	9.20
	24	65.36	58.06	52.43	37.90	18.48	4.34	9	8.35	3.62	6.13	17.56	10.03
	24	77.24	65.04	55.73	38.88	15.30	5.29	11	10.93	2.96	6.45	20.63	10.93
	23	64.65	55.33	47.61	33.44	16.83	4.99	12	8.01	2.55	6.09	17.54	9.24
	22	65.76	54.66	48.86	34.32	14.22	5.70	-	8.76	3.41	6.05	18.30	9.20
	24	74.73	62.81	55.56	38.91	16.71	5.55	11	9.91	3.01	6.39	18.98	11.32
	27	77.06	65.78	59.14	42.34	19.65	5.94	11	10.20	3.59	6.53	20.11	9.91
	22	76.30	63.75	55.44	38.27	16.53	6.52	11	10.29	3.58	6.33	19.94	11.38
	21	67.37	55.60	49.82	34.12	12.84	6.28	12	9.57	3.22	5.97	18.92	10.56
	23	70.59	58.31	52.45	35.71	16.06	6.61	12	9.11	3.91	6.09	19.28	10.01
	24	71.34	59.82	53.20	37.15	16.55	6.64	12	9.57	3.06	6.55	18.94	10.04
	22	66.30	52.83	47.75	32.35	16.98	6.80	-	6.39	2.95	5.82	15.29	9.18
	23	72.65	62.10	53.39	38.35	15.77	6.26	12	10.23	3.58	6.89	20.30	9.93
	23	64.71	56.86	50.12	35.29	15.19	4.53	12	8.11	3.17	5.81	17.32	9.70
	24	65.33	54.67	51.19	35.13	14.00	3.79	-	9.37	2.92	6.14	19.01	8.77
	T31XIBE												
	25	79.14	68.01	59.41	42.81	22.49	5.25	12	9.22	2.20	6.84	18.29	9.93
	-	76.77	66.58	56.86	40.34	22.29	5.83	-	9.36	2.60	7.06	19.02	9.15
	24	69.43	57.82	50.58	36.32	17.43	5.45	-	7.53	2.56	5.91	16.22	8.60
	25	78.95	66.79	57.08	40.52	18.31	5.44	13	8.93	2.83	7.91	19.13	9.70
	24	84.72	72.56	60.06	44.38	22.52	6.15	9	9.88	2.93	6.16	18.88	9.71
	21	65.43	53.55	46.62	32.54	12.66	6.11	12	7.56	3.07	5.21	15.92	9.60
	21	70.91	59.14	53.68	36.62	18.88	5.88	12	8.44	3.12	6.39	17.47	10.20
	23	70.00	61.22	55.00	39.78	19.24	5.29	-	8.07	2.36	5.89	16.64	10.26
	21	66.54	54.70	46.92	33.75	15.80	6.03	-	8.38	2.96	5.44	16.24	9.72

Host species	den	bd	add	tpd	drd	trd	wbm	rad	bl	cpw	rl	dw	ds
Xenopus laevis	-	65.06	51.98	45.78	32.40	16.70	5.42	-	7.23	2.78	6.04	15.72	9.83
T31XIBE (continue)	20	69.22	58.32	50.93	35.49	19.26	5.67	11	7.36	2.75	6.46	16.79	10.50
	21	73.35	60.47	57.66	42.97	21.23	6.83	-	8.79	2.54	5.90	18.10	11.47
	21	62.00	51.01	44.64	31.48	14.71	5.47	11	8.31	2.28	5.77	16.95	9.05
	22	70.26	59.53	53.25	38.01	21.57	4.04	-	8.13	2.05	5.71	15.73	9.79
	21	59.09	48.45	44.57	31.41	15.44	4.74	-	6.13	2.96	5.22	14.24	9.09
	21	71.29	61.90	54.96	41.07	20.10	4.73	10	9.15	2.75	6.40	18.15	11.67
	22	74.27	64.83	56.25	39.82	20.65	4.70	12	9.35	2.73	6.00	17.99	10.40
	21	59.65	49.45	43.46	29.29	12.32	5.49	12	7.32	2.56	5.88	15.91	8.71
	21	72.47	60.66	52.85	36.51	18.65	5.28	12	8.87	2.37	6.90	18.03	10.47
	21	72.71	61.02	51.63	36.64	17.98	5.74	13	9.17	2.61	6.52	18.09	11.68
	23	74.45	63.23	54.56	38.83	18.23	5.73	9	9.13	3.53	5.79	18.40	9.97
	22	69.09	56.47	50.37	35.26	16.54	6.43	11	8.06	3.05	5.58	16.65	9.04
	20	66.25	56.32	48.69	33.64	18.32	5.39	10	7.84	2.13	5.82	15.75	9.75
	23	80.03	67.82	58.56	41.74	22.83	6.42	11	9.00	3.02	6.98	18.78	10.32
	23	75.16	62.85	58.84	41.86	21.64	6.27	11	8.83	3.53	6.42	18.47	10.57
	22	69.45	56.92	48.45	34.14	15.98	5.37	-	7.60	2.20	6.36	16.10	8.73
	22	66.44	55.04	49.72	33.53	14.38	5.67	12	8.79	3.78	5.92	19.22	10.38
	24	81.94	70.69	61.45	43.13	21.29	6.03	12	9.31	3.77	7.20	20.17	10.17
	24	74.05	61.32	53.24	37.22	16.83	4.68	-	8.39	3.64	6.72	18.52	10.03
	27	-	66.97	55.57	40.31	20.97	6.40	11	8.42	3.73	6.29	17.80	8.97
	23	71.80	60.86	50.07	35.60	18.80	5.69	13	7.40	3.09	5.90	16.25	10.36
	25	79.97	74.21	64.28	47.32	22.04	2.40	12	10.86	4.35	6.68	21.08	11.18
	26	81.53	68.90	60.56	42.12	21.09	6.51	12	9.08	4.10	6.36	20.02	9.56
	23	-	68.74	60.34	42.46	19.12	6.01	12	9.58	4.04	7.22	20.98	12.56
	T32XIBB												
	22	70.50	61.34	52.84	38.04	16.26	5.96	12	2.85	5.57	5.49	18.86	8.84

Host species	den	bd	add	tpd	drd	trd	wbm	rad	bl	cpw	rl	dw	ds
Xenopus laevis	21	82.07	71.32	61.69	42.39	17.74	6.42	14	11.80	3.63	6.63	22.05	12.49
T32XIBB (continue)	20	73.79	60.08	52.72	36.87	15.78	6.82	12	9.39	3.05	5.85	18.33	10.13
	T33XIBQ												
	20	66.36	57.32	49.11	37.42	18.50	7.05	9	7.10	3.26	5.08	15.49	7.72
	21	71.45	61.62	54.24	40.51	16.57	4.94	11	9.87	3.15	6.38	19.48	11.16
	22	72.49	62.39	52.97	38.30	16.22	5.16	7	7.98	3.65	5.96	18.28	10.02
	21	77.75	67.34	55.41	39.62	17.75	5.44	9	9.85	4.23	5.71	19.80	9.57
	22	78.53	66.97	58.32	44.30	20.02	6.71	7	10.27	3.56	5.91	19.84	9.45
	19	71.65	60.32	54.45	40.24	20.25	5.50	11	9.01	3.63	5.05	16.85	8.86
	T34XISD												
	22	79.13	69.42	58.87	42.86	19.84	5.18	12	10.10	3.41	7.15	20.41	11.47
	22	76.24	64.59	56.00	39.15	15.84	6.16	10	9.89	4.14	6.10	19.86	10.65
	25	75.13	64.76	57.60	41.31	17.88	4.54	13	10.83	3.58	6.90	21.24	10.64
	25	78.33	66.53	57.03	41.55	22.07	5.66	12	8.48	3.51	6.77	18.67	10.12
	24	83.36	74.25	65.10	45.39	24.19	5.45	11	10.77	3.36	7.92	21.61	9.98
	21	72.45	61.41	55.07	39.48	14.66	4.69	10	10.78	3.75	6.07	20.64	11.28
	-	75.78	63.75	55.42	38.27	15.99	5.91	12	10.47	3.53	6.86	20.72	10.72
	22	79.13	68.17	60.74	42.75	19.77	5.63	10	11.41	3.60	6.74	21.66	11.17
	23	83.05	72.48	63.24	46.73	19.16	5.94	11	12.22	3.80	6.45	22.47	11.92
	26	86.40	75.04	66.32	49.20	28.61	5.65	11	9.70	2.74	7.14	19.55	9.98
	19	73.21	60.85	54.81	37.36	17.95	5.78	12	8.15	3.05	6.62	17.91	10.84
	22	73.38	62.33	55.34	39.55	17.67	6.32	11	9.26	3.36	6.57	18.95	-
	-	78.93	66.17	58.93	41.64	20.07	5.80	12	9.79	3.34	7.05	19.98	9.85
	24	77.35	68.97	58.69	43.27	24.20	4.90	10	8.07	2.92	7.16	17.63	9.88
	20	65.94	54.13	48.90	33.54	15.21	5.71	12	9.54	2.46	6.22	18.03	10.48
	24	71.42	59.70	52.84	37.96	17.96	5.72	11	8.94	2.84	6.30	17.87	9.21
	23	77.90	66.56	55.99	40.22	17.66	6.34	13	10.37	2.53	6.50	19.73	9.88

Host species	den	bd	add	tpd	drd	trd	wbm	rad	bl	cpw	rl	dw	ds
Xenopus laevis **T34XISD (continue)**	20	64.93	53.62	47.23	32.37	13.22	5.09	11	8.69	3.02	5.91	17.67	10.24
	27	75.26	63.91	55.23	39.64	21.61	5.52	10	8.32	2.16	6.95	17.56	8.66
	24	81.60	70.36	57.91	42.71	21.07	5.33	12	8.75	3.22	6.67	18.50	9.86
	24	77.25	65.62	54.38	39.70	22.85	5.64	11	6.98	2.09	6.91	15.86	9.71
	25	75.83	65.36	54.78	38.97	18.35	5.60	9	8.77	2.93	6.73	18.36	9.08

Measurements noted to calculate the area of the blade, central part and ray respectively in a denticle, as well as the area of the denticles within the denticle ring. Please refer to Figure 5.1 and Table 5.1 for more information on the host populations.

Host	Population number	Number of denticles	Diameter (in µm)			Area (in µm²)		
			Tangent point	Denticle ring	Tip of ray	Blade	Central part	Ray
***Amietophrynus* species**	**T4AuBH**	22	42.76	28.54	11.95	17.43	7.60	5.70
						16.28	7.55	5.45
						16.52	7.20	6.42
						17.36	6.25	6.00
						14.18	9.56	7.46
						15.07	8.34	5.66
						16.17	6.96	5.63
		20	43.79	28.45	12.05	19.94	9.08	6.56
						17.92	9.07	6.92
						18.27	9.14	7.68
						19.90	8.96	7.76
						16.22	10.37	7.38
						17.21	8.55	7.22
						15.21	10.37	6.79
						16.57	9.97	6.51
		19	42.60	28.62	10.45	17.52	9.57	6.98
						17.99	10.37	7.77
						18.72	10.41	5.46
						19.47	9.66	7.62
						20.96	8.22	7.94
						19.73	8.75	8.32
						24.21	9.05	7.70
						16.12	8.60	8.28
		20	40.09	26.62	10.49	16.21	7.26	7.46
						15.66	8.13	6.03
						14.72	7.21	5.42
						16.53	8.07	5.06
Amietophrynus poweri	**T9ApCF**	22	47.64	33.03	13.60	17.31	8.02	9.82
						16.82	8.79	8.06
						16.83	9.00	7.31
						16.70	9.98	8.45
		23	57.75	43.19	20.47	20.24	11.15	11.41
						21.62	11.12	12.02
						22.17	11.93	11.03
						20.85	12.33	11.92
						19.88	12.49	10.98

Host	Population number	Number of denticles	Diameter (in µm)			Area (in µm²)		
			Tangent point	Denticle ring	Tip of ray	Blade	Central part	Ray
Amietophrynus poweri (continue)	T9ApCF (continue)					20.54	12.82	11.10
						22.14	10.80	10.77
		24	46.82	31.94	14.69	18.00	7.62	7.62
						18.14	8.48	7.63
						16.34	10.04	8.22
						17.79	8.85	6.82
						17.91	8.10	8.03
	T7ApTB	22	49.99	35.22	15.75	18.61	10.36	9.22
						21.18	12.42	9.07
						17.79	10.56	10.24
						19.09	11.19	9.69
		22	51.89	37.21	17.41	21.66	9.57	12.33
						20.53	9.69	12.84
						19.26	9.04	8.46
		21	54.44	37.71	19.69	23.31	13.25	9.41
						21.99	13.70	10.95
						26.15	12.06	9.67
						25.89	13.68	9.14
						24.17	11.10	7.68
		21	49.42	34.19	17.70	22.10	11.19	8.33
						22.15	9.79	8.20
						25.37	10.62	5.50
Amietophrynus gutturalis	T10AgNC	21	49.42	35.05	16.17	18.69	8.03	8.50
						18.19	9.76	7.80
						16.09	10.61	8.81
						19.48	9.70	8.24
						16.72	10.67	7.02
						18.98	10.41	7.13
		24	52.13	37.26	14.84	20.50	11.01	13.26
						20.52	11.32	14.82
						20.34	11.95	12.98
		20	44.03	30.07	13.15	19.53	8.77	8.91
						17.50	8.46	8.84
						16.95	8.90	9.07
		20	44.90	30.67	13.56	19.30	9.53	7.84
						18.94	9.81	9.33
						21.88	9.01	8.39
		21	47.28	32.55	13.21	17.67	10.04	7.60
						18.58	12.05	7.89

Host	Population number	Number of denticles	Diameter (in µm)			Area (in µm²)		
			Tangent point	Denticle ring	Tip of ray	Blade	Central part	Ray
Amietophrynus gutturalis (continue)	T10AgNC (continue)					18.82	8.10	10.49
						18.81	10.29	11.25
		20	44.26	31.33	14.65	18.17	8.58	6.25
						19.58	9.68	7.72
						18.07	9.95	10.29
		22	50.66	36.29	15.64	20.95	12.62	10.46
						19.93	11.20	8.73
	T12AgND	22	44.09	30.50	13.67	14.83	8.88	5.53
						13.97	9.93	4.98
						14.86	7.93	6.60
						15.09	9.44	8.25
		24	56.31	40.05	17.42	22.45	11.38	11.01
						23.57	13.39	12.09
						26.62	12.25	11.20
						25.16	10.07	14.72
						26.57	12.88	14.30
						25.23	10.86	12.80
						26.38	13.64	17.41
						24.47	12.57	14.51
						23.93	12.93	14.80
		23	49.50	34.40	15.59	15.75	9.17	8.23
						16.42	8.12	10.16
						17.14	7.97	9.67
						17.03	9.54	8.36
						14.56	9.66	8.71
						16.06	10.38	10.39
						15.68	9.82	8.19
		20	49.35	33.98	14.88	21.48	8.98	11.29
						23.19	9.81	9.47
						20.96	10.68	9.00
						20.89	8.71	9.14
						20.07	10.35	11.57
						18.68	9.59	9.69
						20.20	10.10	7.89
						19.25	9.03	6.98
		21	44.52	29.29	12.40	20.91	9.52	4.41
						21.02	9.73	4.35
						19.76	9.93	9.06
						21.16	9.27	10.56

Host	Population number	Number of denticles	Diameter (in µm)			Area (in µm²)		
			Tangent point	Denticle ring	Tip of ray	Blade	Central part	Ray
Amietophrynus gutturalis (continue)	T12AgND (continue)	21	43.67	29.48	9.83	17.61	10.23	6.56
						17.89	10.69	8.77
						17.30	9.91	7.15
						20.99	8.83	10.34
Amietia species	T14AMuNW	19	36.04	25.21	12.22	20.02	17.56	9.52
						20.06	16.39	11.98
						21.14	14.98	11.09
						20.16	12.79	12.32
						19.88	14.23	10.81
		21	39.06	26.26	11.57	16.10	12.61	7.41
						15.85	11.37	7.80
						16.42	11.80	9.13
						17.78	13.58	8.45
						16.26	12.74	9.44
						14.12	13.76	9.10
Amietia angolensis	T18AMaSS	24	46.30	33.41	15.43	14.75	9.17	7.88
						12.22	10.70	7.57
						13.43	10.41	6.87
						13.19	11.35	9.04
						13.20	11.17	9.06
Kassina senegalensis	T21KsZZ	22	61.43	43.72	18.37	29.35	15.74	14.81
						29.99	14.54	16.40
						28.54	14.26	17.44
						26.68	14.87	14.64
		20	58.22	40.81	18.52	26.50	13.89	16.26
						24.72	14.59	14.24
						26.73	13.74	14.87
						28.40	13.44	10.09
						26.46	12.27	11.38
		21	58.48	40.98	19.18	26.93	13.62	14.14
						26.95	13.57	17.43
						26.64	14.80	18.73
						25.85	16.10	17.78
						27.34	16.90	16.23
						28.53	16.52	17.30
						27.61	10.53	17.59
		21	62.44	44.68	21.66	35.93	17.23	14.17
						31.91	18.60	23.89
						31.16	18.36	21.60

Host	Population number	Number of denticles	Diameter (in µm)			Area (in µm²)		
			Tangent point	Denticle ring	Tip of ray	Blade	Central part	Ray
Kassina senegalensis (continue)	T21KsZZ (continue)					32.62	16.82	20.52
		22	59.42	41.15	16.04	29.21	15.85	15.89
						29.68	14.48	15.28
						32.24	13.13	18.64
						29.76	13.47	16.81
		20	58.06	40.32	13.50	27.56	16.40	13.71
						25.34	15.84	14.84
						31.08	13.73	12.86
						22.96	15.33	13.30
						24.21	12.50	12.80
Natalobatrachus bonebergi	T22NbVC	22	61.72	43.63	21.02	26.81	16.24	17.30
						27.66	18.26	18.33
						25.45	17.29	16.13
						26.97	19.06	18.88
						27.34	17.20	13.90
						25.87	20.07	19.75
		25	54.55	39.50	17.22	17.09	13.82	11.75
						13.93	14.76	10.51
						16.63	11.04	11.67
						16.21	12.49	10.27
		21	56.60	41.52	17.52	20.28	18.59	21.52
						18.27	20.50	17.21
						20.40	15.91	18.57
						22.99	19.87	20.94
						22.38	17.51	19.20
		22	63.10	46.40	17.62	22.20	17.26	18.71
						24.58	21.35	18.34
						24.91	22.11	19.55
						23.01	19.55	19.42
		22	61.28	44.48	16.05	28.69	16.31	21.19
						25.65	17.05	21.00
						24.81	17.59	15.77
						20.77	19.21	18.14
Xenopus laevis	T26XlOG	22	50.85	34.36	17.65	27.07	14.06	12.91
						21.14	12.71	9.30
						25.17	11.64	12.23
						29.89	15.41	11.17
						32.19	12.79	13.33
						32.32	13.01	12.43

Host	Population number	Number of denticles	Diameter (in µm)			Area (in µm²)		
			Tangent point	Denticle ring	Tip of ray	Blade	Central part	Ray
Xenopus laevis (continue)	T26XIOG (continue)					30.17	13.36	12.88
						33.50	13.45	13.46
						32.21	16.00	12.05
						28.77	12.31	12.39
		23	52.10	35.60	18.03	27.90	12.92	10.21
						27.76	10.74	10.78
						28.10	11.95	6.25
						26.49	13.43	8.60
						23.89	12.56	7.96
						23.68	12.85	8.04
		21	58.65	41.74	17.82	28.64	11.66	16.02
						24.89	11.24	14.93
						23.06	10.26	14.34
						29.91	10.63	13.93
						25.45	8.78	12.95
						25.17	13.48	14.48
		25	53.45	40.55	18.96	21.79	14.30	14.30
						23.86	17.10	14.59
						22.41	16.41	13.35
						22.93	16.06	14.67
	T27XIHS	20	55.55	39.77	19.97	25.65	13.09	12.71
						24.36	15.29	12.42
						21.48	14.21	12.98
						21.03	15.66	13.47
		22	57.03	40.30	19.78	23.83	14.02	9.77
						23.34	13.68	11.50
						23.73	11.72	12.66
						24.08	14.65	11.87
		20	49.71	34.62	17.12	18.94	10.29	9.81
						21.56	10.64	9.99
						22.60	10.42	9.91
						22.99	8.86	8.91
						24.36	9.32	9.38
						23.03	9.40	12.24
						24.31	8.10	10.31
						24.11	8.73	10.37
						20.13	10.41	10.91
		20	45.59	31.02	13.55	25.83	14.53	12.80
						25.11	17.78	9.97

Host	Population number	Number of denticles	Diameter (in µm)			Area (in µm²)		
			Tangent point	Denticle ring	Tip of ray	Blade	Central part	Ray
Xenopus laevis (continue)	T27XIHS (continue)					25.67	13.87	10.78
						26.34	13.60	12.10
						24.42	17.89	13.99
						27.22	18.28	11.55
						25.54	15.36	15.91
						27.65	14.01	12.24
		23	51.67	37.14	19.21	18.41	8.94	8.34
						18.39	8.73	8.03
						20.85	9.49	8.09
						18.30	8.07	8.51
						16.05	8.50	8.64
		22	46.22	31.65	13.11	15.10	8.96	9.29
						15.14	8.26	8.35
						16.30	7.47	7.12
						16.01	10.06	8.86
		21	48.83	33.28	15.62	17.62	8.56	4.25
						21.58	9.43	4.94
						17.02	10.32	5.39
						20.32	9.99	5.22
						18.77	11.96	8.27
	T28XIBB	20	53.16	37.03	12.87	22.67	12.89	11.09
						23.81	11.32	8.68
						22.87	11.10	13.80
						21.85	13.17	12.48
						18.76	13.99	13.59
		21	53.76	37.04	14.83	21.27	11.91	14.18
						21.30	11.11	12.94
						23.02	11.17	13.14
						20.42	11.91	12.82
						20.80	13.07	7.74
		21	58.59	42.11	17.31	25.03	14.53	17.03
						24.52	15.70	16.07
						24.04	16.89	17.44
						26.54	18.53	12.12
						30.91	17.71	15.58
		22	57.00	40.39	14.71	23.42	13.22	14.39
						20.94	13.23	12.72
						20.95	12.91	13.56
						24.29	14.33	13.40

Host	Population number	Number of denticles	Diameter (in µm)			Area (in µm²)		
			Tangent point	Denticle ring	Tip of ray	Blade	Central part	Ray
Xenopus laevis (continue)	T28XIBB (continue)	20	54.16	38.72	13.35	21.09	16.00	14.26
						23.86	15.99	14.32
						23.85	15.87	13.71
						26.28	17.45	13.60
						25.48	18.92	13.11
	T29XIPC	23	61.73	44.41	21.48	21.10	14.24	12.25
						25.99	13.93	16.91
						25.36	16.36	14.52
						32.95	13.59	11.43
						34.60	15.69	10.73
						32.75	17.28	12.42
						32.06	14.27	13.88
						35.56	14.80	11.88
						33.83	14.08	10.95
	T30XIBE	23	53.55	36.12	14.60	25.98	14.20	10.76
						24.39	16.45	11.49
						24.42	16.13	10.41
		24	56.31	39.89	17.70	23.32	11.69	13.76
						23.63	15.82	10.72
						21.81	17.40	11.34
						22.19	16.47	11.80
						21.43	15.73	9.57
						23.18	14.59	12.23
		23	56.79	40.71	15.52	28.47	16.27	15.41
						29.20	16.08	14.08
						32.45	15.99	16.83
						29.73	21.07	17.60
		23	65.17	47.78	23.41	30.80	21.91	17.19
						32.00	21.12	19.24
						32.79	19.89	16.46
						33.39	20.37	17.07
		21	64.89	44.58	21.12	45.08	19.49	21.55
						44.70	18.81	20.48
						41.41	19.81	21.06
		23	54.46	38.11	15.40	28.87	14.69	14.91
						27.77	19.75	12.07
						27.51	19.57	15.59
						26.86	22.21	14.20
						29.50	20.80	13.41

Host	Population number	Number of denticles	Diameter (in µm)			Area (in µm²)		
			Tangent point	Denticle ring	Tip of ray	Blade	Central part	Ray
Xenopus laevis (continue)	T30XIBE (continue)					28.78	18.86	8.19
		22	58.68	41.27	19.36	31.34	18.13	11.08
						33.11	16.58	13.95
						33.67	16.55	13.39
						34.32	18.68	14.04
						32.11	15.50	13.80
	T31XIBE	24	60.06	44.38	22.52	23.07	11.19	14.44
						24.68	11.98	12.97
						23.05	14.78	10.81
		22	56.25	39.82	20.65	20.34	15.30	12.99
						24.15	13.61	13.18
						22.79	13.73	12.22
						24.85	14.70	10.12
						24.75	14.91	11.90
						22.77	13.02	11.35
		21	51.63	36.64	17.98	19.90	11.50	10.74
						21.76	11.48	10.44
						21.86	10.29	9.70
						23.06	9.60	8.73
		20	48.69	33.64	18.32	23.17	8.60	8.42
						21.00	8.83	9.13
						19.11	9.21	9.02
		23	58.84	41.86	21.64	25.89	13.88	11.82
						28.05	12.69	12.36
						27.84	11.99	10.49
		24	61.45	43.13	21.29	29.60	16.52	15.66
						32.18	16.98	17.25
						30.77	16.51	16.83
	T32XIBB	22	52.84	38.04	16.26	23.02	11.59	8.53
						21.48	12.49	9.95
						21.36	12.14	9.71
						19.73	11.16	9.45
Unknown tadpole species	T35UuBV	23	68.08	49.36	19.43	32.24	19.33	20.22
						30.52	21.90	23.24
						32.08	21.73	17.01
						29.56	21.06	21.48
						29.05	20.65	20.78
		25	63.94	45.72	20.32	23.66	14.30	14.26
						21.60	13.76	15.12

Host	Population number	Number of denticles	Diameter (in µm)			Area (in µm²)		
			Tangent point	Denticle ring	Tip of ray	Blade	Central part	Ray
Unknown tadpole species (continue)	T35UuBV (continue)					22.26	13.83	16.55
						23.03	15.90	17.62
						24.14	15.60	14.09
						22.04	17.66	14.72
						23.82	18.29	13.64
						28.00	18.49	14.34
						24.07	18.35	17.05
		25	68.33	48.20	22.21	33.66	16.26	10.88
						29.80	15.74	14.76
						32.90	15.65	19.46
						30.85	14.55	14.98
						29.33	14.56	16.28
						28.46	13.37	11.05
		23	57.53	40.43	19.83	21.97	21.97	11.26
						23.33	16.55	13.76
						22.57	16.83	10.17
						22.57	14.86	10.55
		27	68.15	49.84	22.76	23.40	15.82	15.62
						22.27	15.50	17.08
						24.46	14.42	17.31
						21.67	15.57	12.86
						22.26	16.32	13.48
		20	62.68	44.19	15.29	35.32	17.54	12.93
						30.12	19.68	16.45
						29.63	19.92	16.76
		21	64.13	44.11	18.43	34.79	17.83	16.14
						29.47	22.34	18.15
						31.47	25.26	15.62
						28.82	23.79	20.58
						27.45	23.74	18.40
	T36UuLF	23	53.48	35.61	18.14	19.85	11.38	7.04
						19.66	9.23	6.10
						19.61	10.42	7.54
						17.85	10.21	8.47
Barbus anoplus	F1BaSD	23	54.50	39.72	22.95	21.78	9.38	9.90
						20.86	10.15	6.92
						25.14	11.10	8.14
		23	69.70	49.78	23.47	30.12	12.86	15.06
						31.56	18.36	14.53

Host	Population number	Number of denticles	Diameter (in µm)			Area (in µm²)		
			Tangent point	Denticle ring	Tip of ray	Blade	Central part	Ray
Barbus anoplus (continue)	F1BaSD (continue)					31.42	21.44	14.00
						26.78	17.94	17.96
	F2BaSS	23	55.04	38.30	20.19	21.51	8.76	10.48
						19.92	8.04	10.34
						20.24	10.68	10.86
						19.76	8.69	10.11
						18.75	9.73	9.62
Barbus species	F3BuBV	28	70.14	51.24	27.99	19.62	15.42	14.34
						22.83	14.63	11.90
						23.21	15.80	13.68
						21.67	16.31	12.63
						21.05	13.73	15.38
	F4BuOG	21	72.42	48.33	26.45	16.71	9.10	5.46
						15.02	9.18	4.65
						14.59	9.32	6.65
						14.86	8.26	4.62
						15.11	8.03	5.62
	F6BuBL	24	44.29	31.97	15.58	13.24	6.39	8.91
						11.60	5.86	8.03
						13.46	4.96	8.44
Barbus barnardi	F5BbTT	23	47.39	32.29	15.40	24.34	12.83	8.87
						23.79	10.58	13.34
						23.10	9.17	10.83
						26.01	12.29	12.44
		22	50.27	34.65	13.00	19.61	10.81	10.22
						18.98	9.74	9.73
						19.13	8.86	12.24
						20.16	12.02	11.14
						21.33	11.48	9.35
						20.66	9.45	11.61
						21.39	11.03	10.41
		22	55.05	38.43	16.28	23.57	14.19	15.47
						24.03	14.52	15.65
						21.21	13.77	17.81
						24.39	14.28	16.72
						24.13	14.57	15.95
						22.78	15.49	14.34
						24.83	13.38	15.96

Host	Population number	Number of denticles	Diameter (in µm)			Area (in µm²)		
			Tangent point	Denticle ring	Tip of ray	Blade	Central part	Ray
Barbus barnardi (continue)	F5BbTT (continue)	24	55.20	37.84	16.09	19.55	11.69	9.21
						18.77	12.05	10.47
						20.47	11.56	11.51
						19.59	9.87	12.09
						17.40	11.69	10.88
						17.52	12.25	11.06
Clarias gariepinus	F7CgUP	23	53.73	37.47	17.31	18.47	9.82	8.31
						17.96	8.43	7.56
						18.35	10.46	9.30
						17.89	8.44	9.24
						19.23	9.07	7.99
Cyprinus carpio	F8CcLF	25	62.74	45.05	22.10	21.91	10.57	9.00
						20.46	14.04	10.72
						17.48	12.79	11.06
						20.32	13.28	10.80
						20.85	11.18	12.09
						21.77	13.16	9.68
		20	49.67	34.99	17.05	17.91	11.93	8.99
						17.07	13.11	7.22
						17.75	11.89	6.27
						17.94	12.76	7.97
						17.93	12.65	6.24
		24	66.97	48.74	20.29	29.69	16.41	16.57
						27.63	16.99	19.72
						29.25	16.12	17.99
						28.91	17.34	19.03
						25.62	15.15	15.66
						27.30	18.17	20.74
						24.68	17.15	17.17
						26.66	15.33	16.36
						28.38	16.42	17.65
Gambusia affinis	F9GaEB	22	53.18	37.58	20.67	18.25	9.08	8.43
						17.96	10.22	6.97
						18.38	12.71	8.49
						19.25	11.59	9.55
						21.45	12.65	10.07
Oreochromis mossambicus	F11OmLF	20	50.93	35.14	14.58	14.96	8.75	6.64
						12.97	10.77	6.61
						13.16	8.76	6.60

Host	Population number	Number of denticles	Diameter (in µm)			Area (in µm²)		
			Tangent point	Denticle ring	Tip of ray	Blade	Central part	Ray
Oreochromis mossambicus (continue)	F11OmLF (continue)					13.13	9.39	7.10
						13.99	9.86	5.51
						13.53	10.54	7.33
Poecilia sphenops	F12PsLV	25	65.45	47.32	20.25	23.02	12.74	19.42
						24.16	16.32	14.07
						21.51	18.05	19.15
						24.59	17.00	16.88
Pseudocrenilabrus philander	F15PpBV	26	72.90	53.25	25.07	37.65	23.79	30.01
						42.10	21.13	28.31
						36.52	21.33	24.36
						39.47	24.04	29.45
						36.85	21.54	20.64
						27.66	24.12	18.92
		24	70.11	50.49	21.67	28.43	14.84	21.87
						26.12	20.54	23.99
						31.85	21.13	17.83
						29.93	18.43	22.35
						30.41	20.30	22.08
						29.09	21.47	26.12
						30.70	20.26	19.91
		23	64.82	45.65	16.51	22.55	17.69	17.88
						27.34	19.28	20.61
						27.81	19.30	19.83
						31.54	22.28	17.78
						27.10	16.84	14.79
						24.85	18.71	16.64
						29.43	17.17	18.47
		24	67.65	49.27	24.32	26.34	17.77	19.59
						26.73	20.99	20.85
						26.63	20.33	17.42
						25.23	18.35	16.28
		25	70.94	51.79	24.57	30.36	20.99	24.45
						27.03	26.35	21.88
						27.68	25.40	21.92
						28.77	21.57	23.93
		23	65.04	45.24	18.18	33.25	16.96	24.43
						33.28	21.17	18.18
						31.77	24.72	22.70
						31.77	20.93	19.83

Host	Population number	Number of denticles	Diameter (in µm)			Area (in µm²)		
			Tangent point	Denticle ring	Tip of ray	Blade	Central part	Ray
Pseudocrenilabrus philander (continue)	F15PpBV (continue)					27.85	23.30	16.43
						32.44	23.11	19.33
						35.07	19.98	18.76
						31.49	23.24	17.14
		23	68.57	41.91	15.43	27.86	15.14	15.41
						24.17	17.39	17.75
						27.61	15.13	16.32
						24.88	16.79	15.31
						29.63	16.85	13.63
						24.96	14.81	16.58
		26	68.82	50.67	20.67	25.04	20.46	20.24
						26.17	20.74	20.44
						28.92	19.44	19.44
						25.35	22.22	18.96
						27.92	18.68	21.18
		27	72.68	52.48	26.14	29.28	16.59	12.50
						27.24	20.88	18.97
						29.43	21.68	16.88
						28.87	19.07	14.67
						29.33	18.94	18.71
	F16PpEB	24	63.21	46.48	23.65	26.26	14.26	15.64
						20.55	15.92	17.13
						25.93	15.19	16.01
						26.36	14.40	16.33
						22.41	16.40	17.45
						27.22	14.19	14.42
		24	65.75	48.34	24.79	29.90	19.44	15.65
						28.62	21.93	12.69
						32.30	22.02	19.95
						31.80	20.06	16.48
						31.33	21.83	13.41
						29.10	21.19	13.56
		26	65.28	46.96	28.05	29.74	20.55	15.15
						30.53	21.87	17.05
						36.36	17.05	17.51
						37.08	20.42	16.86
		24	56.60	39.12	17.28	32.33	12.35	16.28
						26.95	13.56	16.79
						28.79	12.18	15.07

Host	Population number	Number of denticles	Diameter (in µm)			Area (in µm²)		
			Tangent point	Denticle ring	Tip of ray	Blade	Central part	Ray
Pseudocrenilabrus philander (continue)	F16PpEB (continue)					25.75	12.79	17.37
		22	54.78	37.01	15.49	29.86	12.89	14.22
						27.85	14.08	13.95
						26.86	13.70	14.63
Tilapia sparrmanii	F18TsBW	21	52.37	37.50	15.55	19.64	13.45	10.66
						18.06	14.58	8.55
						18.41	16.64	11.39
						19.57	15.57	11.58
						18.43	15.83	9.87
						15.96	16.17	9.64
		24	66.24	47.89	19.83	33.81	16.19	16.05
						31.81	14.48	17.72
						31.48	15.11	14.71
		25	69.28	48.91	24.84	36.27	19.54	14.70
						33.43	17.96	15.03
						34.88	18.32	16.59
						33.97	19.17	20.17
		24	68.41	49.87	21.67	31.02	19.67	17.34
						31.70	19.19	16.24
						32.48	19.31	16.49
						31.05	21.06	17.34
						27.11	19.88	14.93
		24	60.34	43.69	18.15	27.41	13.75	11.80
						22.99	14.55	12.60
						26.40	13.86	12.48
		24	61.61	43.62	19.24	23.70	17.93	13.46
						23.64	19.25	14.83
						23.01	19.47	15.42
		25	66.00	47.95	20.75	32.88	17.00	17.06
						28.00	17.32	16.81
						27.34	17.25	17.93
						26.64	18.78	16.63

Value given for each of the characteristics studied during the examination of three consecutive denticles of various trichodinid populations.

Characteristic	Value given for each of the characteristics									
	1	2	3	4	5	6	7	8	9	10
Blade form ($BlaFor_1$)	Open sickle-shaped									
Distal surface 1 ($DisSur_1$)	Rounded	Rounded, not always smooth								
Distal surface 2 ($DisSur_2$)	Sloping downwards in anterior direction									
Tangent point 1 ($TanPoi_1$)	Rounded	Rounded, sharper in some instances	Rounded in most cases, may be sharper in some instances	Ends sharp mostly, otherwise rounded						
Anterior margin 1 ($AntMar_1$)	Rounded, smooth	Rounded, not smooth	Rounded, not always smooth							
Anterior margin 2 ($AntMar_2$)	Extending less than halfway past y-axis	Extending to or slightly beyond y+1 axis	Extending slightly beyond y+1 axis	Extending towards, but not beyond y+1 axis	Extending to y+1 axis, ending beyond in some instances (last denticle)	Extending to y+1 axis	Extending beyond y+1 axis	Extending beyond y+1 axis, some extending far beyond y+1 axis (see last denticle)		
Blade apophysis ($BlaApo_1$)	Small, rounded	Sometimes visible	Visible in most	Not clearly visible	Small					

Characteristic	Value given for each of the characteristics									
	1	2	3	4	5	6	7	8	9	10
Posterior blade surface 1 ($PosBla_1$)	Smooth	Mostly smooth								
Posterior blade surface 2 ($PosBla_2$)	Moderately, deep semi-lunar curve	Almost L-shaped	Deep semi-lunar curve	Very deep L-shaped curve	Deep L-shaped curve	Moderate to deep L-shaped curve	Deep semi-lunar to L-shaped curve	Deep semi-lunar curve in some cases, mostly almost L-shaped	Very deep semi-lunar to L-shaped curve	Shallow semi-lunar curve
Blade connection 1 ($BlaCon_1$)	Thick	Thick, strong	Thick, robust	Varies between thin and thick	Thin					
Blade connection 2 ($BlaCon_2$)	Extended									
Posterior projection ($PosPro_1$)	Visible in some instances	Clearly visible in some	Not clearly visible	Not clearly visible in all cases, small, when present						
Protrusion on lower central part ($ProLow_1$)	Visible in most instances	Visible in some instances	Not clearly visible							
Central part 1 ($CenPar_1$)	Robust	Slender	Mostly robust	Slender to robust						
Central part 2 ($CenPar_2$)	Rounded tip									

Characteristic	Value given for each of the characteristics									
	1	2	3	4	5	6	7	8	9	10
Central part 3 ($CenPar_3$)	Extending more than halfway past y-axis	Extending less than halfway past y-axis	Extending more than halfway past y-axis, sometimes almost reaching the y-1 axis	Extending less than, or more than halfway past y-axis	Extending halfway or more past y-axis					
Central part 4 ($CenPar_4$)	Fitting tightly into preceding denticle									
Section above and below x-axis ($SecAB_1$)	Similar	Almost similar, section below steeper slope	Section above steeper slope	Section below steeper slope	Top of section above smoother, rounder	Highest point at section above longer than section below, rectangular top at some instances				
Ray connection ($RayCon_1$)	Short, extends almost directly from central part	Short but robust, extends almost directly from central part	Short, same thickness as ray, extends almost directly from central part	Short, sometimes thicker than ray, extends almost directly from central part	Short, narrower than ray, extends almost directly from central part	Short, narrower than ray, extends almost directly from central part				

Characteristic	Value given for each of the characteristics									
	1	2	3	4	5	6	7	8	9	10
Ray apophysis ($RayApo_1$)	Short, extends almost directly from central part	Short, robust, extends almost directly from central part	Short but robust, extends almost directly from central part	Short, robust, extends almost directly from central part	Short, narrower than ray, extends almost directly from central part					
Form of ray 1 ($RayFor_1$)	Straight									
Form of ray 2 ($RayFor_2$)	Curved in posterior direction	Equal in thickness throughout	Parallel to y-axis in most instances	Curved in anterior direction						
Form of ray 3 ($RayFor_3$)	Parallel to y-axis in most instances	Taper slightly to form rounded tip								
Length of denticle above x-axis and denticle below x-axis ($LenAB_1$)	1 : 1.10 - 1.19	1 : 1.20 - 1.29	1 : 1.30 - 1.39	1 : 1.40 - 1.49	1 : 1.50 - 1.59	1 : 1.0 - 1.09				

Measurements noted to calculate the area of the blade, central part and ray respectively in a denticle, as well as the area of the denticles within the denticle ring. Please refer to Figure 5.1 and Table 5.1 for more information on the host populations.

Characteristic	T4AuBH	T6ApCF	T7ApTB	T10AgNC	T12AgND	T14AMsNW	T15AMsOG	T16AMsHs	T17AMsWU	T18AMaSS	T21KsZZ	T22NbVC	T26XIOG	T27XIHS	T28XIBB	T29XIPC	T30XIBE	T31XIBE	T32XIBB	T35UuBV	T36UuLF	F1BaSD	F2BaSS	F3BuBV	F5BbTT	F6BsBL	F7CgUP	F8CcLF	F9GaEB	F12PsLV	F14PpND	F15PpBV	F16PpEB	F18TsBW
$BlaFor_1$	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
$DisSur_1$	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
$DisSur_2$	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
$TanPoi_1$	1	1	1	4	1	3	3	3	2	1	1	1	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	1	1	2
$AntMar_1$	2	1	4	1	2	2	2	1	1	1	1	1	1	2	1	2	1	2	2	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2
$AntMar_2$	1	2	2	2	2	3	4	3	3	5	6	6	3	4	3	3	2	2	3	6	3	2	7	4	7	6	8	2	3	3	3	6	2	7
$BlaApo_1$	1	2	2	3	3	3	2	4	2	5	5	2	4	2	2	2	2	2	2	2	2	2	2	2	3	4	2	4	3	3	2	3	3	3
$PosBla_1$	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1
$PosBla_2$	2	8	1	1	3	4	6	5	4	3	5	1	3	1	3	6	10	7	9	5	3	3	7	5	3	3	5	5	5	3	4	3	7	3
$BlaCon_1$	2	1	1	3	1	2	1	2	1	1	2	1	1	1	1	2	1	2	2	2	1	2	2	1	2	1	1	2	4	5	2	1	2	2
$BlaCon_2$	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
$PosPro_1$	1	4	2	1	3	1	4	3	3	2	1	3	3	2	2	3	3	1	1	1	1	1	1	2	3	3	1	4	2	3	2	3	3	3
$ProLow_1$	1	2	3	2	3	2	2	3	3	3	3	3	3	2	3	3	3	3	3	2	3	3	3	3	3	3	3	3	1	3	1	2	3	3
$CenPar_1$	1	2	2	1	2	1	1	2	1	1	2	3	1	2	2	4	1	1	1	1	1	4	2	1	2	4	1	1	1	1	1	1	1	1
$CenPar_2$	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
$CenPar_3$	1	1	1	1	1	2	1	1	2	3	1	1	1	1	1	5	3	1	1	1	1	2	5	1	4	3	1	1	2	1	1	3	1	1
$CenPar_4$	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
$SecAB_1$	3	1	4	4	2	4	2	4	5	3	5	3	1	4	3	4	4	4	4	4	6	2	3	4	2	4	2	3	4	3	4	4	4	4
$RayCon_1$	2	1	1	2	2	1	2	1	2	2	3	2	3	3	6	4	5	2	3	2	1	1	1	3	3	4	3	2	3	3	1	2	3	3
$RayApo_1$	3	1	1	4	2	2	2	2	2	4	4	2	1	3	5	1	4	4	4	1	1	1	2	1	1	2	1	1	4	2	5	4	2	2
$RayFor_1$	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
$RayFor_2$	1	2	1	2	1	1	2	4	1	1	1	1	1	1	1	1	1	3	2	3	4	2	2	3	4	3	4	3	3	3	2	3	3	1
$RayFor_3$	2	1	2	1	1	2	2	1	3	2	1	1	1	1	1	2	1	1	1	1	3	1	2	1	3	1	3	1	1	1	3	3	1	3
$RayFor_4$	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	3	1	1	1	5	1	4	4	3	3	4	3	4	4	3	4
$LenAB_1$	1	3	3	2	3	2	4	4	1	3	5	3	2	3	4	5	2	2	3	4	2	3	10	1	2	3	3	4	10	3	4	4	2	2

Abstract / **O**psomming

'… in all science, error precedes the truth,
and it is better it should go first than last…'

Hugh Walpole

Abstract

Mobiline ciliophorans belonging to the family Trichodinidae Claus, 1874 are amongst the most common and widely distributed symbionts of fishes. Most of the 260 trichodinid species described from fishes are exclusively associated with fish hosts. A few are also associated with salamanders, crustaceans or coelenterates such as in the case of *Trichodina pediculus* Ehrenberg, 1831 that are hosted by fish or hydras. Juvenile and adult anurans also play host to some trichodinid species. Endotrichodinids such as *T. xenopodos* Fantham, 1924 infect the bladder of adult frogs during amplexus and feed on the debris within the bladder of the host. Ectotrichodinids are hosted on the skin and / or gills of various tadpole species worldwide. Tadpole trichodinids are typically not host specific and occur on fishes in the same water medium and are thus using tadpoles as facultative hosts. Only a few studies concentrated on the collection and / or identification of tadpole trichodinids in southern Africa.The objective of the present study was to determine if trichodinids hosted by tadpoles in southern Africa are fish trichodinids that are temporarily hosted by tadpoles or a different species that are not commonly found on fishes. Cross-transmission experiments indicated that trichodinids from naturally infested tadpoles could form a viable population on naturally uninfested fishes. This indicated that the tadpole trichodinids could be found on fishes in natural conditions. Trichodinids that were collected from various fish and tadpole species in South Africa and Botswana over the past 30 years, were examined. Morphological analysis of the examined trichodinids was undertaken by means of three known methods. These methods aided in describing all examined trichodinids as belonging to *T. heterodentata* Duncan, 1977. *Trichodina heterodentata* is globally distributed and has an apparent affinity for cichlids as fish hosts, but seems to be equally widely distributed on a variety of tadpole hosts, if both these hosts are available in the same water medium.

Keywords: *Trichodina heterodentata*; Tadpoles; Fishes; Southern Africa; Cross-transmission experiments; Morphological analysis

Opsomming

Mobiliene siliofore vorm deel van die familie Trichodinidae Claus, 1874 en is van die mees algemene en wyd verspreide vissimbionte. Die meeste van die 260 trigodinas wat vanaf visse beskryf is, word uitsluitlik met visse geassosieer. Sommiges word ook geassosieer met salamanders, skaaldiere of holtediere soos in die geval van *Trichodina pediculus* Ehrenberg, 1831 wat op visse sowel as hidras voorkom. Jong en volwasse verteenwoordigers van Anura word ook deur verskeie trigodinas as gashere gebruik. Endotrigodinas soos *T. xenopodos* Fantham, 1924 besmet die blaas van volwasse paddas tydens ampleksus en voed op die dooie selle binne die gasheer se blaas. Ektotrigodinas word wêreldwyd op die vel en / of kieue van verskeie paddavisse aangetref. Paddavistrigodinas is gewoonlik nie gasheerspesifiek nie en word ook op visse aangetref wat in die selfde waterbronne as die besmette paddavisse voorkom. Paddavisse word dus as fakultatiewe gashere vir trigodinas beskou. Slegs ‘n paar studies het voorheen op die versameling en / of bestudering van paddavistrigodinas in suider Afrika gekonsentreer. Die doelwit van die huidige studie was om vas te stel of paddavistrigodinas in suider Afrika vistrigodinas is wat slegs tydelik deur paddavisse gehuisves word, of ‘n ander spesie is wat nie op visse voorkom nie. Kruis-besmettingseksperimente het aangedui dat trigodinas wat natuurlik op paddavisse voorkom, ‘n lewensvatbare bevolking op visse kon vorm wat nie natuurlik besmet was nie. Hierdie bevinding dui aan dat die paddavistrigodinas moontlik onder natuurlike toestande op die visse kan voorkom. Trigodinas wat vanaf verskeie vis en paddavis spesies in Suid-Afrika en Botswana oor die laaste 30 jaar versamel is, is bestudeer. Morfologiese ondersoeke van hierdie trigodinas is uitgevoer deur van drie bekende metodes, soos voorgestel deur Lom (1958), Gong *et al.* (2005) en Van As en Basson (1992), gebruik te maak. Deur hierdie metodes te gebruik, is daar vasgestel dat die paddavistrigodinas *T. heterodentata* Duncan, 1977 verteenwoordig. *Trichodina heterodentata* is wêreldwyd verspreid en het vermoedelik ‘n affiniteit vir lede van die Cichlidae as visgashere, hoewel dit voorkom of hierdie trigodinas ook wyd verspreid op verskeie paddavisse voorkom wanneer albei gasheer groepe in dieselfde waterbron beskikbaar is.

Sleutelwoorde: *Trichodina heterodentata,* Paddavisse, Visse, Suider Afrika, Kruis-besmettingeksperimente, Morfologiese ondersoeke

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‘…A flea has smaller fleas upon his back to bite him;
Smaller fleas has lesser fleas
and so ad infinitum…’

J. Swift, On Poetry

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