Phylogeny of the African genus *Ergasilus* (Copepoda: Poecilostomatoida)

by

Ruaan Schlebusch

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Department of Zoology and Entomology

University of the Free State

Supervisor: Prof. L.L. van As

Co-supervisor: Prof. J.G. van As

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Chapter 1

Introduction

- **The why, when, and how**

The Aquatic Ecology Research Group, UFS have been conducting research on the diversity of fish parasites for the past 28 years. The accumulation of raw data on various fish parasite groups, ranging from Protozoa, Myxozoa, helminths, nematodes, branchiurans as well as a variety of parasitic Crustacea, from all over southern Africa and other places in the world, gives an interesting and unparalleled chance to study fish parasites in general. During numerous fish parasitological surveys, many ergasilids have been collected. Although morphological studies have been done on some of this material, nothing has been done regarding the molecular analysis of the African ergasilids, collected by the Aquatic Ecology group. Regarding new morphological studies, this is also lacking and it seems as if there is not a lot of interest in this family of parasitic copepods, particularly in the southern African species. Previous studies conducted in southern Africa was by Oldewage & van As (1987), Douëllou & Ehlwanger (1994) and Andrews (2004). But these are only three studies from southern Africa, whilst most of earlier studies on the ergasilids are only concerned with the northern parts of Africa. The lack of knowledge on this particular group of parasites in southern Africa, is apparent, when taking morphological and molecular studies into account. With more than 180 species (www.marienspecies.org) that are known worldwide, to date only five species DNA sequences (from China) are available on GenBank™.

The present study aims to improve our knowledge of the ergasilids and add value to the already well studied morphological aspects of the group not only in southern Africa, but the family in general. A second aim was to add information regarding the molecular data, focussing on the southern African fauna. This did not only broaden our knowledge on the Ergasilidae, but also started the process for further molecular

studies on the rest of the parasitic Copepoda of Africa, for which there is scant, or almost no information available. The molecular studies proved to be a challenge. However, with every trial conducted during the present study, we added valuable information. Not only apparent new species, but also to the techniques used in molecular analysis of representatives of the Copepoda.

- **Layout of the dissertation**

The layout of this dissertation is as follows: **Chapter 1** is a general introduction to this study. In **Chapter 2** a very broad overview of the order Poecilostomatoida Thorell, 1859 and the family Ergasilidae Burmeister, 1835 in terms of morphology and the use of genetics in the taxonomy of the genus *Ergasilus* von Nordmann, 1832 is given the chapter also includes a compendium. **Chapter 3** gives an overview of the fish hosts and their habitats. **Chapter 4** explains the material and methods used in the study. **Chapter 5** is the results of the four species that were collected, that are morphologically described and the phylogenetic analysis conducted is presented. **Chapter 6** provides a discussion of the results that were found in this study. **Chapter 7** includes all the literature referred to in this dissertation, followed by the abstract and acknowledgements.

Chapter 2

Much Ado About Ergasilids

- **The Order Poecilostomatoida (Copepoda) and the family Ergasilidae – a holistic approach**

To begin to understand the systematics of the copepods, two words come to mind, taxonomy and holistic: two words so broad (in every aspect) that they almost form a paradox in themselves. One has to look (or search) holistically for the answers in a labyrinth of ever changing taxonomic characteristics and other criteria, analysing the whole system before an informed decision can be made. With close to 15 012 accepted species of copepods, analysis of the system seems to be a daunting task, or is it?

The first question should be how one begins to identify these species? The best (and only) place to start is definitively the habitat, and more importantly how the copepods utilise these habitats. The primary division of habitats according to Boxshall & Halsey (2004) is: marine, brackish and fresh water. The utilisation of specific microhabitats by the free-living and parasitic stages of symbiotic copepods within the main aquatic habitat is of the utmost importance. Following Boxshall & Halsey (2004) the identification process can be shortened if one could focus on the likelihood of particular families in the habitat of interest. These families can be categorised as dominant, intermediate, and uncommon or rare, where dominate constitutes about 90% of the total families; intermediate 1 to 10%, and uncommon or rare amounts to less than 1% of the total families in the habitat.

The Copepoda is an exceptionally diverse group of animals. As key producers they play a vital role in the aquatic ecosystems of the world. Therefore they are one of the most abundant and profuse metazoan groups in every aquatic habitat, within all salinity and temperature regimes possible. Ranging from every fresh water

environment imaginable, from the hypersaline conditions of the Dead Sea, Por (1993), to the hot hydrothermal vents and even in the polar regions (www.marinespecies.org). According to Boxshall & Halsey (2004) relatively few copepod families have colonised fresh and inland saline waters. A minimum of 22 independent colonisations have occurred (Boxshall & Jaume, 2000) and this includes the invasion of alien species into various freshwater systems. With such an incredible habitat range it is not surprising that the subclass Copepoda comprises ten orders, with (as stated) more than 15 000 accepted species; nearly half of these species live in a symbiotic relationship with other host organisms. According to Huys & Boxshall (1991) the group is known to have been living in close association with other organisms for almost 144 million years, since the lower Cretaceous Period. Parasitising virtually all the animal phyla, it is those found on fish that are of specific interest in this study. Those that are parasites of fish, are mostly ectoparasites, but there are also endoparasites and interestingly mesoparasite (not endo- or ectoparasites), e.g. some members of the family Lernaeidae, although not all of the representatives are mesoparasites some of the more important ones are the cosmopolitan genus *Lernaea* Linnaeus, 1758 *(Ho, J-S. Personal correspondence). Many of these cause serious problems and can occur in very large numbers on their fish hosts. According to Abdelhalim, Lewis & Boxshall (1991) more than 13 400 individual ergasilids (*Ergasilus sieboldi* von Nordmann, 1832*)* has been reported from a single fish.

The second question is: *what do these copepods look like?* According to Lester & Hayward (2006) the Arthropod parasites of fish have been recorded since the time of Aristotle (300 BCE), with the majority of these belonging to the subclass Copepoda. The diversity of the Copepods creates a problem when one wants to select a typical representative, it is therefore common practice to present a detailed study of a single family or genus and compare it to other groups (Huys & Boxshall, 1991). Of the ten Copepod orders, the Poecilostomatoida is of particular interest. The order can be subdivided into 67 families (www.marinespecies.org).

*Prof. J-S Ho, California State University, Long Beach, USA.

Representatives of the poecilostomatiods are predominantly marine, with virtually all members associated with other animals or parasitic in nature. According to Huys & Boxshall (1991) when one examines the overall morphology, the order shows (possibly) the most diversity in body form of all the orders within the subclass Copepoda.

One of the major families within the order Poecilostomatioda is the Ergasilidae, which is also a major family of freshwater and marine fish parasites in Africa (Oldewage & van As, 1988a; Oldewage & Avenant-Oldewage,1993; & Song, Wang, Yao, Gao & Nie, 2007), with 28 genera.

The Ergasilidae according to Boxshall & Halsey (2004) is a typical example of an intermediate family in a habitat, where the adult females are parasites of fishes, and the naupliar and copepodid stages are free living in the plankton. Boxshall & Halsey (2004) accounts to have observed plankton samples from freshwater and estuarine habitats in southern Africa, where most of the copepods in a collected sample were the developmental stages of ergasilids. According to Lester & Hayward (2006) most of the species in the family belong to the genus *Ergasilus* the type genus of the family. There are currently 154 valid species of *Ergasilus* (www.marinespecies.org) Within the family Ergasilidae there are three genera known from Africa, *Ergasilus* comprising marine, estuarine and freshwater species; *Dermoergasilus* Ho & Do, 1982 from marine and estuarine species; and *Paraergasilus* Markewitsch, 1937 comprising only freshwater species (Fryer, 1964, 1965, 1967, 1968, Oldewage & van As, 1988a & b, Oldewage & Avenant-Oldewage,1993).

According Boxshall & Halsey (2004) and Abdelhalim *et al.* (1993) the body of a typical male and female ergasilid is cyclopiform with a swelling of the prosome somites in females (fig. 2.1). It is a well defined family characterised by the form of the antenna, mandibles, maxillules and maxilla, and most markedly by the loss of the maxilliped in the adult female. The two segmented exopod on the fourth leg (in contrast with the rest of the legs that all have three segmented exopods) is also a characteristic of the genus *Ergasilus* (fig. 2.2). The spine and seta formulae for the swimming legs one to four are also an important characteristic and is usually presented in a tablulated form (table: 2.1).

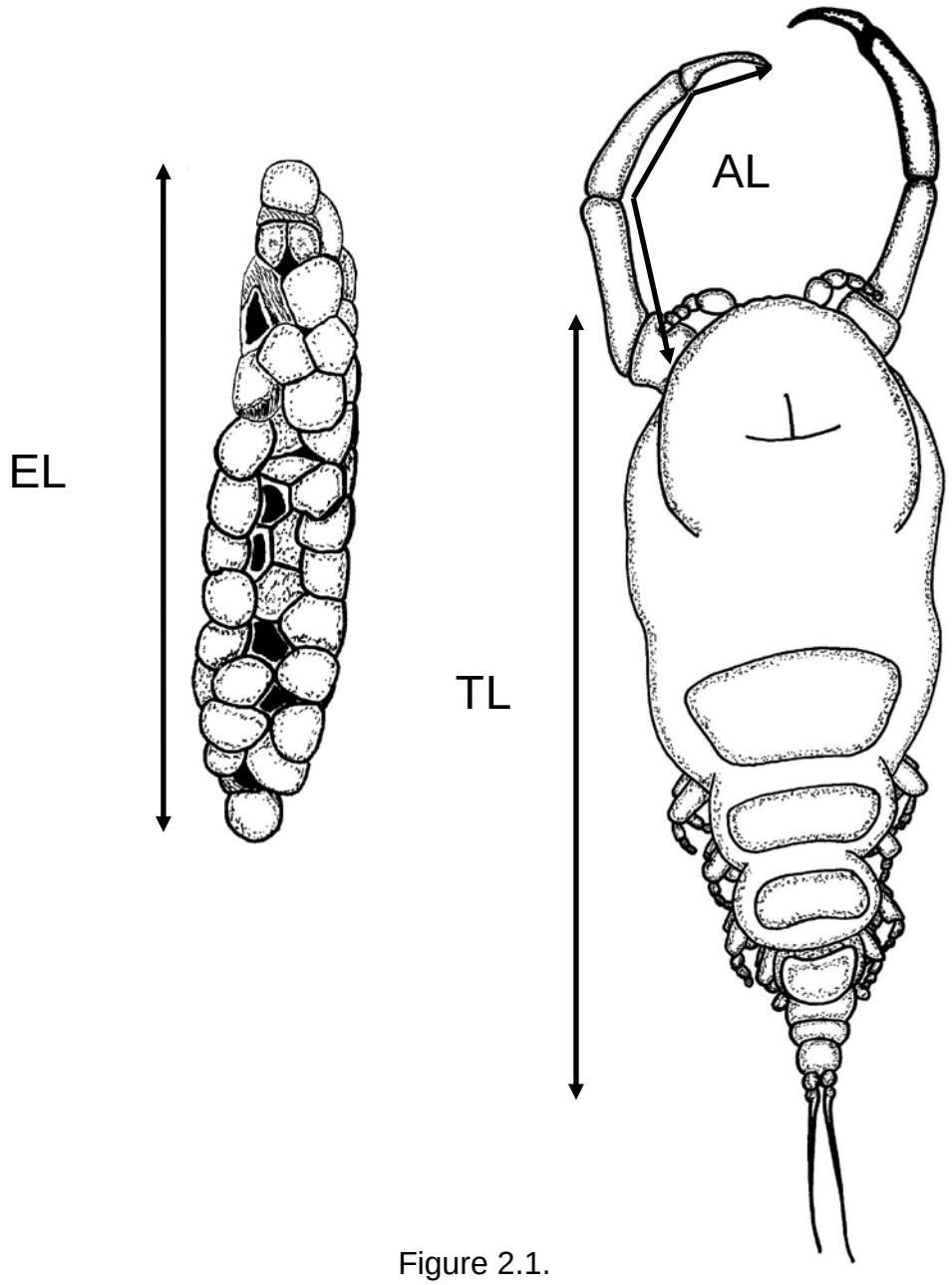


Figure 2.1.

Generalised line drawing of cyclopiform ergasilid and indications of measurements used in the description.

AL. Antenna length

TL. Total length

EL. Egg sac length

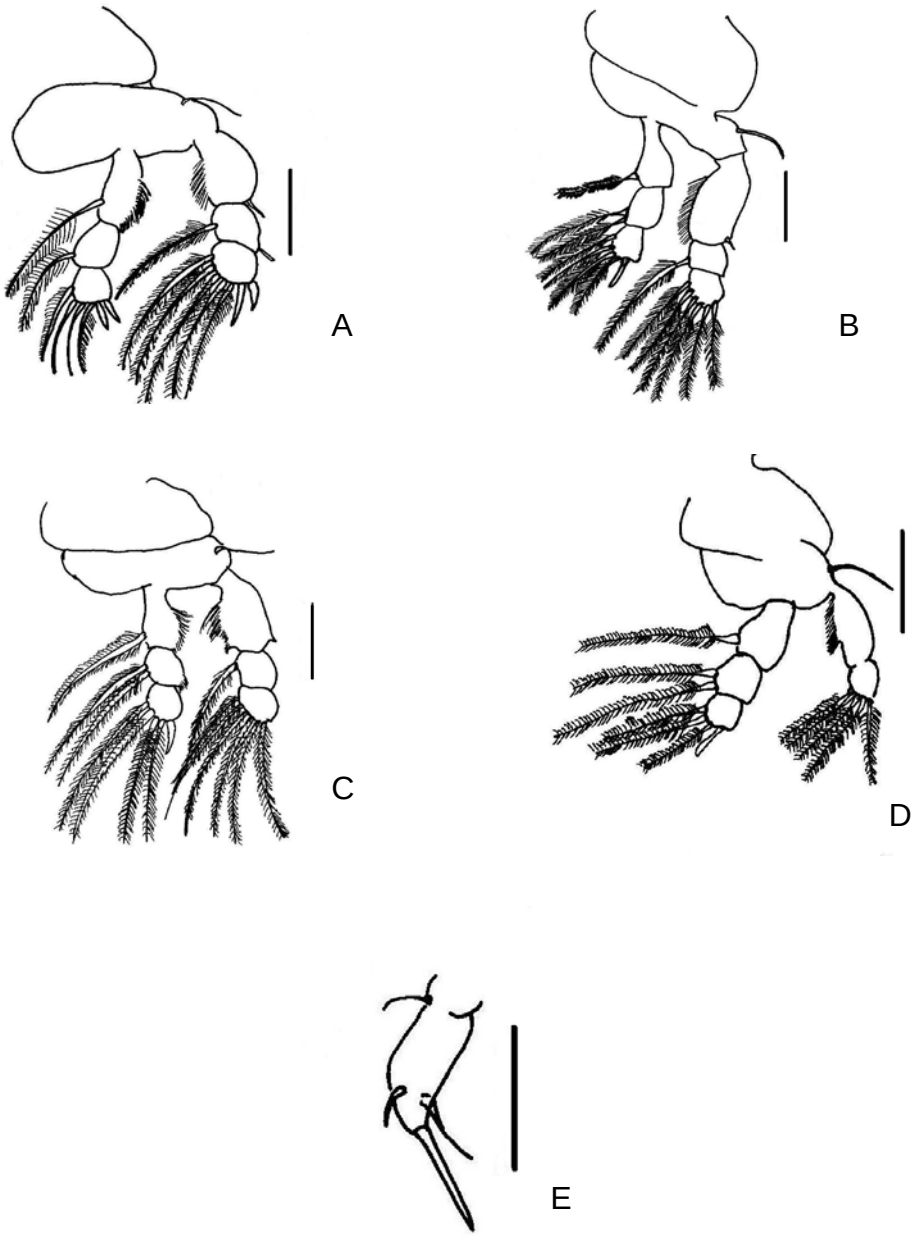


Figure 2.2.

Generalised line drawings of the legs of an *Ergasilus* von Nordmann, 1832

A. Leg 1 (50µm)
B. Leg 2 (50µm)
C. Leg 3 (50µm)
D. Leg 4 (50µm)
E. Leg 5 (50µm)

Table 2.1.: An example of the spine-setae formula of the ergasilid specimen

	Coxa	Basis		*Segment 1	Segment 2	Segment 3
Leg 1	0	I	Exopodite	0 – 1	I – 1	II – 5
			Endopodite	0 – 1	0 – 1	II – 4
Leg 2	0	I	Exopodite	I – 0	0 – 1	0 – 6
			Endopodite	0 – 1	0 – 2	I – 4
Leg 3	0	I	Exopodite	I – 0	0 – 1	0 – 6
			Endopodite	0 – 1	0 - 2	I – 4
Leg 4	0	I	Exopodite	I – 0	0 – 5	-**
			Endopodite	0 – 1	0 – 2	I – 3

* Segment closest to the basis

* *All *Ergasilus* species lack this segment

- **A compendium of ergasilid species (a – v)**

The following compendium include the descriptions of the 11 known species from Africa (a – k), i.e. *Ergasilus macrodactylus* (Sars, 1909), *E. megacheir* (Sars, 1909), *E. kandti* van Douwe, 1912, *E. nodosus* Wilson, 1928, *E. cunningtoni* Capart, 1944, *E. sarsi* Capart, 1944, *E. latus* Fryer, 1961, *E. lamellifer* Fryer, 1961, *E. flaccidus* Fryer, 1965, *E. inflatipes* Cressey & Collette, 1970 and *E. mirabilis* Oldewage & van As, 1987. *Ergasilus sieboldi* and *Ergasilus lizae* Krøyer, 1863 are cosmopolitan species and although these species have only been found on marine fish hosts (Oldewage & Avenant-Oldewage, 1993) they are included because of their world wide distribution as well as potential problems in the aquaculture industry.

Those nine known ergasilids from Asia (l – v), that were also used in the study include: *Ergasilus briani* Markewitsch, 1933, *E. hypomesi* Yamagutim 1936, *E. rostralis* Ho, Jayarajan & Radhakrishnan, 1992, *E. lobus* Lin & Ho, 1998, *E. pararostralis* Amando, 2001, *E. piriformis* El-Rashidy & Boxshall, 2002, *E. sittangensis* El-Rashidy & Boxshall, 2002, *E. danjiangensis* Song, Yao & Nie, 2008, and *E. boleophtalmi* Adday & Ali, 2011.

The species as listed above are in chronological order, but in the compendium they appear alphabetically. The first table of each description contains a remarks column, where the species is compared to each of the other species used in the compendium: first with the African species, starting with *E. cunningtoni* and then the Asian species, starting with *E. briani*. This is followed by the spine and setae formulae and then a summary of the known hosts and distribution, in separate tables. The very last species *E. hypomesi* only has a spine and setae formula as the complete species description could not be found, but it had to be added because it was used in Song *et al.* (2008) in their molecular analysis. The line drawings provided are of those taxonomic characteristics of the authors first mentioned.

(a) *Ergasilus cunningtoni* Capart, 1944 (fig. 2.3)

Total length	0.97mm
Cephalothorax	Width – 0.42mm, length – 0.55mm. Triangular cephalic region with straight anterior border, arched lateral borders. Clearly marked thoracic region, narrower than cephalic region.
Ornamentation	Two lightly marked regions, anterior region circular, posterior region oval. Visible eyespot, lightly pigmented.
Pigmentation	Eyespot lightly pigmented.
Antennule	Six-segmented, with setae on anterior borders.
Antenna	First two segments as long as cephalothorax. Third segment indented on anterior border, indentation formed by two ridges crossing each other, embedded interiorly.
Mouthparts	Posterior border of labrum slightly concave.
Thorax	Four well developed thoracic segments, decreasing in length and width. Fifth segment dorsally visible.
Legs	Legs 1 to 4 characteristic of other *Ergasilus* spp., fifth pair reduced to a single, long segment, with two setae; two terminal, one lateral. Spine-seta formulae in table 2.2.
Abdomen	Four-segmented, genital complex wider than long.
Furcal Rami	Three terminal setae, one very long, two very short setae.
Egg sacs	Long, thin and cylindrical.
Attachment site on host	Attachment to distal end of gill filament, often covered by epithelial tissue.
Remarks	*Ergasilus cunningtoni* differs from *E. kandti* and *E. lamellifer* by having a single segmented fifth leg, whilst the other two have a 2-segmented fifth leg. *Ergasilus cunningtoni* differs from *E. flaccidus* and *E. nodosus* by having a 6-segmented antennule, whilst the other two have a 5-segmented antennule. *Ergasilus cunningtoni* differs from *E. latus*, *E. megacheir*, *E. sarsi* and *E. inflatipes* by having a different number of setae on the fifth leg. *Ergasilus macrodactylus* differs from *E. cunningtoni* by the absence of an inverted T-structure on the cephalic shield; and *E. mirabilis* Oldewage & van As differs by

	having two eye spots on the anterior end of the cephalic shield. *Ergasilus cunningtoni* differs from *E. boleophthalmi*, *E. sittangensis*, *E. rostralis*, *E. sieboldi,* and *E. lizae* by having a single segmented fifth leg, the other 7 have two segmented fifth legs. The number of setae on the fifth leg of *E. danjiangensis*, *E. briani*, *E. pararostralis*, *E. piriformis,* and *E. lobus* differs from *E. cunningtoni.* The spine and setae formulae of *E. hypomesi* differs from *E. cunningtoni.* Tabel 2.3 provides a list of hosts and distribution records for *E. cunningtoni.*

Compiled from Capart (1944).

Table 2.2: Spine-seta formulae for *Ergasilus cunningtoni* Capart, 1944

	Coxa	**Basis**		**Segment 1**	**Segment 2**	**Segment 3**
Leg 1	0	1	Exopodite	I-0	I-1	II-5
			Endopodite	0-1	0-1	II-4
Leg 2	0	1	Exopodite	I-0	0-1	0-6
			Endopodite	0-1	0-2	I-4
Leg 3	0	0	Exopodite	I-0	0-1	0-4
			Endopodite	0-1	0-2	I-4
Leg 4	0	0	Exopodite	0-0	0-5	-
			Endopodite	0-1	0-2	I-3

Compiled from Capart (1944).

Table 2.3: Hosts and localities for *Ergasilus cunningtoni* Capart, 1944

Locality & Reference	**Host**
Lake Tumba, Ubangi River, Democratic Republic of the Congo Capart (1944) Fryer (1964) Fryer (1967)	*Campylomormyrus elephas* (Boulenger, 1898) *Distichodus atroventralis* Boulenger, 1898 *Marcusenius moorii* (Günther, 1867) *Marcusenius greshoffi* (Schilthaus, 1891) *Mormyrops nigricans* Boulenger, 1899 *Pollimyrus isidori* (Valenciennes, 1847) *Pterochromis congicus* (Boulenger, 1897) *Schilbe laticeps* (Boulenger, 1899) *Schilbe tumbanus* (Pellegrin, 1926) *Tylochromis microdon* Regan, 1920
Congo River System	*Hippotamyrus psittacus* (Boulenger, 1897)

Fryer (1964)	*Petrocephalus grandoculis* Boulenger, 1916 *Synodontis nigroventris* David, 1935
Galma River, Nigeria Shotter (1977)	*Barbus macrops* Boulenger, 1911 *Brycinus nurse* (Rüppell, 1832) *Hydrocynus vittatus* Castelnau, 1861 *Mormyrops anguilloides* (Linnaeus, 1758) *Mormyrops macrothalmus* Günther, 1866 *Raiamas senegalensis* (Steindachner, 1870)
Lake Volta, Ghana Paperna (1969)	*Brycinus leuciscus* (Günther, 1867) *Brycinus nurse* (Rüppell, 1832) *Distichodus rostratus* Günther, 1864 *Pellonula leonensis* Boulenger, 1916

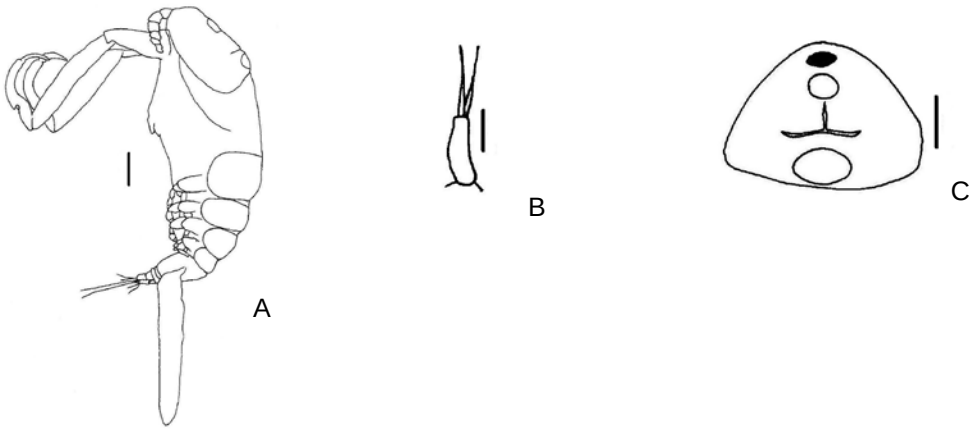


Figure 2.3

Line drawings of *Ergasilus cunningtoni* Capart, 1944

A. Lateral view of whole specimen (female) (100µm)
B. Leg 5 (25µm)
C. Dorsal view of cephalothorax (100µm)

Redrawn from Capart (1944)

(b) *Ergasilus flaccidus* Fryer, 1965 (fig. 2.4)

Total length	0.9mm
Cephalothorax	Longer than wide, bluntly rounded anteriorly.
Ornamentation	Inverted T-structure of thickened chitin, medially situated on dorsal side of cephalothorax, anterior of inverted T is an ovoid area of thin cuticle.
Pigmentation	Unknown.
Antennule	Five-segmented.
Antenna	Prehensile, with small chitinous finger near distal end of second segment. Terminal segment curved with thin walled cuticle.
Mouthparts	Not described.
Thorax	First four thoracic segments distinct, segment 5 narrow with no distinct segmented area visible.
Legs	Only one seta present on second segment of endopod of legs 2 and 3. Leg 5 located dorsally, not visible ventrally, 2-segmented, very small basal segment with single seta, distal segment with 2 terminal setae. First terminal seta slender, longer than entire leg, second seta equals length of leg. Spine-seta formulae provided in table 2.4.
Abdomen	Four-segmented, genital complex wider than long, bulged laterally, widest region in middle. Final segment with group of four to five spinules ventrally on each side, with minute lateral spine on each side.
Furcal Rami	Simple, as wide as long, each with arc of 12 fine spinules ventrally, one minute spinule situated laterally. Furcal rami with four terminal setae, innermost seta longest, swollen near proximal region, very indistinctly demarcated from furcal ramus.
Egg sacs	Equals total body length.
Attachment site on host	Not described.
Remarks	*Ergasilus flaccidus* differs from *E. cunningtoni*, *E. lamellifer*, *E. latus*, *E. macrodactylus*, *E. megacheir*, and *E. sarsi* by having a 5-segmented antennule, the other species have a 6-segmented

	antennule. *Ergasilus flaccidus* differs from *E. kandti* by having a spine on the inner margin of the third antenna segment and it differs from *E. nodosus* by not having plumose setae on the antennule. The fifth leg of *E. danjiangensis*, *E. briani*, *E. pararostralis*, *E. piriformis* and *E. lobus* differs from *E. flaccidus* by having a single segment. The spine and setae formulae of *E. hypomesi* differs from *E. flaccidus.* *Ergasilus lizae*, *E. boleophthalmi* and *E. sittangensis* differs from *E. flaccidus* by having a 6-segmented antennule. *Ergasilus flaccidus* differs from *E. rostralis* by the presence of an inverted T-structure on the cephalothorax. *Ergasilus sieboldi* differs from *E. flaccidus* by having antennae with unadorned inner margins of segments 2 and 3, whilst *E. flaccidus* has adorned segments. Table 2.5 provides a list of hosts and distribution records for *E. flaccidus.*

Compiled from Fryer (1965).

Table 2.4: Spine-seta formulae for *Ergasilus flaccidus* Fryer, 1965

	Coxa	**Basis**		**Segment 1**	**Segment 2**	**Segment 3**
Leg 1	-	-	Exopodite	I-0	I-1	II-5
			Endopodite	0-1	0-1	II-4
Leg 2	-	-	Exopodite	I-0	0-1	0-6
			Endopodite	0-1	0-1	I-4
Leg 3	-	-	Exopodite	I-0	0-1	0-6
			Endopodite	0-1	0-1	I-4
Leg 4	-	-	Exopodite	I-0	0-5	-
			Endopodite	0-1	0-1	I-3

Compiled from Fryer (1965).

Tabel 2.5: Host and locality for *Ergasilus flaccidus* Fryer, 1965

Locality & Reference	**Host**
Lake Tanganyika Fryer (1965)	*Oreochromis tanganicae* (Günther, 1894)

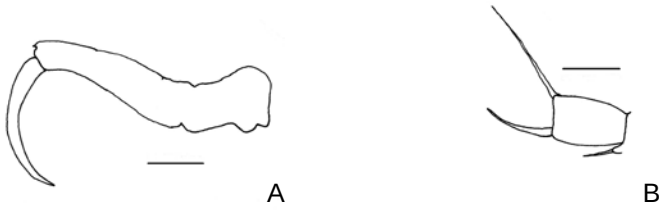


Figure 2.4

Line drawings of *Ergasilus flaccidus* Fryer, 1965

A. Antenna (100µm)
B. Leg 5 (25µm)

Redrawn from Fryer (1965)

(c) *Ergasilus inflatipes* Cressey & Collette, 1970 (fig. 2.5)

Total length	0.625 mm
Cephalothorax	Width – 0.35 mm
Ornamentation	Inverted T-structure on cephalothorax.
Pigmentation	Not described.
Antennule	Six-segmented, segments 4 to 6 with a plumose seta on each segment.
Antenna	Segment 3 with three elongated spines, two on inner margin and one on anterior end of outer margin.
Mouthparts	Mandible with single seta near base of terminal process, first maxilla a small lobe with three setae, second maxilla with single seta on terminal segment near base of spinulose tip.
Thorax	Genital segment slightly wider than longer.
Legs	Legs 1 to 4 typical of genus. Leg 5 single segment with three setae, one near base, terminal 2 setae plumose. Table 2.6 provides spine and setae formulae.
Abdomen	Three-segmented. Ventral surface of segments 1 and 2 with rows of spinules along posterior margin.
Furcal Rami	Short with four setae, innermost longest.
Egg sacs	Length 0.65 mm with about 40 eggs.
Attachment site on host	Not described.
Remarks	This species can be separated from all the other species due to a combination of features, but specifically by the nature of the fifth leg. Tabel 2.7 provides a list of host and distribution records for *E. inflatipes.*

Compiled from Cressey & Collette (1970) and Oldewage & van As (1988a).

Table 2.6: Spine-seta formulae for *Ergasilus inflatipes* Cressey & Collette, 1970

	Coxa	Basis		Segment1	Segment 2	Segment 3
Leg 1	-	-	Exopodite	I-0	I-1	II-5
			Endopodite	0-1	0-1	II-4
Leg 2	-	-	Exopodite	I-0	0-1	0-6
			Endopodite	0-1	0-2	0-6
Leg 3	-	-	Exopodite	I-0	0-1	0-6
			Endopodite	0-1	0-2	I-4
Leg 4	-	-	Exopodite	I-0	0-5	-
			Endopodite	0-0	0-2	I-2

Compiled from Cressey & Collette (1970).

Table 2.7: Hosts and localities for *Ergasilus inflatipes* Cressey & Collette, 1970

Locality & References	**Host**
Volta River, Ghana Ebzia Lagoon, Ivory Coast Oldewage & Avenant-Oldewage (1993)	*Strongylura senegalensis* (Valenciennes, 1864)

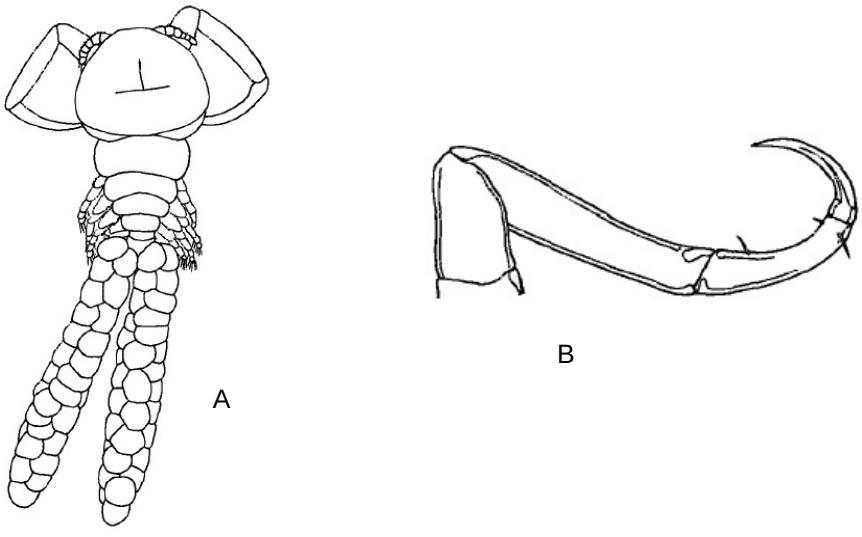


Figure 2.5

Line drawings of *Ergasilus inflatipes* Cressey & Collette, 1970

A. Dorsal view of whole specimen
B. Antenna

Redrawn from Cressey & Collette (1970)

(d) *Ergasilus kandti* van Douwe, 1912 (fig. 2.6)

Total length	0.67mm
Cephalothorax	Width – 0.37mm, length – 0,45mm. Elongated pentagon form. First thoracic segment more narrowly fused than cephalic segment. Frontal margin rounded, medially marked by narrow chitinous reinforcement.
Ornamentation	Circular cephalic structure situated anterior to inverted T, no posterior oval structure present.
Pigmentation	Eyespot not pigmented.
Antennule	Five-segmented.
Antenna	Equals cephalothoracic length, third segment slightly arched with conical tooth-like projection on distal inner side.
Mouthparts	Not described.
Thorax	Segments 2 to 4 decreasing in size. Fifth segment not dorsally visible, fused to anterior margin of genital complex.
Legs	First four pairs well developed, fifth pair reduced, to single short segment. Spine-seta formulae provided in table 2.8.
Abdomen	Four distinct abdominal segments. Posterior borders of genital and abdominal segments with fringe of fine short bristles.
Furcal Rami	Square, terminates in two very long setae and two to three short seta.
Egg sacs	Not described.
Attachment site on host	Not described.
Remarks	*Ergasilus kandti* differs from the following by having a 5-segmented antennule: *E. cunningtoni*, *E. lamellifer*, *E. latus*, *E. macrodactylus*, *E. megacheir, E. mirabilis,* and *E. sarsi*, all seven species have a 6-segmented antennule. *Ergasilus kandti* differs from *E. flaccidus* and *E. nodosus* by having a single segmented fifth leg, and it differs from *E. inflatipes* by not having plumose setae on the fifth leg. *Ergasilus kandti* differs from the following by having a 5-segmented antennule: *E. danjiangensis*, *E. lizae*, *E. boleophthalmi*, *E. briani*, *E.*

	pararostralis, *E. sittangensis*, *E. lobus* and *E, sieboldi*. The spine and setae formulae of *E. hypomesi* differs from that of *E. kandti*. *Ergasilus kandti* differs from *E. rostralis* by having an inverted T-structure on the cephalothorax, and it differs from *E. piriformis* by having a slender second antenna segment. Tabel 2.9 provides a list of hosts and distribution records for *E. kandti.*

Compiled from Capart (1944).

Table 2.8: Spine-seta formulae for *Ergasilus kandti* van Douwe, 1912

	Coxa	**Basis**		**Segment 1**	**Segment 2**	**Segment 3**
Leg 1	-	-	Exopodite	I-0	0-1	II-5
			Endopodite	0-1	0-1	II-4
Leg 2	-	-	Exopodite	I-0	0-1	0-6
			Endopodite	0-1	0-2	I-4
Leg 3	-	-	Exopodite	0-0	0-1	0-6
			Endopodite	0-1	0-2	I-4
Leg 4	-	-	Exopodite	0-0	0-5	-
			Endopodite	0-1	0-2	I-3

Table compiled from Capart (1944).

Table 2.9: Hosts and localities for *Ergasilus kandti* van Douwe, 1912

Locality & Reference	**Hosts**
Lake Albert Fryer (1965), Thurston (1970)	*Lates niloticus* (Linnaeus, 1758) *Bargus bayad* (Forsskål, 1775)
Lake Tumba, Ubangi River, Democratic Republic of the Congo Fryer (1959)	*Pterochromis congicus* (Boulenger, 1897)
Lake Mweru, Democratic Republic of the Congo	*Tylochromis mylodon* Regan, 1920 *Tylochromis bangwelensis* Regan, 1920 *Tylochromis polylepis* (Boulenger, 1900)

Fryer (1967)	
Lake Volta, Ghana Paperna (1969)	*Citharinus citharus citharus* (Geoffroy St. Hilaire, 1808) *Hemisynodontis membranaceus* (Geoffroy St. Hilaire, 1808) *Lates niloticus* (Linnaeus, 1758) *Schilbe intermedius* Rüppell, 1832
Lake Tanganyika Capart (1944) Fryer (1965)	*Limnotilapia dardenii* (Boulenger, 1899) *Lamprologus lemairii* Boulenger, 1899 *Plecodus paradoxus* Boulenger, 1898 *Pseudosimochromis curvifrons* (Poll, 1942) *Oreochromis tanganicae* (Günther, 1894)
Niger River Capart (1956)	*Lates niloticus* (Linnaeus, 1758)

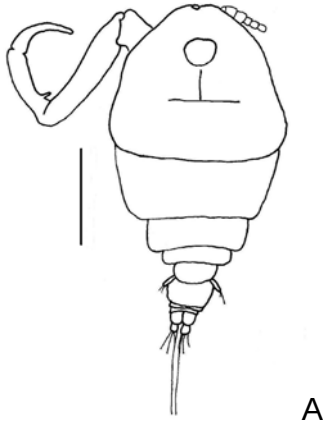


Figure 2.6

Line drawings of *Ergasilus kandti* van Douwe, 1912

A. Dorsal view of adult female (200µm)

Redrawn from Capart (1944)

(e) *Ergasilus lamellifer* Fryer, 1961 (fig. 2.7)

Total length	0.85mm
Cephalothorax	Cephalothorax fused to segment of leg 1, longer than wide, bluntly rounded anteriorly, shallowly indented in posterior third segment.
Ornamentation	Inverted T, anterior to ovoid structure, posterior to circular structure.
Pigmentation	White in colour, with patches of blue pigment.
Antennule	Six-segmented.
Antenna	Prehensile, 4-segmented. Inner margin of segment 2 with thin blade-like chitinous lamella.
Mouthparts	Not described.
Thorax	Not described.
Legs	Segments of legs 1 to 5 distinct, leg 5 2-segmented, with minute basal segment. Distal segment with two terminal setae. Basal segment with one small seta. Spine-seta formulae provide in tabel 2.10.
Abdomen	Four-segmented, genital complex wider than long, bulged laterally, widest anterior to midsection. Remaining abdominal segments very short.
Furcal Rami	Four terminal setae. Innermost seta longest. Longest seta swollen with bend near proximal end.
Egg sacs	Long, up to three quarters of body length.
Attachment site on host	Not described.
Remarks	Differs from all other ergasilid species by having a blade-like lamella on the second antenna segment, except *E. megacheir* which also has lamella, but differs from *E. megacheir* by having 2-segmented fifth leg. Tabel 2.11 provides a list of hosts and distribution records for *E. lamellifer.*

Compiled from Fryer (1961).

Table 2.10: Spine-seta formulae for *Ergasilus lamellifer* Fryer, 1961

	Coxa	Basis		Segment 1	Segment 2	Segment 3
Leg 1	-	-	Exopodite	I-0	I-1	II-5
			Endopodite	0-1	0-1	II-4
Leg 2	-	-	Exopodite	0-0	0-1	0-6
			Endopodite	0-1	0-2	I-4
Leg 3	-	-	Exopodite	0-0	0-1	0-6
			Endopodite	0-1	0-2	I-4
Leg 4	-	-	Exopodite	I-0	0-5	-
			Endopodite	0-1	0-2	I-3

Compiled from Fryer (1961).

Table 2.11: Hosts and localities for *Ergasilus lamellifer* Fryer, 1961

Locality & Reference	**Host**
Lake Volta, Ghana Paperna (1969)	*Parailia pellucida* (Boulenger, 1901)
Lake Victoria Fryer, (1961), Thurston (1970)	*Astatoreochromis alluaudi* Pellegrin, 1904 *Haplochromis guiarti* (Pellegrin, 1904) *Haplochromis longirostris* (Hilgendorf, 1888) *Haplochromis nuchisquamulatus* (Hilgendorf, 1888) *Haplochromis obesus* (Boulenger, 1906) *Haplochromis obliquidens* (Hilgendorf, 1888) *Haplotilapia retordens* (Hilgendorf, 1888) *Macropleurodus bicolor* (Boulenger, 1906) *Platytaeniodus degeni* Boulenger, 1906

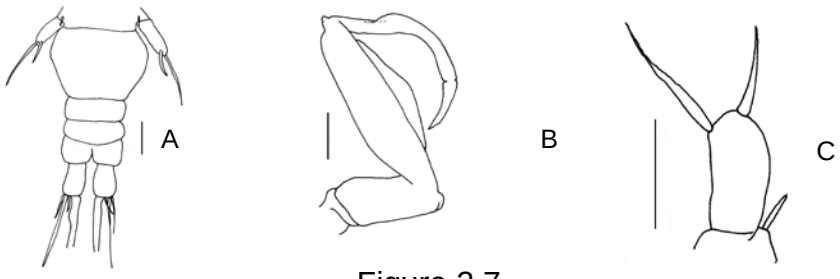


Figure 2.7

Line drawings of *Ergasilus lamellifer* Fryer, 1961

A. Ventral view of the abdomen (25µm)
B. Antenna (50µm)
C. Leg 5 (25µm)

Redrawn from Fryer (1961)

(f) *Ergasilus latus* Fryer, 1960 (fig. 2.8)

Total length	0.9mm
Cephalothorax	Cephalothorax fused to segment of leg 1, longer than wide, bluntly rounded anteriorly, bulged laterally, indented in posterior third half.
Ornamentation	Inverted T-structure present.
Pigmentation	Brown colour, with traces of purple pigment present.
Antennule	Six-segmented.
Antenna	Prehensile, with swollen region on proximal end of third segment.
Mouthparts	Not described.
Thorax	Not described.
Legs	Segments of leg 1 to 5 distinct, typical of members of the genus. Leg 5 very broad with three setae, two terminal setae as long as entire leg, one shorter lateral seta. Spine-seta formulae provided in table 2.12.
Abdomen	Four-segmented, genital complex wider that long, bulged laterally, widest in midsection. Remaining abdominal segments very short.
Furcal Rami	Longer than wide with five terminal setae, innermost longest.
Egg sacs	Long, reaches past longest furcal seta.
Attachment site on host	Not described.
Remarks	*Ergasilus latus* differs from following by having 6-segmented antennule: *E. flaccidus*, *E. inflatipes, E. kandti*, *E. nodosus*. *Ergasilus lamellifer*, *E. macrodactylus*, *E. megacheir*, *E. mirabilis* and *E. sarsi* by either having different number of setae on leg 5 or not having plumose setae on leg 5. *Ergasilus latus* differs from *E. lizae*, *E. boleophthalmi*, *E. sittangensis* and *E. sieboldi* by having single segmented fifth leg. Spine and setae formulae of *E. hypomesi* differs from *E. latus.* *Ergasilus danjiangensis*, *E. briani*, *E. pararostralis*, *E. piriformis* and *E. rostralis* differs from *E. latus* by not having structures on the cephalothorax. Tabel 2.13 provides a list of hosts and distribution records for *E. latus.*

Compiled from Fryer (1960).

Table 2.12: Spine-seta formulae for *Ergasilus latus* Fryer, 1960

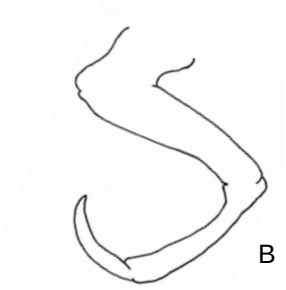
	Coxa	**Basis**		**Segment 1**	**Segment 2**	**Segment 3**
Leg 1	-	-	Exopodite	0-0	I-1	II-5
			Endopodite	0-1	0-1	II-4
Leg 2	-	-	Exopodite	0-0	0-1	0-6
			Endopodite	0-1	0-2	I-4
Leg 3	-	-	Exopodite	0-0	0-1	0-6
			Endopodite	0-1	0-2	I-4
Leg 4	-	-	Exopodite	I-0	0-5	-
			Endopodite	0-1	0-2	I-3

Table compiled from Fryer (1960).

Table 2.13: Hosts and localities for *Ergasilus latus* Fryer, 1960

Locality	**Host**
Lake Turkana, Kenya Fryer (1960)	*Oreochromis niloticus* (Linnaeus, 1758) *Sarotherodon galilaeus galilaeus* (Linnaeus, 1758)
Volta Basin, Ghana Paperna (1969)	*Tilapia zillii* (Gervais, 1848) *Oreochromis niloticus* (Linnaeus, 1758)
Peshi Lagoon, Ghana Paperna (1969)	*Sarotherodon melanotheron heudelotii* (Duméril, 1861) *Tilapia guineensis* (Günther, 1862)
Galma River, Nigeria Shotter (1977)	*Auchenoglanis occidentalis* (Valenciennes, 1840) *Oreochromis niloticus* (Linnaeus, 1758) *Sarotherodon galilaeus galilaeus* (Linnaeus, 1758) *Schilbe mystus* (Linnaeus, 1758) *Tilapia zillii* (Gervais, 1848)
Congo System Fryer (1963) Fryer (1967)	*Sarotherodon melanotheron nigripennis* (Gulchenot, 1861) *Tilapia cabrae* Boulenger, 1899

A



B

Figure 2.8

Line drawings of *Ergasilus latus* Fryer, 1960

A. Leg 5 (10µm)
B. Antenna

Redrawn from Fryer (1960)

(g) *Ergasilus macrodactylus* (Sars, 1909) (fig. 2.9)

Total length	0.97mm to 1.0mm
Cephalothorax	Cephalothorax fused to first segment of leg one. Much longer than wide, bluntly rounded anteriorly. Bulged laterally.
Ornamentation	Well developed eye spot. Circular cephalic structure anterior to inverted T structure, with ovoid cephalic structure posterior.
Pigmentation	White with patches of purple ventrally in cephalothorax region.
Antennule	Six-segmented.
Antenna	Prehensile, long and slender.
Mouthparts	Not described.
Thorax	Segments 2 to 5 distinct, evenly rounded at lateral margins.
Legs	Legs 1 to 4 typical of genus. Leg 5 simple, cylindrical, with 2 terminal setae. Spine-seta formulae provided in table 2.14.
Abdomen	Four-segmented, anterior region of genital complex bulged laterally.
Furcal Rami	Rami with 4 setae. Innermost seta longest.
Egg sacs	Long, reaches beyond furcal setae.
Attachment site on host	Not described.
Remarks	*Ergasilus macrodactylus* differs from the following ergasilids by having a single segmented fifth leg: *E. flaccidus*, *E. kandti*, *E. nodosus*. *Ergasilus cunningtoni*, *E. inflatipes, E. lamellifer*, *E. latus*, and *E. megacheir*, *E. sarsi* differs from *E. macrodactylus* by having a different setae formulae on the fifth leg. *Ergasilus macrodactylus* differs from the following by having structures present on the cephalothorax: *E. danjiangensis*, *E. lizae*, *E. briani*, *E. pararostralis*, *E. sittangensis*, *E. lobus* and *E. rostralis*. *Ergasilus macrodactylus* differs from *E. boleophthalmi* by having a single segmented fifth leg. The spine and setae formulae of *E. hypomesi* differs from *E. macrodactylus.* Tabel 2.15 provides a list of hosts and distribution records for *E. latus.*

Compiled from Sars (1909), and Fryer (1956).

Table 2.14: Spine-seta formulae for *Ergasilus macrodactylus* (Sars, 1909)

	Coxa	Basis		Segment 1	Segment 2	Segment 3
Leg 1	-	-	Exopodite	I-0	I-1	II-5
			Endopodite	0-1	0-1	II-4
Leg 2	-	-	Exopodite	I-0	0-1	0-6
			Endopodite	0-1	0-2	I-4
Leg 3	-	-	Exopodite	0-0	0-2	I-4
			Endopodite	0-1	0-2	I-4
Leg 4	-	-	Exopodite	0-0	0-5	-
			Endopodite	0-1	0-1	I-3

Compiled from Sars (1909) and Fryer (1956).

Table 2.15: Hosts and locality for *Ergasilus macrodactylus* (Sars, 1909)

Locality & Reference	Host
Lake Malawi Fryer (1956)	*Brycinus imberi* (Peters, 1852) *Haplochromis* spp. Hilgendorf, 1888 *Lethrinops* spp. Regan, 1922 *Pseudotropheus* spp. Regan, 1922 *Tilapia* spp. Smith, 1840

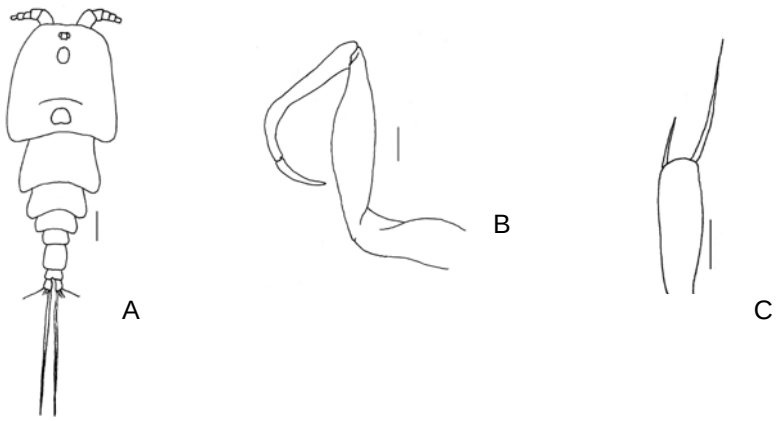


Figure 2.9

Line drawings of *Ergasilus macrodactylus* (Sars, 1909)

A. Dorsal view of adult female (100µm)
B. Antenna (50µm)
C. Leg 5 (10µm)

Redrawn from Fryer (1956)

(h) *Ergasilus megacheir* (Sars, 1909) (fig. 2.10)

Total length	0.62mm. Body short, sub-pyriform in outline when viewed dorsally.
Cephalothorax	Cephalothorax very large, quadrangular in form.
Ornamentation	Inverted T-structure present. Both circular and oval cephalic structures present. Frontal margin of cephalic segment transversely truncated, postero-lateral corners only slightly prominent, rounded.
Pigmentation	Not described.
Antennule	Six-segmented.
Antenna	Large, second segment twice as long as basal segment, oblong in form. Third segment length half of second segment, slightly twisted, terminal hook short with re-curved denticle on inner margin.
Mouthparts	Characteristic of members of genus.
Thorax	First four thoracic segments with lateral regions pointing backwards, obtusely rounded at end, Fifth segment almost entirely concealed.
Legs	Legs 1 to 4 typical of genus. Fifth pair extremely small. Spine-seta formulae provided in table 2.16.
Abdomen	Third of total body length, genital complex dilated, rounded, oval in form.
Furcal Rami	Equal length of last abdominal segment, with setae on inner corner, four setae, innermost longest.
Egg sacs	Not described.
Attachment site on host	Not described.
Remarks	Differs from all 11 African ergasilid species by having a blade-like lamella on the second antenna segment, except *E. lamellifer* which also has the lamella. *Ergasilus megacheir* differs from *E. lamellifer* by having a single segmented fifth leg. Tabel 2.17 provides a list of hosts and distribution records for *E. megacheir.*

Compiled from Sars (1909) and Fryer (1965).

Table 2.16: Spine-seta formulae for *Ergasilus megacheir* (Sars, 1909)

	Coxa	**Basis**		**Segment 1**	**Segment 2**	**Segment 3**
Leg 1	-	-	Exopodite	I-0	I-1	II-5
			Endopodite	0-1	0-1	II-4
Leg 2	-	-	Exopodite	0-0	0-1	0-6
			Endopodite	0-1	0-1	I-4
Leg 3	-	-	Exopodite	0-0	0-1	0-6
			Endopodite	0-1	0-1	I-4
Leg 4	-	-	Exopodite	0-0	0-5	-
			Endopodite	0-1	0-1	I-3

Compiled from Sars (1909) and Fryer (1965).

Tabel 2.17: Hosts and localities for *Ergasilus megacheir* (Sars, 1909)

Locality & Reference	**Host**
Lake Tumba, Congo System Fryer (1964)	*Pterochromis congicus* (Boulenger, 1897)
Lake Tanganyika Capart (1944), Fryer (1965)	*Bathybates minor* Boulenger, 1906 *Bathybates fasciatus* Boulenger, 1901 *Cyphotilapia frontosa* (Boulenger, 1906) *Haplotaxodon microlepis* Boulenger, 1906 *Limnotilapia dardenii* (Boulenger, 1899) *Pseudosimochromis curvifrons* (Poll, 1942) *Synodontis multipunctatus* Boulenger, 1898 *Synodontis granulosus* Boulenger, 1900

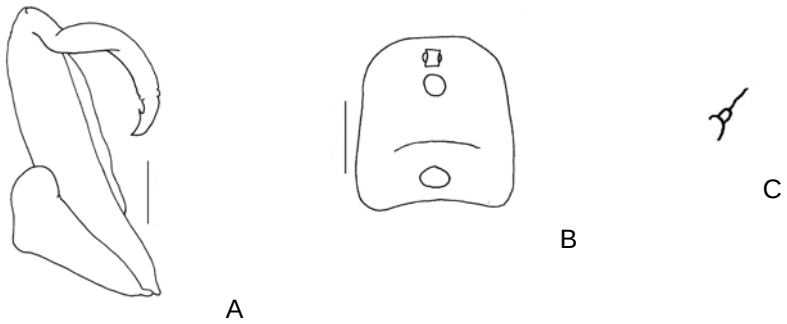


Figure 2.10

Line drawings of *Ergasilus megacheir* (Sars, 1909)

A. Antenna (50µm)
B. Dorsal view of cephalothorax (100µm)
C. Leg 5

Redrawn from Fryer (1956)

(i) *Ergasilus mirabilis* Oldewage & van As, 1987 (fig. 2.11)

Total length	0.93mm
Cephalothorax	Cephalothoracic segment largest, as long as wide.
Ornamentation	Two dorsal oval structures, anterior and posterior to inverted T-structure. Eye spot situated anteriorly to anterior oval structure. Two sensory pits medially situated between inverted T and anterior structure.
Pigmentation	Not described.
Antennule	Six-segmented. Setal formulae: 0-7-4-2-2-4.
Antenna	Slender, smooth and 4-segmented, terminal segment short, curved, pointed and scleritonised.
Mouthparts	Mouth opens posteriorly under ventrally projected, denticulated labrum. First maxilla typical of genus. mandible and second maxilla oppose each other in buccal cavity. Labrum with two lateral glandular projections.
Thorax	First four thoracic segments progressively smaller and wider than long. Paired sensory setae occur dorsally on segments 2 and 4. Fifth thoracic segment compressed, lacks sensory apparatus.
Legs	Fifth leg single-segmented with two terminal setae. Spine-seta formulae provided in table 2.18.
Abdomen	Four-segmented, segments short. First and second abdominal segments with posterior row of ventral bristles. Third segment splits dorso-ventrally with two rows of bristles on posterior, ventral surface of both sides.
Furcal Rami	Four seta.
Egg sacs	Long and slender.
Attachment site on host	Not described.
Remarks	*Ergasilus mirabilis* differs from the following *Ergasilus* spp. by having a single segmented fifth leg: *E. flaccidus*, *E. kandti*, *E. nodosus*. *Ergasilus cunningtoni*, *E. inflatipes, E. lamellifer*, *E. latus*, *E. megacheir* and *E. sarsi* differs from *E. mirabilis* by having a different setae formulae on the fifth leg.

	Ergasilus mirabilis differs from the following by having structures present on the cephalothorax: *E. danjiangensis*, *E. lizae*, *E. briani*, *E. pararostralis*, *E. sittangensis*, *E. lobus* and *E. rostralis*. *Ergasilus mirabilis* differs from *E. boleophthalmi* by having a single segmented fifth leg. The spine and setae formulae of *E. hypomesi* differs from *E. mirabilis.* Tabel 2.19 provides a list of hosts and distribution records for *E. mirabilis.*

Compiled from Oldewage & van As (1987).

Table 2.18: Spine-seta formulae for *Ergasilus mirabilis* Oldewage & van As, 1987

	Coxa	**Basis**		**Segment 1**	**Segment 2**	**Segment 3**
Leg 1	-	-	Exopodite	I-0	I-1	0-6
			Endopodite	0-1	0-1	II-4
Leg 2	-	-	Exopodite	0-0	0-1	0-4
			Endopodite	0-1	0-1	0-6
Leg 3	-	-	Exopodite	I-0	I-1	0-6
			Endopodite	0-1	0-2	0-5
Leg 4	-	-	Exopodite	I-0	0-5	-
			Endopodite	0-0	0-0	0-6

Compiled from Oldewage & van As (1987).

Tabel 2.19: Hosts and localities for *Ergasilus mirabilis* Oldewage & van As, 1987

Locality & Reference	**Host**
Pongola System Oldewage & Van As (1987)	*Synodontis leopardinus* Pellegrin, 1914
Lake Kariba, Zambezi System Douëllou & Erlwanger (1994)	*Hippopotamyrus discorhynchus* (Peters, 1952)

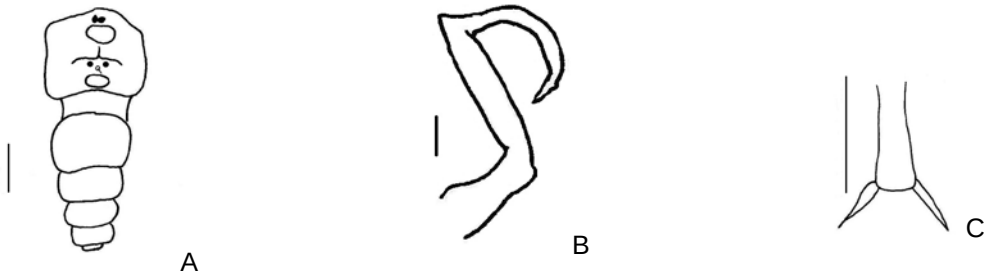


Figure 2.11

Line drawings of *Ergasilus mirabilis* Oldewage & van As, 1987

A. Dorsal view of female (100µm)
B. Antenna (50µm)
C. Leg 5 (25µm)

Redrawn from Oldewage & van As (1987)

(j) *Ergasilus nodosus* Wilson, 1928 (fig. 2.12)

Total length	1mm
Cephalothorax	Width – 0.5mm, length – 0.4mm. Body elongate-obovate, broadest anteriorly, decreasing regularly posteriorly. Cephalic segment distinctly separated from first thoracic segment. Cephalic segment short.
Ornamentation	Not described.
Pigmentation	Not described.
Antennule	Five-segmented. Fourth segment with two long, stout, plumose setae on posterior margin. Setal formulae: 5-2-3-4-5.
Antenna	Enormous - 1.25mm. Four segments, all curved, last two forming half circle.
Mouthparts	Typical of genus.
Thorax	Three thoracic segments diminishing regularly in width, posterior margins of first three slightly invaginated, fourth segment semicircular. Fifth segment entirely concealed in dorsal view.
Legs	Fifth leg entirely lacking. Spine-seta formulae provided in table 2.20.
Abdomen	Four-segmented. Genital complex narrower than fourth thoracic segment. Remaining abdominal segments much narrower than genital complex.
Furcal Rami	Each rami with three plumose setae.
Egg sacs	Long – 1mm. Eggs arranged in six to seven longitudinal rows, approximately 20 eggs in a row.
Attachment site on host	Attach near base of gill filament, buries tip of antennae in filament tissue, as far as swollen joints.
Remarks	Differs from all 11 African ergasilid species by not having a fifth leg. See tabel 2.21 hosts and distribution records for *E. nodosus.*

Compiled from Wilson (1928).

Table 2.20: Spine-seta formulae for *Ergasilus nodosus* Wilson, 1928

	Coxa	Basis		Segment 1	Segment2	Segment 3
Leg 1	-	-	Exopodite	-	-	-
			Endopodite	-	-	-
Leg 2	-	-	Exopodite	-	-	-
			Endopodite	-	-	-
Leg 3	0	0	Exopodite	I-0	0-1	0-5
			Endopodite	0-1	0-2	0-5
Leg 4	0	1	Exopodite	I-0	0-4	-
			Endopodite	0-0	0-0	I-2

Compiled from Wilson (1928).

Table 2.21: Host and locality for *Ergasilus nodosus* Wilson, 1928

Locality & Reference	**Host**
White Nile, Omdurman	*Bargus bajad* (Forsskål, 1775)

Compiled from Wilson (1928).

Figure 2.12

Line drawings of *Ergasilus nodosus* Wilson, 1928

A. Antennule (25µm)
B. Dorsal view of cephalothorax and antenna (200µm)

Redrawn from Wilson (1928)

(k) *Ergasilus sarsi* Capart, 1944 (fig. 2.13)

Total length	0.65mm.
Cephalothorax	Width – 0.33mm, length – 0.39mm. Anterior border evenly rounded, posterior border straight. Gap between cephalic segment and first thoracic segment clearly marked.
Ornamentation	Cephalic segments with distinct pattern. Eye spot visible.
Pigmentation	Not described.
Antennule	Six-segmented. Setae formulae: 2-8-2-2-2-6.
Antenna	Long and robust, second segment much longer than third. Anterior border of third segment marked by slight but wide depression.
Mouthparts	Not described.
Thorax	First four segments well developed, decreasing in length and width posteriorly. Fifth segment dorsally visible.
Legs	First four pairs typical of genus. Fifth pair reduced to single segment, with three setae. Two short terminal setae, one short postero-lateral seta. Spine-seta formulae table 2.22.
Abdomen	Four-segmented.
Furcal Rami	Longer than final abdominal segment, each ramus with three setae.
Egg sacs	Length – 0.22mm, cylindrical and short.
Attachment site on host	Not described.
Remarks	*Ergasilus sarsi* differs from the following species by having a single segmented fifth leg with three setae: *Ergasilus cunningtoni*, *E. flaccidus*, *E. macrodactylus*, and *E. mirabilis*, all these species have different number of setae and segments. *Ergasilus lamellifer* and *E. megacheir* differs from *E. sarsi* by having a lamella on the second antenna segment. *Ergasilus inflatipes* and *E. latus* differ from *E. sarsi* by not having a curved third antenna segment. *Ergasilus kandti* differs from *E. sarsi* by having a 5-segmented antennule. *Ergasilus sarsi* differs from *E. danjiangensis*, *E. lizae, E. boleophthalmi*, *E. lobus*, *E. rostralis* and *E. sieboldi* by the nature of the fifth leg. *Ergasilus pararostralis*, *E. sittangensis* and *E. piriformis*

	differ from *E. sarsi* by having aesthetascs on the antennule. *Ergasilus briani* differs from *E. sarsi* by not having an inverted T-structure on the cephalothorax. The spine and seta formulae of *E. hypomesi* differ from that of *E. sarsi.* Tabel 2.23 provides a list of hosts and distribution records for *E. sarsi.*

Compiled from Capart (1944).

Table 2.22: Spine-seta formulae for *Ergasilus sarsi* Capart, 1944

	Coxa	**Basis**		**Segment 1**	**Segment 2**	**Segment 3**
Leg 1	-	-	Exopodite	0-0	I-1	II-5
			Endopodite	0-1	0-1	II-4
Leg 2	-	-	Exopodite	0-0	0-1	0-6
			Endopodite	0-1	0-2	I-4
Leg 3	-	-	Exopodite	0-0	0-1	0-6
			Endopodite	0-1	0-2	I-4
Leg 4	-	-	Exopodite	I-0	0-5	-
			Endopodite	0-1	0-2	I-3

Table compiled from Capart (1944).

Tabel 2.23: Hosts and localities for *Ergasilus sarsi* Capart, 1944

Locality & References	**Host**
Lake Mweru, Congo Basin Capart (1944), Fryer (1967)	*Haplochromis moeruensis* (Boulenger, 1899) *Tylochromis mylodon* Regan, 1920 *Tylochromis bangweulensis* Regan, 1920
Lake Bangweula, Congo System Fryer (1959)	*Clarias ngamensis* Castelnau, 1861 *Marcusenius macrolepidotus* (Peters, 1852) *Synodontis nigromaculatus* Boulenger, 1905
Volta Basin, Ghana Paperna (1969)	*Clarias gariepinus* (Burchell, 1822)
Galma River, Nigeria Shotter (1977)	*Clarias anguillaris* (Linnaeus, 1758) *Heterobranchus bidorsalis* Goeffroy St. Hilaire, 1809

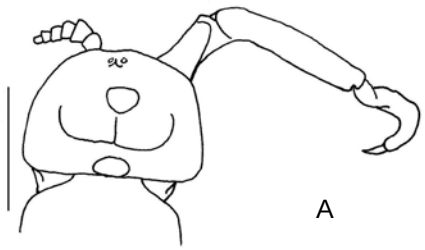


Figure 2.13

Line drawings of *Ergasilus sarsi* Capart, 1944

A. Dorsal view of cephalothorax and antenna (200µm)

Redrawn from Capart (1944)

(l) *Ergasilus briani* Markewitsch, 1933 (fig. 2.14)

Total length	0.64mm.
Cephalothorax	Cephalothorax oblong, tapering posteriorly, with slight indentation on medial margin.
Ornamentation	Oval structure on dorsal surface of cephalothorax.
Pigmentation	Not described.
Antennule	Six-segmented.
Antenna	Four-segmented. Segment two with two short spines, one posterior end of inner margin, the other on distal end of inner margin.
Mouthparts	Typical of genus.
Thorax	Four-segmented.
Legs	Legs 1 to 4 typical of genus. Fifth leg with three setae, one on posterior margin and two on anterior end. Spine-seta formulae provided in table 2.24.
Abdomen	Three-segmented. Genital complex sub-spherical.
Furcal Rami	Furcal rami with four seta.
Egg sacs	Short and stubby.
Attachment site on host	Not described.
Remarks	*Ergasilus briani* differs from *E. cunningtoni*, *E. flaccidus*, *E. inflatipes, E. kandti*, *E. lamellifer*, *E. latus*, *E. macrodactylus*, *E. megacheir*, *E. mirabilis* and *E. sarsi* by not having an inverted T-structure on the cephalothorax. *Ergasilus briani* differs from *E. briani* by having plumose setae on the antennule. *Ergasilus briani* differs from *E. dangiangensis* by not having a enlarged second antenna segment. *Ergasils briani* differs from *E. boleopthalmi*, *E. sittangensis*, *E. piriformis* and *E. sieboldi* by not having an inverted T-structure on the cephalothorax. *Ergasilus pararostralis* differs from *E. briani* by having spines on the inner margin of segment 2 of the antenna. The spine and setae formulae of *E. hypomesi* differ from *E. briani.*

Distribution	*Ergasilus briani* Markewitsch, 1933 is distributed throughout Europe and Asia.

Compiled from Halisch (1939) and Alston, Boxshall & Lewis (1996).

Table 2.24: Spine-seta formulae for *Ergasilus briani* Markewitsch, 1933

	Coxa	**Basis**		**Segment 1**	**Segment 2**	**Segment 3**
Leg 1	0-0	I-0	Exopodite	I-0	I-1	II-5
			Endopodite	0-1	0-1	II-4
Leg 2	0-0	I-0	Exopodite	I-0	0-1	I-6
			Endopodite	0-1	0-1	I-4
Leg 3	0-0	I-0	Exopodite	I-0	0-1	I-6
			Endopodite	0-1	0-1	I-4
Leg 4	0-0	I-0	Exopodite	I-0	0-4	-
			Endopodite	0-1	0-1	I-3

Compiled from Halisch (1939) and Alston, Boxshall & Lewis (1996).

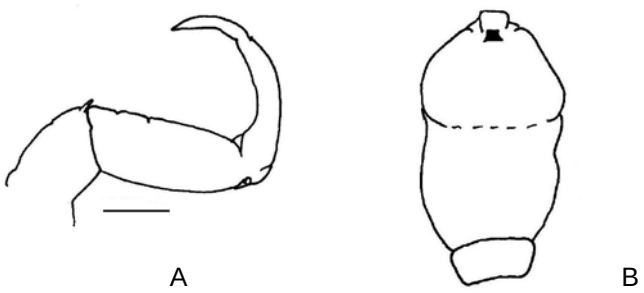


Figure 2.14

Line drawings of *Ergasilus briani* Markewitsch, 1933

A. Antenna (50µm)
B. Dorsal view of female (100µm)

B redrawn from Halisch (1939) and A from Alston, Boxshall & Lewis (1996).

(m) ***Ergasilus boleophthalmi*** **Adday & Ali, 2011** (fig. 2.15)

Total length	0.73mm to 0.83mm.
Cephalothorax	Width – 0.33mm to 0.44mm, length - 0.31mm to 0.51mm. Cephalothorax oval with anterior end tapering, completely incorporating first thoracic segment.
Ornamentation	Inverted T-structure on dorsal surface of cephalothorax.
Pigmentation	Not described.
Antennule	Six-segmented. Seta formula: 3-11-3-2-2+aesthetasc-7+aesthetasc.
Antenna	Long and slender, with single short spine on basal part of second segment.
Mouthparts	Mandible with two interiorly situated blades and one posterior blade. Maxillule small lobed with three long setae on posterior margin.
Thorax	Second to fifth segments narrowing posteriorly.
Legs	Fifth leg two-segmented, basal segment with single outer segment, terminal segment with long seta on posterior end and one seta on lateral margins. Spine-seta formulae provided in table 2.25.
Abdomen	Abdomen three-segmented, each segment with single row of short spines on ventral surface, near posterior margin.
Furcal Rami	Furcal rami with four setae.
Egg sacs	Shorter than body.
Attachment site on host	Not described.
Remarks	*Ergasilus boleophthalmi* differs from the following species by having a two-segmented fifth leg: *E. cunningtoni, E. latus, E. macrodactylus, E. megacheir, E. mirabilis* and *E. sarsi.* These species have a single segmented fifth leg. *Ergasilus lamellifer* differs from *E. boleophthalmi* by having a lamella-like strip on the second antenna segment. *Ergasilus flaccidus, E. kandti* and *E. nodosus* differ from *E. boleophthalmi* by having a five-segmented antennule. *Ergasilus boleophthalmi* differs from *E. pararostralis* and *E. piriformis*

	by having a 6-segmented antennule, whilst the later two species have a 5-segmented antennule. *Ergasilus sittangensis* differs from *E. boleophthalmi* by having a plumose seta on the terminal end of the second segment of fifth leg. *Ergasilus boleophthalmi* differs from all the other species by having aesthetascs on the antennule, lacking in the other species. Table 2.26 provides a list of hosts and distribution records for *E. boleophthalmi.*

Compiled from Adday & Ali (2011).

Table 2.25: Spine-seta formulae for *Ergasilus boleophthalmi* Adday & Ali, 2011

	Coxa	**Basis**		**Segment 1**	**Segment 2**	**Segment 3**
Leg 1	0-0	I-0	Exopodite	I-0	0-1	II-5
			Endopodite	0-1	0-1	II-4
Leg 2	0-0	I-0	Exopodite	I-0	0-1	0-6
			Endopodite	0-1	0-2	I-4
Leg 3	0-0	I-0	Exopodite	I-0	0-1	I-6
			Endopodite	0-1	0-2	I-4
Leg 4	0-0	I-0	Exopodite	I-0	I-5	-
			Endopodite	0-1	0-2	I-3

Compiled from Adday & Ali (2011).

Table 2.26: Hosts and localities for *Ergasilus boleophthalmi* Adday & Ali, 2011

Locality & References	**Host**
Shatt Al-Basrah Canal, Iraq Adday & Ali (2011)	*Boleophtalmus dussumieri* Valenciennes, 1837 *Bathygobius fuscus* (Rüppell, 1830)

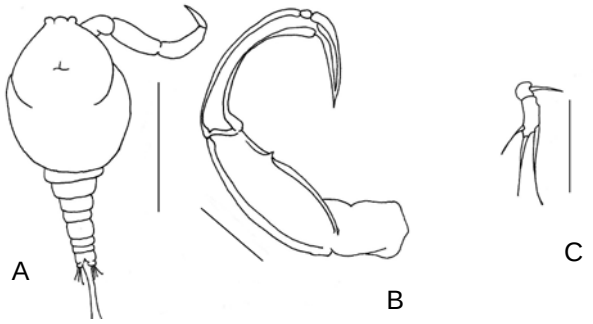


Figure 2.15

Line drawings of *Ergasilus boleophthalmi* Adday & Ali, 2011

A. Dorsal view of female (500µm)
B. Antenna (110µm)
C. Leg 5 (450µm)

Redrawn from Adday & Ali (2011)

(n) *Ergasilus danjiangensis* Song, Yao & Nie, 2008 (fig. 2.16)

Total length	1.11mm to 1.27mm.
Cephalothorax	Width – 0.28mm to 0.43mm, length – 0.57mm to 0.66mm. First pedigerous somite incorporated into cephalothorax.
Ornamentation	Eye spot visible on anterior end of cephalothorax.
Pigmentation	Not described.
Antennule	Six-segmented. Seta formulae: 1-7-5-3-2-5.
Antenna	Five-segmented, short and stout. First segment short, terminal hook curved.
Mouthparts	In centre of cephalothorax. Mandible un-segmented. Maxillule with transversely oval knobs with two stout setae. Maxilla with two segments.
Thorax	Not observed.
Legs	Legs 1 to 4 typical of genus. Fifth leg one segmented with single long terminal seta. Spine-seta formulae provided in table 2.27.
Abdomen	Genital complex barrel-shaped and narrowing posteriorly
Furcal Rami	Rami with four setae, innermost the longest.
Egg sacs	Short.
Attachment site on host	Not described.
Remarks	Differs from all other species by possession of enlarged second antenna segment. The spine and setae formulae of *E. hypomesi* differ from *E. danjiangensis.* Table 2.28 provides a list of hosts and the distribution record for *E. boleophthalmi.*

Compiled from Song, Yao, & Nie (2008).

Table 2.27: Spine-seta formulae for *Ergasilus danjiangensis* Song, Yao & Nie, 2008

	Coxa	**Basis**		**Segment 1**	**Segment 2**	**Segment 3**
Leg 1	0	I-0	Exopodite	I-0	0-1	II-5
			Endopodite	0-1	0-1	II-4
Leg 2	0	I-0	Exopodite	I-0	0-1	I-6
			Endopodite	0-1	*0-1*	I-4
Leg 3	0	I-0	Exopodite	I-0	0-1	I-6
			Endopodite	0-1	0-1	I-4
Leg 4	0	I-0	Exopodite	I-0	I-5	-
			Endopodite	0-1	0-2	I-3

Compiled from Song, Yao, & Nie (2008).

Tabel 2.28: Hosts and locality for *Ergasilus danjiangensis* Song, Yao & Nie, 2008

Locality & References	**Host**
Danjiangkou Reservoir, Hubei Province, China Song, Yao, & Nie (2008)	*Opsariichthys bidens* Günther, 1873 *Zacco platypus* (Temminck & Schlegel, 1846)

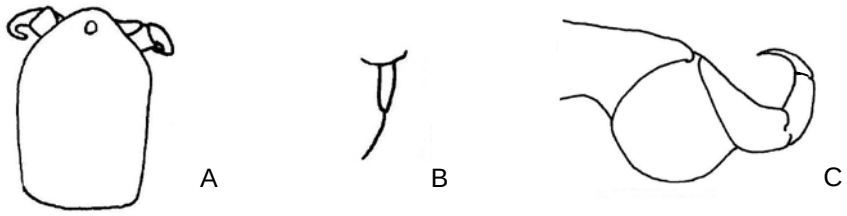


Figure 2.16

Line drawings of *Ergasilus danjiangensis* Song, Yao & Nie (2008)

A. Dorsal view of female cephalothorax
B. Leg 5
C. Antenna

Redrawn from Song, Yao, & Nie (2008)

(o) *Ergasilus lizae* Krøyer, 1963 (fig. 2.17)

Total length	0.8mm to 1mm.
Cephalothorax	Cephalothorax oblong, slightly narrower posteriorly.
Ornamentation	Dorsal eye spot visible on anterior end of cephalothorax.
Pigmentation	Not described.
Antennule	Six-segmented. Seta formula: 3-11-4-4-3-8.
Antenna	Slender, third segment with short spine on posterior margin and two short spines on anterior margin.
Mouthparts	Ventrally, somewhat protruding, typical of genus.
Thorax	Second to fourth segments gradually diminishing in width, with fifth segment short and narrow.
Legs	Legs 1 to 4 typical of genus, fifth leg 2-segmented, with short basal segment with one seta on postero-lateral corner, second segment oblong with two setae on anterior margin. Spine-seta formulae provided in table 2.29.
Abdomen	Abdomen 3-segmented. Genital complex sub-spherical.
Furcal Rami	Caudal ramus longer than wide, bearing four un-adorned setae, one very long, other three short and slender.
Egg sacs	Long.
Attachment site on host	Not described.
Remarks	*Ergasilus lizae* differs from *E. cunningtoni*, *E. flaccidus*, *E. inflatipes, E. kandti*, *E. lamellifer*, *E. latus*, *E. macrodactylus*, *E. megacheir*, *E. mirabilis* and *E. sarsi* by not having inverted T structure on the cephalothorax. *Ergasilus nodosus* differs from *E. lizae* by having plumose setae on the antennule. *Ergasilus lizae* differs from *E. dangiangensis* by not having an enlarged second antenna segment. *Ergasils lizae* differs from *E. boleopthalmi*, *E. sittangensis*, *E. piriformis* and *E. sieboldi* by not having an inverted T-structure on the cephalothorax. *Ergasilus briani* and *E. pararostralis* differ from *E. lizae* by having spines on the inner margin of segment 2 of the antenna. The spine and setae formulae

	of *E. hypomesi* differ from *E. lizae.*
Distribution	*Ergasilus lizae* is a cosmopolitan species found on 30 hosts from coastal waters of North America, South America, Europe, Asia and Australia. It is however restricted largely to members of the Mugilidae, although it has been found on eels and tilapia as well as mullet in aquaculture ponds in Israel.

Compiled from Kabata (1992) and Lester & Hayward (2006).

Table 2.29: Spine-seta formulae for *Ergasilus lizae* Krøyer, 1963

	Coxa	**Basis**		**Segment 1**	**Segment 2**	**Segment 3**
Leg 1	-	-	Exopodite	I-0	0-1	II-5
			Endopodite	0-1	0-1	II-4
Leg 2	-	-	Exopodite	I-0	0-1	0-6
			Endopodite	0-1	0-2	0-5
Leg 3	-	-	Exopodite	I-0	0-1	0-6
			Endopodite	0-1	0-2	0-5
Leg 4	-	-	Exopodite	I-0	0-5	-
			Endopodite	0-1	0-2	0-4

Compiled from Kabata (1992).

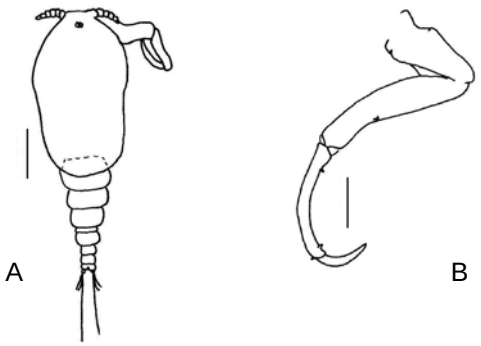


Figure 2.17

Line drawings of *Ergasilus lizae* Krøyer, 1963

A. Dorsal view of female (200µm)
B. Antenna (100µm)

Redrawn from Kabata (1992)

(p) *Ergasilus lobus* Lin & Ho, 1998

Total length	0.53mm.
Cephalothorax	Cephalothorax and first thoracic segment greatly inflated.
Ornamentation	Not described.
Pigmentation	Not described.
Antennule	Six segmented. Setae formula: 3-12-4-4-2-7
Antenna	Short and curved.
Mouthparts	Typical of genus.
Thorax	Intercoxal bar with prominent postero-ventral plate in leg 1, less developed in leg 2 and 3, completely absent in leg 4.
Legs	Legs 1 to 4 typical of genus. Fifth leg extremely reduced, small knob with single seta. Spine-seta formulae provided in table 2.30.
Abdomen	Genital complex, with row of fine spines on ventral surface. Rows of spines at posterior end on ventral surface of all segments.
Furcal Rami	Four unadorned setae.
Egg sacs	Distinctly longer than body.
Attachment site on host	Not described.
Remarks	*Ergasilus lobus* differs from all other Asian species with respect to the fifth leg, which is not reduced in the other species. *Ergasilus lobus* differ from *E. kandti*, *E. piriformis* have a five segmented antennules and, *E. megacheir* which has a lamella on the second antenna segment. See tabel 2.31 for host and distribution records for *E. lobus.*

Compiled from Lin & Ho (1998).

Table 2.30: Spine-seta formulae for *Ergasilus lobus* Lin & Ho, 1998

	Coxa	**Basis**		**Segment 1**	**Segment 2**	**Segment 3**
Leg 1	0-0	1-0	Exopodite	I-0	0-1	II-4
			Endopodite	0-1	0-1	II-4
Leg 2	0-0	1-0	Exopodite	I-0	0-1	I-5
			Endopodite	0-1	0-2	I-4
Leg 3	0-0	1-0	Exopodite	I-0	0-1	I-5
			Endopodite	0-1	0-2	I-4
Leg 4	0-0	1-0	Exopodite	0-0	I-0	I-4
			Endopodite	0-1	0-2	I-3

Compiled from Lin & Ho (1998).

Table 2.31: Host and locality for *Ergasilus lobus* Lin & Ho, 1998

Locality & References	**Host**
Chi-ku Village, Tainan County, Taiwan Lin & Ho (1998)	*Epinephelus malabaricus* (Bloch & Schneider, 1801)

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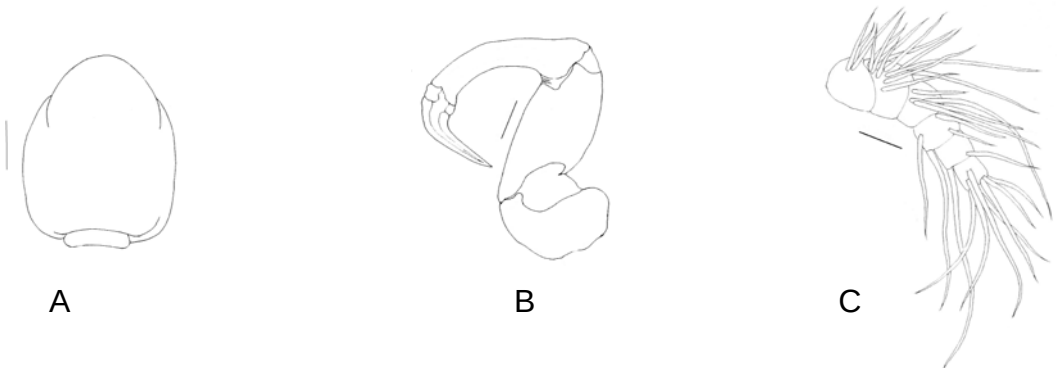


Figure 2.18

Line drawings of *Ergasilus lobus* Lin & Ho, 1998

A. Cephalothorax (100µm)
B. Antanna (30 µm)
C. Antennule (20 µm)

Redrawn form Lin & Ho (1998)

(q) *Ergasilus pararostralis* Amado *et al.*, 2001 (fig. 2.19)

Total length	0.70mm.
Cephalothorax	Cephalothorax clearly separate from first thoracic segment. Rostrum well defined with 13 sensory pits and setae.
Ornamentation	None.
Pigmentation	None.
Antennule	Five-segmented. Setae formula: 16-6-4+aesthetasc-7+aesthetasc.
Antenna	Long and slender, four segmented. Second segment with spine on inner margin. Third segment with two short spines on inner margin.
Mouthparts	Typical of genus.
Thorax	Thoracic sternites smooth.
Legs	Typical of genus. Fifth leg, one segmented with two setae at apex and one seta on thoracic segment. Spine-seta formulae provided in table 2.32.
Abdomen	Three segmented.
Furcal Rami	Caudal ramus with one long and three short setae. Innermost seta, longest and spinulose.
Egg sacs	Shorter than body.
Attachment site on host	Not described.
Remarks	*Ergasilus pararostralis* differs from *E. piriformis* by not having an enlarged first antenna segment, and *E. boleophthalmi* by having a 5-segmented antennule. *Ergasilus sittangensis* differs from *E. pararostralis* by having a plumose seta on the fifth leg. The species description in the original article is by Pinto Da Motta Amado, Da Rocha, Piasecki, Al-Daraji, & Mhaisen (2001) but the taxon author for *E. pararostralis* is given as only Amado, this technically incorrect, following Zoological Nomenclature regulations. See tabel 2.33 for host and distribution records for *E. pararostralis.*

Compiled from Pinto Da Motta Amado, Da Rocha, Piasecki, Al-Daraji, & Mhaisen (2001).

Table 2.32: Spine-seta formulae for *Ergasilus pararostralis* Amado, 2001

	Coxa	Basis		Segment 1	Segment 2	Segment 3
Leg 1	0-0	I-0	Exopodite	I-0	0-1	II-5
			Endopodite	0-0	0-1	II-4
Leg 2	0-0	I-0	Exopodite	0-0	0-1	I-5
			Endopodite	0-1	0-1	I-5
Leg 3	0-0	I-0	Exopodite	0-0	0-1	I-5
			Endopodite	0-1	0-1	I-5
Leg 4	0-0	I-0	Exopodite	0-0	0-6	-
			Endopodite	0-1	0-1	I-4

Compiled from Pinto Da Motta Amado, Da Rocha, Piasecki, Al-Daraji, & Mhaisen (2001).

Table 2.33: Host and localities for *Ergasilus pararostralis* Amado, 2001

Locality & References	**Host**
Khor al-Zubair Lagoon, Iran Pinto Da Motta Amado, Da Rocha, Piasecki, Al-Daraji, & Mhaisen (2001)	*Liza subviridis* (Valenciennes, 1836)

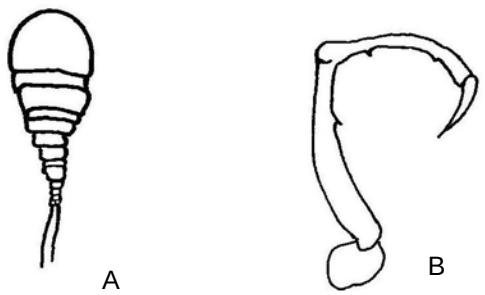


Figure 2.19

Line drawings of *Ergasilus pararostralis* Amado, 2001

A. Dorsal view of female
B. Antenna

Redrawn from Pinto Da Motta Amado *et al.* (2001)

(r) *Ergasilus piriformis* El-Rashidy & Boxshall, 2002 (fig. 2.20)

Total length	0.68mm.
Cephalothorax	Cephalothorax inflated and pear-shaped.
Ornamentation	Inverted T-structure on dorsal surface of cephalothorax.
Pigmentation	Rostrum ornamented ventrally with four sensilla and three sensory pits.
Antennule	Five-segmented. Seta formula: 16-5+ aesthetasc-4-2+aesthetasc-7+aesthetasc.
Antenna	Four-segmented. First segment with large sub-spherical process extending laterally around basis of segment.
Mouthparts	Mandible with three blades; anterior blade small, with teeth on anterior margin; middle blade with teeth anteriorly and posteriorly; posterior blade with teeth only on posterior margin. Maxillule lobate, bearing three outer setae and small medially situated process.
Thorax	Second to fourth segments narrowing posteriorly.
Legs	Leg 1 to 4 typical of genus, with plumose setae on exopodal and endopoda segments. Fifth leg represented by papilla bearing single seta. Spine-seta formulae provided in table 2.34.
Abdomen	Genital complex sub-spherical with two curved rows of spines on ventral surface.
Furcal Rami	Caudal rami with oblique rows of spinules and 4 unadorned setae.
Egg sacs	Not described.
Attachment site on host	Not described.
Remarks	Differs from all other Asian species by having an enlarged first antennal segment. Table 2.35 provides a list of host and distribution records for *E. piriformis.*

Compiled from El-Rashidy & Boxshall (2002).

Table 2.34: Spine-seta formulae for *Ergasilus piriformis* El-Rashidy & Boxshall, 2002

	Coxa	**Basis**		**Segment 1**	**Segment 2**	**Segment 3**
Leg 1	0-0	I-0	Exopodite	I-0	0-1	I-5
			Endopodite	0-1	0-1	II-4
Leg 2	0-0	I-0	Exopodite	I-0	0-1	0-6
			Endopodite	0-1	0-1	I-4
Leg 3	0-0	I-0	Exopodite	I-0	0-1	0-6
			Endopodite	0-1	0-1	I-4
Leg 4	0-0	I-0	Exopodite	0-0	0-5	-
			Endopodite	0-1	0-2	I-3

Compiled from El-Rashidy & Boxshall (2002).

Table 2.35: Host and locality for *Ergasilus piriformis* El-Rashidy & Boxshall, 2002

Locality & References	**Host**
Calcutta, and Delhi India El-Rashidy & Boxshall (2002)	*Sicamugil cascasia* (Hamilton, 1822)

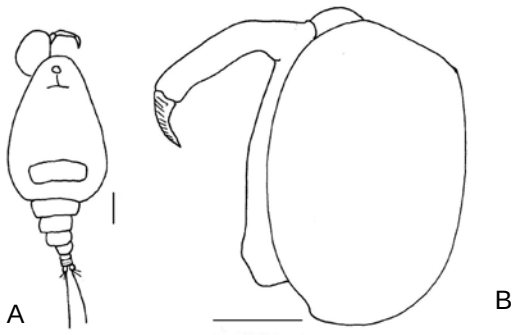


Figure 2.20

Line drawings of *Ergasilus piriformis* El-Rashidy & Boxshall, 2002

A. Dorsal view of female (100µm)
B. Antenna (50µm)

Redrawn from El-Rashidy & Boxshall (2002)

(s) *Ergasilus rostralis* Ho, Jarayajan & Radhakrishnan, 1992 (fig. 2.21)

Total length	0.84mm.
Cephalothorax	Rostrum with two anteriorly pointed setules.
Ornamentation	None.
Pigmentation	None.
Antennule	Five-segmented. Seta formula; 15-6-3-2-7.
Antenna	Long, slender with very visible curve. Spine present on second and third segment on inner margin.
Mouthparts	Not described.
Thorax	Plate on intercoxal bar of legs 1 to 3 with small protrusions on postero-lateral corners, plate absent in leg 4.
Legs	Typical of genus, with plumose setae on exopodal and endopodal segments. Fifth leg single segment with two setae on apex. Spine-seta formulae provided in table 2.36.
Abdomen	Three-segmented with row of spines on posterior margin on each segment.
Furcal Rami	Caudal ramus, with two patches of spinules on distal part of ventral surface and four setae.
Egg sacs	Shorter than body.
Attachment site on host	Not described.
Remarks	*Ergasilus rostralis* differs from *E. cunningtoni*, *E. flaccidus*, *E. inflatipes, E. kandti*, *E. lamellifer*, *E. latus*, *E. macrodactylus*, *E. megacheir*, *E. mirabilis* and *E. sarsi* by not having an inverted T-structure on the cephalothorax. *Ergasiuls nodosus* differs from *E. rostralis* by having plumose setae on the antennule. *Ergasilus rostralis* differs from *E. dangiangensis* by not having an enlarged second antennal segment. *Ergasils rostralis* differs from *E. boleopthalmi*, *E. piriformis, E. sittangensis* and *E. sieboldi* by not having an inverted T-structure on the cephalothorax. *Ergasilus pararostralis* differs from *E. rostralis* by having spines on the inner margin of segment 2 of the antenna. The spine and setae formulae

	of *E. hypomesi* differs from *E. rostralis.* Table 2.37 provides a list of hosts for *E. rostralis.*

Compiled from Ho, Jarayajan & Radhakrishnan (1992).

Table 2.36: Spine-seta formulae for *Ergasilus rostralis* Ho, Jarayajan & Radhakrishnan, 1992

	Coxa	**Basis**		**Segment 1**	**Segment 2**	**Segment 3**
Leg 1	0-0	I-0	Exopodite	I-0	0-1	II-5
			Endopodite	0-1	0-1	II-4
Leg 2	0-0	I-0	Exopodite	I-0	0-1	I-5
			Endopodite	0-1	0-2	I-4
Leg 3	0-0	I-0	Exopodite	I-0	0-1	I-5
			Endopodite	0-1	0-2	I-4
Leg 4	0-0	I-0	Exopodite	I-0	0-5	-
			Endopodite	0-1	0-2	I-3

Compiled from Ho, Jarayajan & Radhakrishnan (1992).

Table 2.37: Hosts and locality for *Ergasilus rostralis* Ho, Jarayajan & Radhakrishnan, 1992

Locality & References	**Host**
Lake Veli, Trivandrum, India Ho, Jarayajan & Radhakrishnan (1992)	*Liza macrolepis* (Smith, 1846) *Valamugil seheli* (Forskål, 1775)

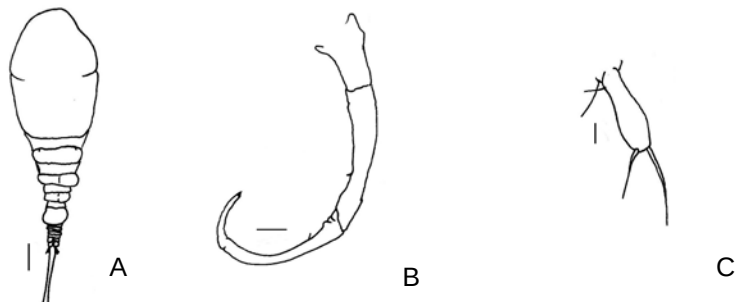


Figure 2.21

Line drawings of *Ergasilus rostralis* Ho, Jarayajan & Radhakrishnan (1992)

A. Dorsal view of female (100µm)
B. Antenna (50µm)
C. Leg 5 (10µm)

Redrawn from Ho, Jarayajan & Radhakrishnan (1992)

(t) *Ergasilus sieboldi* Von Nordmann, 1832 (fig. 2.22)

Total length	0.87mm.
Cephalothorax	Cephalothorax tapering at posterior end, with indentation on medial margin.
Ornamentation	Inverted T-structure on anterior dorsal surface of cephalothorax, with circular structure posterior to inverted T-structure, and eye spot anteriorly to inverted T structure.
Pigmentation	Not observed.
Antennule	Six-segmented.
Antennae	Four-segmented, short. Second segment with short spine on inner surface, third segment with two short spines on inner surface, posteriorly and anteriorly.
Mouthparts	Maxilliped absent as is typical of group.
Thorax	Four-segmented.
Legs	Legs typical of genus. Spine-seta formulae provided in table 2.38.
Abdomen	Genital complex with longitudinally orientated genital apertures, and three free abdominal segments.
Furcal Rami	Four unadorned setae.
Egg sacs	Long.
Attachment site on host	Indiscriminate on position on gill filaments.
Remarks	*Ergasilus sieboldi* differs from *E. flaccidus*, *E. kandti* and *E. nodosus* by having an inverted T-structure on the cephalothorax. *Ergasilus cunningtoni*, *E. inflatipes, E. lamellifer*, *E. latus*, *E. macrodactylus*, *E. megacheir*, *E. mirabilis*, and *E. sarsi* differ from *E. sieboldi* by the nature of the number of setae and segments on the fifth leg. *Ergasilus sieboldi* differs from *E. briani, E. dangiangensis*, *E. lizae*, by having an inverted T structure on cephalothorax. *Ergasilus sittangensis*, *E. piriformis*, and *E. pararostralis* differs by having aesthetascs on the antennule. The spine and setae formulae of *E. hypomesi* differs from *E. sieboldi.*
Distribution	*Ergasilus sieboldi* is an cosmopolitan species that in Russia alone

	infests over 60 species of fish and 60 other hosts all over the world.

Compiled from Halisch (1939), Fryer (1982), and Abdelhalim *et al.* (1991).

Table 2.38: Spine-seta formulae for *Ergasilus sieboldi* von Nordmann, 1832

	Coxa	**Basis**		**Segment 1**	**Segment 2**	**Segment 3**
Leg 1	-	-	Exopodite	I-0	I-1	II-5
			Endopodite	0-1	0-1	II-4
Leg 2	-	-	Exopodite	I-0	0-1	I-6
			Endopodite	0-1	0-2	I-4
Leg 3	-	-	Exopodite	I-0	0-1	I-6
			Endopodite	0-1	0-2	I-4
Leg 4	-	-	Exopodite	I-0	0-5	-
			Endopodite	0-1	0-2	I-3

Compiled from Fryer (1982) and Abdelhalim *et al.* (1991).

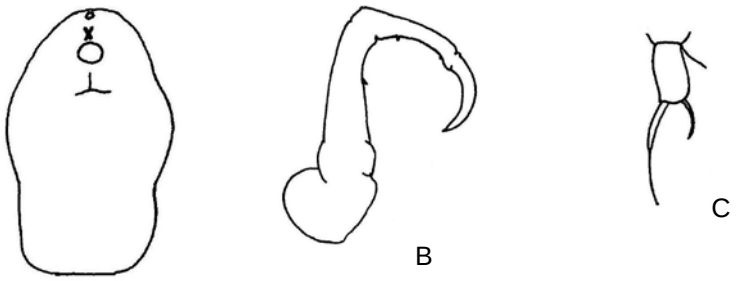


Figure 2.22

Line drawings of *Ergasilus sieboldi* von Nordmann, 1832

A. Dorsal view of caphalothorax
B. Antenna
C. Leg 5

Redrawn from Fryer (1982)

(u) *Ergasilus sittangensis* El-Rashidy & Boxshall, 2002 (fig. 2.23)

Total length	0.78mm.
Cephalothorax	Cephalothorax oblong with first thoracic segment incorporated into cephalosome.
Ornamentation	Inverted T-structure on dorsal surface of cephalothorax.
Pigmentation	None.
Antennule	Six-segmented. Setae formulae: 3-13-5+aesthetasc-4+aesthetasc-2+aesthetasc-7+aesthetasc.
Antenna	Four-segmented. First segment with setae on inner margin. Second segment with spine near mid-point of inner margin. Third segment with small spines on proximal and distal n concave margin.
Mouthparts	Typical of genus.
Thorax	Second to fourth segments narrowing posteriorly.
Legs	Leg 1 to 4 typical of genus, with plumose setae on exopodal and endopodal segments. Fifth leg, 2-segmented. Basal segment with one seta on inner margin, terminal segment with two setae on apex. Spine and setae formulae in table 2.39.
Abdomen	Genital complex wider than long, with five rows of spinules on ventral surface. Segments with rows of spines on posterior margin.
Furcal Rami	Caudal rami with four furcal setae, longest two spinulose, shortest two unadorned.
Egg sacs	Long, as long as body.
Attachment site on host	Not described.
Remarks	*Ergasilus sittangensis* differs from all other species by having plumose setae on the fifth leg, whilst all other species have unadorned setae. See tabel 2.40 for host and distribution records for *E. sittangensis.*

Compiled from El-Rashidy & Boxshall (2002).

Table 2.39: Spine-seta formulae for *Ergasilus sittangensis* El-Rashidy & Boxshall, 2002

	Coxa	**Basis**		**Segment 1**	**Segment 2**	**Segment 3**
Leg 1	0-0	I-0	Exopodite	I-0	0-1	II-5
			Endopodite	0-1	0-1	II-4
Leg 2	0-0	I-0	Exopodite	I-0	0-1	0-6
			Endopodite	0-1	0-1	I-4
Leg 3	0-0	I-0	Exopodite	I-0	0-1	0-6
			Endopodite	0-1	0-2	I-4
Leg 4	0-0	I-0	Exopodite	I-0	0-5	-
			Endopodite	0-1	0-2	I-3

Compiled from El-Rashidy & Boxshall (2002).

Table 2.40: Hosts and localities for *Ergasilus sittangensis* El-Rashidy & Boxshall, 2002

Locality & References	**Host**
Sittang River, Burma El-Rashidy & Boxshall (2002)	*Sicamugil hamiltoni* (Day, 1870)

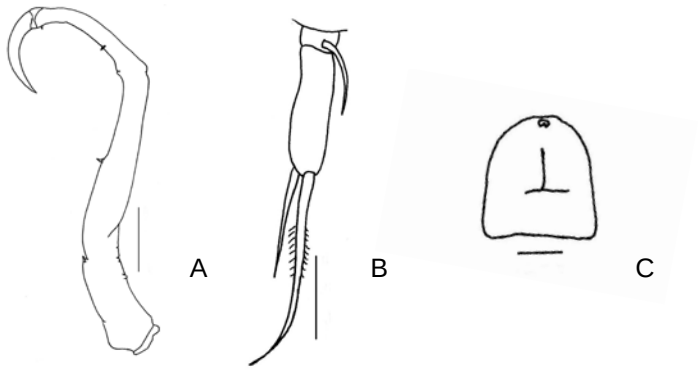


Figure 2.23

Line drawings of *Ergasilus sittangensis* El-Rashidy & Boxshall, 2002

A. Antenna (100µm)
B. Leg 5 (25µm)
C. Dorsal view of cephalothorax (100µm)

Redrawn from El-Rashidy & Boxshall (2002)

(v) *Ergasilus hypomesi* Yamaguti, 1936

The following *Ergasilus* species do not have a complete description, only spine-seta formulae are available.

Table 2.41: Spine-seta formulae for *Ergasilus hypomesi* Yamaguti, 1936

	Coxa	**Basis**		**Segment 1**	**Segment 2**	**Segment 3**
Leg 1	-	-	Exopodite	I-0	0-1	II-5
			Endopodite	0-1	0-1	II-4
Leg 2	-	-	Exopodite	I-0	0-1	0-6
			Endopodite	0-1	0-2	I-4
Leg 3	-	-	Exopodite	I-0	0-1	0-6
			Endopodite	0-1	0-2	I-4
Leg 4	-	-	Exopodite	I-0	0-5	-
			Endopodite	0-1	0-2	I-3

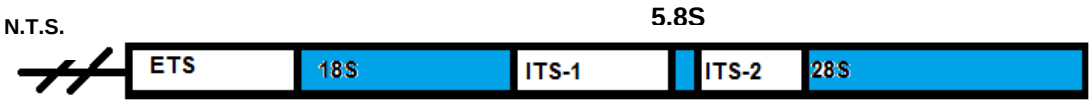
Compiled from Pinto Da Motta Amado *et al.* (2001).

- **The use of molecular studies in ergasilid taxonomy**

Most of what is known about the Family Ergasilidae is based on morphological studies only (Song *et al.*, 2007), and only very recently has phylogenetics been used to better understand the relationships among the members of the family. Using 18S and 28S ribosomal deoxyribonucleic acid (rDNA) sequences, Song *et al.* (2007) studied the phylogenetic relationships of 14 species from four genera in the Ergasilidae, which included the genera; *Ergasilus, Paraergasilus* Markevich, 1937*, Sinergasilus* Yin, 1949 and *Pseudergasilus* Yamaguti, 1936.

The use of rDNA is evident from the fact that these sequences encode for ribosomal ribonucleic acid (rRNA), and that ribosomes are ubiquitous in all life forms, and are the cornerstones in the synthesis of proteins. Ribosomal DNA is a collective noun for rRNA and their associated spacer regions (Hillis & Dixon, 1991) (fig. 2.23). In the eukaryote nuclear genome the rDNA array comprises several hundred tandemly repeated copies of the transcription unit and non-transcribed spacer. The number of these copies varies greatly, and can be anything from one to several thousand. In the eukaryote genome the two internal transcribed spacers (ITS-1 and ITS-2) separate the 18S, 5.8S and 28S gene (fig. 2.23). Upstream of the 18S gene is a external transcribed spacer (ETS) (fig. 2.23). These transcribed spacers contain signals for the processing of the rRNA transcript. Adjacent copies of the rDNA repeat units are separated by a non-transcribed spacer (N.T.S.). This region contains sub-repeating elements that serves as enhancers of the transcription process.

Figure 2.23. Ribosomal deoxyribonucleic acid (rDNA) array of an Eukaryote, redrawn from Hillis and Dixon (1991).



The present study aims to use sequences derived from the 28S rDNA gene, using the designed primers in Song *et al.* (2007), to construct a phylogeny for the *Ergasilus*

species from southern Africa, and to compare it with the results obtained from the morphological study on the same species.

- **The use of *Lernaea* as an outgroup**

The choice of a suitable outgroup was easy and following the study by Song *et al.* (2007) *Lernaea cyprinacea* Linnaeus, 1758*,* collected from South Africa, was used as an out-group.

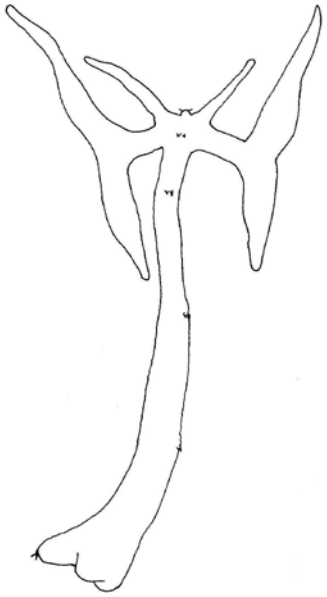


Figure 2.24: Line drawing of *Lernaea* sp.

Redrawn from Garbda (1963)

According to Ho (1998) there are currently 110 species of lernaied copepods that are known from 332 species of freshwater fishes belonging to 161 genera in 41 families. Almost all the information on the lernaieds is based on the morphology of the highly modified females. According to Ho (1998) some of the members of the family, e.g. *Lernaea* Linnaeus, 1758, are mesoparasites. Attachment to the host is via a transformed cephalothorax comprising an elongated, un-segmented trunk, neck and embedded, branched anchor-like head (Boxshall & Halsey, 2004) (fig. 2.24) . Following the work of Grabda (1963) on *Lernaea cyprinacea* the modifications in the male cephalothorax are not as pronounced as it is with the females. The two largest genera in the family Lernaeidae Cobbod, 1879, that together account for 71% of the known species, are *Lamproglena* von Nordmann, 1832 and *Lernaea*. According to Fryer (1968) a few species from the genus *Lernaea* show no signs of host specificity (e.g. *Lernaea barnimiana* (Hartmann, 1865)), most of the members seem to be restricted to one fish family and in some cases (e.g. *Lernaea bargi* Harding, 1950) to one host genus. As for the distribution of the family the majority of the lernaeids are from Africa and Asia (Ho, 1998, van As & van As, 2007). The use of *Lernaea* from Africa in the present study is evident from the fact that both of the

genera used in the study, *Ergasilus* (as the main focus group) and *Lernaea* (as the out-group) are parasites of fish.

- **What is in a name?**

For the sake of interest and etymology, the names of all the organisms are important, it brings clarity to what would, certainly, have been an absolutely chaotic system, without these scientific names. Kabata (1988) stated the following; "I cannot resist classifying names by their types. As I see them they can be grouped in the following manner:

Names taken from the mythological pantheon of antiquity, or from classical authors."

According to Kabata (1988), 18th century gentry studied classical lore and with much enthusiasm "moved with ease amongst the Olympians". It is therefore no surprise that the copepods described from that time have names derived from the Greek gods, goddesses and monsters.

An excellent example of this is Carolus Linnaeus (1707 – 1778) himself: the universally known little cnidarian *Hydra* is one of these creatures. Its namesake the mythical monster lived in an equally mythically swamp called Lerna, Greece. In 1758 he found a parasitic copepod that - to him - appeared similar to *Hydra.* Since that name was already taken, he used the next best thing. Thus another creature reminiscent of the monster from Lerna was named *Lernaea,* a parasitic copepod of fish (Kabata, 1988).

The parasitic copepod that is the main focus of this dissertation, *Ergasilus,* is another excellent example. When Alexander Davidovič von Nordmann (24 May 1803 - 25 June 1866), a Finnish biologist, described the genus in 1832 he probably already knew the following line taken from the Latin play titled *Captivi* by Titus Maccius Plautus.

"Ergasilus: No parasite now am I, but a right royal king of kings; so large a stock of provisions for my stomach is there at hand in thee harbour."

The comic relief in the play, is provided by the sponger (or the Latin term parasitus), Ergasilus, looking for a free dinner from his host.

Chapter 3

Hosts and Habitats

- **Host Habitats**

With more than 70% of the Earth's surface covered by water, it is estimated that only 35,029,000 km^3 (De Silva, Abery & Nguyen, 2007) of it is freshwater, or about 2.5%. Only 23.5% is habitable (De Silva *et al.,* 2007) and only 0.01% (113,000 km^3) is naturally available as drinking water. This incredible resource is home to nearly 25% of the global vertebrate diversity; supported through a number of freshwater ecosystems, e.g. rivers, lakes, marshes and seasonal or ephemeral wetlands (De Silva *et al.,* 2007). Of all the vertebrates, fish are the most diverse, comprising nearly 50% of all the known vertebrate species (van As, du Preez, Brown & Smit, 2012). The current estimate of the number of fish species, following De Silva *et al.* (2007) and www.fishbase.org, is 32 700. This incredible number of species is represented by 482 fish families (van As *et al.,* 2012). According to De Silva *et al.* (2007), 40% (13 080) of these families are freshwater species and close to 600 species more need freshwater at some stage to complete their life cycle. It is therefore necessary to elaborate a little on the freshwater systems and fish species of Africa and Asia, to completely comprehend the complexity of host and parasite interactions.

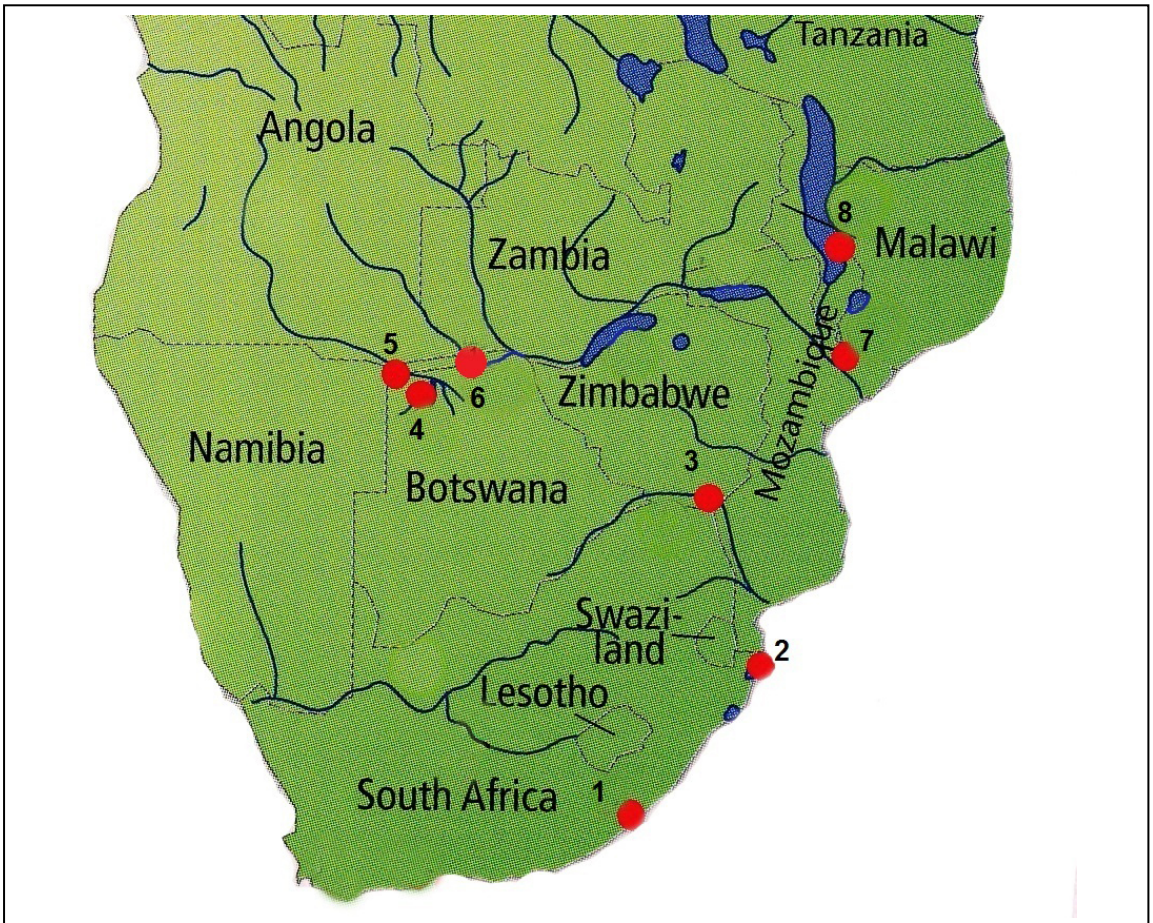
- **Africa**

According to van As *et al.* (2012) Africa has been shaped by 60 international river basins, with 677 natural and man-made lakes or reservoirs that supports an estimated 30 000km^3 of fresh water. The continent is drained by 12 large river systems, with nearly 100 coastal rivers that drain the escarpments. The important collection sites for this study include Lake Malawi, the Okavango River and Delta

(Botswana), the Zambezi River, the Limpopo River and the Orange River. Figure 3.1. gives a map and collections sites.

The largest diversity of fish species in Africa is found towards the equator, with more than 700 known species described from the Congo system. Lake Malawi boasts 800 species, the Zambezi 134, the Limpopo 50 and the Orange system only 16 species (van As *et al.,* 2012). Table 3.1. summarises the fish hosts (from Africa) used in the study and indicates the localities where each species was collected. All together 29 fish species from 10 families were investigated in the study.

Figure 3.1. Map of southern African river systems indicating the collection sites.



1. Kowie Estuary
2. Pongola River
3. Limpopo River
4. Okavango Delta
5. Okavango Delta
6. Lake Liambezi
7. Zambezi System
8. Lake Malawi

Table 3.1: The fish hosts examined in the study and the collection locality of each species [Compiled using Konings (1990), Skelton (2001), www.fishbase.org].

Family	Collection locality
Family Mormyridae	
Genus *Hippopotamyrus* Pappenheim, 1906	
Hippopotamyrus ansorgii (Boulenger, 1905)	Zambezi (Mozambique)
Genus *Petrocephalus* Marcusen, 1854	
Petrocephalus wesselsi Kramer & van der Bank, 2000	Okavango River (Botswana)
Family Cyprinidae	
Genus *Labeo* Cuvier, 1817	
Labeo rosae Steindachner, 1894	Pongola River (South Africa)
Genus *Barbus* Cuvier & Cloquet, 1816	
Barbus afrohamiltoni Crass, 1960	Pongola River (South Africa)
Family Characidae	
Genus *Hydrocynus* Cuvier, 1816	
Hydrocynus vittatus Castelnau, 1861	Pongola, Shalala Pan (South Africa), Okavango River (Botswana)
Genus *Rhabdalestes* Hoedeman, 1951	
Rhabdalestes maunensis (Fowler, 1935)	Okavango River (Botswana)
Genus *Brycinus* Valenciennes, 1849	
Brycinus lateralis (Boulenger, 1900)	Okavango River (Botswana)
Family Hepsetidae	
Genus *Hepsetus* Swainson, 1838	
Hepsetus odoe (Bloch, 1794)	Okavango River (Botswana), Zambezi (Mozambique)

Table 3.1 (continued): The fish host localities.

Family	Locality
Family Schilbeidae	
Genus *Schilbe* Oken, 1817	
Schilbe intermedius Rüppell, 1832	Okavango River (Botswana)
Family Clariidae	
Genus *Clarias* Scopoli, 1777	
Clarias gariepinus (Burchell, 1822)	Pongola River (South Africa), Zambezi (Mozambique), Okavango River (Botswana)
Clarias ngamensis Castelnau, 1861	Pongola, Shalala Pan (South Africa), Okavango River (Botswana)
Family Mochokidae	
Genus *Synodontis* Cuvier, 1816	
Synodontis leopardinus Pellegrin, 1914	Chobe (Namibia), Zambezi System (Mozambique), Pongola (South Africa), Okavango River (Botswana)
Synodontis nigromaculatus Boulenger, 1905	Zambezi (Mozambique), Okavango River (Botswana)
Synodontis zambezensis Peters, 1852	Zambezi (Mozambique)
Synodontis macrostigma Boulenger, 1911	Zambezi (Mozambique), Okavango River (Botswana)
Synodontis vanderwaali Skelton & White, 1990	Okavango River (Botswana)
Synodontis thamalakanensis Fowler, 1935	Okavango River (Botswana)

Table 3.1(continued): The fish host localities.

Family	Locality
Family Cichlidae	
Genus *Tyrannochromis* Eccles & Trewavas, 1989	
Tyrannochromis macrostoma (Regan, 1922)	Lake Malawi
Genus *Pseudotropheus* Regan, 1922	
Pseudotropheus tropheops Regan, 1922	Lake Malawi
Genus *Petrotilapia* Trewavas, 1935	
Petrotilapia nigra Marsh, 1983	Lake Malawi
Petrotilapia genalutea Marsh, 1983	Lake Malawi
Petrotilapia tridentiger Trewavas, 1935	Lake Malawi
Genus *Maylandia* Meyer & Föster, 1984	
Maylandia barlowi (Mckaye & Stauffer, 1986)	Lake Malawi
Family Gobiidae	
Genus *Glossogobius* Gill, 1862	
Glossogobius giuris (Hamilton-Buchanan, 1822)	Pongola, Shalala Pan & Mengo Pan (South Africa)

- **Asia**

The endemic freshwater fish species numbers in East, South and South-East Asia is estimated at 559, belonging to 47 families. The six largest families, with the percentage of species it constitutes is given in table 3.2. The other 41 families constitutes at least one endemic species each. Nation-wide the most number of endemic freshwater fish species occur in India (191), followed by China (88), Indonesia (84), and Myanmar (60) (De Silva *et al.,* 2007).

Table 3.2: Table indicating the six largest endemic fish host families and percentage of species the family constitutes from Asia (adapted from De Silva *et al.* (2007)).

Fish host family	Persentage of species
Cyprinidae	43
Balitoridae	16
Sisoridae	25
Gobiidae	20
Melanotaeniidae	19
Bagridae	16

Only the families Cyprinidae and Gobiidae, in table 3.2, are found in both Asia and Africa (www.fishbase.org). Within these families no fish host species was used in the present study that occur on both continents. Table 3.3 summarises the Asian fish species used in the present study.

Table 3.3: The fish hosts from Asia that were used in the present study.

Host	**Locality**
Opsariichthys bidens Günther, 1873	China
Zacco platypus (Temminck & Schlegel, 1846)	China
Boleophtalmus dussumieri Valenciennes, 1837	Iraq
Bathygobius fuscus (Rüppell, 1830)	Iraq
Liza subviridis (Valenciennes, 1836)	India
Sicamugil hamiltoni (Day, 1870)	Burma
Sicamugil cascasia (Hamilton, 1822)	India
Epinephelus malabaricus (Bloch & Schneider, 1801)	Taiwan
Liza macrolepis (Smith, 1846)	India
Valamugil seheli (Forskål, 1775)	India

Chapter 4

Material and Methods

- **Collection of fish hosts**

The Aquatic Ecology Research Group, University of the Free State, has collected fish parasites from different locations all over the world for the last 28 years. This collection includes a variety of parasites from different taxonomic groups. The collection of ergasilids includes material from southern Africa and includes locations such as South Africa, Namibia, Botswana and Malawi. During the present study additional field work was done in South Africa as well as, Botswana and Namibia. Figure 4.1 indicates photographs of collection sites, collection methods and field laboratories in Botswana and Namibia.

- **Study and preparation of the material for morphological identification**

Light and dissection microscopy

At the Aquatic Ecology laboratory in Bloemfontein the ergasilid specimens were sorted and numbered, according to the host species which they parasitised. In this case the ergasilids, which occur on the gills of the fish, were removed with the gills and examined using a Wild dissection microscope. A datasheet with the name of the fish, the parasite, the date and the location was placed with each of the specimens for identification purposes. Once the ergasilids were removed from the gills they were fixed in 70% (for morphological purposes) and 100% (for molecular purposes) ethanol. The fixed specimens were cleaned using an Ultrasonic water bath, after which they were placed in Lactic acid to clear. The latter step was done for approximately 15 minutes. The Lactic acid plays a second role in that it strengthens the setae, preventing it from breaking. Following this, the specimens were placed in Lignin Pink (Lignin Pink powder is dissolved in lactic acid) for another half an hour, to

stain it; making it easier to examine the specimens. A Leitz Laborlux D compound light microscope and a dissecting microscope were used to examine the specimens. A drawing tube, attached to the light microscope, was used to draw the specimens. Between four and 20 specimens were drawn from each fish host species. Body measurements (see fig. 2.1) were compiled and are provided as a mean value followed by the minimum and maximum values. All measurements are given in milli-meters (mm). At the end of each species description in Chapter 5, is a table providing the measurements of the *Ergasilus* sp. from each host. The spine-seta formula of the legs was determined by counting the number of spines and setae on each segment of the endo- or exopodite of the legs, as wel as the presence or absence of setae on the coxa and basis. The spines are represented by roman numerals, while the setae are represented by Arabic numerals, this was done by using the standard method explained by Huys & Boxshall (1991). The endopodal and exopodal segments are numbered one to three, with one furthest away from the body and three at the base of the basis.

Scanning Electron Microscopy

The specimens selected for scanning electron microscopy were dehydrated from 70% to 100% ethanol and critical point dried using the standard techniques. Once the specimens were critically point dried they were mounted onto Scanning Electron Microscopy stubs using double-sided tape. The specimens were then sputter coated with gold and studied using the JEOL WINSEM JSM 6400 Scanning Electron Microscope at the Centre for Confocal and Electron Microscopy, University of the Free State. The electronic photographs of the specimens were sorted and examined for detailed morphological information on the specimens.

- **Molecular analysis**

Various extraction methods and kits were used without positive results, and therefore only those methods that did provide positive results will be provided or briefly explained.

DNA extraction

The extraction of DNA from the specimens were at first attempted by using DNA extraction kits, without success. According to Song *et al.* (2007) the genomic DNA

from their study was prepared by the standard phenol/chloroform extraction method. Following the procedure in Carvalho, Dadour, Grith & Harvey (2005) the DNA extraction was preformed on the samples:

Phenol-Chloroform extraction method:

1. Add 400µl Tris-EDTA (pH 7.5) to a 1.5mL Eppendorf tube, clearly marked, add 10µL Protease K. Incubate at 55°C for 2 hours.
2. Add 400µl Phenol and centrifuge at 14 000 x g for 5 minutes.
3. Remove the supernatant to a new, marked Eppendorf tube and add 800µL Chloroform. Centrifuge for 5 minutes at 14 000 x g.
4. Remove the supernatant to a new, marked Eppendorf tube and add 800µL Chloroform. Centrifuge for 10 minutes at 14 000 x g.
5. Remove the supernatant to a new marked tube and add an equal volume of ice-cold Isopropanol (70 – 100%). Incubate at -70°C for 1 hour.
6. Centrifuge the tube for 10 minutes at 14 000 x g. Remove the supernatant and keep the pellet.
7. Wash the pellet with 70% ethanol and air dry for 15 minutes.
8. Resuspend the pellet in 15µL 1x TE-buffer (pH 7.5).

The DNA quality and quantity was measured using a Thermo Scientific Nano Drop Lite Spectrophotometer at the Genetics Department, UFS and The Pesticide Tick Resistance Laboratory, Department of Zoology and Entomology, UFS.

Polymerase Chain Reaction (PCR)

After the successful extraction of DNA from the samples, a PCR reaction was attempted using the primers used in the study by Song *et al.* (2007) (table 4.1) for the 28S rDNA gene using the Qiagen TopTaq PCR DNA Polymerase kit. The primers were obtained from Inqaba Biotech (Pretoria, South Africa). The Palm-Thermal Cycler was used to run the PCR reaction at the different running temperatures.

Table 4.1: The sequences for primers used for amplification of 28S rDNA geneby PCR from *Ergasilus* sp. from southern Africa.

Primer	**Sequence**
28SF	**5’ – ACA ACT GTG ATG CCC TTA G – 3’**
28SR	**5’ – TGG TCC GTG TTT CAA GAC G – 3’**

The PCR conditions used by Song *et al.* (2007) as well as those advised by the Qiagen TopTaq DNA Polymerase kit were used. Agarose gel electrophoresis (using a 1% Agarose gel) was used to visualise the PCR reaction. After various trials where the PCR reaction failed, all the samples of extracted DNA were sent to Inqaba Biotech for PCR and Sequencing analysis, this was successful and the sequences received from Inqaba Biotech were used to study the phylogenetic relationships between the species. The 28S rDNA sequences from the 9 *Ergasilus* and 2 *Lernaea* species were analyzed using different tree-building methods, i.e., neighbour-joining (NJ), maximum parsimony (MP), maximum likelihood (ML). Nodal support was assessed using bootstrap resampling with 1000 bootstraps. The analysis was done using Geneious R7 and Mega 5.2.2 computer software.

- **The use and comparison of *Ergasilus* species**

In the present study the ergasilids collected from southern Africa are compared to the known species from Africa and some of the ergasilids species from China. The addition of the Chinese species is because of the molecular section of this study: the only published molecular results attainable (at present) are from Chinese ergasilid species. It was therefore prudent to use the Chinese ergasilids in the morphological description of the southern Africa species. In the descriptions only those characters of the species that are different to the rest are mentioned. The species descriptions were done following the most recent publications.

Figure 4.1

Photographs of the collection sites, collection methods and field laboratories.

A. The use of scoop net to catch small fish in Botswana

B. Using fishing rods in the Okavango Delta, Botswana

C. The lab in Leseding Research Camp, Samochima, Botswana

D. Using a cast net to catch fish in Namibia

E. The field lab in Namibia

F. At the start of an incredible “Trans-Okavango” boat trip

Images courtesy of the Aquatic Ecology Research Group, UFS.

Chapter 5

Results

- **Morphological results**

- ***Ergasilus* sp. A**

Type host

Family: Mochokidae

Synodontis leopardinus Pellegrin, 1914

Type locality

Lake Liambezi (17° 54'41.83"S; 24° 22'20.79"E)

Other hosts and distributions

Family: Mochokidae

Synodontis nigromaculatus Boulenger, 1905 (Okavango River, fig. 5.6)

S. zambezensis Peters, 1852 (Okavango River and Delta, fig. 5.6)

S. thamalakanensis Fowler, 1935 (Okavango River and Delta, fig. 5.6)

S. macrostigma Boulenger, 1911 (Pongola River, Okavango River and Delta, fig. 5.6)

S. vanderwaali Skelton & White, 1990 (Okavango River, fig. 5.6)

Family: Schilbeidae

Schilbe intermedius Rüppell, 1832 (Okavango River and Zambezi System, fig. 5.6)

Family: Clariidae

Clarias ngamensis Castelnau, 1861 (Pongola River and Zambezi System, fig. 5.6)

C. gariepinus (Burchell, 1822) (Pongola River, Limpopo River and Zambezi System, fig. 5.6)

Family: Gobiidae

Glossogobius giuris (Hamilton-Buchanan, 1822) (Pongola River, fig. 5.6)

Family: Cyprinidae

Labeo rosae Steindachner, 1894 (Pongola River, fig. 5.6)

Family: Characidae

Hydrocynus vittatus Castelnau, 1861 (Pongola River, Okavango River and Zambezi System, fig. 5.6)

Family: Cichlidae

Serranochromis macrocephalus (Boulenger, 1899) (Okavango Delta, fig. 5.6)

S. angusticeps (Boulenger, 1907) (Zambezi System, fig. 5.6)

Oreochromis andersonii (Castelnau, 1861) (Zambezi System, fig. 5.6)

Family: Mormyridae

Hippotamyrus ansorgii (Boulenger, 1905) (Okavango River, fig. 5.6)

Family: Hepsetidae

Hepsetus odoe (Bloch, 1794) (Okavango River, fig. 5.6)

Species description of female (Based on 19 specimens from *Synodontis leopardinus* Pellegrin, 1914)

Total Length: Mean length 1.44mm (1.35 – 1.52mm range), measured from anterior end of cephalothorax to posterior margin of caudal rami, excluding furcal setae (figs. 5.1 A & 5.5 E). Body gradually narrowing posteriorly.

Cephalothorax: Prosome 5-segmented. Cephalosome as wide as long, distinctly separated from first free pedigerous somite. Distinct distribution of sensory setae and pits on dorsal surface of cephalic shield (fig. 5.1 A). Rostrum, on ventral side, with distinct sensory pits and setae. Two small oval cephalic structures situated anterior to sensory pits and setae on rostrum (fig. 5.1 B).

Ornamentation: Dorsal surface of cephalic shield, with circular cephalic structure anterior to inverted T, oval cephalic structure posterior to inverted T. Inverted T only visible using light microscopy (fig. 5.1 A).

Pigmentation: Dark blue pigments all over dorsal surface of prosome and urosome.

Mouthparts: Maxilla, second segment ending in two spinulose areas (fig. 5.2 B). Mandibles with 2 blades, anterior with rows of bristles, posterior blade slender with row of bristles along anterior margin (figs. 5.2 A & 5.5 E).

Thorax: Four-segmented, narrowing posteriorly, with less sclerotinised regions between segments (fig. 5.1 B). First pedigerous somite wide and broad, nearly twice as broad as second somite. Dorsal surface of segments with distinct distribution of sensory setae and pits (fig. 5.1 A). Ventral surface with row of comb-like setae situated medially, extending from left to right on intercoxal plate of first leg pair (fig. 5.1 D), plate with sensory pits at each end.

Legs: Legs 1 to 4 biramous, all legs 3-segmented, except 2-segmented exopod of leg 4 (figs. 5.4 A). Spine and setae formulae similar to other *Ergasilus* species, summarised in table 5.1. Legs 1 to 4 with sensory pit on dorsal outer margin of coxa.

Leg 1 - first exopodal segment with row of long fine setae on inner margin. First endopodal segment with row of long fine setae on outer margin (fig. 5. 4 A).

Leg 2 - first exopodal segment with row of long fine setae on inner margin. First endopodal segment with row of long fine setae on inner margin (fig. 5.4 B).

Leg 3 - first exopodal segment with row of long fine setae on inner margin. First endopodal segment with row of long fine setae on outer margin (fig. 5.4 C).

Leg 4 - first exopodal segment with row of sort fine setae on inner margin. First and second endopodal segment with row of short setae seta on inner margin (fig. 5.4 D).

Leg 5 - 2-segmented with single short seta on basal segment, and two terminal setae and one lateral seta on the distal segment (fig. 5. 4 E).

Table 5.1: Spine and seta formulae for *Ergasilus* von Nordmann, 1832 sp. A collected from *Synodontis leopardinus* Pellegrin, 1914 from Lake Liambezi.

	Coxa	Basis		*Segment 1	Segment 2	Segment 3
Leg 1	0	1	Exopodite	0 – 1	I – 1	II – 5
			Endopodite	0 – 1	0 – 1	II – 4
Leg 2	0	1	Exopodite	I – 0	0 – 1	0 – 6
			Endopodite	0 – 1	0 – 2	I – 4
Leg 3	0	1	Exopodite	I – 0	0 – 1	0 – 6
			Endopodite	0 – 1	0 - 2	I – 4
Leg 4	0	1	Exopodite	I – 0	0 – 5	-**
			Endopodite	0 – 1	0 – 2	I – 3

*Segment 1 closest to basis.

**All *Ergasilus* species lack this segment.

Abdomen: Urosome 5-segmented, comprising short fifth pedigerous somite, rounded genital somite, with rows of fine pin-like scales on ventral surface. Posterior margin of first free abdominal segment bordered by row of comb-like spines. Posterior margin of second free abdominal segment bordered by row of comb-like spines. Third free abdominal segment bi-lobed, with group of clustered comb-like spines on each lobe (fig. 5.3 B).

Furcal rami: Furcal rami longer than anal segment. Two sensory pits near base of each ramus. Group of spines posterior to each sensory pit. Four furcal setae present on each ramus, inner most longest, longer than abdomen (figs. 5.3 B; 5.5 D). Longest seta adorned with tooth-like spines (fig. 5.5 F).

Antennule: Six-segmented (figs. 5.3 C; 5.5 B), tapering distally. Seta formula from proximal to distal segments: 3 – 11 – 4 – 4 – 2 – 4.

Antenna: Four-segmented, all segments slender, unadorned. Third segment visibly twisted medially. Mean length 0.75mm (600 – 860mm) (figs. 5.3 A; 5.5 C).

Egg sacs: Egg sacs elongated 1.0 - 1.1mm (fig. 5.1 C).

Remarks: *Ergasilus* sp. A. differs from *E. flaccidus* and *E. lamellifer* by having a fifth leg with two terminal setae and one lateral seta, while these two species have no lateral setae. *Ergasilus cunningtoni, E. nodosus* and *E. sarsi* have three furcal setae whilst *Ergasilus* sp. A has four furcal setae. *Ergasilus macrodactylus, E. megacheir, E. mirabilis, E. latus* and *E. kandti* have single segmented fifth legs and *Ergasilus* sp. A has a 2-segmented fifth leg. *Ergasilus briani* and *E. lobus* differ from *Ergasilus* sp. A. by not having an inverted T structure on the dorsal surface of the cephalothorax. *Ergasilus pararostralis*, *E. pyriformis* and *E. rostralis* differs by having a 5-segmented antennule. *Ergasilus* sp. A. differs from *E. lizae*, *E. boleopthalmi* and *E. sieboldi* by not having any ornamentation on any of the antenna segments. The first antenna segment of *E. danjiangensis,* which is swollen, differs completely from that of *Ergasilus* sp. A, which is slender. The spine and seta formulae of *E.* sp. A differs from that of *E. hypomesi*.

Based on the description above and the differences between *Ergasilus* sp. A and the known species there is little doubt that *Ergasilus* sp. A is a new species. The combination of the following characteristics distinguish this species from the others:

- Two-segmented fifth leg, with single short seta on basal segment, two terminal setae and one lateral seta on the distal segment.
- Antennal third segment with a large twisted area near its centre.
- Longest furcal seta spinulose

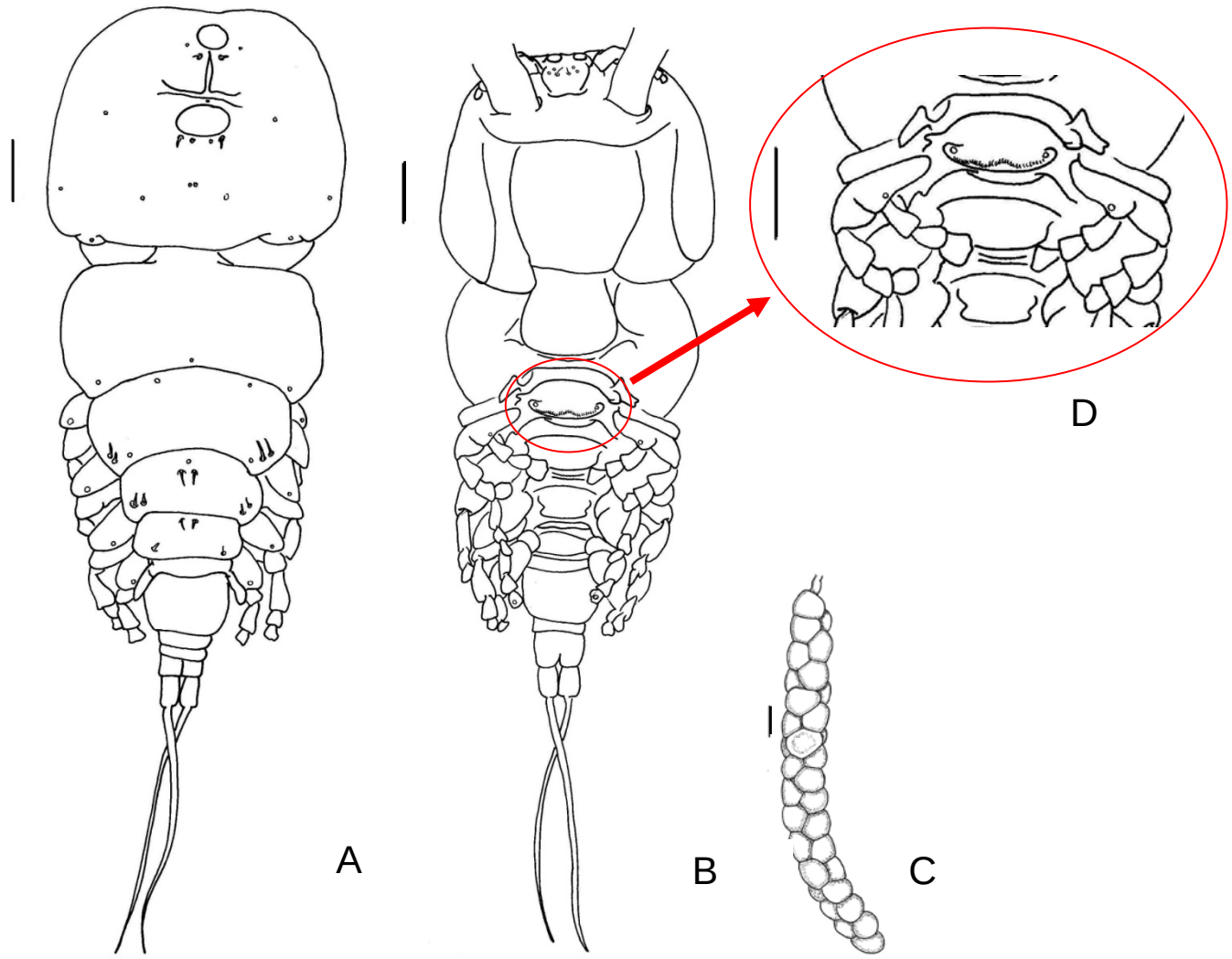


Figure 5.1

Line drawings of *Ergasilus* van Nordmann, 1832 sp. A collected from *Synodontis leopardinus* Pellegrin, 1914 from Lake Liambezi

A. Dorsal view of whole specimen (100µm)
B. Ventral view of whole specimen (100µm)
C. Egg sac (100µm)
D. Intercoxal plate (100µm)

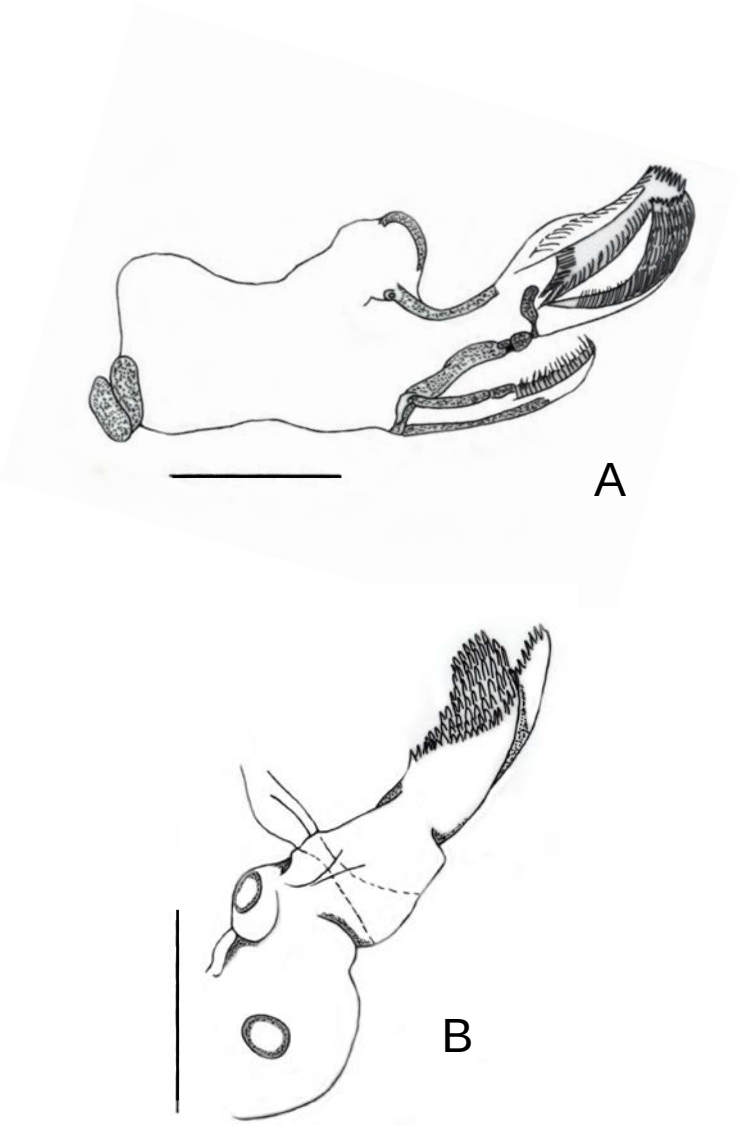


Figure 5.2

Line drawings of *Ergasilus* van Nordmann, 1832 sp. A collected from *Synodontis leopardinus* Pellegrin, 1914 from Lake Liambezi

A. Mandible (10µm)
B. Maxilla (10µm)

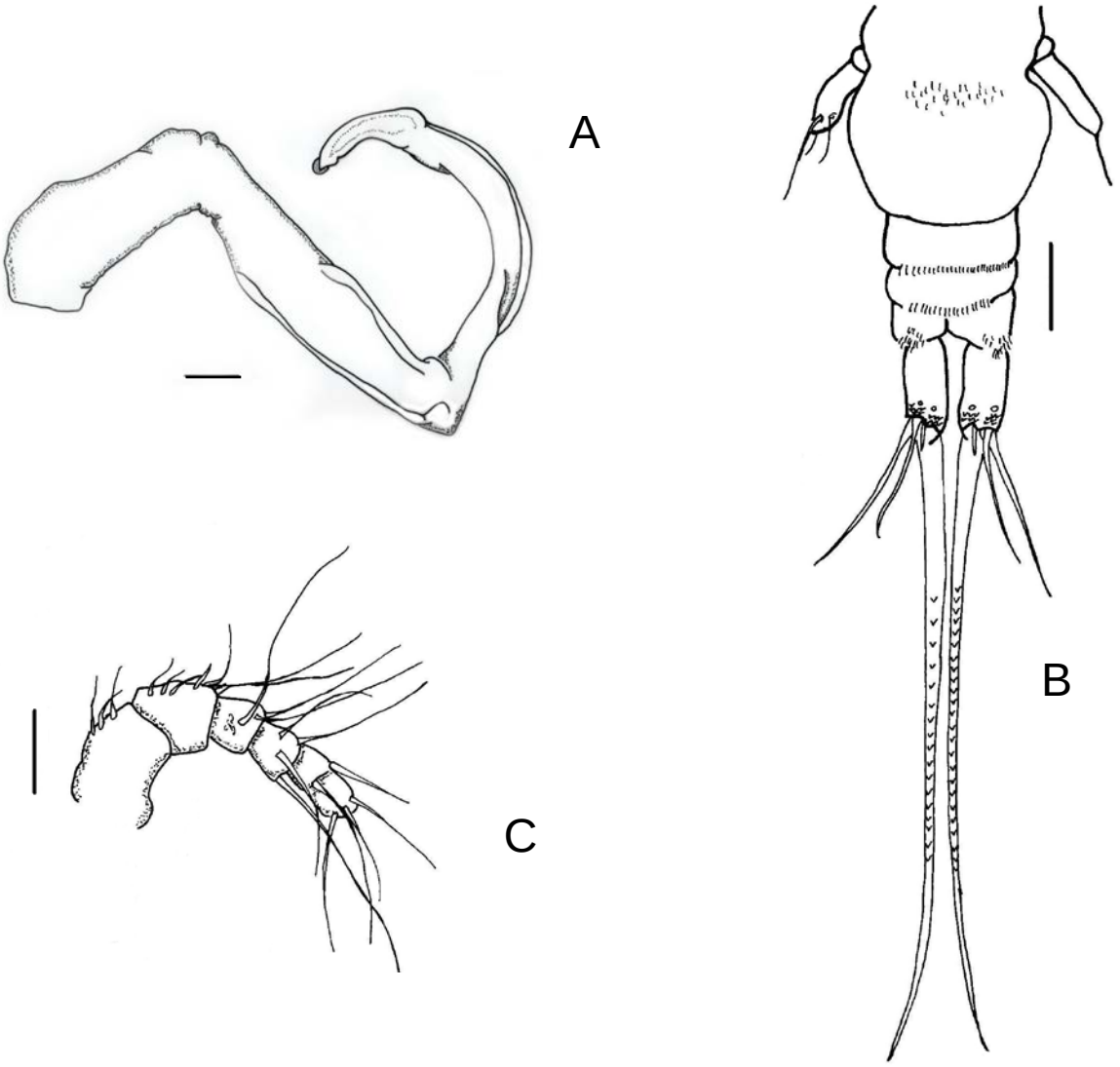


Figure 5.3

Line drawings of *Ergasilus* van Nordmann, 1832 sp. A collected from *Synodontis leopardinus* Pellegrin, 1914 from Lake Liambezi

A. Antenna (100µm)
B. Dorsal view of abdomen and furcal rami (100µm)
C. Antennule (100µm)

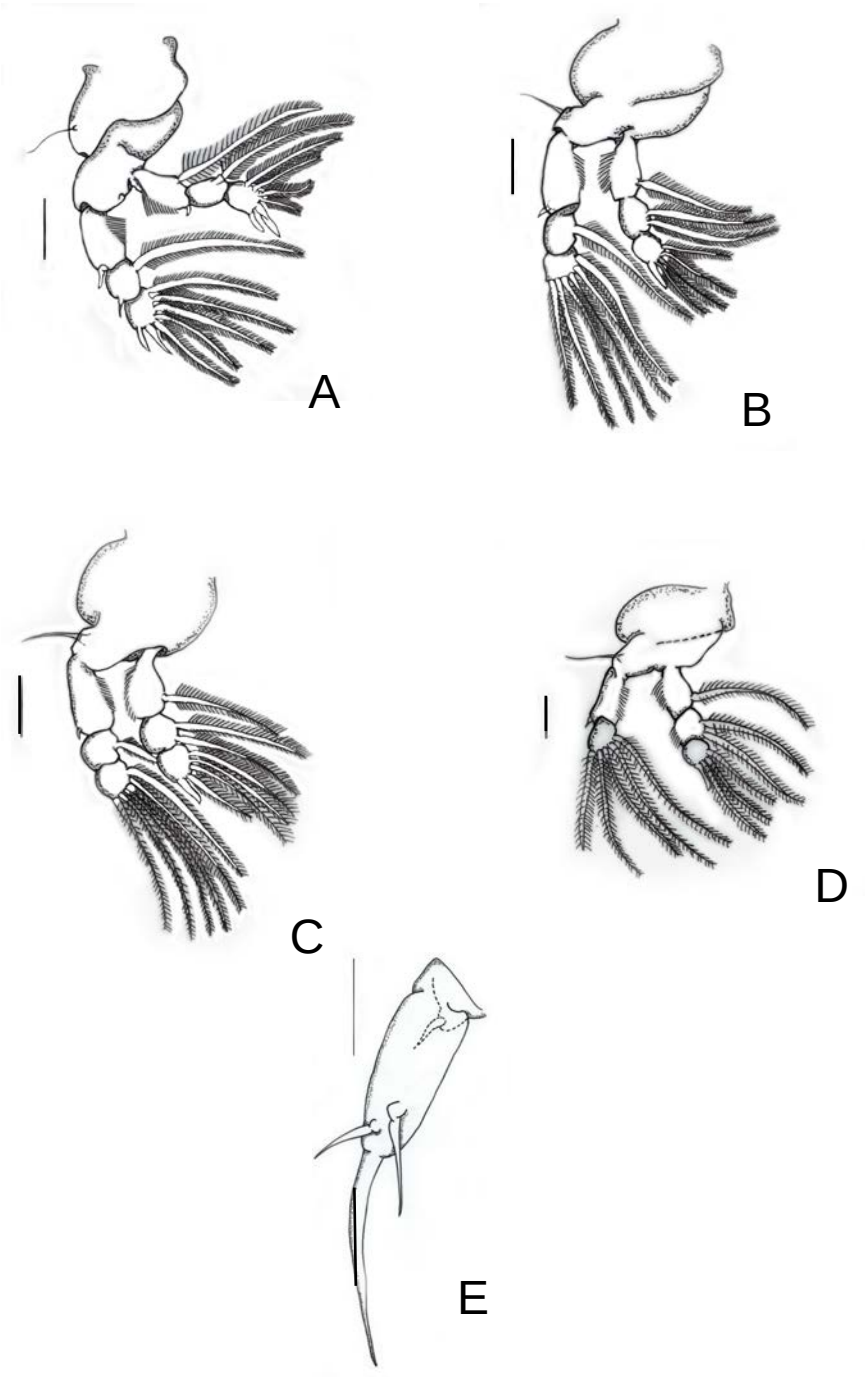


Figure 5.4

Line drawings of *Ergasilus* van Nordmann, 1832 sp. A collected from *Synodontis leopardinus* Pellegrin, 1914 from Lake Liambezi

A. Leg 1 (50µm)
B. Leg 2 (50µm)
C. Leg 3 (50µm)
D. Leg 4 (50µm)
E. Leg 5 (10µm)

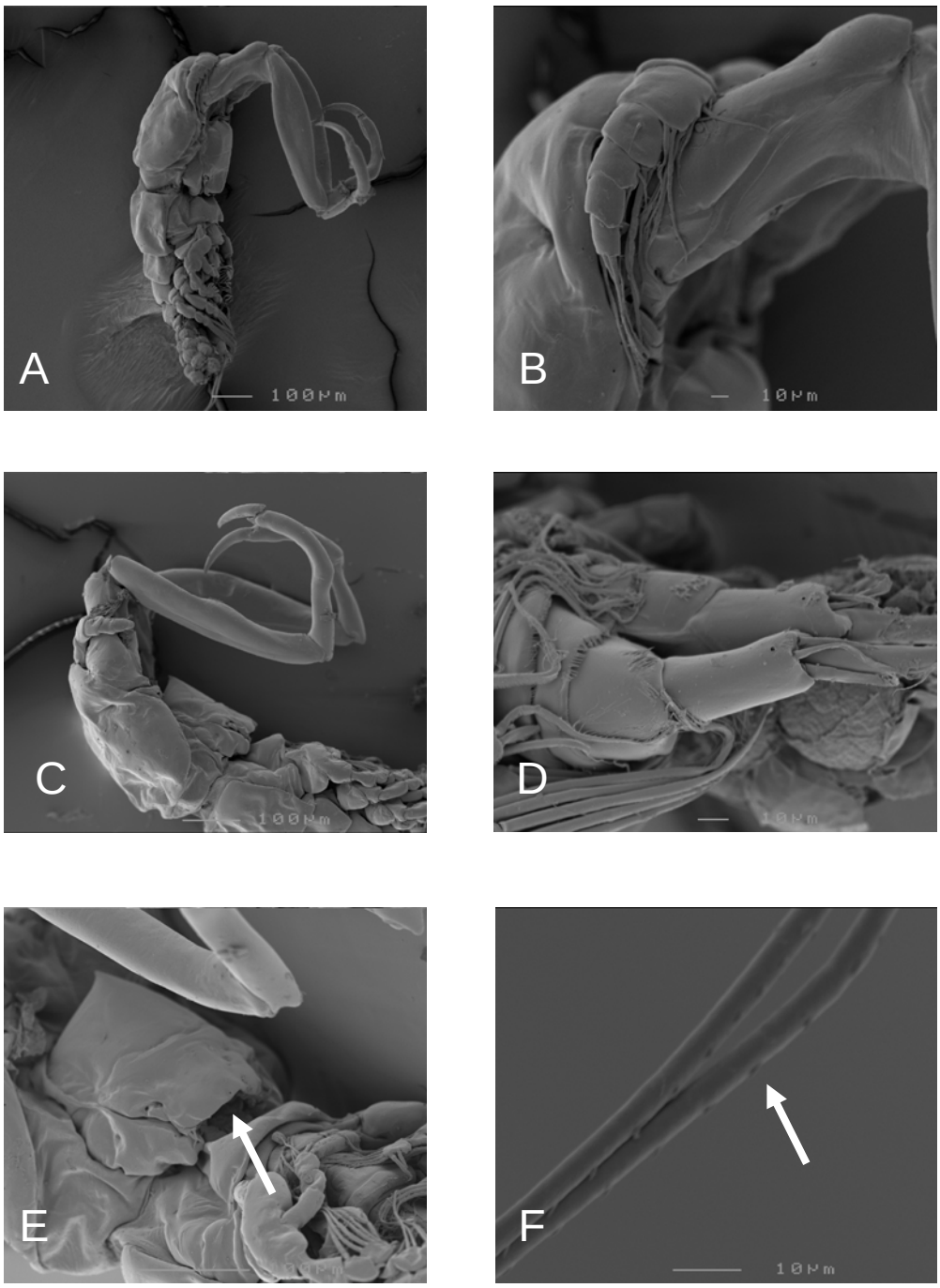
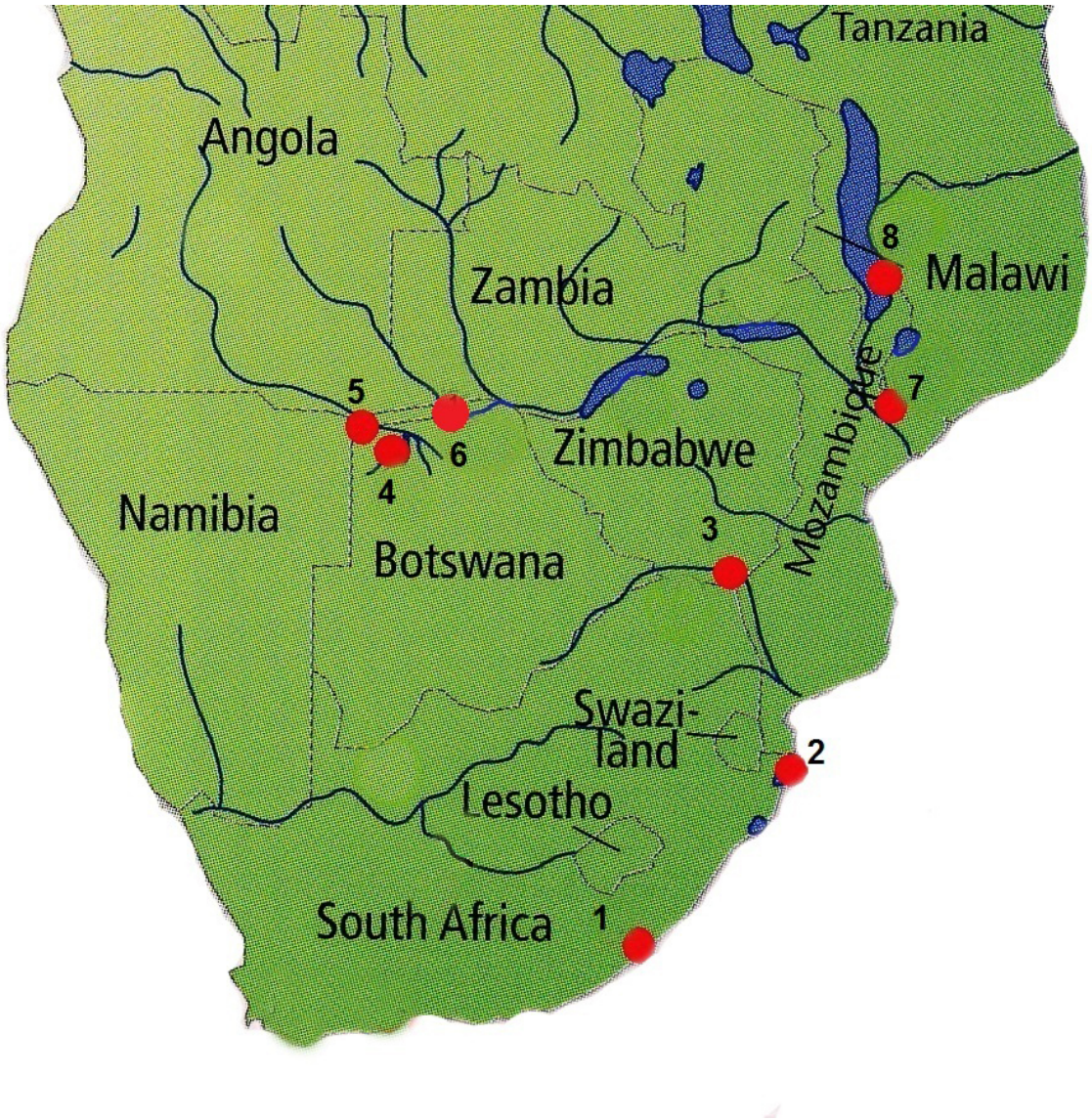


Figure 5.5

Scanning electron micrographs of *Ergasilus* van Nordmann, 1832 sp. A collected from *Synodontis leopardinus* Pellegrin, 1914 from Lake Liambezi

A. Lateral view of whole specimen
B. Antennule
C. Antenna and lateral view of cephalothorax
D. Furcal rami
E. Mouth parts (arrow)
F. Longest furcal seta with short spinules (arrow)

Figure 5.6: Map of southern African river systems indicating the distribution where *Ergasilus* sp. A was collected from the fish hosts.



2. Pongola River
3. Limpopo River
4. Okavango Delta
5. Okavango River
6. Lake Liambezi
7. Zambezi System

- ***Ergasilus* sp. B**

Type host

Family: Characidae

Rhabdalestes maunensis (Fowler, 1935)

Type locality

Shakawe, Okavango Delta (S18°26'05.0" ; E021°54'23.0")

Other hosts and distributions

Family: Characidae

Brycinus lateralis (Boulenger, 1900) (Okavango River and Delta, fig. 5.12)

Hydrocynus vittatus Castelnau, 1861 (Pongola River, fig. 5.12)

Family: Mormyridae

Petrocephalus wesselsi Kramer & van der Bank, 2000 (Okavango River, fig. 5.12)

Species description of female (Based on 10 specimens from *Rhabdalestes maunensis* (Fowler, 1935))

Total Length: Mean length 0.5mm (0.45 – 0.56mm range), measured from anterior end of cephalothorax to posterior margin of caudal rami, excluding furcal setae. Body gradually narrowing posteriorly.

Cephalothorax: Prosome 5-segmented, longer than wide, distinctly separated from first free pedigerous somite. Distinct distribution of sensory seta and pits on dorsal surface of cephalic shield (fig. 5.7 A).

Ornamentation: Dorsal surface of cephalic shield, with circular cephalic structure anterior to inverted T, oval cephalic structure posterior to inverted T. Inverted T only visible using light microscopy (fig. 5.7 A).

Pigmentation: Blue pigmentation present in cephalosome, only visible in unstained specimens.

Mouthparts: Maxilla, with one blade, with spinulose area (fig. 5.9 B). Mandible with three blades, anterior with row of distal bristles, medial blade with row of bristles on

outer margin, posterior blade slender with row of bristles along inner margin (fig. 5.9 A).

Thorax: Four-segmented, segments overlapping posteriorly, with narrow, less sclerotinised regions between segments. First pedigerous somite wide and broad, nearly twice as broad as second somite (fig. 5.11 B). Dorsal surface of segments with distinct distribution of sensory setae and pits (fig. 5.7 C). Ventral surface with row of comb-like setae situated medially, extending from left to right on intercoxal sclerite of first leg pair, plate with sensory pits at each end (fig. 5.8 A).

Legs: Legs 1 to 4 biramous, all legs 3-segmented, except 2-segmented exopod of fourth leg (fig. 5.10 A - D). All setae plumose, spine and setae formulae summarised in table 5.3.

Leg 5: Two-segmented with single short seta on basal segment, and two terminal setae on distal segment (fig. 5.10 E).

Table 5.3: Spine and seta formulae for *Ergasilus* von Nordmann, 1832 sp. B collected from *Rhabdalestes maunensis* (Fowler, 1935), from Shakawe, Okavango River.

	Coxa	Basis		*Segment 1	Segment 2	Segment 3
Leg 1	0	1	Exopodite	0 – 1	I – 1	II – 5
			Endopodite	0 – 1	0 – 1	II – 4
Leg 2	0	1	Exopodite	I – 0	0 – 1	0 – 6
			Endopodite	0 – 1	0 – 2	I – 4
Leg 3	0	1	Exopodite	I – 0	0 – 1	0 – 6
			Endopodite	0 – 1	0 - 2	I – 4
Leg 4	0	1	Exopodite	I – 0	0 – 5	-**
			Endopodite	0 – 1	0 – 2	I – 3

*Segment 1 closest to basis.

**All *Ergasilus* species lack this segment.

Abdomen: Urosome 5-segmented, with short fifth pedigerous somite, rounded genital somite, with three rows of fine pin-like scales on ventral surface and row of comb-like spines on posterior border. Posterior margin of first to second free

abdominal segment bordered by row of comb-like spines. Anal segment bilobed, with row of posterior comb-like spines on each lobe (fig. 5.8 C).

Furcal rami: Furcal rami longer than anal segment, two sensory pits near base of each ramus. Group of spines posterior to each sensory pit, four furcal setae present on each ramus, inner most longest, longer than abdomen (figs. 5.8 A; 5.11 C). Longest seta spinulose (fig. 5.11 D).

Antennule: Six-segmented (figs. 5.8 B; 5.11 A), tapering distally, seta formula from proximal to distal segments: 2 – 6 – 3 – 4 – 1 – 3.

Antenna: Mean length 0.3mm (0.3 – 0.31mm). Four-segmented, segments 1 and 2 slender, segment 3 broader with thin vellum-like strip on inner surface, all segments unadorned. Terminal hook capped (fig. 5.8 D).

Egg sacs: Shorter than total body length. Mean length 0.3mm (0.25 – 0.37mm) (figs. 5.7 B).

Remarks: *Ergasilus* sp. B differs from *E. cunningtoni*, *E. latus*, *E. sarsi*, *E. macrodactylus* and *E. mirabilis* by having a two-segmented fifth leg, while all five species have one segment. The following seven species differ from *Ergasilus* sp. B in the form and adornment of the antennae: *E. nodosus* has a swollen joint between segments 2 and 3. The first antennal segment of *E. danjiangensis* differs completely from that of *Ergasilus* sp. B, by being very broad in both segments. *Ergasilus kandti*, *E. lizae*, *E. boleopthalmi*, *E. sieboldi*, and *E. sittangensis* by having unadorned segments. *Ergasilus pararostralis*, *E. piriformis* and *E. rostralis* differs from *Ergasilus* sp. B by having a 5-segmented antennule, whilst *Ergasilus* sp. B has six segments. *Ergasilus briani* and *E. lobus* differs from *Ergasilus* sp. B by not having an inverted T structure on the dorsal side of the cephalothorax. The spine and seta formula from *Ergasilus* sp. B differ from *E. hypomesi*. *Ergasilus lamellifer* and *Ergasilus* sp. B are very similar, except for the indentation in the third antenna segment of *E. lamellifer,* that is not present in *Ergasilus* sp. B and the differences in the spine and seta formulae. *Ergasilus* sp. B differs from *Ergasilus* sp. A by the presence of a lamella-like strip on the second antenna segment, and by having a 6-segmented antennule. But the spine and seta formulae for these two species are the same.

Based on the above description and the differences between *Ergasilus* sp. B and the known species there is little doubt that this is a new species. The following outstanding characteristic distinguishes this species from the others:

- Antenna with a very broad second segment with a lamella-like strip.

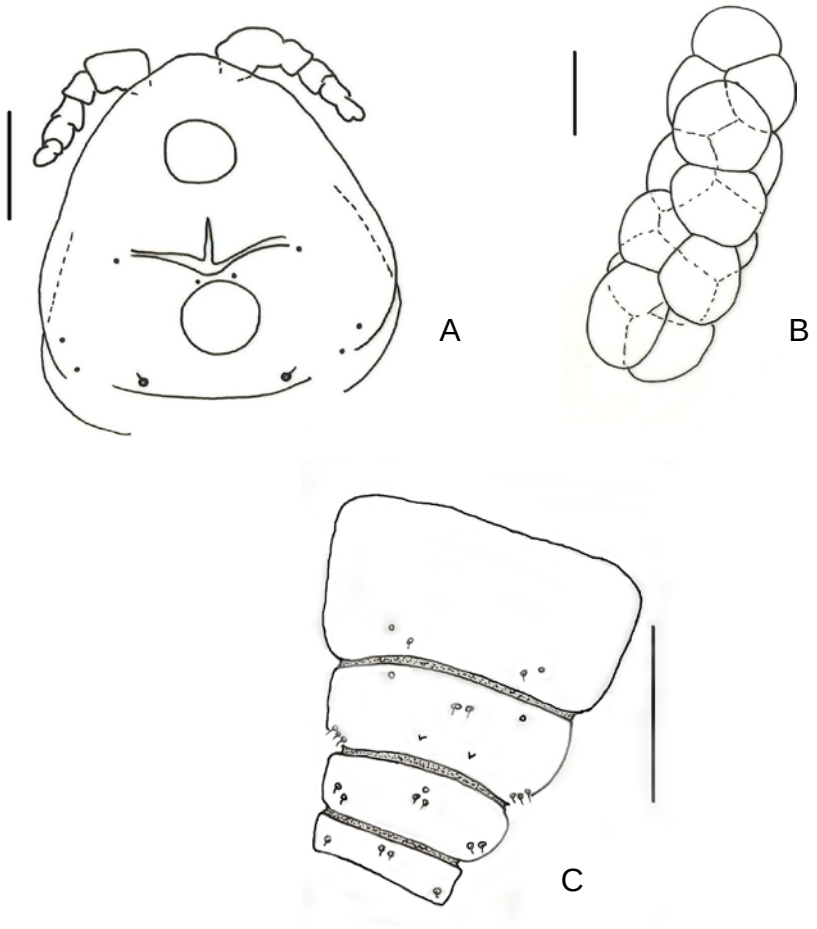


Figure 5.7

Line drawings of *Ergasilus* van Nordmann, 1832 sp. B collected from *Rhabdalestes maunensis* (Fowler, 1935) from Shakawe, Okavango River.

A. Dorsal view of cephalic shield (50µm)
B. Egg sac (100µm)
C. Dorsal view of thoracic segments (100µm)

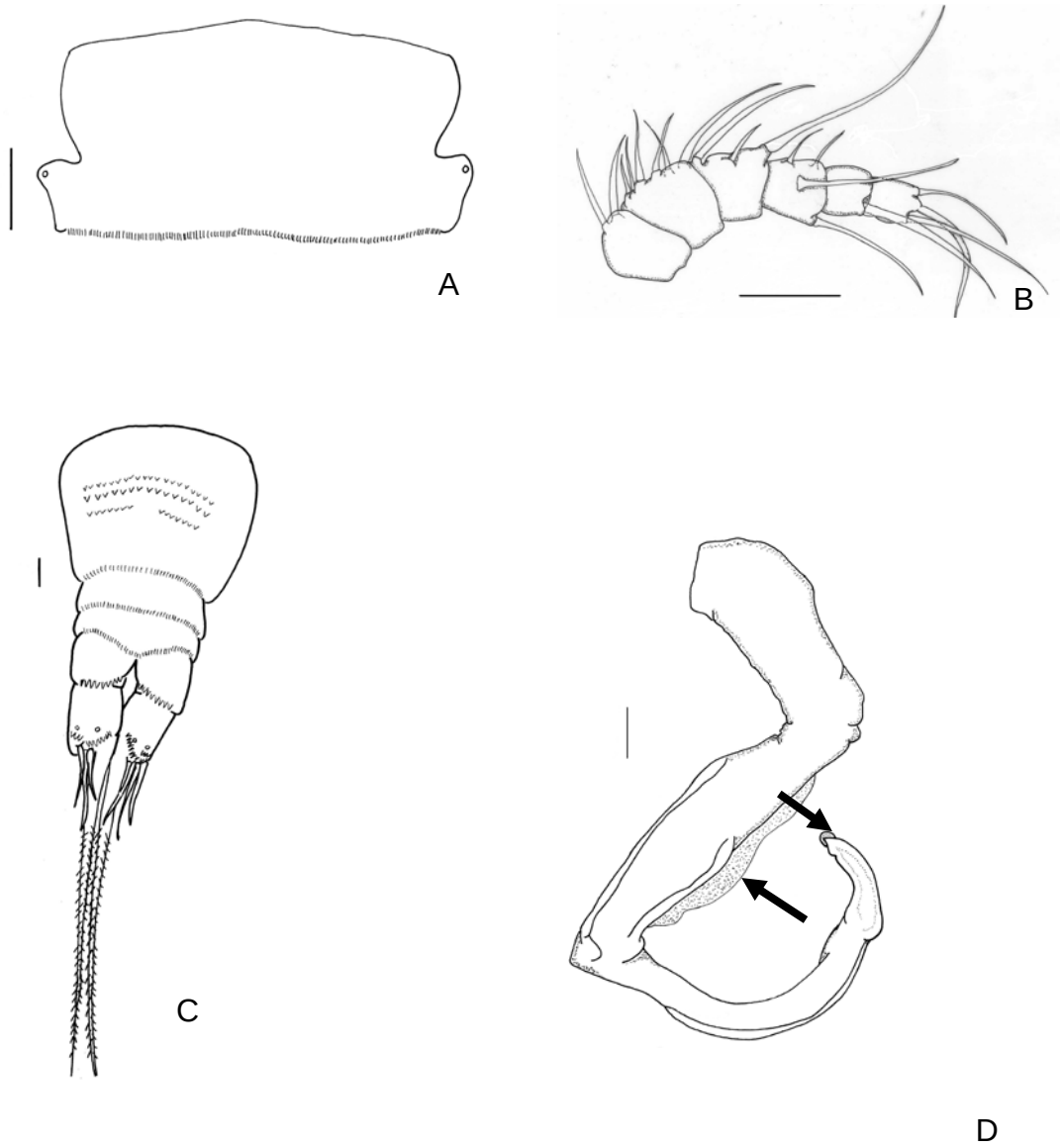


Figure 5.8

Line drawings of *Ergasilus* van Nordmann, 1832 sp. B collected from *Rhabdalestes maunensis* (Fowler, 1935) from Shakawe, Okavango River.

A. Intercoxal sclerite of first leg pair (10µm)
B. Antennule (10µm)
C. Dorsal view of abdomen and furcal rami (10µm)
D. Antenna with vellum-like stip and capped hook (arrows) (10µm)

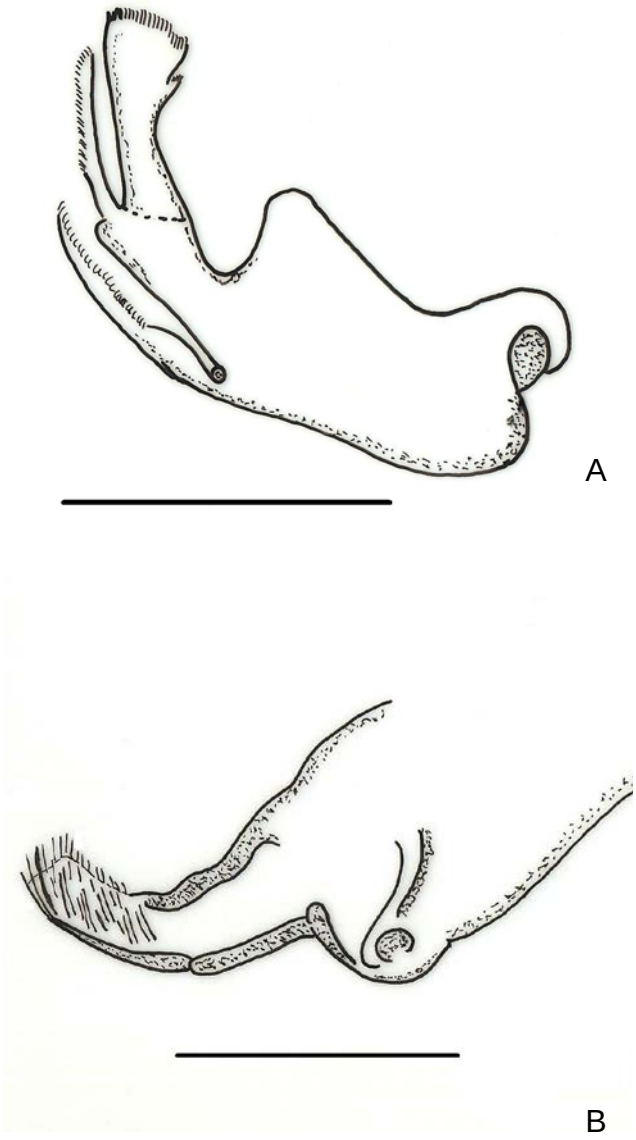


Figure 5.9

Line drawings of *Ergasilus* van Nordmann, 1832 sp. B collected from *Rhabdalestes maunensis* (Fowler, 1935) from Shakawe, Okavango River.

A. Mandible (10µm)
B. Maxilla (10µm)

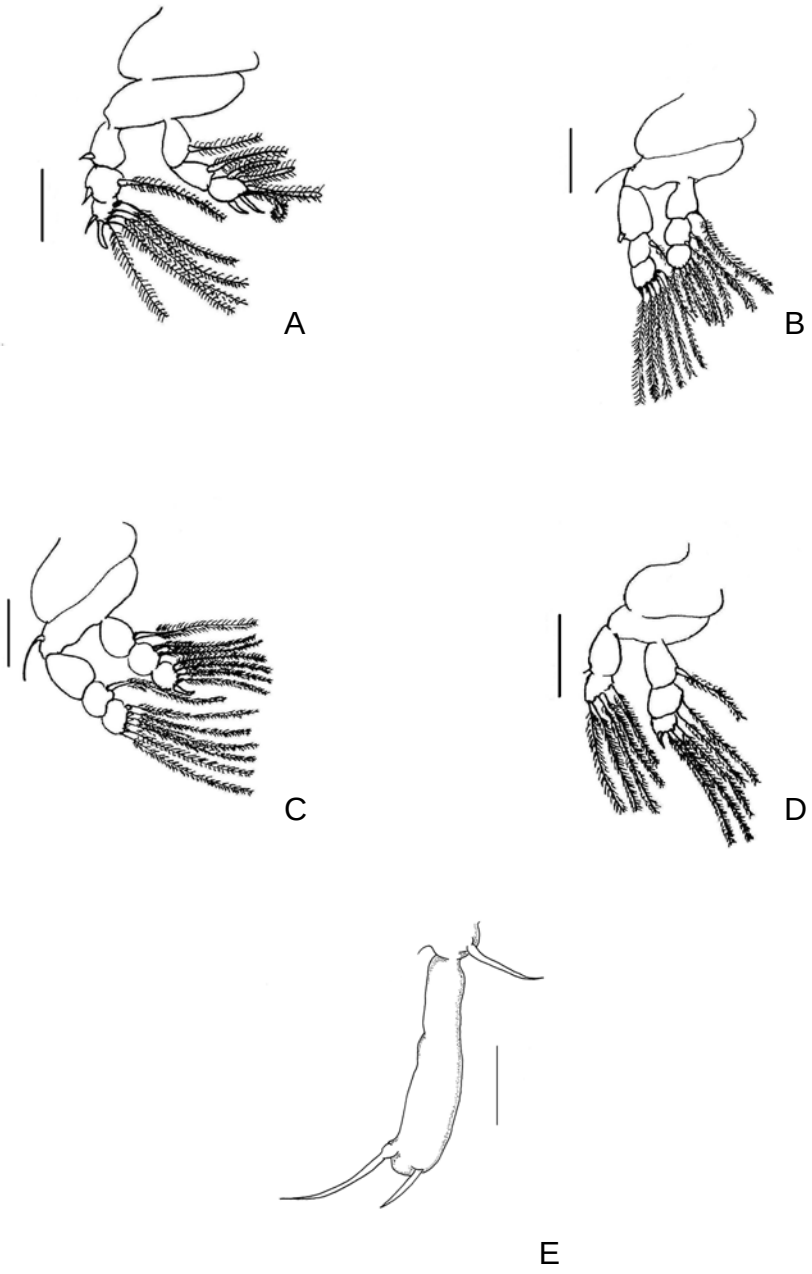


Figure 5.10

Line drawings of legs of *Ergasilus* van Nordmann, 1832 sp. B collected from *Rhabdalestes maunensis* (Fowler, 1935) from Shakawe, Okavango River.

A. Leg 1 (50µm)
B. Leg 2 (50µm)
C. Leg 3 (50µm)
D. Leg 4 (50µm)
E. Leg 5 (10µm)

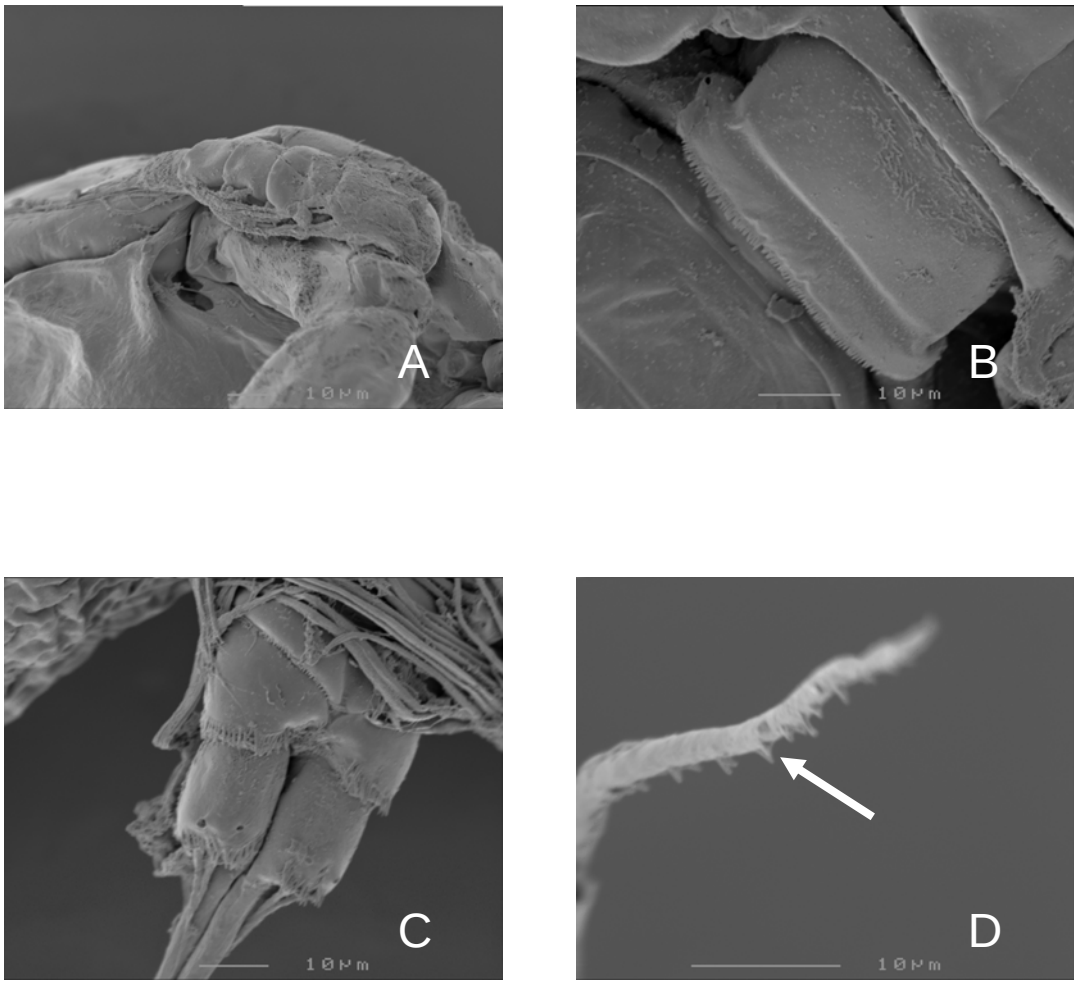
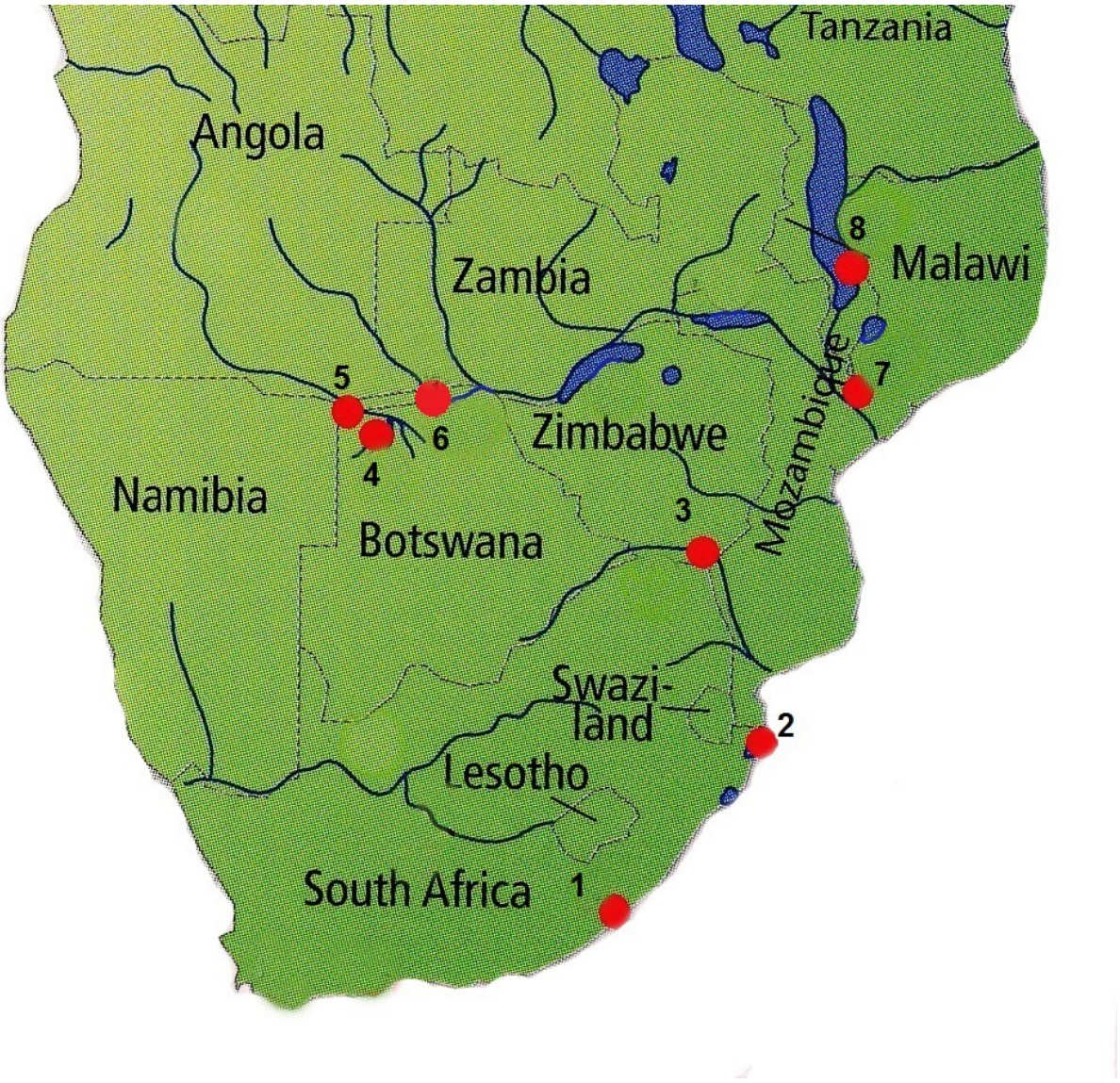


Figure 5.11

Scanning electron micrographs of *Ergasilus* van Nordmann, 1832 sp. B collected from *Rhabdalestes maunensis* (Fowler, 1935) from Shakawe, Okavango River.

A. Antennule
B. Intercoxal sclerite of first leg pair
C. Furcal rami
D. Longest furcal seta with spines (arrow)

Figure 5.12: Map of southern African river systems indicating the distribution where *Ergasilus* sp. B was collected from the fish hosts.



2. Pongola River

5. Okavango River

4. Okavango Delta

- ***Ergasilus* sp. C**

Host:

Family:

Micropterus punctulatus Rafinesque, 1819

Locality:

Kowie Estuary (S 34° 42’ 36.04” ; E 20° 06’ 23.47”) (fig. 5.17)

Species description of female: (Based on 15 specimens from *Micropterus punctulatus* Rafinesque, 1819)

Total length: Mean length 0.55mm (0.53 – 0.6mm) measured from anterior end of cephalothorax to posterior margin of caudal rami, excluding furcal setae (figs. 5.13 A; 5.16 A). Body gradually narrowing posteriorly.

Cephalothorax: Prosome 5-segmented. Cephalothorax as long as wide, distinctly separated from first free pedigerous somite, with lob-like indentations on the anterior side of the shield (figs. 5.13. A ; 5.16 B).

Ornamentation: Dorsal surface of cephalic shield, with inverted T structure (fig. 5.13. A).

Pigmentation: No pigmentation observed.

Mouthparts: Maxilla with very large basis and single blade with anterior spinulose area (fig. 5.14 C). Mandible with three blades, all with anterior spinulose area. Large spine-like protrusion on basis anterior to third blade (fig. 5.14 B).

Thorax: Four-segmented, segments with distinct dorsal shields, decreasing in size and overlapping posteriorly (fig. 5.13 A).

Legs: Legs 1 to 4 biramous, all legs 3-segmented, except 2-segmented exopod of fourth leg, all setae plumose (fig. 5.15 A-D); spine and seta formulae summarised in table 5.5.

Leg 1 - first to third exopodal segments with ridge of short spines on inner margin. Third endopodal segment with single long sharp spine on outer margin (fig. 5.15 A).

Leg 2 - with single short seta on outer margin. First exopodal segment with row of short setae on outer margin. First to third endopodal segments with ridge of short spines on outer margin. Third endopodal segment with elongated spine on outer margin (fig. 5.15 B).

Leg 3 and **Leg 4** typical of genus.

Leg 5 - one-segmented with two distal setae and one medial seta.

Table 5.5. Spine-seta formulae for *Ergasillus* von Nordmann, 1832 sp. C collected from *Micropterus punctulatus* Rafinesque, 1819 from the Kowie Estuary.

	Coxa	**Basis**		***Segment 1**	**Segment 2**	**Segment 3**
Leg 1	0	0	Exopodite	I-0	I-0	II-5
			Endopodite	0-1	0-1	II-4
Leg 2	0	1	Exopodite	0-0	0-1	0-6
			Endopodite	0-1	0-2	I-5
Leg 3	0	0	Exopodite	0-0	I-1	0-6
			Endopodite	0-1	0-2	I-4
Leg 4	0	0	Exopodite	I-0	0-5	-**
			Endopodite	0-1	0-1	0-4

* Segment 1 closest to basis.

**All *Ergasilus* species lack this segment.

Abdomen: Four-segmented, fifth leg closely associated with genital complex. Third, free abdominal segment and furcal ramus fused, not distinct. Short seta on anterior end of dorsal surface (figs. 5.13 B; 5.16B).

Furcal rami: Four furcal setae, inner most longest and spinulose (figs. 5.13 B ; 5.16 C).

Antennule: Six-segmented tapering distally. Seta formula from proximal to distal segments: 5 – 5 – 2 – 2 - 5 (fig. 5.14 A).

Antennae: Mean length 0.37mm (0.31 – 0.4mm), Segments slender and unadorned. Third segment with characteristic indentation (fig. 5.13 A).

Egg sacs: Mean length 0.43mm (0.36 - 0.51mm). Shorter than average body length (fig. 5.13 A).

Remarks: *Ergasilus* sp. C differs from *E. cunningtoni* by having four furcal setae and from *E. flaccidus, E. latus, E. macrodactylus, E. megacheir, E. mirabilis* as well as *E. lamellifer* by having different spine and seta formulae. *Ergasilus* sp. C differs from *E. nodosus* by having a fifth leg, whilst *E. nodosus* does not. *Ergasilus kandti* differs by having a short spine on the third antennal segment. The first antenna segment of *E. danjiangensis* differs completely from that of *Ergasilus* sp. C, by being very broad. *Ergasilus kandti*, *E. lizae*, *E. boleopthalmi*, *E. sieboldi*, and *E. sittangensis* differ from *Ergasilus* sp. C by having adorned antennal segments, whilst *Ergasilus* sp. C has unadorned segments. The cephalic shield of *Ergasilus* sp. C differs from all the mentioned species as well as *E. latus*, *E. sarsi*, *E. danjiangensi, E. lizae*, *E. briani*, *E. pararostralis*, *E. piriformis*, *E. lobus*, *E. rostralis*, and *Ergasilus* sp. A by having lob-like indentations on the anterior side of the shield. *Ergasilus* sp. C differs from *E. hypomesi* by having a different spine and seta formula. *Ergasilus* sp. C differs from *Ergasilus* sp. B by the absence of a lamella-like strip on the second antenna segment. *Ergasilus* sp. C differs from *E. inflatipes* by not having plumose setae on the antennule segments, whilst *E. inflatipes* have plumose setae on antennule segments 4 to 6.

Based on the above description and the differences between *Ergasilus* sp. C and the known species there is little doubt that this is a new species. It has a number of outstanding morphological characteristics that distinguishes this species from the other known species. *Ergasilus* sp. C differ from *E.* sp. A and *E.* sp. B in that *E.* sp. A and *E.* sp. B have circular cephalic structures and *E.* sp. C does not.

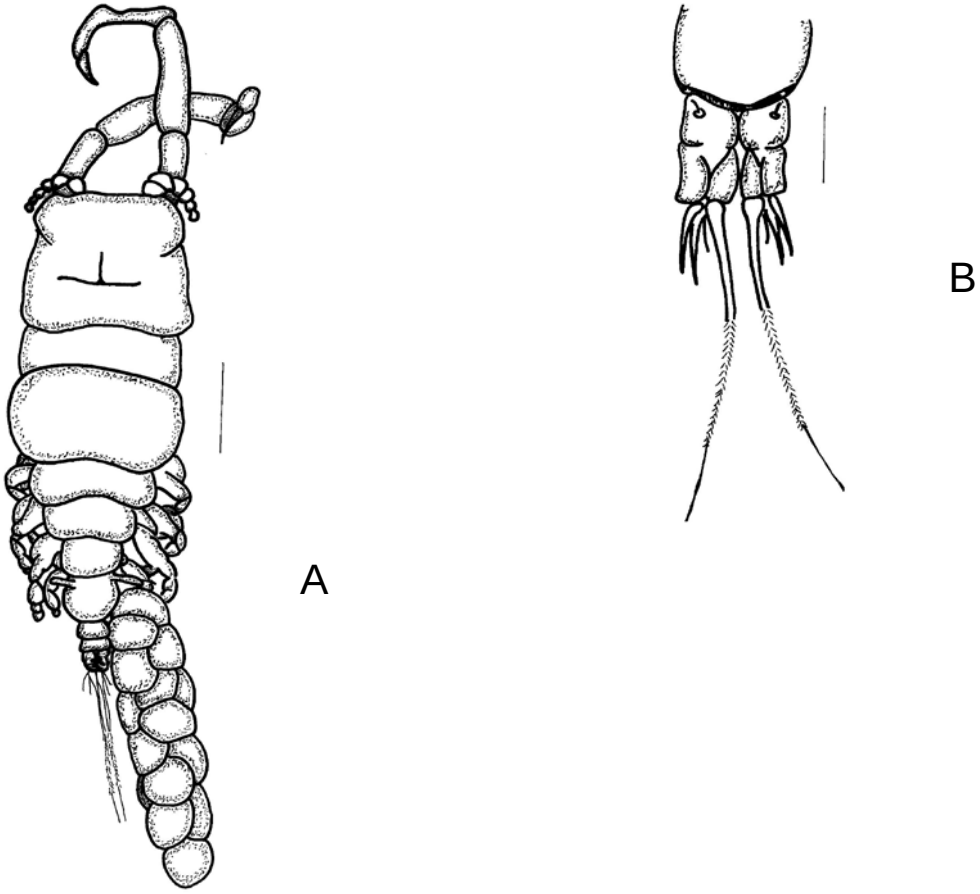


Figure 5.13

Line drawings of *Ergasilus* van Nordmann, 1832 sp. C collected from *Micropterus punctulatus* Rafinesque, 1819 from the Kowie Estuary.

A. Dorsal view of whole specimen (100µm)
B. Dorsal view of third abdominal segment and furcal ramus (50µm)

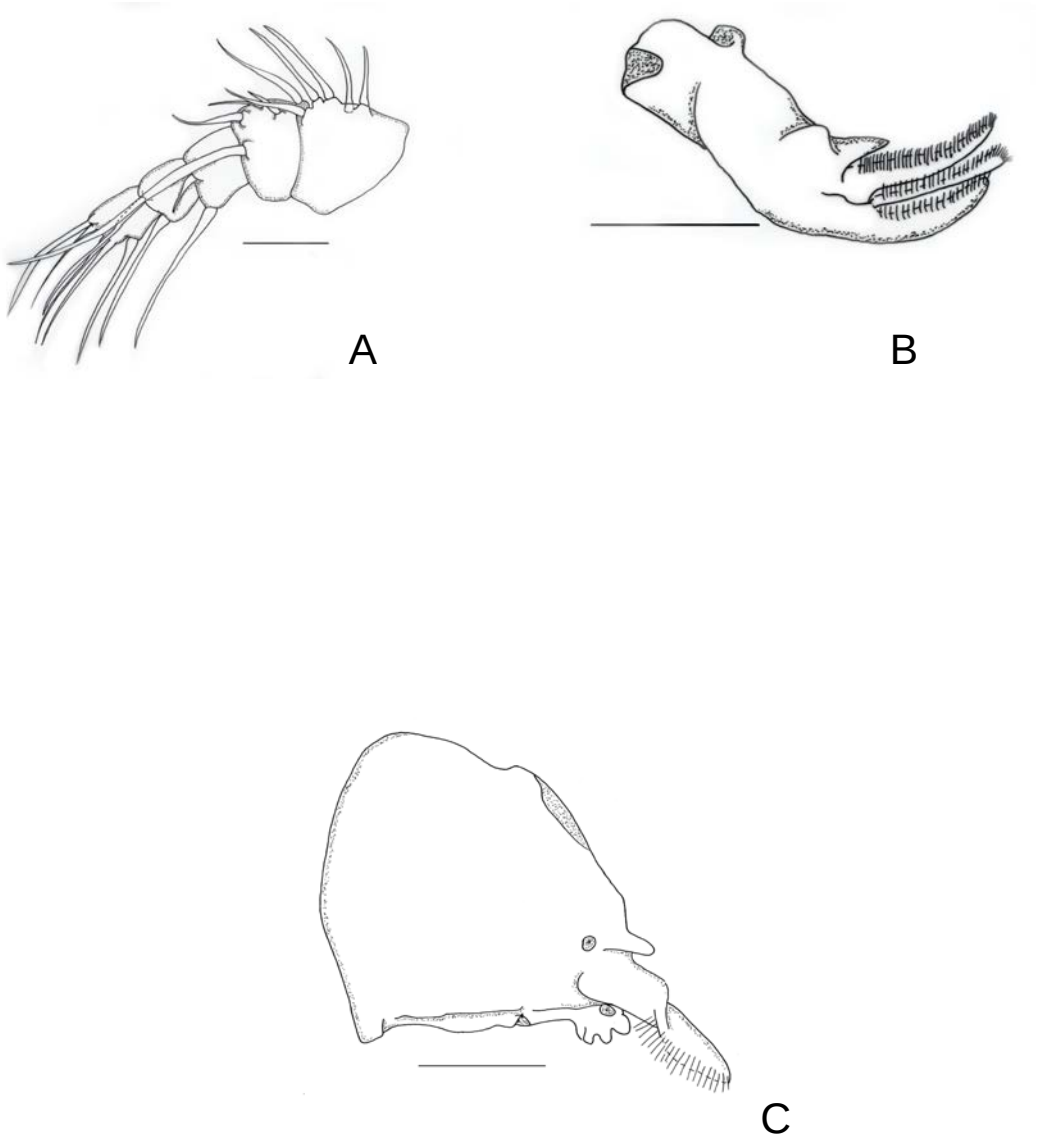


Figure 5.14

Line drawings of *Ergasilus* van Nordmann, 1832 sp. C collected from *Micropterus punctulatus* Rafinesque, 1819 from the Kowie Estuary.

A. Antennule (10µm)
B. Mandible (10µm)
C. Maxilla (10µm)

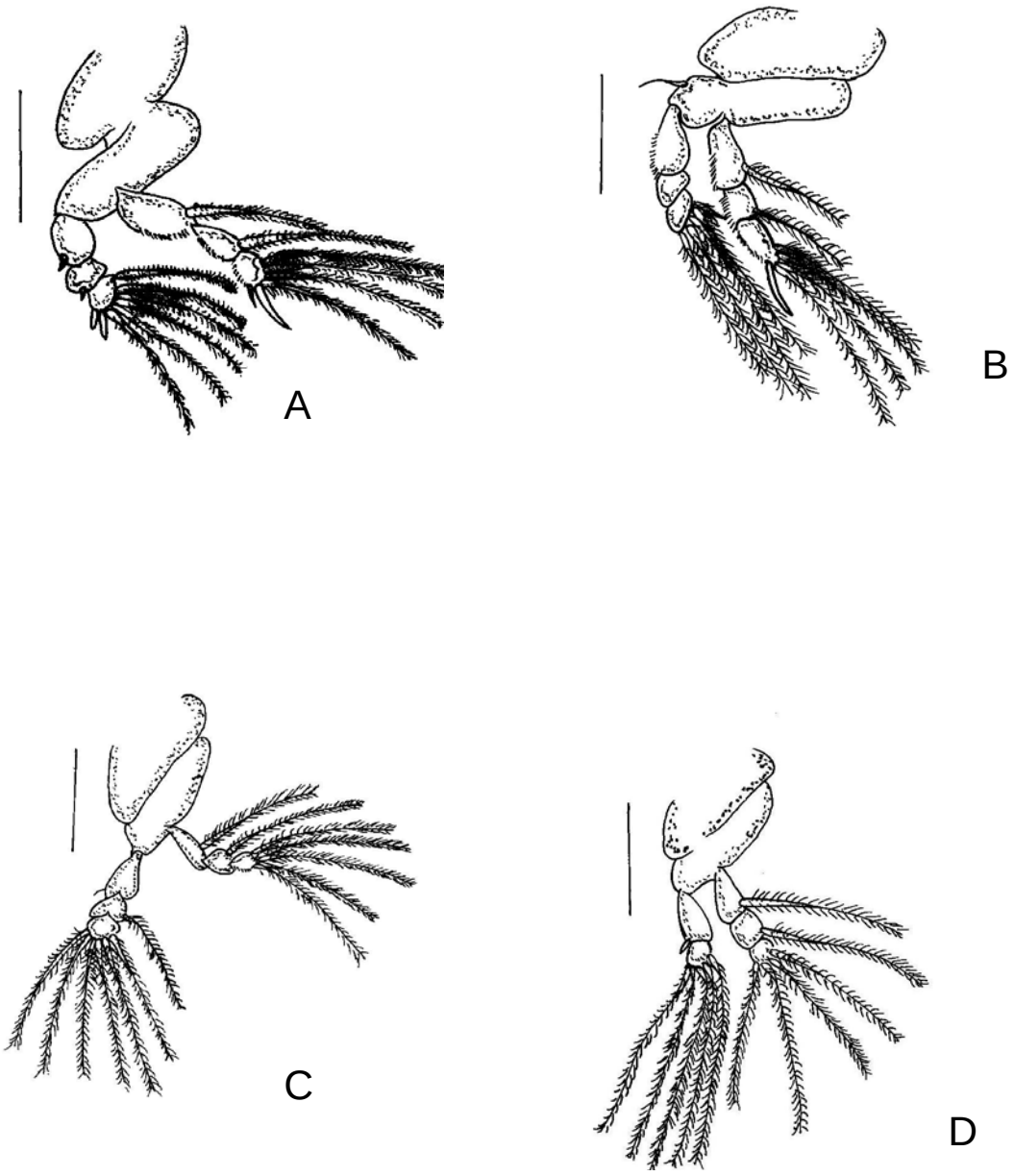


Figure 5.15

Line drawings of *Ergasilus* van Nordmann, 1832 sp. C collected from *Micropterus punctulatus* Rafinesque, 1819 from the Kowie Estuary.

A. Leg 1 (50µm)
B. Leg 2 (50µm)
C. Leg 3 (50µm)
D. Leg 4 (50µm)

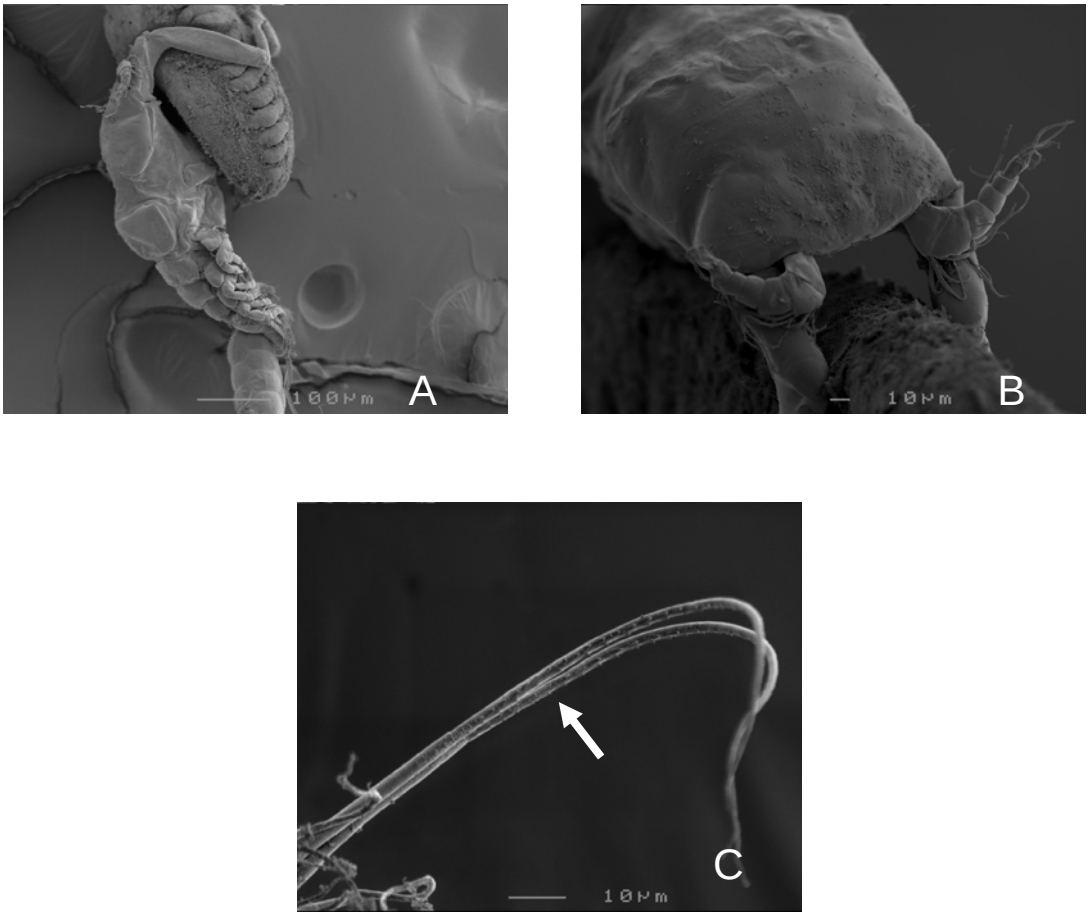
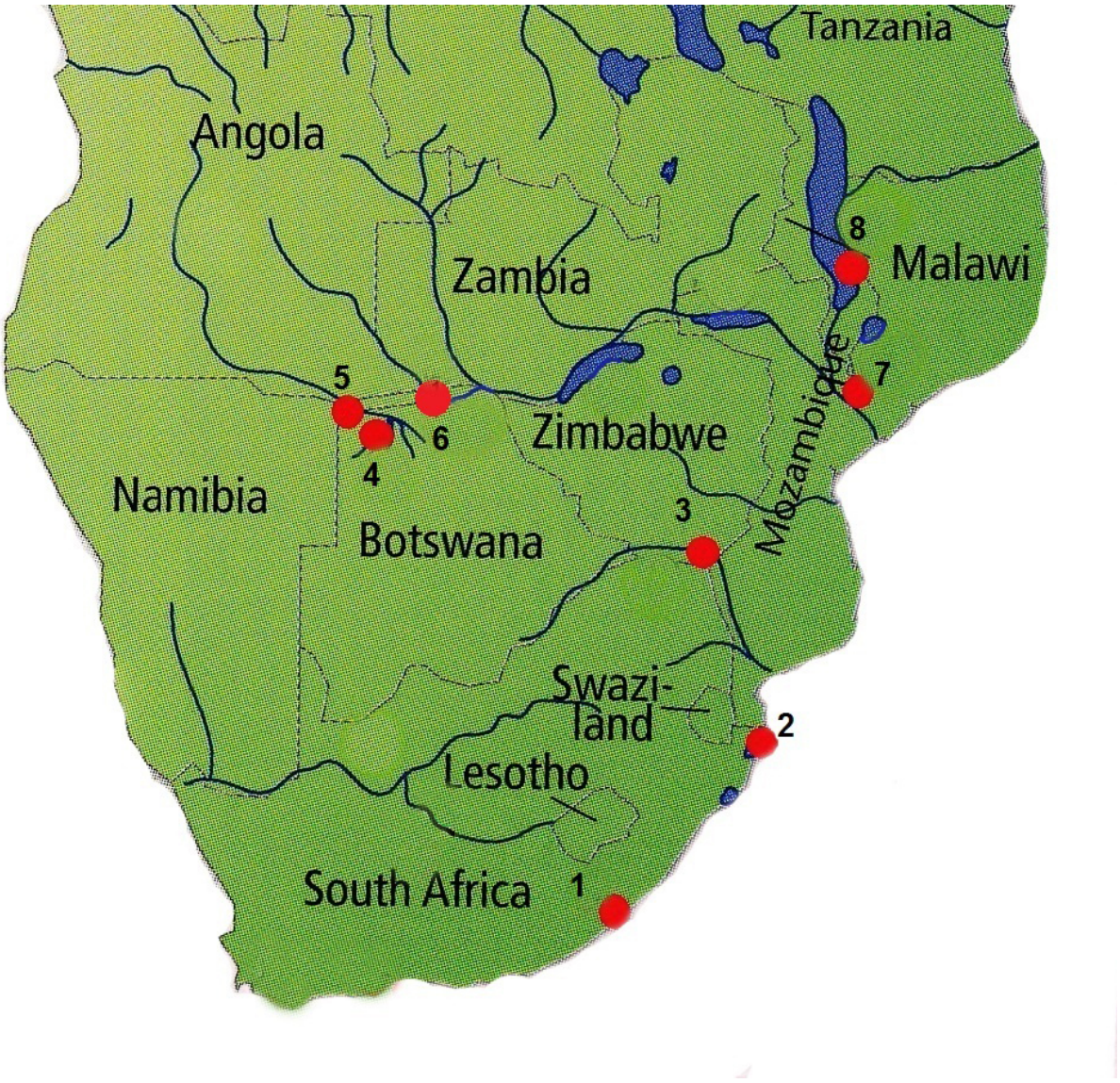


Figure 5.16

Scaning electron micrograph of *Ergasilus* van Nordmann, 1832 sp. C collected from *Micropterus punctulatus* Rafinesque, 1819 from the Kowie Estuary.

A. Lateral view of whole specimen
B. Dorsal view of cephalic shield and antennulae
C. Furcal setae with short scales (arrow)

Figure 5.17: Map of southern African river systems indicating the distribution where *Ergasilus* sp. C was collected from the fish host.



1. Kowie Estuary.

- ***Ergasilus* sp. D**

Type host:

Family: Chichlidae

Petrotilapia tridentiger Trewavas, 1935

Locality:

Lake Malawi (S 34º 42' 36.04" ; E 20º 06' 23.47") (fig. 5.21)

Other hosts and distributions

Family: Chichlidae

Petrotilapia genalutea Marsh, 1983 (Lake Malawi, fig. 5.21)

P. nigra Marsh, 1983 (Lake Malawi fig. 5.21)

Tyrannochromis macrostoma Regan, 1922 (Lake Malawi, fig. 5.21)

Species description of female (Based on 11 specimens)

Total length: Mean length 0.76mm (0.57 – 0.8mm) measured from anterior end of cephalothorax to posterior margin of caudal rami, excluding furcal setae (figs. 5.18 A & B). Body gradually narrowing posteriorly.

Cephalothorax: Prosome 5-segmented. Cephalothorax slightly tapering anteriorly, distinctly separated from first free pedigerous somite, with slight indentations on anterior side of the cephalic shield (figs. 5.18 D).

Ornamentation: Dorsal surface of cephalic shield, with circular cephalic structure anterior to inverted T, oval cephalic structure posterior to inverted T. Distinct distribution of small circular indentations, anterior and poster of oval structure (fig. 5.18 D).

Pigmentation: No pigmentation observed.

Mouthparts: Maxilla with single blade covered by spinules (fig. 5.19 D). Mandible with three blades, all with anterior spinulose area (fig. 5.19 C).

Thorax: Four-segmented, distinct dorsal shields, decreasing in size. Narrow, less sclerotinised region between 2 and 3 and segments 3 and 4. Sensory setae and pits present on dorsal side of thoracic segments 1 to 4, absent on segment 5. Intercoxal sclerite of legs 1 to 3 with fine comb-like spines along posterior margin, single sensory pit on both posterior corners (fig. 5.18 A).

Legs: Legs 1 to 4 biramous, all legs 3-segmented, except 2-segmented exopod of fourth leg, all setae plumose (fig. 5.20 A-D); spine and setae formulae similar to other *Ergasilus* species, summarised in table 5.6.

Leg 1 - first exopodal segment with fine spine on inner margin. First endopodal segment with short setae on outer margin. Second and third endopodal segments with ridge of very fine spines on outer margins. Third endopodal segment with two very broad spines each with ragged outer edge (fig. 5.20 A).

Leg 2 - basis of leg 2 with row of comb-like spines on ventral surface. Third exopodal segment with short, fine spines on outer margin. Second and third endopodal segment with short, fine spines on outer margin. Third endopodal segment with very broad ragged edged spine on outer margin (fig. 5.20 B).

Leg 3 - third exopodal segment with row of very fine spines on outer margin. Second and third endopodal segments with rows of very fine spines on outer margin. Third endopodal segment with very broad ragged edged spine (fig. 5.20 C).

Leg 4 - basis of leg 2 with row of tooth-like spines on ventral surface. All endopodal segments with row of very fine spines on outer margin (fig. 5.20 C).

Leg 5 - two-segmented with single seta on basal segment, and two terminal setae on distal segment (fig. 5.20 E).

Table 5.6. Spine-seta formulae for *Ergasilus* von Nordmann, 1832 sp. D collected from *Petrotilapia tridentiger* Trewavas, 1935 from Lake Malawi.

	Coxa	Basis		*Segment 1	Segment 2	Segment 3
Leg 1	0	0	Exopodite	I-0	I-1	II-5
			Endopodite	0-1	0-1	II-4
Leg 2	0	1	Exopodite	I-0	0-1	0-6
			Endopodite	0-1	0-2	I-5
Leg 3	0	0	Exopodite	I-0	0-1	0-6
			Endopodite	0-1	0-2	I-4
Leg 4	0	0	Exopodite	I-0	0-5	-**
			Endopodite	0-1	0-2	I-3

* Segment 1 closest to basis.

**All *Ergasilus* species lack this segment.

Abdomen: Abdomen 4-segmented, genital complex with small comb-like spines along posterior ventral margin. Segments 2 and 3 with small comb-like spines along posterior ventral margin. Segment 4 with line of approximately eight larger tooth-like spines (fig. 5.18 B).

Furcal rami: Longer than wide with two sensory pits near posterior end, posterior to sensory pits two groups of tooth-like spines. Each ramus with 4 furcal setae, inner most longest (figs. 5.18 B; 5.18 C).

Antennulae: Six-segmented tapering distally. Seta formula from proximal to distal segments 2 – 8 – 5 – 4 – 2 – 6 (fig. 5.19 A).

Antennae: Mean length 0.67mm (0.6 – 0.72mm), Segments slender and unadorned (fig. 5.19 A).

Egg sacs: Mean length 0.47mm (0.41 - 0.5mm), almost length of total body length (fig. 5.18 C).

Remarks: *Ergasilus* sp. D differs from *E. cunningtoni*, *E. kandti*, *E. latus, E. macrodactylus, E. megacheir, E. mirabilis* and *E. sarsi* by having a 2-segmented fifth leg, whilst these seven species all have a single-segmented fifth leg. *Ergasilus* sp. A, *E. nodosus*, *E. flaccidus*, *E. lamellifer*, *E. danjiangensis*, *E. kandti*, *E. lizae*, *E. boleopthalmi*, *E. sieboldi* and *E. sittangensis* differ in the form of their antennae.

Ergasilus sp. A has an unadorned antenna with a twisted third segment, and *E. nodosus* has a swollen joint between segments 2 and 3. *Ergasilus flaccidus* has a chitinous lamella on the inner margin of the second antennal segment. The first antenna segment of *E. danjiangensis* differs completely from that of *Ergasilus* sp. D, by being very broad. *Ergasilus kandti*, *E. lizae*, *E. boleopthalmi*, *E. sieboldi*, and *E. sittangensis* differs from *Ergasilus* sp. D by having adorned antennal segments, whilst *Ergasilus* sp. D has unadorned segments. *Ergasilus* sp. D differs from *Ergasilus* sp. B by the absence of a lamella-like strip on the second antenna segment. *Ergasilus* sp. D differs from *Ergasilus* sp. C by the presence of circular cephalic structures. *Ergasilus* sp. D differs from *E. hypomesi* by having a different spine and setae formula.

Based on the above description and the differences between *Ergasilus* sp. D and the known species there is little doubt that *Ergasilus* sp. D is a new species. The following character distinguish this species from the others:

- Legs 2, 3 and 4 have distinct ridges of comb-like spines on the outer margin, and ragged edged spines on the exopodal and endopodal segments of legs 1 to 4.
- The distinct distribution of small circular indentations, anterior and poster, of the oval structure.
- The cephalothorax with slight indentations on the anterior side of the cephalic shield.

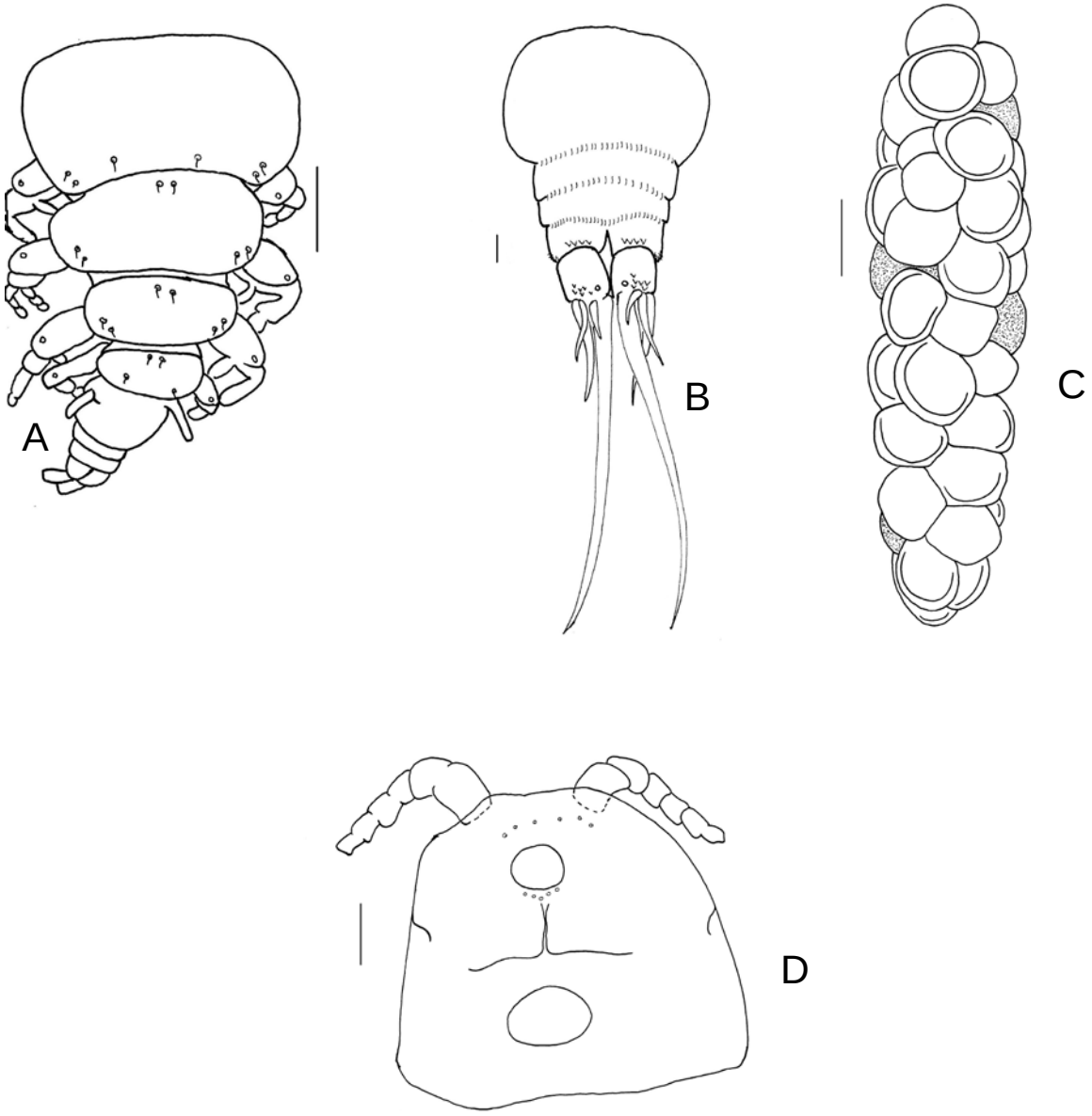


Figure 5.18

Line drawings of *Ergasilus* van Nordmann, 1832 sp. D collected from *Petrotilapia tridentiger* Trewavas, 1935 from Lake Malawi.

A. Dorsal view of thorax (100µm)
B. Ventral view of abdomen and furcal ramus (50µm)
C. Egg sac (100µm)
D. Dorsal view of cephalic shield (50µm)

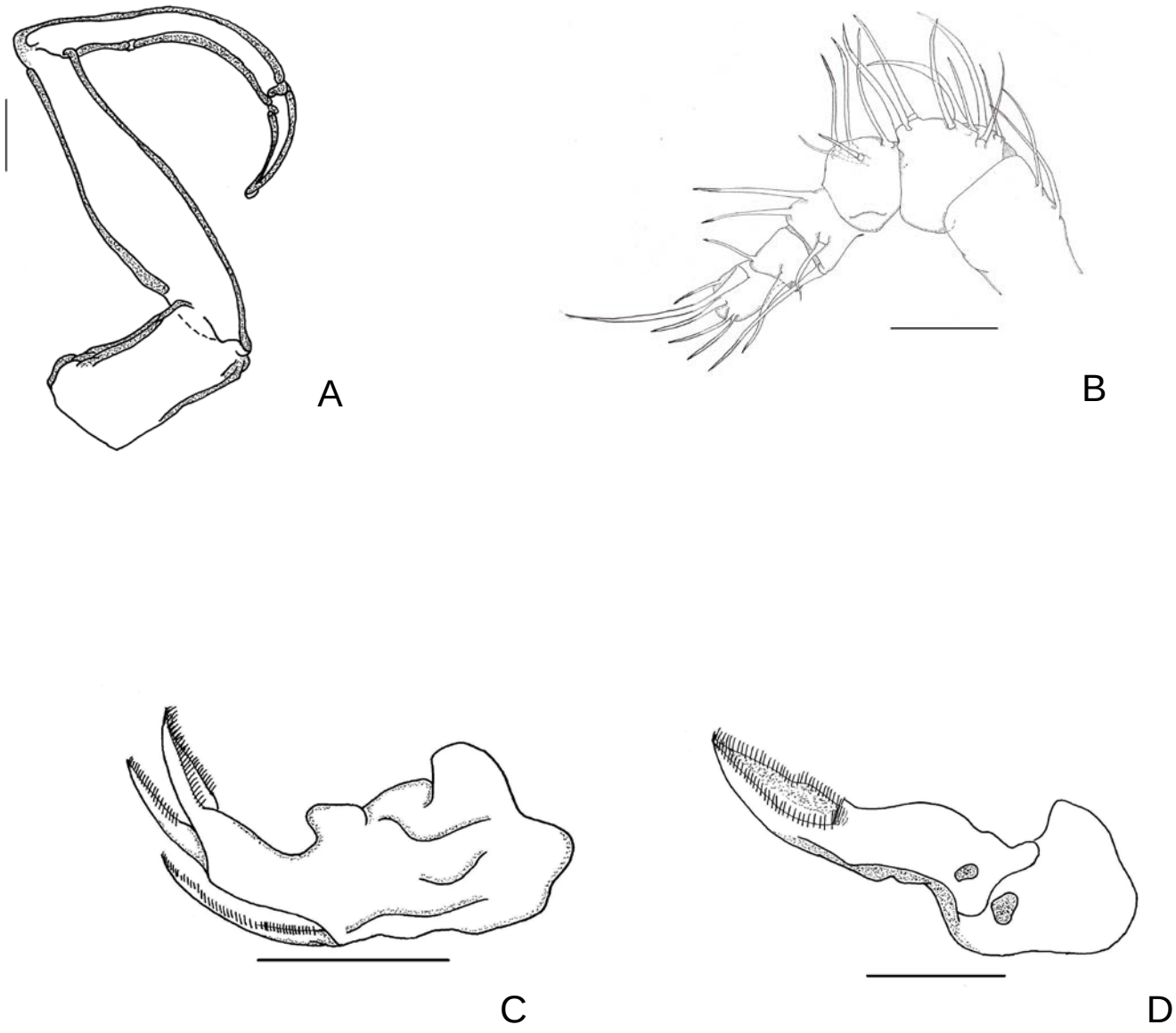


Figure 5.19

Line drawings of *Ergasilus* van Nordmann, 1832 sp. D collected from from *Petrotilapia tridentiger* Trewavas, 1935 from Lake Malawi.

A. Antenna (50µm)
B. Antennule (10µm)
C. Mandible (10µm)
D. Maxilla (10µm)

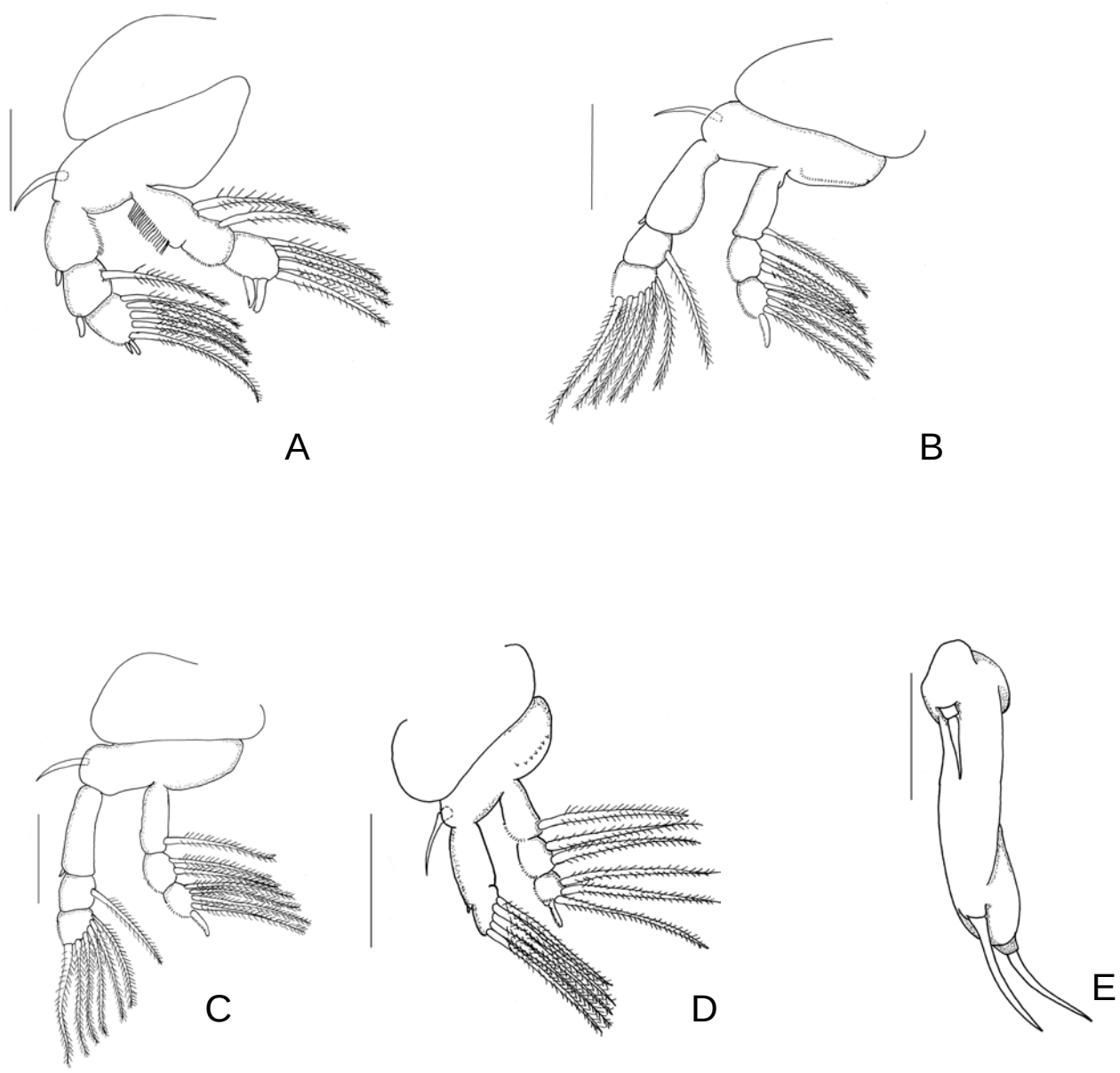
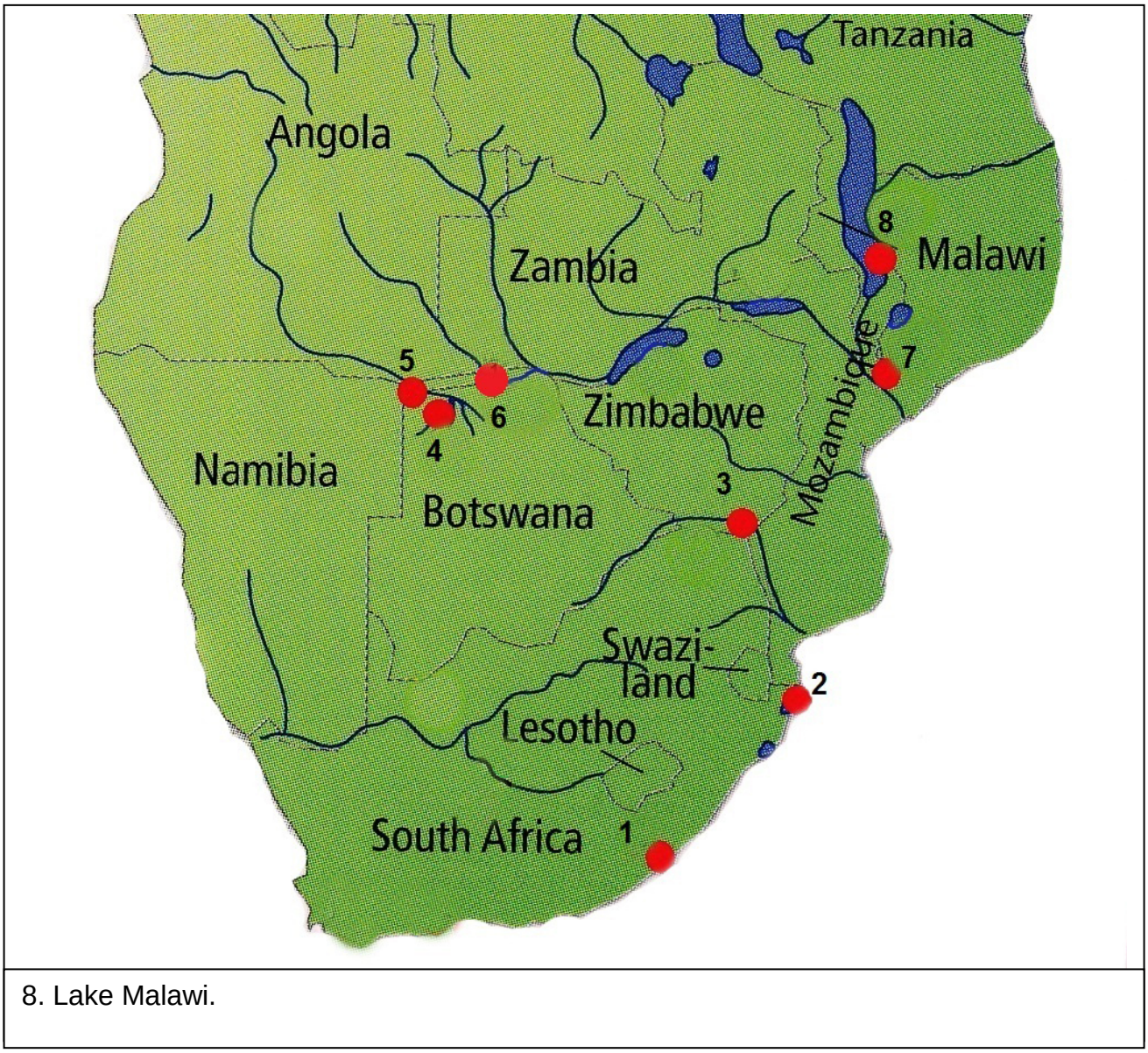


Figure 5.20

Line drawings of *Ergasilus* van Nordmann, 1832 sp. D collected from *Petrotilapia tridentiger* Trewavas, 1935 from Lake Malawi.

A. Leg 1 (50µm)
B. Leg 2 (50µm)
C. Leg 3 (50µm)
D. Leg 4 (50µm)
E. Leg 5 (50µm)

Figure 5.21: Map of southern African river systems indicating the distribution where *Ergasilus* sp. D was collected from its fish host.



8. Lake Malawi.

- **Molecular results – Phylogenetic analysis using the sequences**

A total of 11 samples, nine *Ergasilus* and two *Lernaea* specimens, were used in the DNA extraction, done with the phenol/chloroform extraction method. The extracted DNA samples were analysed using a Thermo Scientific Nano Drop Lite Spectrophotometer. The spectrometry results readings are given in table 5.8.

Table 5.8: The spectrometry readings from the DNA extraction process, indicating the A260, A260/A280 and concentration values.

Sp. No.	Parasite host	Collection date	A260 (10mm)	A260/ A280	Concentration ng/µL
1	*Synodontis nigromaculatus* Boulenger, 1970	27/7/2013	2.766	1.65	138.3
2	*Rhabdalestes maunensis* (Fowler, 1935)	25/12/2002	9.692	1.83	484.6
3	*Brycinus lateralis* (Boulenger, 1900)	11/01/2003	0.248	1.7	12.4
4	*Clarias gariepinus* (Burchell, 1822)	5/10/2006	3.469	1.65	173.5
5	*Synodontis vanderwaali* Skelton & White, 1990	15/12/2006	4.262	1.65	213.1
6	*Synodontis nigromaculatus* Boulenger, 1970	3/01/2007	2.775	1.67	138.7
7	*Serranochromis angusticeps* (Boulenger, 1970)	07/12/2003	1.201	1.6	60.1

Table 5.8 (continued): The spectrometry readings from the DNA extraction.

Sp. No.	Parasite host	Collection date	A260 (10mm)	A260/ A280	Concentration ng/µL
8	*Tyrannochromis macrostoma* (Regan, 1922)	18/12/2006	2.742	1.59	137.1
9	*Cyprinus carpio* Linnaeus, 1758 (*Lernaea cyprinacea* Linnaeus, 1758)	19/02/2013	6.645	1.71	332.3
10	*Cyprinus carpio* Linnaeus, 1758 (*Lernaea cyprinacea* Linnaeus, 1758)	19/02/2013	5.575	1.84	278.7
11	*Tyrannochromis macrostoma* (Regan, 1922)	-	3.359	1.66	167.9

These samples were sent to Inqaba Biotech for PCR and sequencing. In total four of the 11 28S rDNA (table 5.8) sequences gave positive results and were employed in the tree building process. These four sequences only represent one of the species described in the morphological analysis (*Ergasilus* sp. A), but it occurs on different hosts (see table 5.2). The samples from Lake Malawi (*Ergasilus* sp. D) and those from *Rhabdalestes maunensis* (Fowler, 1935) (*Ergasilus* sp. B) did not give positive results and were not used in the phylogenetic analysis. The samples from *Ergasilus* sp. C was not suitable to use in the molecular study. Six sequences from Chinese ergasilids, available from GenBank™, were used in the tree building process, presented in table 5.9. The 28S rDNA, from the four southern Africa sequences, ranges from 652 to 657 base pairs, with a G + C content among 55.98%-59.94%. The sequence alignment was done in Geneious R7, using the following consensus alignment methods: Nucleotide Alignment Geneious, ClustalW, and Muscle. To analyse the phylogenetic relationships among the nine ergasilid species the

Neighbor-Joining (NJ), Maximum Parsimony (MP) and Maximum Likelihood (ML) phylogenetic trees were reconstructed based on their 28S rDNA sequences, using *Lernaea cyprinacea* as an out-group.

Table 5.9: The six 28S rDNA sequences, from the Chinese ergasilids used in the present study with their GenBank™ database accession numbers.

***Ergasilus* sp.**	**Accession number**
E. anchoratus	DQ107528
E. peregrinus	DQ107531
E. hypomesi	DQ107539
E. scalaris	DQ107538
E. briani	DQ107532
E. tumidus	DQ107535

The NJ, MO and ML trees all gave different phylogenies for the different alignment methods. However, alignment by ClustalW gave results with high bootstrap values and that indicated a best fit scenario with the morphological analysis. Therefore the results for only the trees done with the ClustalW alignments will be discussed (figs. 5.22, 5.23 and 5.24). Almost all of the three tree-building methods gave vastly different phylogenies, although the results from the NJ and ML trees are most alike in branching and bootstrap values. In both trees (figs. 5.22, 5.23) the four ergasilids from southern Africa are grouped together in a clade, with a ML bootstrap value of 99%. It appears that specimens 1, 4 and 6 (table 5.8) are the same species, with 5 being a different species. The MP tree separates all four of the southern Africa ergasilids as different species, clustering specimens 1 and 4 together, with a bootstrap value of 33% (fig. 5. 24). What is evident is that specimen 5 is, as with the ML tree, completely different, with a bootstrap value of 100% (fig. 5.22, 5.23 and 5.24). In both the ML and NJ trees the four ergasilids from southern Africa are nestled together in a clade, with a ML bootstrap value of 99% (fig. 5.23). In the ML tree, the four ergasilid species from southern Africa are grouped away from the Chinese species, but with a very low bootstrap value of 48% (fig. 5. 23). In both the NJ and ML trees *Ergasilus peregrinus* is the closest related Chinese species to the southern African species. In addition in the NJ, MP and ML trees *Lernaea*

cyprinacea forms an out-group. It is evident that the ML tree gave the best results. The low bootstrap value of 48% branching the four southern African ergasilid species from the Chinese species may be ascribed to the low number of species used in the present analysis. This means that nine ergasilid specimens may not be enough to assign a higher consensus value.

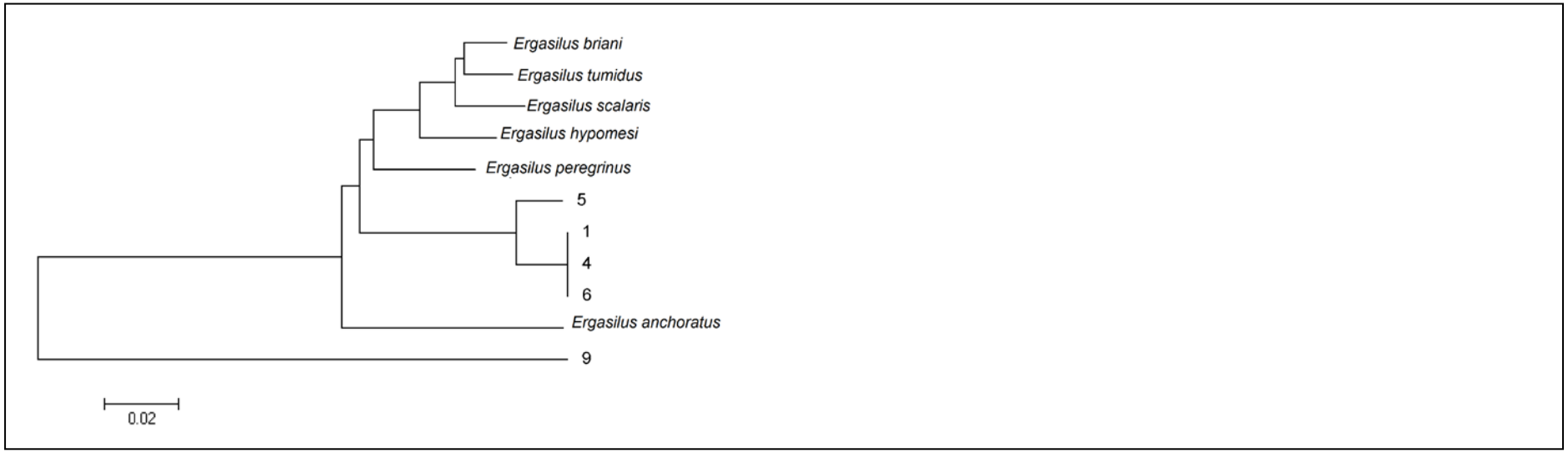


Figure 5.22.

The Neighbor-Joining consensus tree for ClustalW alignment

1. *Synodontis nigromaculatus* Boulenger, 1970
4. *Clarias gariepinus* (Burchell, 1822)
5. *Synodontis vanderwaali* Skelton & White, 1990
6. *Synodontis nigromaculatus* Boulenger, 1970
9. *Cyprinus carpio* Linnaeus, 1785 (*Lernaea cyprinacea* Linnaeus, 1758)

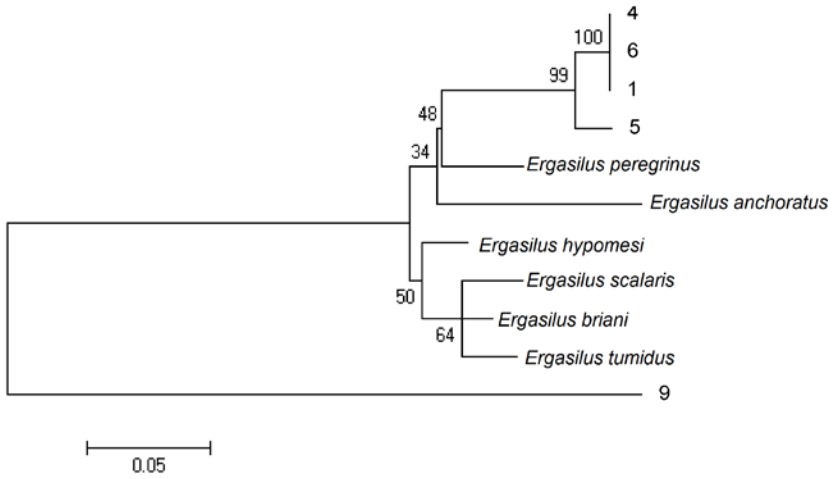


Figure 5.23.

The Maximum Likelihood bootstrap consensus tree for ClustalW alignment

1. *Synodontis nigromaculatus* Boulenger, 1970
4. *Clarias gariepinus* (Burchell, 1822)
5. *Synodontis vanderwaali* Skelton & White, 1990
6. *Synodontis nigromaculatus* Boulenger, 1970
9. *Cyprinus carpio* Linnaeus, 1785 (*Lernaea cyprinacea* Linnaeus, 1758)

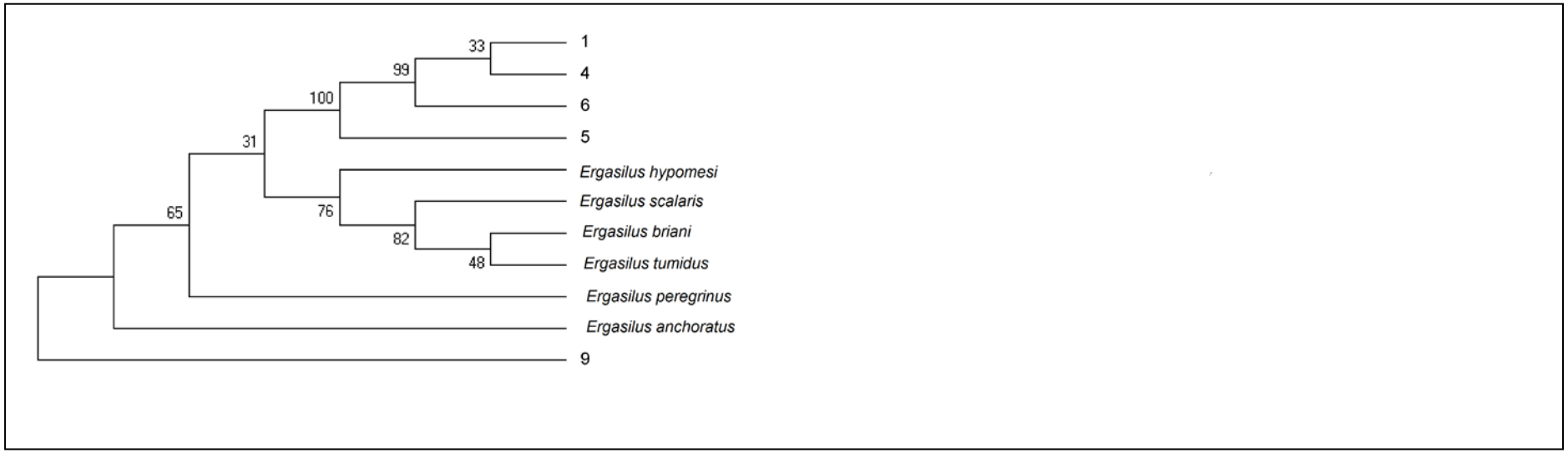


Figure 5.24.

The Maximum Parsimony bootstrap consensus tree for ClustalW alignment

1. *Synodontis nigromaculatus* Boulenger, 1970
4. *Clarias gariepinus* (Burchell, 1822)
5. *Synodontis vanderwaali* Skelton & White, 1990
6. *Synodontis nigromaculatus* Boulenger, 1970
9. *Cyprinus carpio* Linnaeus, 1785 (*Lernaea cyprinacea* Linnaeus, 1758)

Chapter 6

Discussion

- **A comparison between the morphological and molecular data**

The ergasilids are not only a major family in the order Poecilostomatoida, but also a major family of freshwater parasites in Africa (Oldewage & Avenant-Oldewage, 1993 and Song *et al.*, 2008). Four *Ergasilus* species, from southern African freshwaters, were used in the morphological analysis. Morphologically the results are unique and discernible. *Ergasilus* sp. A, B, C and D are possible new species. *Ergasilus* sp. A is unique in various ways, but the 2-segmented fifth leg singles it out from the other African species. *Ergasilus* sp. B is small compared to the other three species, 0.5mm (table 5.4), and can be singled out by the lamella-like strip on the broad second antenna segment. These two species should have a different spine and seta formulae, as the rest of the more than 180 ergasilid species of the world, but that is not the case. Tables 5.1 and 5. 3 clearly indicate that the spine and seta formulae are exactly the same. Morphologically this usually is a very good indication of these being the same species. But from the morphological results in Chapter 5 it is clear that they are not. In all other respects these two ergasilids differ completely. In size eg. the total length for *Ergasilus* sp. A is 1.4mm (table 5.2) and that of *Ergasilus* sp. B is only 0.51mm (table 5.4). Another difference is the presence of a lamella-like strip on the third antenna segment of *Ergasilus* sp. B, which is absent from *Ergasilus* sp. A. The seta formula for the antennule, and the nature of the fifth leg differ in both species, all of this clearly distinguishes them as different species, except as mentioned for the spine and seta formulae of legs 1 to 4. *Ergasilus* sp. C does not have a single outstanding characteristic to distinguish it from other ergasilid species, but it is rather a combination of morphological differences that makes it unique. *Ergasilus* sp. D, like *Ergsilus* sp. C, has small differences, that is difficult to observe e.g. the ridges of comb-like spines on the outer margin of the exopodal and

endopodal segments of legs 2, 3 and 4; as well as the small circular indentations on the cephalothorax. Although all very distinct, they are easily missed. These results from all four of the species stipulate the importance and need for close and accurate morphological identification and other accompanying techniques when a single species is not as readily discernible from the others. When morphological characters are as difficult to discern, other aspects of the parasites biology could help in the identification process, host specificity is one example.

Ergasilids vary in their level of host specificity, with some that are specific to their host genus, notably those infesting the fish family Cichlidae, but the majority are less opportunistic (Paperna, 1996). Examples of these opportunistic species include *E. cunningtoni,* that have been recorded from seven fish families (Fryer, 1968), as well as *E. kandti, E. sarsi* and the cosmopolitan species *E. lizae* (Paperna, 1996). Taking the four possible new species from the present study into account the statement and information above is reiterated. *Ergasilus* sp. A is clearly not host specific as it was found on nine fish host families (Chapter 5), this is further supported by the body measurements from each fish in these families (table 5.2) that do not vary greatly. *Ergasilus* sp. B although very small was found on two fish host families, Characidae and Mormyridae, that are quite discernible from each other not only in biology but also morphologically. *Ergasilus* sp. C was only described from a single host species, but this is due to the lack of samples and cannot be used as an indication of host specificity. In the case of *Ergasilus* sp. D it may seem that the species shows specificity for the family Chichlidae (see table 5.6), but here as with *Ergasilus* sp. C the lack of enough data discourages any ideas or indications to this species being host specific.

These small differences in the four species creates an excellent platform for the use of molecular analysis as an added tool in identification. The phylogenetic tree constructed suggested that samples 1, 4 and 6 (see table 5.8) are the same species. Thus the ergasilids collected from *Synodontis nigromaculatus, S. leopardinus* and *Clarias gariepinus* are the same species. This reflects the results from the morphological analysis. Although all four of the species were used in the molecular analysis, the only positive results were obtained from *Ergasilus* sp. A. Four

sequences were obtained from three different hosts, i.e. *S. nigromaculatus, S. vanderwaali* and *C. gariepinus.* In the present study the four sequences and the six sequences from China, done by Song *et al.* (2008) were used to determine the phylogenetic relationship of the results obtained for *Ergasilus* sp. A from Africa based on 28S rDNA sequences. Sample number 5 (table 5.8) is a problem, this specimen was collected from *S. vanderwaali.* Morphologically this species is the same as samples 1, 4 and 6, but this was not reflected in the molecular analysis. When comparing the measurements (see table 5.2) of *Ergasilus* sp. A one can see that there is no significant difference in any of the body measurements between the different fish host species. The morphological data for the ergasilid from *S. vanderwaali* is based on one specimen and the body measurements are similar to not only the ergasilid collected from *S. nigromaculatus, S. leopardinus* and *C. gariepinus,* but the 13 other hosts that this species was collected from (see table 5.2). These small, almost insignificant, differences are not enough to single it out as a morphologically different species. This could be rectified if there were more specimens to dissect and use in the morphological analysis, to affirm the molecular results with a more detailed morphological analysis.

The results give an excellent example to outline the differences and difficulties in the use of only morphology and or only genetics in taxonomy. If one is to take a closer look at morphology and its uses, it is plain that it is a system that has worked extremely well for a very long time. But there are problems, and the morphological results in this study prove it beautifully. Following Huys & Boxshall (1991) the spine and seta formulae for each species in the order Poecilostomatoida should be different, but the morphological results of this study clearly show that this is not always the case. An excellent example is the spine and seta formulae for legs 1 to 4 of *Ergasilus* sp. A (see table 5.1) and *Ergasilus* sp. B (see table 5.3) that are the same. This is where molecular data could have provided us with an answer, but the extracted DNA did not produce positive results and *Ergasilus* sp. B could not be used in the phylogenetic analysis. For the 180 *Ergasilus* sp. (www.marinespecies.org) the spine and seta formula has always been different, but as these spines and setae are difficult to observe, especially with an ergasilid as small as *Ergasilus* sp. B, making an error with the the number of spines and setae is not impossible. There is always the possibility that more species could have the

same formulae, but this needs more detailed examination and can include larger sample sizes.

When comparing the morphological data for specimens 1, 4 and 6 (table 5.8) and the ML phylogenetic tree it is clear that the used of morphology and genetics to supplement each other can and does work. *But how accurate can this process really be?* According to DeSalle, Egan & Siddal (2005) one of the major shortcomings in the use of DNA is that all classical studies and taxonomic schemes that accomplish the same thing as DNA are character based, whereas the methods normally used in DNA studies are done by utilising distance measures. Character based methods have the logical advantage that when diagnostic character data are lacking, they will fail to diagnose a species, allowing for a degree of hypothesis testing not available when using distances. Another shortcoming involves the lack of an objective set of criteria to delineate taxa when using distances. This is evident from the fact that mostly the use of single gene trees are used as evidence for phylogenetic relationships (DeSalle *et al.,* 2005). A further controversial aspect of DNA studies is the number of individuals of each species to include in the analysis. In taxonomy one normally examine numerous individuals, from multiple localities (and hosts) across the range of a given species to distinguish variation within the particular population in order to identify those characters that are uniquely shared among all members of a species (DeSalle *et al.,* 2005).

One other problem with the use of DNA in species delineation is how to read and understand the trees, that are the end product. Following Gregory (2008) these trees are the subject of detailed, rigorous analysis that seeks to reconstruct the patterns of branching that have led to the diversity of all life on Earth. This statement brings one of the previous points back into consideration: the lack of an objective set of criteria to outline taxa when using distances. *Should all phylogenetic studies not be based on the same genes or the entire genome for that matter?* When looking at processes such as later gene transfer and gene duplication, it is evident that the path of individual genes may not be the same as the paths of those of the species in which these individual genes reside (Gregory, 2008). This is evident from the fact that different gene sequences give different phylogenetic outputs. But as with the present study genes that are used to infer phylogenetic history are more suitable than others. In the study done by Song *et al.* (2008) only the 18S and 28S rDNA

were used and different results were obtained for the different genes. This shows that some genes may be more suitable for phylogenetic studies than others, just as not all characters are suitable or used for taxonomical purposes are suitable in morphological analyses.

- **Why go through all the trouble?**

Taking all of this into account, a particular question comes to mind, and maybe even the most asked question by the layman to any scientist. *Why?* The fact of the matter is that everywhere in the world the problems of human overpopulation are too obvious, and food shortages are the main topic of every debate. The depletion of natural resources funnels the most creative minds to innovative ideas to solve the problems that we are facing. Aquaculture can be the answer to many problems. But there is a down side. Parasitism of these farmable fish species, and the introduction of the fish host to different countries because of their aquaculture potential is or should be a great concern. According to Van As *et al.* (2012) the common Carp *Cyprinus carpio,* was responsible for the introduction of two alien and invasive crustaceans ectoparasites, i.e. the anchor worm *Lernaea cyprinacea* and the fish louse *Argulus japonicus* Thiele, 1900*. Lernaea* plays an important part in the present study and has been used as the outgroup in the molecular analysis, as has been done by Song *et al.* (2008). As a branchiuran, *Argulus* sp. forms part of four genera, that presently comprises 113 valid species in the single family Argulidae (Polly, 2008). Molecular studies have been done on these branchiurans and are of interest, but does not form part of the present study, thus with the Branchiura aside the Copepoda can be seen as an exceptionally diverse group of animals, with close to 15 012 accepted species (www.marinespecies.org). Understanding the parasitic copepod diversity, should make it easier to counteract the problems caused by these parasitic species.

When taking the family Ergasilidae into account, it is evident right from the start that not all the species are a great threat to the aquaculture industry. According to Paperna (1996) all the freshwater species are endemic to Africa and none of these 12 species can be singled out as a major threat to the aquaculture industry. The two cosmopolitan species, *E. sieboldi* and *E. lizae* only described from coastal regions or brackish waters do not present a great threat either (Paperna, 1996). Although *E.*

lizae is a problem in mullet culture in the Mediterranean and Middle East (Lester & Hayward, 2006). According to Zimmerman (2003) no ergasilid is listed indicating problem alien aquatic animals in South Africa.

In the family Ergasilidae only the females are parasitic, and is found on the gills of fish. Where the ergasilids attach to the gill filaments it produces a small foci of erosion and feeding apparently involves the excretion of proteolytic enzymes for external digestion (Paperna, 1996). Such erosion processes have been observed in infections of *E. megacheir* and *E. lizae.* These erosion and degradation processes may extend beyond the epithelial lining, resulting in obstructed branchial blood vessels, with a resulting decrease in the respiratory function of the gills (Kabata, 1970). No information is available for African freshwater fish species, but according to Paperna (1996) pond reared *Mugil cephalus* infested with 100 to 200 and 1500 to 2000 individuals of *E. lizae* were severely emaciated. Losses at harvest reached 50% compered with 10% in uninfected ponds.

In order to control these parasitic species, a rapid identification process seems to be the way forward. The underlying problem is that for most of the copepods any real eradication should be done before the parasitic adults are observed, and herein lays the challenge. To do this one needs to understand the life-cycle of these organisms. According to Alston *et al.* (1996) accounts of ergasilid life-cycles are often fragmentary and numerous discrepancies exist in the number of nauplius stages recognised. Studies on the life-cycle of any copepod takes not only time but considerable experience. Therefore more accurate identification processes should be the focus of future studies. Morphologically accurate species identification keys could be of considerable value, however to date only keys that lead to genus level exist. On the molecular side quick identification processes by means of rapid PCR, sequencing and phylogenetic analysis could help in the identification process. As the ergasilids do not show considerable host specificity (Paperna, 1996) it can cause problems as more information on the various ergasilid species will be needed to ease the identification processes, morphologically and molecular alike.

All the information available for any of the African ergasilid or any other ergasilid species is based on information in published articles, from recognised and accredited journals. *The information gathered here should be trustworthy, or is it?*

When taking an article like the one by Song *et al.* (2008) into account one can clearly see that it is a molecular study for some of the ergasilids from China. The information in the article is clearly laid out in an accepted scientific manner. However, with closer inspection some problems surface. Starting with GenBank™ accession numbers of some of the species. In figure 2 of the article depicting the Bayesian inference tree inferred from analysis of partial 28S rDNA sequences the accession numbers for *E. danjiangensis*, as supplied by the authors, are in fact those of *E. tumidus* (Song *et al.* 2008). There is no *E. danjiangensis* in the GenBank™ database but only *E. danjiangs,* and *E. briani* is given as *E. branini.* Although there can be typing errors in the manuscript, these are critical errors that should not be there at all. Where are the control mechanisms, or did the scientists become so complacent that generating and output of data has become more important than accuracy and trusted data and resources? The article by Song *et al.* (2008) also leaves one final question, without giving any evidence to support their claims, without any diagrams, sketches or photos, they identified the various ergasilid species morphologically and then did molecular analysis on the said species. Taking into account that there are mistakes in the article, what reassurances do we have that the morphological identifications of the species used are correct? This leads to a very important point made earlier, morphological and molecular studies should aid one another, and should therefore be in the same study, verification and supportive data is of crucial importance to form a holistic taxonomic picture: morphology and genetics cannot stand alone.

- **Why? – the answers**

The present study provides evidence, although partial, that traditional taxonomy using morphological characteristics, coupled with molecular analysis, can be used as supportive evidence for species delineation, as is the case with the results from *Ergasilus* sp. A taking specimens 1, 4 and 6 into account. Both the morphological identification and the molecular analysis supports the conclusion that these specimens are the same species. But it is also clear that morphology can stand on its own, with minor alteration here and there. Genetics has a long way to go to reach the same level of accuracy, which is also clear with the results obtained from

Ergasilus sp. A. When taking specimen 5 into account, morphologically it is the same species as specimens 1, 4 and 6, but this is not mirrored in the molecular analysis.

Although only four new South African *Ergasilus* species, out of a possible 180 species, have been morphologically identified in the present study and described and only one of these four species gave positive results in the molecular analysis, the outcome makes a significant contribution to the phylogenetic knowledge of the Ergasilidae from Africa. It also contributes to the parasitic Copepoda in general, e.g. family Lernaeidae, with *L. cyprinacea* that was used as the outgroup, with regards to this species and its distribution in Africa. This dissertation also add valuable knowledge with regards to the different techniques used during the molecular analysis. This information will be stored on GenBank™, after the completion of the planned scientific papers to be written, thus adding a little more clarity to the whole conundrum of Copepoda diversity and taxonomy.

Chapter 7

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* Not seen in the original form.

Abstract

The exactness of species descriptions in taxonomy, taking only morphology into account, has long been debated over. This is evident in the systematics of parasitic copepods that are mostly based on morphological characteristics. The gap in molecular information of the class Copepoda leaves a hole in the understanding of crustacean systematics and their ecological importance. Due to work done in China by a group of Chinese scientists on the genus *Ergasilus* (Family Ergasilidae). molecular studies on genera of this family from Africa were used to do the same analysis. Although only some representatives of the family is a threat to the fish populations of Africa, the availability of samples and information on morphological traits make this family a good starting point in the use of molecular work on the parasitic copepods of Africa. The focus of this study is the phylogeny of southern African ergasilids, based on the morphological characteristics coupled with the 28S rDNA sequences, with specifically designed primers (used in the Chinese study). The family Lernaeidae (using the genus *Lernaea*), closely related to the genus *Ergasilus*, was used as the out-group. Using the morphological analysis four new species of *Ergasilus* from southern Africa were found, i.e. *Ergasilus* sp. A from *Synodontis leopardinus* Pellegrin, 1914; *Ergasilus* sp. B from *Rhabdalestes maunensis* (Fowler, 1935); *Ergasilus* sp. C from *Micropterus punctulatus* Rafinesque, 1819 and *Ergasilus* sp. D from *Petrotilapia tridentiger* Trewavas, 1935. The molecular study proved to be a challenge and only results for *Ergasilus* sp. A could be obtained and were used in a tree-building analysis with the sequences from the Chinese study.

Keywords: Ergasilidae, southern Africa, 28S rDNA, morphology, taxonomy, copepod

Opsomming

Die debat rakende die gebruik van slegs morfologiese kenmerke en die akkuraatheid hiervan, word al lank bespreek. Dit spruit uit die feit dat die huidige sistematiek van die Copepoda slegs op morfologie gegrond is. Die tekort in molekulêre studies van die klas Copepoda laat ‘n gaping om die breër krustasee-sistematiek en om hul ekologiese belangrikheid ten volle te verstaan. Volgens ’n molekulêre studie deur ‘n groep Sjinese wetenskaplikes op die genus *Ergasilus* (Familie Ergasilidae) gedoen is, is die gaping nou oorbrug en kon soortgelyke werk op die spesies van Afrika uitgevoer word. Alhoewel die familie net sekere spesies bevat wat probleme by die visbevolkings van Afrika veroorsaak, maak die beskikbaarheid van eksemplare en die morfologiese inligting van die groep ‘n uitstekende begin vir molekulêre studies van parasitiese krustaseë van Afrika. Die fokus van hierdie studie was die filogenie van verteenwoordigers van die suidelike-Afrika Ergasilidae, gebaseer op morfologies kenmerke wat gerugsteun word die molekulêre analise deur van die 28S rDNS geenvolgordes gebruik te maak. The familie Lernaeidae en die genus *Lernaea,* wat naby verwant aan die genus *Ergasilus* is*,* is as buitegroep gebruik. Na aanleiding van die morfologiese resultate wat verkry is, word vier nuwe spesies van die genus *Ergasilus* van suidelike-Afrika beskryf, nl. *Ergasilus* sp. A vanaf *Synodontis leopardinus* Pellegrin, 1914; *Ergasilus* sp. B vanaf *Rhabdalestes maunensis* (Fowler, 1935); *Ergasilus* sp. C vanaf *Micropterus punctulatus* Rafinesque, 1819 en *Ergasilus* sp. D vanaf *Petrotilapia tridentiger* Trewavas, 1935. Die molekulêre studie was problematies sowel as ‘n uitdaging en slegs resultate van *Ergasilus* sp. A is verkry en is in die boom-bou analise tesame met die geenvolgordes vanaf die Sjina studie gebruik.

Sleutelwoorde: Ergasilidae, suidelike-Afrika, 28S rDNS, morfologie, taksonomie, kopepood

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Appendix

Table 5.2: Table of body measurements of *Ergasilus* sp. A collected from the various fish hosts.

***Synodontis nigromaculatus* Boulenger, 1905**			
	n	**Mean**	**Min. – Max.**
Total length	18	1.4	1.3 – 1
Cephalothorax			
- Length	18	0.33	0.29 – 0.35
- Width	18	0.3	0.28 – 0.32
Antenna	14	0.97	0.69 – 1.15
Furcal setae	2	0.24	0.22 – 0.26
Egg sacs	12	1.0	0.67 – 1.50
***Synodontis zambezensis* Peters, 1852**			
	n	**Mean**	**Min. – Max.**
Total length	2	0.91	0.91
Cephalothorax			
- Length	2	0.32	0.3 – 0.35
- Width	2	0.36	0.36 – 0.37
Antenna	4	0.95	0.89 – 1.1
Furcal setae	2	0.37	0.37
Egg sacs	-	-	-
***Synodontis thamalakanensis* Fowler, 1935**			
	n	**Mean**	**Min. – Max.**
Total length	3	1.18	1.1 – 1.2
Cephalothorax			
- Length	3	0.37	0.3 – 0.41
- Width	3	0.4	0.36 – 0.43
Antenna	3	0.95	0.89 – 1.1
Furcal setae	2	0.37	0.37
Egg sacs	-	-	-
***Synodontis macrostigma* Boulenger, 1911**			
	n	**Mean**	**Min. – Max.**
Total length	3	1.1	1.0 – 1.2
Cephalothorax			
- Length	3	0.3	0.27 – 0.33
- Width	3	0.38	0.38
Antenna	6	0.88	0.86 – 0.9
Furcal setae	6	0.3	0.25 – 0.32
Egg sacs	4	0.87	0.74 – 1.1
***Synodontis vanderwaali* Skelton & White, 1990**			
	n	**Mean**	**Min. – Max.**
Total length	1	1.1	1.1
Cephalothorax			
- Length	1	0.29	0.29
- Width	1	0.29	0.29
Antenna	2	0.77	0.77 – 0.78
Furcal setae	2	0.27	0.25 – 0.3
Egg sacs	1	0.81	0.81

Table 5.2 (continued): Table of body measurements of *Ergasilus* sp. A collected from the various fish hosts.

***Schilbe intermedius* Rüppell, 1832**			
	n	**Mean**	**Min. – Max.**
Total length	5	1.09	1.0 – 1.1
Cephalothorax			
- Length	5	0.27	0.27 – 0.29
- Width	5	0.29	0.25 – 0.33
Antenna	5	0.87	0.80 – 0.97
Furcal setae	5	0.27	0.2 – 0.35
Egg sacs	4	0.90	0.85 – 0.95
***Clarias ngamensis* Castelnau, 1861**			
	n	**Mean**	**Min. – Max.**
Total length	3	1.25	1.22 – 1.28
Cephalothorax			
- Length	3	0.31	0.3 – 0.32
- Width	3	0.41	0.4 – 0.43
Antenna	6	1.36	0.85 – 1.66
Furcal setae	6	0.32	0.31 – 0.35
Egg sacs	5	1.36	0.85 – 1.66
***Clarias gariepinus* (Burchell, 1822)**			
	n	**Mean**	**Min. – Max.**
Total length	2	0.91	0.91
Cephalothorax			
- Length	2	0.25	0.25 – 0.28
- Width	2	0.37	0.36 – 0.39
Antenna	4	0.95	0.89 – 1.1
Furcal setae	2	0.37	0.37
Egg sacs	-	-	-
***Glossogobius giuris* (Hamilton-Buchanan, 1822)**			
	n	**Mean**	**Min. – Max.**
Total length	3	1.14	1.08 – 1.25
Cephalothorax			
- Length	3	0.3	0.27 – 0.33
- Width	3	0.38	0.38
Antenna	6	1.36	0.85 – 1.66
Furcal setae	6	0.32	0.31 – 0.35
Egg sacs	6	1.46	0.9 – 1.64
***Labeo rosae* Steindachner, 1894**			
	n	**Mean**	**Min. – Max.**
Total length	3	1.12	1.07 – 1.24
Cephalothorax			
- Length	3	0.31	0.301 – 0.33
- Width	3	0.37	0.37
Antenna	6	0.88	0.86 – 0.9
Furcal setae	6	0.3	0.25 – 0.32
Egg sacs	4	0.87	0.74 – 1.1

Table 5.2 (continued): Table of body measurements of *Ergasilus* sp. A collected from the various fish hosts.

***Hydrocynus vittatus* Castelnau, 1861**			
	n	**Mean**	**Min. – Max.**
Total length	1	1.1	1.1
Cephalothorax			
- Length	1	0.3	0.3
- Width	1	0.36	0.36
Antenna	2	0.77	0.77 – 0.78
Furcal setae	2	0.27	0.25 – 0.3
Egg sacs	1	0.81	0.81
***Serranochromis macrocephalus* (Boulenger, 1899)**			
	n	**Mean**	**Min. – Max.**
Total length	4	1.38	1.35 – 1.41
Cephalothorax	-	-	-
- Length	4	0.5	0.48 – 0.51
- Width	4	0.46	0.45 – 0.49
Antenna	8	0.93	0.91 – 0.96
Furcal setae	-	-	-
Egg sacs	-	-	-
***Serranochromis angusticeps* (Boulenger, 1907)**			
	n	**Mean**	**Min. – Max.**
Total length	3	1.2	1.2 – 1.2
Cephalothorax			
- Length	3	0.31	0.3 – 0.32
- Width	3	0.41	0.4 – 0.43
Antenna	6	1.3	0.85 – 1.66
Furcal setae	6	0.31	0.31 – 0.35
Egg sacs	5	1.3	0.85 – 1.66
***Oreochromis andersonii* (Castelnau, 1861)**			
	n	**Mean**	**Min. – Max.**
Total length	3	1.1	1.1 – 1.2
Cephalothorax			
- Length	3	0.3	0.27 – 0.33
- Width	3	0.38	0.38
Antenna	4	0.95	0.89 – 1.1
Furcal setae	2	0.37	0.37
Egg sacs	-	-	-
***Hippotamyrus ansorgii* (Boulenger, 1905)**			
	n	**Mean**	**Min. – Max.**
Total length	3	1.15	1.15
Cephalothorax			
- Length	3	0.31	0.30 – 0.33
- Width	3	0.37	0.37
Antenna	5	1.09	1.0 – 1.2
Furcal setae	2	0.31	0.31 – 0.32
Egg sacs	2	0.78	0.78 – 0.79

Table 5.2 (continued): Table of body measurements of *Ergasilus* sp. A collected from the various fish hosts.

***Hepsetus odoe* (Bloch, 1794)**			
	n	**Mean**	**Min. – Max.**
Total length	3	1.15	1.15
Cephalothorax			
- Length	3	0.31	0.30 – 0.33
- Width	3	0.37	0.37
Antenna	5	1.09	1.0 – 1.2
Furcal setae	2	0.31	0.31 – 0.32
Egg sacs	2	0.78	0.78 – 0.79

Table 5.4: Table of body measurements of *Ergasilus* sp. B from the various fish hosts.

***Brycinus lateralis* (Boulenger, 1900)**			
	n	**Mean**	**Min. – Max.**
Total length	8	0.51	0.47 – 0.56
Cephalothorax			
- Length	8	0.19	0.17 – 0.2
- Width	8	0.16	0.15 – 0.17
Antenna	16	0.3	0.30 – 0.31
Furcal setae	16	0.12	0.09 – 0.19
Egg sacs	14	0.3	0.25 – 0.37
***Hydrocynus vittatus* (Castelnau, 1861)**			
Total length	1	0.55	0.55
Cephalothorax			
- Length	1	0.17	0.17
- Width	1	0.12	0.12
Antenna	2	0.29	0.29
Furcal setae	2	0.17	0.16 – 0.19
Egg sacs	-	-	-
***Petrocephalus wesselsi* Kramer & van der Bank, 2000**			
Total length	4	0.72	0.66 – 0.79
Cephalothorax			
- Length	4	0.22	0.20 – 0.23
- Width	4	0.2	0.19 – 0.21
Antenna	6	0.49	0.41 – 0.55
Furcal setae	4	0.19	0.16 – 0.22
Egg sacs	2	0.38	0.37 – 0.4

Table 5.7: Table of measurements of *Ergasilus* sp. D from the various fish hosts.

***Petrotilapia genalutea* Marsh, 1983**			
	N	**Mean**	**Min. – Max.**
Total length	4	0.68	0.68 – 0.71
Cephalothorax			
- Length	4	0.19	0.17 – 0.2
- Width	4	0.16	0.15 – 0.17
Antenna	8	0.49	0.45 – 0.51
Furcal setae	8	0.12	0.09 – 0.19
Egg sacs	6	0.46	0.45 – 0.52
***Petrotilapia nigra* Marsh, 1983**			
Total length	1	0.95	0.95
Cephalothorax			
- Length	1	0.37	0.37
- Width	1	0.32	0.32
Antenna	2	1.21	1.21
Furcal setae	2	0.72	0.72 – 0.73
Egg sacs	-	-	-
***Tyrannochromis macrostoma* Regan, 1922**			
Total length	4	0.72	0.66 – 0.79
Cephalothorax			
- Length	4	0.22	0.20 – 0.23
- Width	4	0.2	0.19 – 0.21
Antenna	6	0.49	0.41 – 0.55
Furcal setae	4	0.19	0.16 – 0.22
Egg sacs	2	0.38	0.37 – 0.4