

Review of the African Fish Louse Genus *Dolops* (Branchiura)

by

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ABSTRACT

The Aquatic Parasitology Research Group has been doing research on parasites in the Okavango Delta and Panhandle since 1997. Representatives of the Branchiura are present on several different fish hosts in the Delta and Panhandle. *Dolops ranarum* (Stuhlman, 1891) is a branchiuran that has a Pan-African distribution. In contrast with the other branchiurans present in Africa; *Argulus* (Müller, 1785) has 32 species and *Chonopeltis* (Thiele, 1900) has 14 species present in freshwater habitats in Africa, *D. ranarum* has only one representative in freshwater habitats on the continent. This leads to the question whether there may have been a speciation event in which new species may have developed. This study investigates the possibility of a speciation event using both morphological and molecular methods. Specimens were collected from different fish host genera such as *Clarias* Scopoli, 1777, *Serranocromis* Regan, 1920, *Oreochromis* Günther, 1889, *Synodontis* and *Tilapia* A. Smith, 1840, as well as *Schilbe intermedius* Rüppel, 1832 and *Hepsetus odoe* (Bloch, 1794). Subsequently, the fish lice were visually examined for characters that indicated significant differences. Specimens were also utilised for DNA sequencing. It was clear from the morphological results that there has been no speciation event as a result of the utilisation of different hosts. However, the molecular analysis was less successful, possibly due to the low quality of DNA as a result of the storage method.

ABSTRAK

Die Akwatiese Parasietologie Navorsingsgroep doen al navorsing in die Okavango Delta en Pypsteel sedert 1997. Verteenwoordigers van die Branchiura is aanwesig op verskeie verskillende visgashere in die Delta en Pansteel. *Dolops ranarum* (Stuhlman, 1891) het 'n pan-Afrikaanse verspreiding. In teenstelling met die ander branchiura aanwesig in Afrika; *Argulus* (Müller, 1785) het 32 spesies en *Chonopeltis* (Thiele, 1900) het 14 spesies aanwesig in varswater habitate in Afrika, *D. ranarum* het slegs een verteenwoordiger in varswater habitate op die kontinent. Dit lei tot die vraag of daar nie moontlik 'n spesiasie gebeurtenis plaasgevind het waar nuwe spesies ontwikkel het nie. Hierdie studie ondersoek die moontlikheid van 'n spesiasie gebeurtenis met die gebruik van beide morfologiese en molekulêre metodes. Monsters is versamel van verskillende visgasheergenera soos byvoorbeeld (*Clarias* Scopoli, 1777, *Serranocromis* Regan, 1920, *Oreochromis* Günther, 1889, *Synodontis* en *Tilapia* A. Smith, 1840, sowel as *Schilbe intermedius* Rüppel, 1832 en *Hepsetus odoe* (Bloch, 1794). Voorts is die visluise visueel ondersoek vir karakters wat betekenisvolle verskille aandui. Monsters is ook gebruik om die opeenvolging van DNA te bepaal. Dit was duidelik uit die morfologiese resultate dat daar geen spesiasie gebeurtenis plaasgevind het as 'n gevolg van die benutting van verskillende gashere nie. Ongelukkig was die molekulêre analise minder suksesvol, moontlik te wyte aan die lae kwaliteit van die DNA as gevolg van die stoormetodes.

Chapter 1: General Introduction

Since 1997 the Aquatic Parasitology Research Group at the University of the Free State has been concerned with the Okavango Fish Parasite Project. This project aims (amongst other things) to better understand the Okavango Delta, the parasites occurring on fish and their life cycles.

The Okavango Delta situated in north-western Botswana, spreads a fan of floodplains and waterways over a large part of the arid Kalahari Desert. While it is a unique wetland-area, beautiful and breathtaking to the tourist, it is also the beating heart of a large community of people that depends on it for all its needs, from nourishment to housing. Additionally, it also supports an intricate and almost unknown ecology of fish, birds and their attendant parasites.

Water is the single most important commodity in southern Africa, and its importance is only rivalled by its scarcity. The practical reason for doing a study such as this in the Okavango Delta specifically, is because it presents an opportunity not found in South Africa. In South Africa, the rivers have been tainted with exotic fish, birds and of course parasite species, as well as the activities of an industrially active nation. The near pristine nature of the Delta, makes it ideal as a model for what rivers in southern Africa are ideally supposed to look like. It presents the opportunity to study a river where the natural balance of fauna can be seen and studied. The knowledge gained can be applied to rivers in other parts of southern Africa. An attempt can be made to improve the quality of the water and the ability of a river to exist optimally, providing a habitat for all its attendant creatures and still be a valuable resource to people. Research on the natural state of rivers before human intervention, is vital to the subsequent conservation of water resources worldwide. Before the unique nature of rivers (in southern Africa as opposed to other geographical areas) is understood, no successful attempt can be made to improve the state of rivers in southern Africa.

In the course of the research done by the Fish Parasitology Research Group, much has been discovered, amongst others, about branchiurans in Africa. The

class Branchiura Thorell, 1846 comprises a single family (the Arguilidae) consisting of four genera, *Argulus* Müller, 1785, *Chonopeltis* Thiele, 1900, *Dolops* Audouin, 1837, and *Dipteropeltis* Calman, 1912. While having a worldwide distribution, the dispersal pattern of the different genera is more interesting still. While *Argulus* is found worldwide, *Dolops* is found only in the southern hemisphere, and *Chonopeltis* is an endemic genus to Africa. Current information on *Dipteropeltis* suggests that this genus is endemic to South America. More information on the distribution of branchiurans will be provided in Chapter 2 of this dissertation.

One of the many questions that arose was whether *Dolops ranarum* (Stuhlman, 1891), with a Pan-African distribution, truly was just one species, as this species occurred on several divergent hosts, and representatives of the species were found throughout most of the African rivers, south of the Sahara.

Therefore, the aim of this study was to determine whether the specimens of *D. ranarum* parasitising different fish hosts might be members of different species due to a speciation event.

In an attempt to determine whether all specimens belong to *D. ranarum*, the following methods were applied. Firstly, specimens from various hosts were collected and compared morphologically. Secondly, a molecular comparison of the mitochondrial gene COI of the collected specimens was done. The different biologies of the hosts seemed to indicate that isolation might have occurred. Unfortunately many of the hosts of *D. ranarum* still seem to be much of a mystery to science as, according to Skelton (1993; 2001), the biology and ecology of many members of the African fish fauna have not been fully studied at this time. Consequently, little information is available on many aspects of the biology of the hosts. This was one of the difficulties encountered during the course of this project.

1.1 Species concepts and taxonomy

- **What is a Species?**

This question has been one of the most difficult to answer because of two problems. Firstly, there is what people want a species to mean, since there are as many opinions as there are biologists interested in discussing them (Quicke 1993; Meffe & Carroll 1994). Secondly there is the question of whether an objective definition, satisfying everyone will ever be found. In my opinion this is very unlikely, simply because of the dual nature of the word “species”. It is important to realise that species designations are nothing more than a testable hypothesis based on the best information available at the time. Species designations can change when more accurate information becomes available, causing the hypothesis to be rejected in future (Meffe & Carroll 1994).

The term, species, has a different meaning to students of evolution and to students of systematics (Mayr 1999):

1. The direct characteristics of the individuals that comprise the species (Quicke 1993),

The systematist merely sees the species as a category, a practical device to help in pigeonholing the bewildering variety of living organisms. The species is only one of the many members of the hierarchical systematic categories (Mayr 1999). It follows that a change in the species definition will have only minimal practical consequences for the systematist.

2. The process that leads to the forming of a species (Quicke 1993).

The evolutionist is interested in the dynamic qualities of a species (Mayr 1999): in effect the process of speciation. The evolutionist sees the species as a stage in the flowing river of evolution. Therefore, the

processes needed for the actual separation of species from one another, both genetic and ecological, need to be clear. This cannot be clear unless a precise definition of a species is formulated. Therefore, the evolutionist will face a major dilemma if there were to be a change in the meaning of the word species (when discussed in evolutionary terms). Consequently, if this definition changes, so do all the other parameters involved in speciation (Mayr 1999).

According to Benton & Werner (1966) taxonomic groupings are in general artificial constructs set up for the convenience of the biologist. However, the species is a natural unit. Usually the members of a particular population resemble each other more closely than the members of the same species in other populations.

It is also important to see that the concept “species” is always relative (Mayr 1969). If the diversity of nature were continuous it would not be possible to sort individuals into groups, that is to say species. In the animal world the diversity is indeed discontinuous existing in the local fauna in groups that are indeed more or less discrete (Mayr 1969). However the diversity in a population is not discrete. Most characteristics that vary geographically in a given species change at diverse places in different directions.

The concept species is the first term that should be specified. What is meant when one is using the word “species”? This differs depending on the species concept one supports. Next it is important to say what exactly defines the parameters of a species, and what criteria are used to define it. The species question has been exacerbated by the confusion surrounding the definitions of words used in the debate (Mayr 1969).

- **Species Concepts**

It is important that we take note of species concepts. The way the concept “species” is defined can influence our perceptions of; i) how many species

there might be ii) their importance relative to populations and iii) their interactions and changes over time. And this may impact on how we manage conservation and also what we eventually conserve (Meffe & Carroll 1994).

In recent years, the new implication of different species concepts has been noticed and called taxonomic inflation (Isaac, Mallet & Macel 2004). It refers to the elevation of subspecies (already described as part of a species) to species level because of the different definition of a species, rather than the discovery of a new species (finding an organism that has not been described before) (Isaac *et al.* 2004). This phenomenon seriously impacts species lists that many biologists consider accurate and stable.

According to de Meeus, Durand & Renaud (2003) it seems that most of the species concepts concentrate on organisms (sexually reproducing organisms) that do not represent global diversity. It is interesting to note that only now are unicellular organisms mentioned in relevant literature. Even more relevant to the present study, is the fact that parasitic organisms that comprise 30% of described eukaryotes and which have often before challenged conventional species concepts are often completely disregarded. It may indeed be that parasitic organisms largely outnumber their free-living counterparts in biological diversity (de Meeus *et al.* 2003). Therefore, species concepts should take the larger number of organisms into account. Another problem with finding a species concept that truly describes species is that we can only see a small fraction (a few individuals) of a species at a time (Meffe & Carroll 1994). This hinders in finding a true impression of the total scope of diversity in a species.

It raises the question whether the definition of the concept species should not also be specifically applied to the higher taxonomic groupings. Perhaps different criteria should be used for vertebrates than for invertebrates. Since the biological species concept only takes into

account sexually reproducing species (most of the vertebrates) while the invertebrates include many species that do not reproduce sexually. In this case the specific meaning and the parameters involved in the discussion of a certain group would be implicit in literature pertaining to a certain group of organisms. It cannot be denied that more clarity exists in the higher classification of organisms than in the lower (barring a few exceptions).

- **Systematics and Taxonomy**

The definition of taxonomy is the theory and practice of classifying organisms (Mayr 1969). It is important, as it is the only science that supplies information about the diversity of life (Mayr 1969). Classification provides the information necessary to reconstruct the phylogeny of life. It reveals evolutionary phenomena making it available to science for study. The classification supplied by taxonomy is of heuristic and explanatory value in other branches of biology.

Systematics is the scientific study of the kinds and diversity of organisms and of any and all relationships among them (Mayr 1969). It is singular amongst the biological sciences in not being a reductionist science. Other fields of biology have the tendency to reduce anything to the level above it. The basic mechanisms and processes underlying all life are indeed similar, but in looking only at this similarity the unique diversity of life is often forgotten (Mayr 1969).

In contrast systematics aims to redress the balance (in biological science) in emphasising the diversity of life. According to Mayr (1969) one of the important concerns of systematics is to establish by comparison what the distinctive properties of every species and higher taxon is. A taxon is a taxonomic group of any rank that is sufficiently distinct to be worthy of being assigned to a definite category (Mayr 1969).

Systematics and taxonomy are important, as many factors are present in the environment that impact on the organisms that inhabit it. Even today scientists cannot agree on the definition of a species and yet it is imperative to have an idea of a species before issues of a biological nature can be discussed. Human classification constructs were developed to make it easier for us to understand the natural world around us; however nature is very unwilling to fit into our compartments. Therefore, there are always exceptions to any rule imposed by humans. The different species concepts and disagreement on which concept is correct, have lead to taxonomic inflation, and inaccurate species lists. This leads to serious consequences in conservation and the estimation of biodiversity in some taxa.

Species concepts have in general concentrated on organisms, like vertebrates, that do not represent the true diversity of life. Therefore, it does not represent the diversity in, especially, reproductive strategies. When considering the most effective system of classifying organisms, the best system would be the one incorporating the maximum information content with the easiest retrievability. The biological species concept, popular as it is, has the drawback that it depends only on the breeding potential of a population. In comparison, the cladistic species concept depends on the branching hierarchy of evolution. Cladism is practical because shared derived characters exist as a result of a common ancestor.

A *Biological species concept*

The biological species concept, formulated by Ernst Mayr in 1942, depends on the fact of reproductive isolation. It also underlines the populational aspect and genetic unity of a species. This means that the species obtains a reality from the historically evolved shared information content of its gene pool. The words themselves, evolution and variation, indicate change and that the only way that we could possibly hope to show an accurate representation is if it were to be dynamic (Mayr 1969 & 1999). This concept circumscribes a species as “*groups*

of actually or potentially interbreeding populations which are reproductively isolated from other such groups" (Mayr 1969). This concept has been the one favoured by most zoologists in the 20th century (Meffe & Carroll 1994). According to Meffe & Carroll (1994) the biological species concept is genetically based, a solid base for sexually reproducing species if they are sympatric. Consequently, this is a good definition only for bi-parental organisms that interbreed sexually; this excludes strict-selfers and clones (de Meeus *et al.* 2003). Asexual species and chronospecies are also hard to classify as species using the biological species concept. It is very hard to prove whether all the members of the different populations of a certain species would be able to necessarily interbreed, if they were to come into contact (de Meeus *et al.* 2003). According to Benton and Werner (1966) this criterion is generally useful (for the identification of species) if one keeps in mind that isolation may break down and hybridisation occurs commonly.

Mayr (1969) himself stated that "*...it is important to focus on the basic biological meaning of a species: a species is a protected gene pool.*" Perhaps this is a better description of what a species is than his well-known phrase quoted earlier. This definition would not exclude as many organisms. Unfortunately it leads to the question of how the gene pool is protected, and this leads to a discussion on the mechanisms of isolation.

Mayr (1969) also stated that the more distant two populations become in space and in time the more difficult it becomes to test their species status in relation to one another, which is obviously true. He then proceeded to say that this also makes this knowledge more irrelevant biologically. However, my opinion is that, the validity of this statement only holds up for some fields of study. In the study of evolution, species related over time would most certainly be biologically relevant in order to trace the ancestry of species present today to species that existed in the past. Especially in the case of this particular study, where relevance over space is also an issue. According to Fryer (1968) all the *Dolops* specimens collected in Africa so far are *D. ranarum*, and in the interim no new species of *Dolops* have been described in Africa. If this were not true it would hold interest for biodiversity. From the Pongola to the Nile Rivers is a large

area for any animal, but for a crustacean a few millimetres long, is surely very far. Just because the habitats of hosts are disjunct it does not mean that it is not the same species. However, it would be hard to prove that two individuals from disjunct ranges could result in viable offspring due to the fact that these individuals would never in nature come into contact with one another.

According to de Meeus *et al.* (2003) species identification and speciation will not be resolved as long as the biological species concept is used to describe life. While this species concept circumscribes exactly how a species is supposed to behave and lists the criteria, the intrinsic nature of nature is such that it is fluid and changing. All species concepts basically fail because they try to sort life into neat categories and life itself defeats that (de Meeus *et al.* 2003).

As mentioned, this is particularly true for parasites such as cestodes and trematodes, because these organisms are more often found in isolation in the host and must therefore be self-fertilising. There are simple organisms, such as unicellular protozoans and some cnidarians that reproduce by division. The fact that there are more avian species than species of bacterium described is a clear indication of the skewed picture of biodiversity obtained by this species concept (de Meeus *et al.* 2003).

It is necessary to have a grasp of species for conservation and research purposes. The biological species concept is inadequate in some cases, but is a good starting point for the definition of species.

The criticisms levelled at this concept naturally lead to new concepts that do not depend on the knowledge of breeding potential (Quicke 1993) for example, the phylogenetic species concept.

B *The Phylogenetic / Cladistic species concept*

The phylogenetic / cladistic species concept states that classification should reflect shared characters (similarities) due to common ancestry, in other words the branching hierarchy of evolution (Ridley 1986). Wiley (1981) defines

characters as a feature of an organism, which is the product of an ontogenetic or cytogenetic sequence of previously existing parental organisms. In this concept, species are seen as basal units of classification. Here the relationships of the species are dependant on a strict hierarchy of monophyletic groups. However, it is important to remember that shared derived characters do not define cladistic classification. Instead, shared derived characters exist because of the phylogenetic relationships among the groups that have an independent existence (Ridley 1986). According to Meffe & Carroll (1994) classification should reflect genealogical relations correctly and unequivocally at all levels. This may mean the elevation of many subspecies to full species level.

In phylogenetic studies both morphological and molecular data are used. In the case of the latter the question arises what a significant difference would be in the genetic makeup of a species. Consequently estimates of expected genetic divergence is needed to proclaim two separate species. A yardstick has been devised by estimating sequence divergence between and within taxa for a variety of vertebrates. Studies on invertebrates and other groups may now be compared to the study on vertebrates by Johns & Avise (1998) to determine a fitting yardstick for these groups as well.

It is unfortunate that the majority of phylogenetic studies were done in Europe and the USA, because although these regions may possess the economical strength needed for phylogenetic study, the ecological riches do not compare with tropical areas in the world. This means that the typical levels of divergence in such areas poor in biodiversity may be biased downwards (Harris & Froufe 2004).

As with all other species concepts, this concept is also relative. A species can only be defined in the presence of other species, never as an independent perception. There are even those who argue that the definition of species must be dissociated from evolutionary concepts and other methods used to circumscribe taxa (Rapini 2004). This may be a possible solution that resolves Mayr's (1999) question of where in the stream of evolution should we draw the

line to delineate a species. He excused the inadequacy of the biological species concept to explain all the different types of animals by claiming that there is not enough information available in all taxa, therefore that question should be postponed until more information is available (Mayr 1999).

Arguments issued against the cladistic species concept seem to have become less convincing, as this species concept developed independently from the biological species concept or even evolutionary thought. According to Brower (2000) a great innovation for cladistics came about in the late 20th century. Cladists were able to combine both the positive points of Hennig's 1966 cladism (Hennig 1966) and phenetics to form a new formula, discarding the metaphysical Hennigian shell of common ancestry, to group organisms by parsimony and shared character state change (Brower 2000). This was a great improvement on the previous schools of thought. The evolutionary systematists and Hennig did not have a method to realise their phylogenetic theories and pheneticists had no theory to discriminate among the many possible methods of grouping based on similarity (Brower 2000). Even now, this does not mean that all the inadequacies in the cladistic species concept have been resolved. For instance, the weighting of characters makes the trees drawn subjective to the scientist's view of what is important.

Mayr (1969) made an argument regarding the ambiguity of terms used when discussing the biological species concept, and suggested that if this misunderstanding could be cleared up, many more scientists would come around to thinking that the biological species concept is indeed the most logical and well thought out concept that has been advanced up to date. However, Brower (2000) pointed out the same problem with regard to the cladistic species concept. He equated some of the debates raging as a Tower of Babel where no one spoke the same language and therefore the arguments were useless.

It is possible that at the time when Mayr articulated his criticism of cladism, it was merited. Cladism is a branch of biological science that has seen intensive development over the last forty years. Ideas that were in the rough phrased by Hennig (1966) spawned a theory that is in many ways unlike the original that he

expounded. What we really need from systematics is a system that allows us to unearth an orderly pattern in nature, i.e. a cladistic approach (Brower 2000).

The fact that biological evolution is a complex process, encompassing many factors makes it almost impossible to predict the impact of different influences, biotic and abiotic. According to Gavrillets (2004) regarding speciation, an easier way to look at speciation is to use the metaphor of fitness landscapes. This makes the idea of speciation visible on paper, and easier to deal with. The theory of the rugged fitness landscape is generally seen to be more correct than flat or single peak fitness landscapes (Gavrillets 2004) (Figure 1.1).

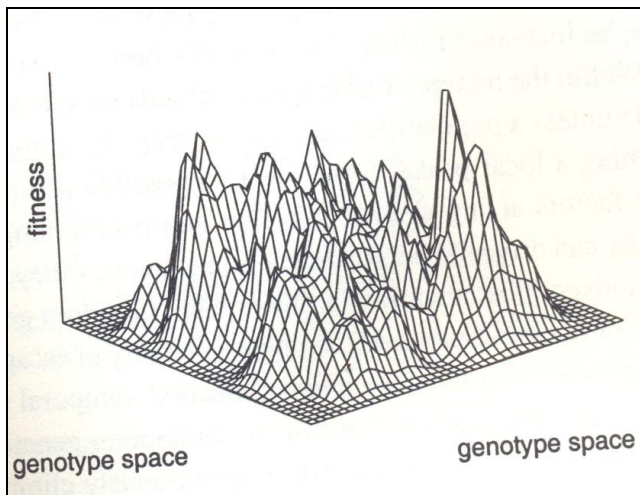


Figure 1.1: An example of the rugged fitness landscapes scanned from Gravilets (2004).

The peaks represent the different high-fitness combinations of several genes, or “co-adapted gene complexes”. This means that the peaks show the several possibilities of the solution of survival for a species. According to Gravilets (2004) the closely grouped peaks can be seen as different species within a genus. It also makes sense that fitness peaks are important because all agents of evolution, mutation, recombination and genetic drift should drive speciation in the direction of the fitness peaks.

This makes sure that once the fitness peaks are approached, selection will prevent any movement away from it. The question arises how changes then still appear if the local fitness peaks are reached? Speciation would then basically involve movement from one fitness peak to another as seen in Figure 1.1.

However, the question arises, how the population can possibly breach the valleys from one fitness peak to another. The possible agents for change may be random genetic drift or possibly, and in my opinion the more effective agent, change in the environment. It may be possible that the different hosts used by the parasite represents different fitness peaks in this case. The possibility also exists that the parasites diversified at the same pace as the fish that they are found on today. Present on a common ancestor, the parasites may have encountered new hosts as they evolved.

In the case of *Dolops ranarum*, the most likely form of speciation (if speciation had occurred) is adaptive radiation. The different hosts utilised by *D. ranarum* each present a unique challenge to the fish louse to survive and reproduce. In effect, each of the different populations of *D. ranarum* present on different hosts, are exploiting a separate habitat.

At some point during the evolution of *D. ranarum*, this lineage developed haemoglobin (Fox 1957), making it possible for this specific species of branchiuran to survive on hosts living in niches with low oxygen content, such as *Clarias gariepinus*, while *D. ranarum* is less common on species which live in areas with abundant oxygen, apparently out-competed by its sister species *Argulus africanus* (Fryer 1961).

Basically we could describe a species as a group that is isolated from other such groups by morphological, physiological or geographic factors. Therefore each species has at least one unique evolutionary event that sets it apart from other such groups. It is possible that this one evolutionary event (the development of haemoglobin) is the secret to the success of *D. ranarum* in Africa. This enables *D. ranarum* to utilise niches that are inhospitable to

competitors, even while the other branchiurans evolved in different aspects of their biology such as:

- Developing new methods of reproduction, the members of the genus *Dolops* are the only branchiurans in which reproduction is achieved through means of a spermatophore,
- Developing new means of attachment to the host, the members of the genus *Dolops* are the only branchiurans which did not develop suckers instead of hooks for attachment, and
- Developing new methods of obtaining nutrition, the members of the genus *Argulus* developed a stylet for more effective feeding, and it is speculated that the members of the genus *Chonopeltis* do not feed on particles of tissue like members of the genus *Dolops* either, but possibly on slime from the skin of the host.

It is possible that the development of haemoglobin is the most important evolutionary development for *Dolops ranarum*, as this trait may have greatly contributed to its success and survival. The haemoglobin made it possible for *D. ranarum* to parasitise hosts that are not available to competitors from its own family (i.e. *Argulus* and *Chonopeltis*).

1.2 Comparison in the strategies of several crustacean fish parasites

The subphylum Crustacea includes organisms with widely varying morphology, biology and life strategies. The genera *Lamproglana* von Nordman, 1832 and *Ergasilus*, von Nordman, 1832 are both also part of the subphylum Crustacea. Like *Dolops ranarum*, both of these genera also occur in the Okavango and will be used for life strategy comparison. While the subphylum Crustacea is very diverse, the trends observed within these two genera present the opposite sides of the spectrum of possibility. While ergasilids parasitise any available host, *Lamproglana* species are very specific in the choice of a host.

- ***Ergasilus* von Nordman, 1832**

Ergasilus species falls into the Class Copepoda, the Order Poecilostomatoida and the Family Ergasilidae. Ergasilids are some of the most common copepod parasites of fishes. While ergasilids can swim fairly well when detached, the first pair of legs are supplied with heavy bladelike spines, and in some species the first and second endopodal segments are fused, giving the leg greater rigidity. This appendage is used for the rasping of mucus and epithelial cells from the gill where it is attached. This rasping of the gill often leads to secondary infection and when a host is heavily infected with ergasilids, this may result in death. The ergasilids have no species specificity whatsoever and colonise the hosts in enormous numbers. It is possible to find from a single individual, to hundreds of individuals on the gills of an infected host.

- ***Lamproglena* von Nordman, 1832**

The genus *Lamproglena* is included in the Class Copepoda, Order Cyclopoida, Family Lernaecidae. According to Van As & Van As (2007) all members of this genus are gill parasites of freshwater fishes, except one species. These parasites occur on cichlid hosts and are widely distributed in all four of the major river systems of Africa (Fryer 1961). *Lamproglena* species are very group specific. For instance, according to Tsotetsi *et al.* (2004) only *L. clariae* was collected from *Clarias gariepinus*. In the case of *L. hepseti*, Van As & Van As, 2007 it was found only on the African Pike (Van As & Van As 2007). Both records indicate absolute host specificity.

- ***Branchiura* (Thorell, 1864)**

Chonopeltis is even more host specific than the species mentioned above, most occurring on only one single host species.

It is a well-known fact by now that the flow of the Okavango River has changed over the past millennia. While it does not reach the sea today,

terminating in the alluvial fan in the Kalahari Desert, it used to flow to the Orange River in the west, and more recently into the Limpopo River in the east. It is possible that the reason *D. ranarum* is not found in the rivers south of the Limpopo, is because when the Okavango River reached the ocean through the Orange River, the fish species (which are hosts to *D. ranarum*) had not yet reached the Okavango, and therefore never reached the Orange River.

It is also important to notice the influence of the host on the evolution of the parasite. The migration and subsequent evolution in the related genus *Chonopeltis* can be seen with the variation in parasites found in each successive river from north to south down the east coast of Africa. Avenant-Oldewage & Knight (1994) noted that according to current knowledge, only two species of *Chonopeltis* occur in more than one river system. The geological distribution of *Chonopeltis* in Africa is illustrated by the information listed in Table 1.1 related to Figure 1.2.

Table 1.1: List of the *Chonopeltis* Thiele, 1900 species present in Africa and their distribution, information from Van As & Van As (1999a)

Species	River systems	Numbers on map
<i>Chonopeltis schoutedeni</i> (Brian, 1940)	Great Lakes of Central Africa	11, 12, 13, 16 & 17
<i>Chonopeltis congicus</i> Fryer, 1979	Great Lakes of Central Africa	11, 12, 13, 16 & 17
<i>Chonopeltis flaccifrons</i> Fryer, 1960	Great Lakes of Central Africa	11, 12, 13, 16 & 17
<i>Chonopeltis elongatus</i> Fryer, 1974	Great Lakes of Central Africa	11, 12, 13, 16 & 17
<i>Chonopeltis brevis</i> Fryer, 1961	Great Lakes of Central Africa	11, 12, 13, 16 & 17
<i>Chonopeltis koki</i> Van As, 1992	Zambezi River	18

Table 1.1 continued		
Species	River systems	Numbers on map
<i>Chonopeltis lisikli</i> Van As & Van As, 1996	Okavango Delta	23
<i>Chonopeltis liversedgei</i> Van As & Van As, 1999	Okavango Delta	23
<i>Chonopeltis inermis</i> Thiele, 1900	Lake Malawi, Limpopo River System	17 & 24
<i>Chonopeltis meridionalis</i> Fryer, 1964	Limpopo River	24
<i>Chonopeltis fryeri</i> Van As, 1986	Olifants River, Mpumalanga	
<i>Chonopeltis victori</i> Avenant-Oldewage, 1991	Olifants River, Mpumalanga	
<i>Chonopeltis australis</i> Boxhall, 1976	Orange-Vaal System	25
<i>Chonopeltis minutus</i> Fryer, 1977	Olifants River, South Western Cape	

As can be seen from the information included in Table 1.1 and Figure 1.2, *C. brevis* occurs in the Tana River and Lake Victoria in the Nile River System, while *C. inermis* occurs in Lake Malawi and the Limpopo System. In comparison, a wide variety of *Chonopeltis* species occur in the Zaire and Limpopo River systems. In Figure 1.2 the position of *Chonopeltis* species found in the successive rivers can be seen. *Chonopeltis fryeri* and *C. inermis* occurred only on clariid hosts in the Limpopo System. *Chonopeltis meridionalis*, *C. victori* and *C. australis* occurred on cyprinids and on *Labeo rosae*.

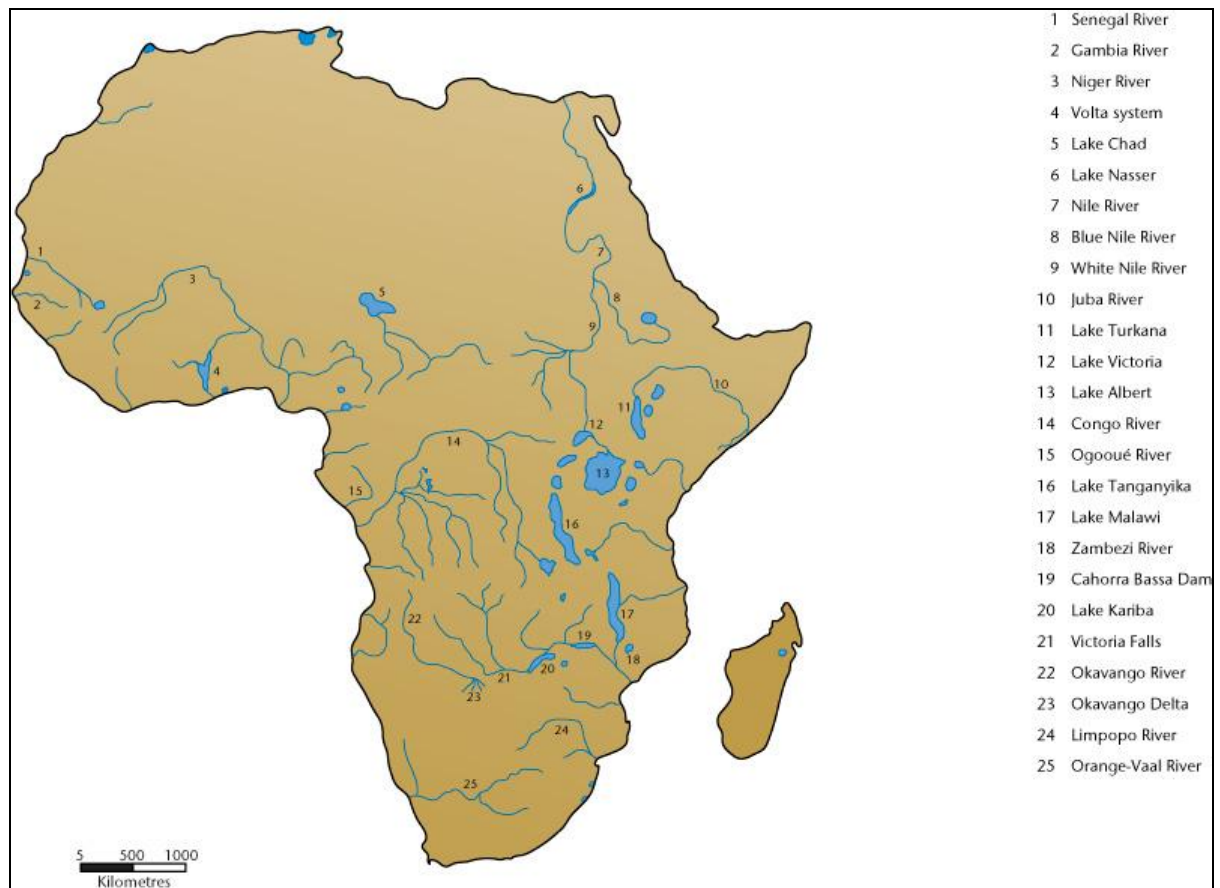


Figure 1.2: The rivers down the east coast of Africa (courtesy of the Aquatic Parasitology Research Group)

The relationship between the parasite and the host is obligate and therefore it is clear to see that the distribution of the parasite and the host (that must occur sympatrically in the same river system) generally overlap. However, *Clarias* does occur naturally in the rivers north of the Limpopo and was imported into the Cape-rivers (Van As & Van As 1999).

1.3 The Cytochrome Oxidase I gene

The Cytochrome Oxidase I gene occurs within the mitochondrial DNA (mtDNA). According to Dawnay *et al.* (2006) mitochondrial genes have a higher number of copies, making the yield of mtDNA higher than that of genomic DNA. mtDNA genes also characteristically lack recombination promoting the loss or fixation of

mtDNA haplotypes. This reduces within species diversity and thus enables species identification.

Herbert *et al.* (2003) give the following advantages why the COI gene should be used for DNA barcoding:

- The robust nature of the universal primers, which should enable the recovery of its 5' end from the representatives of most if not all animal phyla, and
- COI appears to have a greater range of phylogenetic signal than any other mitochondrial gene.

The COI gene is likely to provide deeper phylogenetic insights because changes in the amino-acid sequence occur more slowly than those in other mitochondrial genes. Due to this fact, the study of amino-acid substitutions may make it possible to assign unidentified organisms to a higher taxonomic group before examining the nucleotide substitutions to determine its species identity (Herbert *et al.* 2003).

Dasmahapatra & Mallet (2006) described the methodology used in DNA barcoding as straightforward. Sequences of the selected barcoding region are acquired from a range of individuals. A phylogenetic tree is constructed from the obtained sequence data, and a distance-based 'neighbour-joining' method. In such a tree, similar (and therefore putatively related) individuals are clustered together. The term 'DNA barcode' implies that each species is characterised by a unique sequence. However, there is considerable genetic variation within a species and between different species. Genetic distances between species are, of course, usually greater than those within species. Consequently, the phylogenetic tree shows clusters of closely related individuals. According to the DNA barcoding method each of these clusters is assumed to represent a separate species.

Herbert *et al.* 2003 concentrated on making a COI profile for the seven most diverse animal phyla, founded on the analysis of 100 representative species. This study showed that the baseline information designated 96% of the newly analysed taxa to the correct phylum. This test was repeated for the hexapods, because this is the most ubiquitous group on the planet. Finally, the ability of COI sequences to identify up to species level was tested using lepidopterans. This group was chosen because sequence divergences are low among the families in this order. This makes species identification a challenge, particularly because this is one of the orders of insects containing the most species. All of the 150 specimens analysed were assigned to the correct species.

Herbert *et al.* (2003) created COI profiles, which were calculated to give an indication of COI diversity contained by each taxonomic collection and were afterwards used as the basis for identifications of the phylum, ordinal or species level by determining the sequence similarity between each unknown taxon and the species included in a specific profile.

The study confirmed the viability of building up a COI-based identification system for all organisms. PCR products were collected from all species and there was no indication of complications, indels were uncommon. Aside from the fact that these sequences were easy to obtain and align, they had a high level of diversity. It was so high in fact that it was sufficient to enable the reliable assignment of organisms to higher taxonomic categories. According to Herbert *et al.* (2003) the measure of success for any taxonomic system was its capability to provide correct species identification. This test has provided evidence that if a comprehensive database of sufficient sequences could be created, this could be a feasible taxonomic system. Therefore, the COI gene was judged a viable place to start in the amplification of DNA of branchiurans.

This dissertation will deal with the study in the following order: Chapter 2 is a review of the Branchiurans in Africa in general, and *Dolops ranarum* in particular. The taxonomy, distribution and biology (including the life cycle) of *D. ranarum* are discussed. This is followed by information on the evolutionary

history of crustaceans (very briefly) branchiurans and related fish hosts. This was included to indicate that during evolutionary history of the hosts and the parasites, there has been ample time for speciation. The early representatives of branchiurans may have been parasitising early representatives of the fish host genera. This may have lead to isolation.

Chapter 3 includes a brief overview of the major river basins of Africa with specific reference to the Okavango Delta (the study locality). This information is included to give emphasis to the unique nature of the Okavango River when compared with the other major river systems in Africa. In addition a brief description is given of the biodiversity of the Okavango system, with emphasis on the fish fauna.

Chapter 4 indicates the materials and methods that were employed during the morphological study, while Chapter 5 gives the results of the morphological study. Chapter 6 is an overview of molecular methods in taxonomy, Chapter 7 and 8 are the molecular materials and methods and molecular results respectively. Chapter 9 is a general discussion on both the morphological and molecular studies, including concluding remarks and indications of further study. Ending with the references in Chapter 10, which is followed by the acknowledgements and attached appendices.

Chapter 2: A review of the African Branchiura

Branchiurans are commonly known as fish lice and have representatives in fresh, brackish and marine habitats (Van As & Van As 2001). Three genera of Branchiura are present in Africa namely *Argulus*, *Dolops* and *Chonopeltis*, of which *Argulus* is the most commonly known. Although found on the skin and fins of the fish, branchiurans most often occur in the gill chambers of the host (Schram 1986).

There are approximately 32 species of *Argulus* present on hosts in Africa, 14 species of *Chonopeltis*, but only one species of *Dolops* has been reported; i.e. *D. ranarum*, which has a Pan-African distribution (see Table 2.1). Nevertheless, *Dolops ranarum* is the most common argulid in Africa and a very early component of the African fauna (Douellou & Erlwanger 1994).

Table 2.1: List of representatives of the Branchiura Thorell, 1864 in Africa information obtained from Avenant, Loots & van As (1989b); Tam & Avenant-Oldewage (2008); Van As, Van Niekerk & Olivier (1999); Van As & Van As (1999a); Van As & Van As (1999b)

<i>Dolops</i> Audouin, 1837
<i>D. ranarum</i> (Stuhlman, 1891)
<i>Argulus</i> Müller 1875**
<i>A. purpureus</i> Risso, 1826
<i>A. melita</i> Beneden, 1891
<i>A. japonicus</i> Thiele, 1900
<i>A. africanus</i> Thiele, 1900
<i>A. belones</i> Van Kampen, 1909
<i>A. arcassonensis</i> Cuénot, 1912
<i>A. angusticeps</i> Cunningham, 1913
<i>A. personatus</i> Cunningham, 1913
<i>A. exiguus</i> Cunningham, 1913
<i>A. incisus</i> Cunningham, 1913
<i>A. rubecens</i> Cunningham, 1913
<i>A. rubropunctatus</i> Cunningham, 1913
<i>A. striatus</i> Cunningham, 1913
<i>A. ambloplites</i> Wilson, 1920

Table 2.1 continued
<i>A. confusus</i> Wilson, 1920
<i>A. reticulatus</i> Wilson, 1920
<i>A. schoutedeni</i> Monod, 1921
<i>A. alexandrensis</i> Wilson, 1923
<i>A. zeii</i> Brian, 1924
<i>A. trachynoti</i> Brian, 1927
<i>A. otolithi</i> Brian, 1927
<i>A. rhipidiophorus</i> Monod, 1931
<i>A. rijkmansii</i> Brian, 1940
<i>A. dartevelli</i> Brian, 1940
<i>A. wilsonii</i> Brian, 1940
<i>A. capensis</i> Barnard, 1955
<i>A. multipocula</i> Barnard, 1955
<i>A. ambloplites jollymani</i> Fryer, 1956
<i>A. brachypeltis</i> Fryer, 1959
<i>A. monodi</i> Fryer, 1959
<i>A. dageti</i> Dollfus, 1960
<i>A. cunningtoni</i> Fryer, 1964
<i>A. fryeri</i> Rushton-Mellor, 1994
<i>A. gracilis</i> Rushton-Mellor, 1994
<i>A. kosus</i> Avenant-Oldewage, 1994/ <i>A. smalei</i> Avenant-Oldewage & Avenant, 1995*
<i>A. izintwala</i> Van As & Van As, 2001
<i>Chonopeltis</i> Thiele. 1900
<i>C. intermis</i> Thiele, 1900
<i>C. schoutedeni</i> (Brian, 1940)
<i>C. congicus</i> Fryer, 1959
<i>C. flaccifrons</i> Fryer, 1960b
<i>C. brevis</i> Fryer, 1961
<i>C. meridionalis</i> Fryer, 1964
<i>C. elongatus</i> Fryer, 1974
<i>C. australis</i> Boxhall, 1976
<i>C. minutus</i> Fryer, 1977/ <i>C. australissimus</i> Fryer, 1977*
<i>C. fryeri</i> Van As, 1986
<i>C. victori</i> Avenant-Oldewage, 1992
<i>C. koki</i> Van As, 1992
<i>C. lisikili</i> Van As & Van As, 1996
<i>C. liversedgei</i> Van As & Van As, 1999

*Note: The species that are considered by some authors to be synonymous are indicated together.

** Table 2.1 has been compiled from the sources quoted. Therefore, only species deemed synonymous by these particular authors have been indicated as such.

2.1 Taxonomy of the Branchiura

First included in the Copepoda in the early 1800's this group was reassigned to the Branchiopoda, now a different taxon altogether (Van As & Van As 2001). According to Van As & Van As (2001), Thorell created the name Branchiura in 1864, but agreed that it belonged with the Branchiopoda and in 1875, Clauss reassigned the Branchiura to the Copepoda where it remained until 1932 when Martin established it as a separate group, a subclass of Maxillopoda. However, some taxonomists do not recognise Maxillopoda as a Class, and therefore all the subclasses under Maxillopoda are recognised as separate Classes. In this instance Branchiura is seen as a class as in Van As & Van As (2001).

All the representatives of the Branchiura are external parasites of fish, found in the gill chambers and on the skin of the fish (Fryer 1968). The Crustacea is a subphylum of the Arthropoda. According to Schram (1986) branchiurans are a class of parasitic crustaceans possibly related to the copepods. In the class Branchiura there is only one family the Argulidae Leach, 1819 consisting of four genera i.e. *Argulus*, *Chonopeltis*, *Dolops* and *Dipteropeltis*.

2.2 Distribution of Branchiura

Branchiurans are found globally in marine, freshwater and brackish habitats (Schram 1986). Eleven species of branchiurans are present in the rivers of southern Africa (Table 2.2).

Table 2.2 Branchiuran species present in freshwater habitats in southern Africa – host species and distribution (Adapted from Van As & Van As 2001)

Parasite species	Hosts	Distribution
<i>Dolops ranarum</i> *	<i>Clarias gariepinus</i> <i>Oreochromis mossambicus</i> <i>O. mortimeri</i> <i>Labeobarbus marequensis</i>	Occurs only in habitats where <i>Clarias gariepinus</i> and <i>Oreochromis mossambicus</i> are present sympatrically: Limpopo and Orange-Vaal Systems Zambezi System (Lake Kariba and Pongola Floodplain) Okavango River
<i>Argulus capensis</i>	<i>Labeobarbus aeneus</i> <i>L. kimberleyensis</i> <i>C. gariepinus</i> <i>Labeo capensis</i> <i>L. umbratus</i> <i>Tilapia sparrmanii</i> <i>Carassius auratus</i> (exotic) <i>Cyprinus carpio</i> (exotic)	Established throughout southern Africa and the coastal rivers on the west and south coast.
<i>Chonopeltis australis</i>	<i>Labeo capensis</i> <i>Labeobarbus aeneus</i>	Orange-Vaal System
<i>C. minutus</i> / <i>C. australissimus</i> *	<i>Barbus calidus</i> <i>B. erubescens</i> <i>Pseudobarbus burgi</i>	Olifants System (W. Cape) Great Berg River (W. Cape)
<i>C. fryeri</i>	<i>C. theodora</i> <i>C. gariepinus</i>	Limpopo System (Limpopo River and the Olifants River, Mpumalanga)
<i>C. inermis</i>	<i>C. theodora</i>	Limpopo System
<i>C. victori</i>	<i>L. rosae</i> <i>L. ruddi</i> <i>L. rubropunctatus</i> <i>Labeobarbus marequensis</i>	Limpopo System (Olifants and Letaba Rivers)

Table 2.2 continued		
Parasite species	Hosts	Distribution
<i>C. koki</i>	<i>L. cylindricus</i>	Zambezi System (Eastern Caprivi)
<i>C. meridionalis</i>	<i>L. rosae</i>	Limpopo System (Nuanetzi River)
<i>C. lisikili</i>	<i>Synodontis leopardinus</i> <i>S. macrostigma</i>	Thamalalakane, Shashi, Okavango and Kavango Rivers (Botswana)
<i>C. liversedgei</i>	<i>Mormyrus lacerda</i>	Okavango River (Botswana)

*Note: The species that are considered by some authors to be synonymous are indicated together.

- Distribution of the genus *Argulus* Müller, 1875**

According to Rushton-Mellor (1994b) *Argulus* is the largest of the class Branchiura containing nearly 150 species worldwide. So far 32 species have been reported from African rivers (Rushton-Mellor 1994a). However, this number has been disputed, and while Rushton-Mellor (1994c) indicated that 37 species have been described, certain doubts existed about the validity of some of the species. For instance, Rushton-Mellor (1994c) indicated that in 1928 Monod thought that *A. arcassonensis* Cuénot, 1912 might be synonymous to *A. zeii* Brian, 1924 and that *A. otolithi* Brian, 1927 and *A. arcassonensis* Cuénot, 1912 might be similar. In addition, Van As *et al* (1999) indicated that *A. kosus* Avenant-Oldewage, 1994 and *A. smalei* Avenant-Oldewage & Avenant, 1995 might be the same species. However, only two, *A. japonicus* Thiele, 1900 and *A. capensis* Barnard, 1955 are found in South Africa south of the Limpopo River (Van As & Van As 2001). Rushton-Mellor (1994c) also mentioned that *Argulus* species in general, are found in marine and freshwater habitats, and that some species in particular, are found in both fresh and salt water. This was in contrast to *Dolops* and *Chonopeltis* present in Africa that are found in freshwater habitats only.

- **Distribution of the genus *Chonopeltis* Thiele, 1900**

The genus *Chonopeltis* is endemic to Africa, and so far 14 species have been described, eight from southern Africa (Van As & Van As 2001).

- **Distribution of the genus *Dipteropeltis* Calman, 1912**

The fourth genus, *Dipteropeltis*, may be endemic to South America, as no representatives have been found on the other continents (Van As & Van As 2001). The only other report of *D. hirundo* was by Carvalho *et al.* (2003) collected from piranhas in the Pantanal in Brazil.

- **Distribution of the genus *Dolops* Audouin, 1891**

There are 14 known species of *Dolops*, all from freshwater habitats (Van As & Van As 2001). *Dolops* species all occur in the southern hemisphere, one species is present in Africa south of the Sahara, 12 species in found in South America and one in Tasmania (Van As & Van As 2001) (Table 2.3). Due to the nature of the distribution of *Dolops* in the rest of the landmasses located in the southern hemisphere, it seems likely that representatives of the genus *Dolops* would also be present in Australia, as yet undiscovered.

Schram (1986) noted that the distribution makes it probable that the Branchiura is an ancient group “*with strong connections to Gondwanaland*”. This can be inferred from the distinct ranges of the Branchiuran genera as described above.

Table 2.3: Summary of *Dolops* Audouin, 1837 (Branchiura) species distribution and hosts

Species	Locality	Host
Africa		
<i>Dolops ranarum</i> (Stuhlman, 1891)	See Table 2.5	See Table 2.5
South America		
<i>Dolops longicauda</i> (Heller, 1857)		
<i>Dolops kollari</i> (Heller, 1857)	Brazil	Host unknown
<i>Dolops lacordairei</i> (Audouin, 1857)		
<i>Dolops doradis</i> (Cornalia, 1860)	Central America	<i>Doras niger</i>
<i>Dolops intermedia</i> (Da Silva, 1878)		
<i>Dolops geayi</i> (Bouvier, 1897)	Guinea	Free Swimming
<i>Dolops discoidalis</i> Bouvier, 1899	Brazil	<i>Platysoma</i> sp.
<i>Dolops reperta</i> (Bouvier, 1899)	Guinea	<i>Aymara</i>
<i>Dolops striata</i> (Bouvier, 1899)	Guinea	<i>Anguilla</i> sp
<i>Dolops bidentata</i> (Bouvier 1899)	Guinea	<i>Anguilla</i> sp
<i>Dolops carvalhoi</i> De Castro, 1949		
<i>Dolops nana</i> De Castro, 1950		
Tasmania		
<i>Dolops tasmanianus</i> Fryer, 1969	Lake Surprise, Tasmania	<i>Galaxias</i> sp

According to current information different branchiuran genera are very orderly in distribution, either globally, hemispherically or endemically to a continent (Table 2.4). While representatives can be found in freshwater and brackish environments, all the marine species (genus *Argulus*) are found in coastal waters and not in the open ocean. Consequently, it is likely that branchiurans evolved during the Triassic, when all the continents were united in the supercontinent Pangaea, spreading from an original site of evolution before the continents were divided by continental drift. In addition, the most primitive of the genera of Branchiura would appear to be *Dolops* due to two main reasons namely:

- The manner of reproduction in *Dolops*. While the other genera use specialised copulation structures on the legs, *Dolops* uses a spermatophore (Fryer 1960), and

- The fact that *Dolops* retains the maxillule as hooks throughout the life cycle. The other members of the genera are born with maxillulae as hooks, but these hooks develop into suckers during the ontogeny (Avenant *et al* 1989a).

Table 2.4 : Number of species per genus of representatives of the Branchiura occurring in freshwater habitats in each of the biogeographic regions of the world (adapted from Poly 2008)

Branchiuran genera	Zoogeographical Regions								
	Paleartic	Nearctic	Neo-tropical	Afro-tropical	Oriental	Austra-lasian	Islands Oceanic Pacific	Ant-arctic	World
<i>Argulus</i>	8	18	21	25	16	2	1		85
<i>Chonopeltis</i>				14					14
<i>Dipteropeltis</i>			1						1
<i>Dolops</i>			9	1		1			11
<i>Total Argulidae</i>	8	18	31	40	16	3	1	0	111

- **Distribution of *Dolops ranarum* (Stuhlman 1891)**

Dolops ranarum is endemic to Africa, occurring on several freshwater fish species and less often on tadpoles (Avenant *et al* 1989a, Fryer 1968). In contrast to the genus *Argulus*, *Dolops ranarum* is very specific in its habitat, in that it is only found in African river systems where clariids and cichlids occur simpatrically. However, *D. ranarum* is opportunistic as to its hosts, being found on fishes of diverse families and on amphibian hosts (Table 2.5).

Table 2.5: Summary of the known information on the distribution, host preference and position preference for *Dolops ranarum* (Stuhlman, 1891) in Africa (courtesy of the Aquatic Parasitology Research Group)

Reference	Locality	Host*†	Position on host*
Stuhlman (1891)	Mouth of Bukoba West Nyanza	Tadpole (possibly related to <i>Hyperolius</i>)	Body surface and entrance to gills
Wilson (1902)	Bukoba, West Nyanza	Tadpoles	Skin
Cunnington (1913)	Kala, Tanzania	<i>Lates microlepis</i>	Mouth and gill chamber
	Bukoba Victoria Nyanza	Large representative of the Siluridae. Local name “Nfui”	Body surface, mouth and gill chambers
		<i>Protopterus aethiopicus</i>	Body surface and mouth
		Representative of Siluridae Local name Nshonzi	Body surface, mouth and gill chambers
		Possibly a catfish	Skin
	Lake Malawi	Local name “Sungwa”	
Monod (1928)	White Nile	Local name “Hala” (<i>Heterobranchus bidorsalis</i>)	Mouth
	Kala, Tanzania	In zooplankton	
Brian (1940)	Lake Mweru, Lukonzolwa	<i>Eutropius</i> sp	Mouth and Gill chambers
	Lake Albert	Unidentified fish	Gills

Table 2.5 continued			
Reference	Locality	Host*†	Position on host*
Barnard (1955)	Hartebeespoort Dam	<i>Oreochromis mossambicus</i>	
	Apies River	<i>Labeobarbus marequensis</i>	
	Olifants River (Near Middelburg)	<i>Labeobarbus marequensis</i>	
	Matopo Dam (Bulawayo)	<i>Micropterus salmoides</i>	
Fryer (1956)	Region of Lake Malawi, Banga River	<i>Clarias gariepinus</i>	
	Bana Lagoon	Free swimming larva	
	Bana Lagoon	<i>Clarias gariepinus</i>	
	Mouth of stream in Nkata Bay	<i>Clarias gariepinus</i>	
Fryer (1959a)	Lake Bangweulu (Zambia)	Most commonly on smooth-skinned fish (<i>Chrysichtys</i> , <i>Eutropius</i> , <i>Schilbe</i> , <i>Clarias</i> and <i>Heterobranchus</i> for instance) but also occasionally on fish with scales	
Fryer (1959b)	Congo River	<i>Ophiocephalus obscurus</i>	
	Upper Zambezi River	<i>Clarias gariepinus</i>	
	Mid Zambezi River, near Chibobama	<i>Oreochromis mossambicus</i>	
	Kafue River near Mazabuka	<i>Serranochromis</i> sp	
	Fort Johnstone, Lake Malawi		

Table 2.5 continued			
Reference	Locality	Host*†	Position on host*
Fryer (1959b)	Lake Nabugabo, Uganda		
	Lake Baringo, Kenya		
Fryer (1961)	Victoria Nile	<i>Bagrus docmac</i>	
	Lake Victoria	<i>Protopterus aethiopicus</i> <i>Clarias gariepinus</i> <i>Bagrus docmac</i> <i>Tilapia esculenta</i> <i>Tilapia variabilis</i>	
Fryer (1964)	Niger River (Upper-Senegal)	<i>Tetraodon fahaka</i>	
	Vila Perreira de Eça (Angola)		
	Lake George (Uganda)	<i>Bagrus docmac</i>	
	Lake Mweru	<i>Labeo altevelis</i>	
Fryer (1965a)	Lake Mweru	<i>Tilapia macrochir</i>	
Fryer (1965b)	Lake Tana (Ethiopia)	<i>Tilapia nilotica</i> <i>Varichorhinus</i> sp	
	Koka Dam (Ethiopia)	<i>Tilapia</i> sp	
	Lake Albert	<i>Auchenoglanis occidentalis</i> <i>Bagrus bayad</i> <i>Bagrus docmac</i> <i>Clarias lazera</i> <i>Lates niloticus</i>	

Table 2.5 continued			
Reference	Locality	Host*†	Position on host*
Fryer (1968)	Nile River system	<i>Dinopterus filicibarb</i>	
	Niger River system		
	Congo River System		
	Zambezi River System		
	Limpopo River System		
	Awash River (Ethiopia)		
	Angola		
	Lake Nyasa		
	Lake Bangweulu		
	Lake Mweru		
	Lake Malawi		
	Lake Edward/George		
	Lake Albert		
	Lake Victoria		
	Lake Kyoga		
	Lake Tana		
Thurston (1970)	Lake Victoria	<i>Bagrus docmac</i> <i>Clarias gariepinus</i> <i>Astatoreochromis alloudi</i> <i>Mormyrus kannume</i>	
	Lake Albert	<i>Lates alberianus</i>	
	Lake Kyoga	<i>Lates alberianus</i>	
	Victoria Nile	<i>Lates alberianus</i>	
Bruton (1979)	Lake Sibaya	<i>Clarias gariepinus</i>	
Mbahinzireki (1980)	Lake Victoria	<i>Bagrus docmac</i>	

Table 2.5 continued			
Reference	Locality	Host*†	Position on host*
Avenant & van As (1985)	Limpopo River System Orange-Vaal River System	<i>Labeobarbus marquensis</i> <i>Barbus mattozi</i> <i>Labeo rubropunctatus</i> <i>Duopius depressirostris</i> <i>Clarias gariepinus</i> <i>Micropterus dolomieu</i> <i>Chetia flaviventris</i> <i>Oreochromis mossambicus</i>	Mouth cavity, gills body surface and fins
Douellou & Erlwanger (1993)	Lake Kariba	<i>Oreochromis mortimeri</i> <i>Pseudocrenilabrus philander</i> <i>Serranochromis codringtonii</i> <i>Serranochromis macrocephalus</i> <i>Tilapia rendalli</i> <i>Clarias gariepinus</i> <i>Synodontis zambezensis</i> <i>Mormyrops deliciosus</i> <i>Mormyrus longirostris</i>	
Boane et al, (2008)	Limpopo River and Chuali Lagoon	<i>Cyprinus carpio</i>	

*Note: If the information is not included it was not supplied

†Note: If the local name is given it was used by the original author

2.3 Branchiuran morphology

• General morphology

According to Schram (1986) the morphology of the branchiurans can be defined in the following manner: the body is particularly characterised by the greatly flattened shape. Branchiurans are organisms with a carapace as a bi-lobed dorsal shield.

The eyes are paired and compound and naupliar eyes persist in the adult. The antennules are short and typically have spines or hooks on the basal segments. The antennae, which are also small, bear terminal spines and basal hooks and are closely associated with the antennules. The maxillulae are highly modified as either hooks or suckers for attachment. Four trunk somites are present, each with a biramous, setose pair of appendages. Modifications may be present on the second through fourth thoracopods on the males for copulation. The abdomen is unsegmented. The anus is situated in the cleft between the two lobes and is flanked by the caudal rami.

The internal anatomy of branchiurans consists of a digestive system, which is relatively simple bar the caeca; the caeca arise from the anterior of the midgut and are very branched. This branching increases with age. The caeca are present within the carapace, and during feeding, the caeca become engorged and fill the volume of the carapace (Schram 1986). Subsequently, excretion is achieved using the maxillary glands.

The circulatory system is equally simple, consisting of a triangular heart situated in the last thoracic segment and an anterior aorta. The blood moves freely within the large body sinuses assisted by the beating of the heart and ordinary body movement. Respiration occurs when the blood moves over the thin cuticle of the respiratory areas on the ventral surface of the carapace (Schram 1986).

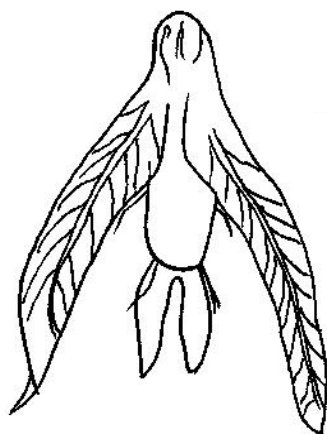
The female reproductive system generally comprises a median ovary, which opens on the last thoracic sternite by means of a short oviduct. The vaginal opening is flanked by two seminal papillae connected by ducts to two seminal receptacles in the anterior part of the abdomen. According to Schram (1986) the male reproductive system is more complex. A pair of testes is present in the abdomen and is connected by vasa efferentia to a median seminal vesicle in the thorax. A pair of vasa deferentia leads from the anterior end of the vesicle and proceeds to the posterior of the organism. The vasa deferentia are joined by the ducts, which drain glands. The vasa differentia then turn medially and join into a single ejaculatory duct, which opens on the last thoracic sternite.

Schram (1986) described the nervous system of the branchiurans as centralized. The large brain is connected to the antennules and eyes. The antennal nerves originate from the circumesophageal commissures. The posterial ganglia are fused. However, six lobes can still be recognised. This ganglionic mass innervates the remaining appendages as well as the carapace and the abdomen.

- **Specific morphological traits of branchiuran genera**

The morphological traits that are important in the classification of branchiurans differ among the genera. *Dipteropeltis* is not discussed, as no representative of this genus is present in Africa. However, a representation of *Dipteropeltis* is included in Figure 2.1 for a complete picture of the family Argulidae. The general representatives utilised are according to Van As & Van As (2001).

Dorsal



Ventral

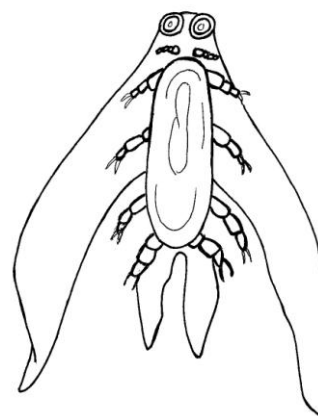


Figure 2.1 Representation of *Dipteropeltis hirundo* Calman, 1912 redrawn from Calman (1912), dorsal and ventral view

➤ *Argulus* Müller, 1785

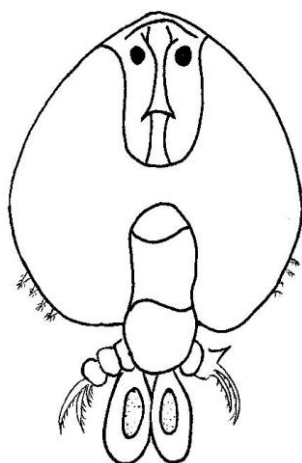
Cunnington (1913) also remarked that the accurate diagnoses of *Argulus* species are becoming increasingly difficult, because the differences are often minor in nature and concerned several parts of the external anatomy. In addition, Rushton-Mellor (1994c) indicated that the keys to the species of *Argulus* from the African

region no longer included all the species now known from that area. In addition many of the keys included characters, which exhibit intraspecies variation.

The representative species of the genus chosen was *Argulus japonicus* Thiele, 1900 genus according to Van As & Van As (2001) (Figure 2.2). In the case of *Argulus* the genus is diagnosed by the following traits according to McLaughlin (1980) and Van As & Van As (2001):

- The carapace is trifoliate with a distinct anterior lobe,
- Pigmentation present in most species (though less prominent than in *Dolops*),
- The size and shape of the abdomen varies between species,
- Antennules with strong sickle formed terminal hooks,
- Antennae are present,
- Maxillulae are suckers,
- Stylet is present on front of proboscis,
- Mouth in proboscis,
- Respiratory areas may be of specific taxonomic importance, they consist of two to three clearly distinguishable areas on the ventral side of the lateral carapace lobes, and
- The number of sclerites within the suckers and their shape are both used to differentiate among species.

Dorsal



Ventral

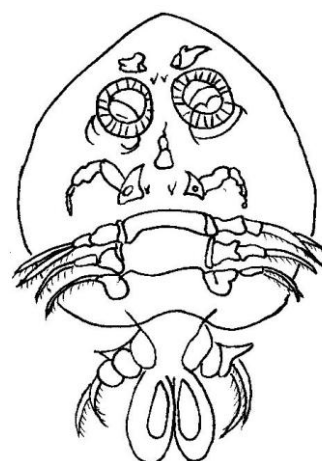


Figure 2.2: Dorsal and ventral views of *Argulus japonicus* Müller, 1785 as a general representative species of the genus according to Van As & Van As (2001) redrawn from Van As & Van As (2001)

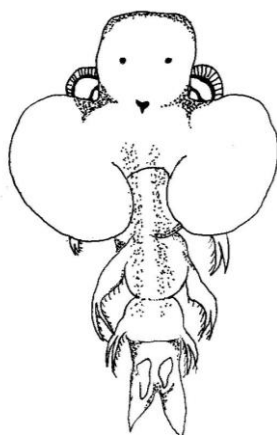
➤ *Chonopeltis* Thiele, 1900

In the case of *Chonopeltis* the genus is characterised by the following traits according to Van As & Van As (2001). *Chonopeltis australis* Boxshall, 1976 was chosen as the general representative species of the genus according to Van As & Van As (2001).

- The carapace is trifoliate and covers at most the first two pairs of legs,
- The anterior lobe is narrow, rectangular and is supported by a thickened rim at the anterior margin,
- A medial indentation is present in the thickened margin for some species,
- In general the anterior lobe is further supported by chitinous rods,
- Antennae are uniramous and have four segments, this characteristic does not vary greatly between different species,
- Antennules are absent, but a cluster of 12-15 setae are found at the base of each antenna possibly representing a rudimentary antennule,
- No stylet or mouth tube is present,

- The maxillulae develop into a large pair of suckers in adults. In the case of *Chonopeltis*, the number and shape of sclerites and rods do not seem to be taxonomically significant,
- The peg and socket copulation structures on the legs are relevant in species descriptions,
- Shape and size of the abdomen,
- Length of the abdomen relative to the body,
- The absence or presence of pigmentation, and
- The size and shape of the testes and spermathecae.

Dorsal



Ventral

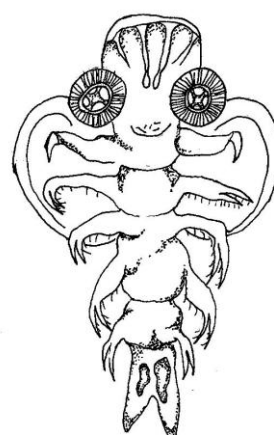


Figure 2.3: Dorsal and ventral views of *Chonopeltis australis* Boxshall, 1976 as a general representative species of the genus according to Van As & Van As (2001), redrawn from Van As & Van As (2001)

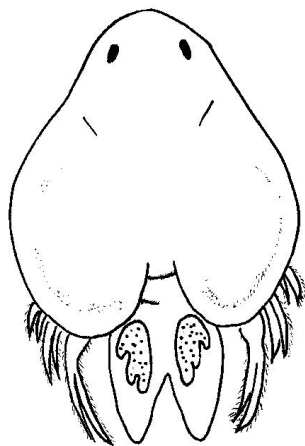
➤ *Dolops* Audouin, 1837

Stuhlman (1891) first described *Dolops ranarum* under the name of *Gyropeltis ranarum*. This name was given because the first representatives of the species were collected from tadpoles in Bukoba, Tanzania. However, fish lice are much more common on fish than on tadpoles.

The representative species chosen was *D. ranarum* (Stuhlman, 1891) (Figure 2.3). In the case of *Dolops* the genus is typified by the following characteristics according to Fryer (1969).

- Carapace covers all or at least the first three pairs of the swimming legs,
- Antennae and antennules are both present, (see Figure 2.5)
- Maxillae short and strong, terminating in strong sclerotised hooks, suckers are absent,
- Maxillulae also terminates in hooks (Figure 2.5), and
- Spermatophore used for reproduction.

Dorsal



Ventral

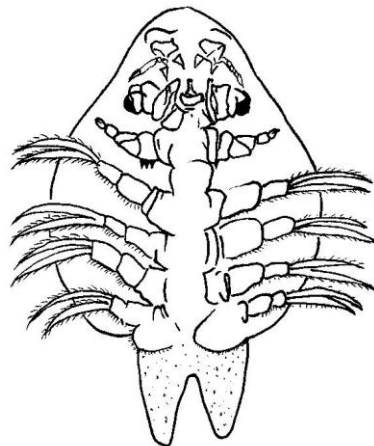


Figure 2.4: Dorsal and ventral views of *Dolops ranarum* (Stuhlman 1891) as a general representative species of the genus according to Van As & Van As (2001)



Figure 2.5: (A) Antennule and antenna (B) maxilla of *Dolops ranarum* (Stuhlman 1891) redrawn from Van As & Van As (2001).

- **Specific morphological traits of *Dolops ranarum* (Stuhlman, 1891)**

The species, *Dolops ranarum* was re-described by Avenant *et al.* (1989b) with the following characteristic attributes:

- The carapace is almost round, the relationship between the width and the length of the carapace is ± 1 ,
- The carapace has a deep incision posteriorly,
- Two sharp tapering spines are present at the base of the antennulae and antennae (the presence of these two spines specifically is also stated by Wilson (1902) to be characteristic of the species), and
- Maxillulae are well-developed hooks.

2.4 Branchiuran biology

As mentioned previously, branchiurans fasten to hosts utilising the antennal hooks and the maxillulae. Despite their small size branchiurans are competent swimmers, and leave their hosts to locate mates, lay eggs and acquire new hosts (Schram 1986). However, Schram (1986) also notes that *Argulus* and *Dolops* appear to be more competent swimmers than *Chonopeltis* and *Dipteropeltis*.

According to Schram (1986) branchiurans utilise their hosts in different ways to acquire nourishment. Some species insert a proboscis into a blood vessel of the host, while others feed by rasping away at the host's integument, feed on the mucus and epithelial cells as well as extra cellular fluids.

- **General biology of *Argulus* Müller, 1785**

According to Van As & Van As (2001), all stages of development of this parasite are found throughout the year. The copulation process in *Argulus* is similar to that of *Dolops* except that a spermatophore is produced (in the case of *Dolops*). Eggs develop in the ovaries of the females in an unsynchronised manner. One to nine strings of eggs are laid with up to 226 eggs per string arranged in rows.

Since *Argulus japonicus* is an exotic species, originally from the Far East, it is associated with cyprinid hosts. *A. japonicus* has been distributed throughout the world by humans trading in ornamental fish and aquaculture. The stylet is utilised to penetrate the epidermis of the fish, disrupting the host epithelium. The mouth is then placed over the lesion to draw blood (Van As & Van As 2001).

- **General biology of *Chonopeltis* Thiele, 1900**

According to Van As & Van As (2001) little is known about the mating and breeding habits of *Chonopeltis*, but it is expected that it will be similar to those of other branchiuran species. Egg clusters of up to 26 adhering to specimen bottles were observed during laboratory experiments. The eggs began to hatch after 28 days.

After hatching, the carapace is undivided, but develops to the typical three-lobed shape towards the pre-adult stage. The antennae are large, extending far beyond the carapace margin. The maxillulae are pincer-like appendages. The base of the third podomere of the maxilla is indented with a cluster of brush-like setae present in the depression. This hollow becomes more prominent during subsequent larval stages and the larvae utilise this prehensile joint for grooming. This function is only present in the juvenile stages as the setae disappear in the last moult prior to adulthood (Van As & Van As 2001).

It has been observed by Van As & Van As (2001) that the mouth is functional in the first larval stage, suggesting that the larvae feed actively. The undifferentiated legs are not functional and the abdomen is a single lobe with terminal furcal rami. As with *Argulus* species, the maxillule develops into a functional sucker. In *Chonopeltis* species the rudiment of the hook loses its functionality during the fifth moult and is lost in the last moult.

Chonopeltis species are highly selective to the host and even the location of attachment on the host (Van As & Van As 2001). The branchiurans are never found on the host in large numbers and no reports exist of mortalities or pathological effects on hosts caused by *Chonopeltis* infections.

Presently, little is known about the feeding habits of *Chonopeltis* species. However, it is unlikely that they feed on blood, because red blood cells would be easily seen after feeding within the alimentary canal in their transparent bodies. In addition the morphology of the mouth makes it unlikely for the organism to feed through suction. It is possible that *Chonopeltis* feeds on the mucus excreted by the fish host (Van As & Van As 2001).

- **Biology of *Dolops ranarum* (Stuhlman, 1891)**

Dolops ranarum does not remain permanently attached to a particular site on the host, but moves around on the surface (Van As & Van As 2001). As a result of the use of maxillulae formed into hooks to attach to the host, clear lesions can be observed on the integument of the host. It has been suggested that *D. ranarum* feeds on tissue particles that have been extracted from the host rather than from blood. Despite attachment lesions, it is unlikely that infestations of this parasite will have serious impacts on the host if infestations are low (Van As & Van As 2001).

According to Fryer (1961) *D. ranarum* is capable of parasitising many different hosts, and the parasite seems to be influenced more by the habitat preferences of the host than their specific properties. In addition Fryer also noted in 1961 that *D. ranarum* could withstand conditions with relatively low oxygen concentrations, possibly due to the presence of haemoglobin within the organism. As a result of

this adaptation, *D. ranarum* is the most common parasite on hosts that dwell in areas with low oxygen tension within Lake Victoria (*Protopterus aethiopicus*, *Clarias mossambicus*, and *Bagrus docmac*) whilst *Argulus africanus* (which occurs commonly in Lake Victoria as well) is found most often on hosts that do not frequent such areas (*Tilapia* sp) (Fryer 1961).

The most obvious difference in the reproductive biology between *D. ranarum* and the other species of Branchiura is that *D. ranarum* reproduces with the transference of a spermatophore (Fryer 1956). According to Van As & Van As (2001) seasonal investigation on the biology of *D. ranarum* indicated that this species does not breed throughout the year like most tropical species. A single female *D. ranarum* can produce up to 566 eggs, which are deposited in untidy rows (Fryer 1959a, Avenant *et al.* 1989b). Gonadal development starts in early spring, making it likely that copulation takes place during this time (Van As & Van As 2001).

During the study undertaken by Avenant *et al.* (1989b) it was also found that the females prefer to lay their eggs on a substrate such as rocks, if rocks are present in the aquariums. The eggs are covered in a gelatinous substance, which hardens (within a few hours) to cement the eggs to the substrate. Eyes were visible within 47 days. The eggs hatched at room temperature (20°C) within 57 days, with the larva freeing itself from the egg within a few minutes. All of the data recorded during the study undertaken by Avenant *et al.* (1989b) differed from the data found by Fryer (1964), as noted in Avenant *et al.* (1989b). Also, none of the larvae hatched during the study settled on a host. Avenant *et al.* (1989b) suggested that this was due to the presence of an intermediate host when hatched in natural circumstances, or merely due to the laboratory conditions. This is merely one of the questions that still remain with regard to the biology of *D. ranarum*.

The effect of *D. ranarum* on the host is minimal. The damage caused by a light infestation of *D. ranarum* is slight, but there is always the risk of secondary infection as the host's skin is pierced (Fryer 1968).

There has been more information gathered on the biology of *Argulus* than that of *Dolops* species, but the fact that there is already a significant difference in their

mating (use of a spermatophore instead of direct copulation using copulatory structures on the legs (Fryer 1968)) could possibly indicate that other differences exist between the genera as well.

It is a probability that the behaviour of the host during the breeding season would have an effect on the parasites' ability to find mates and for the offspring to acquire hosts when the fingerlings emerge. This would indicate that the utilisation of different hosts would cause different strategies in mating, which may (or may not) have lead to isolation of populations of *D. ranarum* utilising different hosts.

2.5 Fish representatives in the geological record of Africa

Fish species are more numerous than all other vertebrates combined, and yet are among the least known of the vertebrates (Skelton 1993; 2001). The diversity of fish families (especially Cichlidae) has sparked evolutionary debates since the 70's (Stewart 2001). For some reason (as also remarked by Stewart 2001) there have been few studies on African ichthyofauna. This means that we have no better understanding of the biogeographic history of Africa today than we had in the 19th century. Consequently, we also have a very incomplete understanding of the biogeographic history of the attendant parasite fauna.

Two very different oceans border southern Africa: in the east, the warm Mozambique-Agulhas Current, flowing south, in the west the cold, northerly flowing Benguela Current. The continent consists of a low-lying coastal plain, the escarpment and the higher interior plateau (Skelton 1993; 2001). The valleys of the larger rivers penetrate deep into the interior.

According to Lowe-McConnel (1987) the biogeography of Africa provides information on the history of the fauna in Africa. Various connections with other continents are indicated as well as the isolation of Africa subsequent to the break-up of Gondwanaland.

In addition, Lowe-McConnel (1987) provides examples of the taxa found in the geological record in Africa and the distribution of the relevant taxa worldwide:

- Representatives of archaic taxa distributed globally e.g. Dipnoi,
- Endemic families that evolved on the continent after the break-up of Gondwanaland,
- Representatives of taxa found on both Africa and South America indicating a common origin on Gondwanaland e.g. characids and siluroids,
- Representatives of taxa found in Africa and Asia, indicating common origin in Gondwanaland, or later migration, and
- The offspring of immigrants from marine environments.

Representatives of all the families of hosts utilised by *D. ranarum* have been around for several million years, even up to genus level, ever since the Miocene, more than 5.4 million years ago (Stewart 2001). The fossil record as well as the species present today in the southern African rivers indicates that fish species migrated from north to south in Africa. However, whilst information on the fish fauna in Africa today is reasonably accessible, information on the fish fossil record is not so easily found. This is particularly true for West and South Africa (Stewart 2001).

Stewart (2001) provides a visual representation of the geological record, which is adapted to only include the fish species relevant to this (Table 2.6). However, this information only applies from Miocene to Pleistocene, indicating the presence of fish species in the Cenozoic. The presence of fossilised remains of Clariidae, *Synodontis* Cuvier, 1816 species and representatives of the Cichlidae are indicated using red, blue and yellow respectively. Table 2.7 is the geological time Table included in order to put the fossil record shown in Table 2.6 in geological perspective.

Table 2.6: Geological record of fossils of host families of *Dolops ranarum* (Stuhlman, 1891) dating from the Cenozoic. Clariidae is indicated with red, Cichlidae with yellow and Mochokidae (*Synodontis* sp) with blue

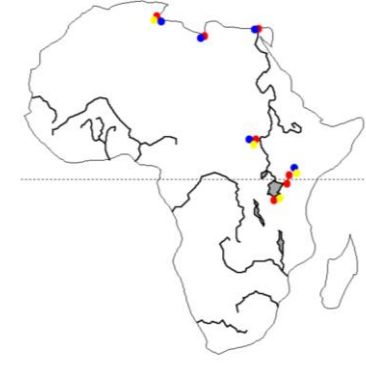
Fish fossils dating from the Cenozoic	
Miocene 25-5 Million years ago	
Early and middle Miocene	
Late Miocene	

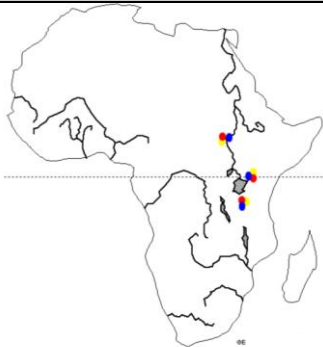
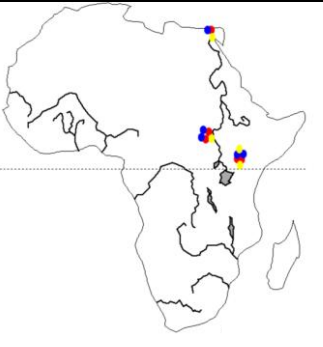
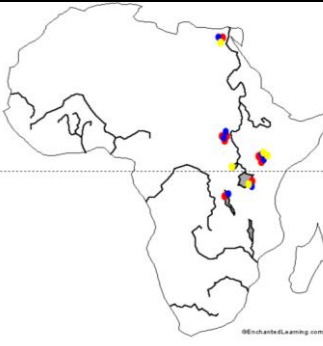
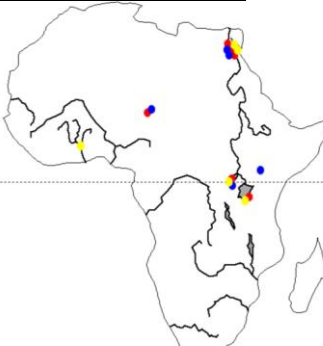
Table 2.6 continued	
Pliocene 5-2 Million years ago	
Early Pliocene	
Late Pliocene	
Pleistocene 1.6-0.01 Million years ago	
Early and middle Pleistocene	
Late Pleistocene	

Table 2.7: The geological time scale where the white blocks indicate the time periods during which fish fossils were found in Africa as indicated in Table 2.6 adapted from Long (1995)

Era	Period	Epoch	Time span
Cenozoic	Quaternary	Holocene	10 000 years ago till present
		Pleistocene	1.6-0.01 Million years ago
	Tertiary	Pliocene	5-2 Million years ago
		Miocene	25-5 Million years ago
		Oligocene	38-25 Million years ago
		Eocene	55-38 Million years ago
		Palaeocene	65-55 Million years ago
Mesozoic	Cretaceous		144-66.4 Million years ago
	Jurassic		208-144 Million years ago
	Triassic		254-208 Million years ago
Palaeozoic	Permian		286-245 Million years ago
	Carboniferous		360-286 Million years ago
	Devonian		408-360 Million years ago
	Silurian		438-408 Million years ago
	Ordovician		505-438 Million years ago
	Cambrian		540-505 Million years ago
Precambrian	Proterozoic		2500-540 Million years ago
	Archean		3960-2500 Million years ago

The Order Characiformes is present in the fossil record, but no members of the Family Hepsetidae have been found. This is the only one of the possible hosts of *D. ranarum* not found (so far) in the fossil record sometime during the Neogene. The Order Siluriformes, with the Family Clariidae are well represented in the fossil record in Africa. Today *Clarias* is represented by 32 species (Stewart 2001). Another member of the Family Clariidae, *Heterobranchus* Saint-Hillaire, 1809 has cranial features nearly indistinguishable from *Clarias*, but fossil representatives are mostly placed in the genus *Clarias* because these fishes are much more common than their relatives. Fossilised clariid remains are the most common fish fossils reported in Africa, most likely because of the easily recognisable cranial ornamentation (Stewart 2001). These fossils date from the middle-Miocene to the Pleistocene.

Another possible, but perhaps only an incidental host is the Family Mochokidae, particularly the genus *Synodontis* that is represented by many species throughout Africa today. Fossil remains of this genus have been found from the early Miocene onwards.

Of the Order Perciformes, the Family Cichlidae (for instance *Oreochromis*) is also present in the fossil record during this time period. According to Skelton (1993; 2001) the cichlids can be divided into the tilapiini and the haplochromini. Most of the fossil remains from this family are of tilapine origin; Stewart (2001) suggested this because the tilapiini are generally larger fish. However ancient members of both tribes were found in beds from the early Miocene period.

2.6 Evolution of crustaceans – specifically pertaining to the branchiurans

Fossil evidence of crustaceans have been discovered dating from the Cretaceous and more recent (Table 2.8). In order to put the time period in perspective, Table 2.7 is a summary of the general geological timetable.

Table 2.8: Geological record of the Crustacea (adapted from Green 1961)

Geological period	First positive records of Crustacea
Cretaceous	True Crabs (Brachyura)
Jurassic	Dromiid Crabs Many Decapoda Natantia (Cirripedia)
Triassic	Eryonidae Penaeid Prawns
Permian	Notostraca
Carboniferous	Anaspidacea
Devonian	Earliest malacostracan Conchostraca
Silurian	
Ordovician	Ostracods

Table 2.8 continued	
Geological period	First positive records of Crustacea
Cambrian	Ostracods Concostraca (in the) Burgess Shales Pseudocrustacea Trilobites

There is currently no fossil record as yet for representatives of the Branchiura as a group. According to Walossek & Müller (1998) the Branchiura is a group that has no fossil record as yet, but several morphological traits indicate that they might belong to the group Maxillopoda. However, in the past the taxonomic position of the Branchiura has been changed, and it is not impossible that it will be changed again as new information is uncovered.

2.7 Evolution of fish and attendant parasites in Africa

The co-evolution of parasites and hosts has been central to the study of parasitology since the inception of the discipline. This includes the interpretation of basic aspects including host-specificity, the adaptations of parasites, the phylogeny of the groups of parasites and the defences of both the hosts and parasites alike. Phylogenetic data of host-parasite assemblages have confirmed co-speciation (Tinsey & Jackson 1998). Unfortunately this research has generally not been done on crustacean parasites. Host-parasite assemblages of cestodes and lice in birds have been well documented (Tinsey & Jackson 1998).

According to Tinsey & Jackson (1998) the case of the host-parasite relationships in *Xenopus laevis* Daudin, 1802 have been well studied. In this group, there was an ancient speciation event, where the group was divided into two distinct parts. One has multiples of 20 chromosomes, and the other has 36 chromosomes exactly. The parasites found on each of these groups are specific to that group. In other words certain cestode parasites are found to be associated with a specific segment of the population. This segment is determined by the number of chromosomes.

When the history of a species is discussed, the changing nature of the earth itself is the most fundamental part of the discussion (Long 1995). The drift of the continents on the mantle of the earth causes some of the plates to be pushed under other plates where they melt in the molten mantle. The positions of the continents in the past give us an inkling of how species were spread over the globe. According to Long (1995) the movement of the continents, together with the changing atmosphere and constantly changing sea levels and other climatic factors drive the evolutionary mechanism.

- **Crustacean phylogeny**

The phylogeny of the crustaceans shows the enormous diversity of this sub-phylum. Crustacea also show a remarkable morphological plasticity. Many forms evolved over time, since the development of the group in the Cambrian (Wills 1998). According to Wills (1998) the segmented nature of crustaceans (as with all arthropods) gives endless opportunity for specialisation adapting to specific environments. This is one of the reasons why morphology cannot always be used to determine phylogenetic relationships.

According to Giribet *et al.* (2005) in the last century the phylogenetic scenarios speculated upon by scientists have been extremely diverse. While the position of representatives of the Branchiura has been changed often, even the position of the crustaceans has been hotly debated between different groups of scientists. According to Giribet *et al.* (2005) palaeontologists support a closer relationship between the crustaceans and the chelicerates, while the neontologists mostly agree on a monophyletic origin for the representatives of the Mandibulata with crustaceans as a sister group to the representative of the Tracheata or Atelocerata representatives. In later years the molecular analysis of the group seems to indicate a closer relationship between hexapods and crustaceans is possible (Giribet *et al.* 2005).

- **Evolution of fish**

The evolution of fishes is one of the greatest moments in the history of life on earth, because fish were the first organisms to develop a backbone. This means that their fossilised remains still offer valuable clues to their behaviour, feeding and habitat. In comparison, many of their invertebrate ancestors left no trace.

Fishes do not only play an important role in our past, but also in our present. Even today fish is one of the main ingredients of our diet as humans. Getting back to the past, 500 million years ago when the first fish evolved, they were the highest achievement of evolution up to that date for 150 million years (Long 1995). Fishes are the most diverse of the vertebrates with more than 23 000 known species existing today (Long 1995).

Bony fishes (osteichthyans) first appeared during the Devonian and later in the same period the earliest lobe-finned fishes appeared (Long 1995). A striking feature of the fossil record at this time is that the fish seem to be restricted to certain areas (Long 1995). Looking at the distribution of fish in the fossil record gives us a clearer picture of what the earth was like at that time (Table 2.9).

In the Witteberg group in southern Africa (during the Carboniferous period, 365 million years ago) identifiable fish fossils in this part of Africa were found (Jubb 1967). These Palaeozoic fish could have been either freshwater fishes, or marine fish that could tolerate freshwater conditions. However, it was not until the Mesozoic (150 – 175 million years ago), that a diverse fish fauna appeared in Africa. Numerous deposits from the Pleistocene were found in Kenya, Lower Egypt, Uganda, parts of the Sahara, southern Nigeria and the Congo Basin (Jubb, 1967).

According to Jubb (1967) these deposits from the Pleistocene contain several genera such as *Clarias*, *Tilapia* and *Synodontis*. Members of these genera act as hosts for *D. ranarum*.

Table 2.9: Geological time scale with indication of the rise of fish groups adapted from Long (1995) and Stewart (2001) and the evolution of *Dolops* Audouin, 1837

Era	Period	Epoch	Major events in the evolution of fish	Events in the evolution of <i>Dolops</i>
Cenozoic	Quaternary	Recent		
		Pleistocene		
	Tertiary	Pliocene		
		Miocene	Fossils of fish families present in Africa include: Clariidae, Mochokidae (<i>Synodontis</i> sp) and Cichlidae	
		Oligocene		
		Eocene		
		Palaeocene		Last time South American and African ranges of <i>Dolops</i> are in direct contact (Fryer 1969)
Mesozoic	Cretaceous			Last time a South American–Australian connection existed, if it ever existed at all (Fryer 1969).
	Jurassic		First teleost fishes	
	Triassic			Rise of representatives of Crustacea

Table 2.9 continued				
E r a	Period	Epoch	Major events in the evolution of fish	Events in the evolution of <i>Dolops</i>
P a l e o z o i c	Permian			
	Carboniferous			
	Devonian		First osteichthyans, actinopterygians	
	Silurian		First fishes with jaws	
	Ordovician			
	Cambrian			
	Precambrian			

2.8 Branchiuran evolution

Fryer (1969) noted that the areas where *Dolops* species occur have not been in contact since the Tertiary (60 million years ago). Fryer (1969) also speculates that the distribution of *Dolops* can be explained by the following:

- Continental drift or migration along former land bridges.
- *Dolops* had a more widespread distribution and that it has retreated from the northern hemisphere. This still implies that the genus is at least 60 million years old. However, this option depends on the distribution of fish crossing the late Pliocene land bridge. This makes the scenario unlikely as very few fish species (perhaps none) crossed this bridge, making the crossing of *Dolops* unlikely. In addition, South American fish species are in general closely related to African fish fauna.
- *Dolops* migrated across the oceans.

However, no species of *Dolops* are present in marine environments, the only member of the family to have marine representatives are members of the genus *Argulus*, making the last option unlikely.

The distribution of the fish hosts in the past determines the distribution of parasites in the present. According to Skelton (1993; 2001) the rise and retreat of the oceans have a great impact on water basins in Africa and indeed around the world. During the history of Africa the rise and fall of the oceans happened several times with the general temperature of the earth rising and falling with the ages. This would have influenced the connections between rivers in Africa, as on the other continents.

From a previous study of Jansen van Rensburg (2004) it was concluded that the most ancient of the branchiurans in Africa is *D. ranarum*. While *Argulus* is more widespread, this may only point to the fact that the mutual ancestor of all *Argulus* species had already evolved and therefore spread to the other continents before the break-up of the supercontinent Pangaea. After this geological event, the genetic isolation of the species on the respective continents gave rise to the more than 150 species of *Argulus* present today.

Due to the endemism of *Chonopeltis* in Africa, it is probable that it is the youngest of the genera, and that the genus evolved after Gondwanaland broke up to form the continents as we see them today. It is possible that the same criteria may apply to *Dipteropeltis*.

Dolops ranarum is not highly host specific, but it is specific to systems where the clariids and cichlids occur sympatrically. This is because of the changing nature of the drainage channels in southern Africa, whilst the Okavango River first drained to the west through the Orange River (during the geological past) and then drained to the east through the Limpopo River, it today however, ends in a swamp in the middle of the Kalahari Desert.

All the fish present in Africa today originated in Asia (Stewart 2001), spreading through Africa down to the river systems in South Africa. Clariids are present in the Orange and Vaal Rivers; however, cichlids are not. This could be because the dispersal of the clariids occurred at a time when the Okavango was draining to the west and the dispersal of the cichlids occurred only later when the Okavango was draining to the east

as mentioned previously. *Dolops ranarum* is only found as far south as the Limpopo River, the southernmost system where cichlids and clariids occur together.

If clariids were the original hosts of *D. ranarum*, it is most likely that *D. ranarum* would be endemic in the Orange and Vaal River Systems as well as its present distribution. However, due to the fact that *D. ranarum* does not occur naturally in the systems where only clariids are present, it is probable that the original hosts of *D. ranarum* were likely the cichlids and their forerunners and that the parasites later adopted the clariids as hosts. In any case more than enough geological time passed for the parasites and the hosts to enter into a symbiotic relationship.

According to the dispersal of branchiurans in the world today, it seems likely that they originated during the Triassic or before when the super continent Pangaea was the only landmass. This is indicated by the fact that no branchiurans are found in oceanic waters, even if some *Argulus* species may occur in coastal waters offshore. It is possible that some hosts traversed the oceans, however it is much more likely that the association dates from the Triassic, as mentioned previously. Here it is worth mentioning that with the origin of representatives of the Crustacea in the Cambrian (Wills 1998), and fish in the Silurian, there was ample time before the Triassic for evolutionary dependence to occur in the case of the parasite, and hosts were around for the parasites to occur on. Fryer (1969) stated that the parasitic habits of *Dolops* do not make the dispersal to Tasmania by continental drift impossible, as the crustacean may well be far older than its teleost host. The possibility of an Antarctic route makes it possible for *Dolops* to have reached Tasmania without ever having to cross an ocean. This means that all the representatives of the genus *Dolops* have possible routes to the present range of the genus without having to traverse a marine habitat.

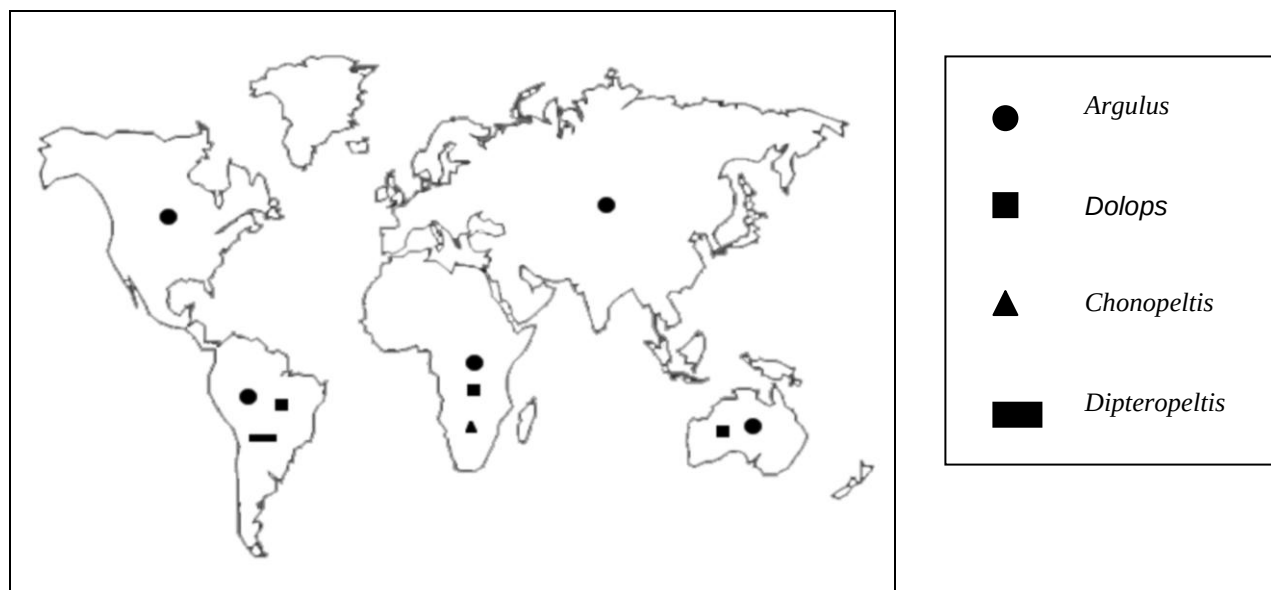
The evidence for this statement is problematic, because in truth very few organisms fossilise at all, and the small size of the parasites make finding them even more unlikely. According to Huys & Boxshall (1991) copepods do not fossilise well, and while a harpacticoid copepod was described from a Neolithic excavation site in Kent (southern England) it was found that this specimen was desiccated rather than fossilised and it was possible to rehydrate the specimen prior to study. True copepod fossils were found in North and South America (harpacticoids and cyclopoids) found in Miocene and Pleistocene lake deposits (Huys & Boxshall 1991). The localities of all records of fossil

and subfossils are noted in Figure 2.6. The first fish parasite fossil that was found was from the Lower Cretaceous named *Kabatarina pattersoni* Cressey & Boxshall, 1989. In this case both sexes of this siphonostomatoid parasite were found on the gills of a fossil teleost fish (*Cladocycclus gardneri* Agassiz) preserved in calcareous nodules in the Santana Formation at Serra do Araripe, Brazil.



Figure 2.6: Localities of all records of fossil and subfossil copepods with inset geological time scale (Huys & Boxshall 1991)

Therefore, because fossils of these small creatures are rare, their distribution is the best indication of the time when the parasites first evolved. As mentioned previously, the distribution of the representatives of the Branchiura is very discrete, with *Dolops* species only occurring on the continents that were part of Gondwanaland (excluding Australia) (Figure 2.7). As mentioned previously 14 species of *Dolops* have been described at this time.



*Due to the small size of Tasmania, the presence of *Dolops* on this island is indicated on Australia

Figure 2.7: Present distribution of known species of Branchiura occurring in freshwater habitats on each of the continents

Chapter 3: Overview of African River Basins, with specific reference to the study locality (the Okavango Delta) and its biodiversity particularly fish fauna.

In the middle of the Kalahari Desert lies the 15 000 to 18 000-square kilometre alluvial fan that is the Okavango Delta (Stuart & Stuart 1995; Bailey 1998) (Figure 3.1). It consists of floodplains, waterways, forests and islands, all of this in the largest continuous stretch of sand in the world (Haddon & McCarthy 2004). According to Bailey (1998) there is no great certainty about exactly when the Okavango Delta was formed, but it is certain that it formed over millions of years, in conjunction with the neighbouring desert the Kalahari, and the large masses of water that covered this area in the past. However, McCarthy (2005) indicated that the ancestral Okavango drainage system dates from the early Cenozoic. Figure 3.1 shows the location of the Okavango Delta.

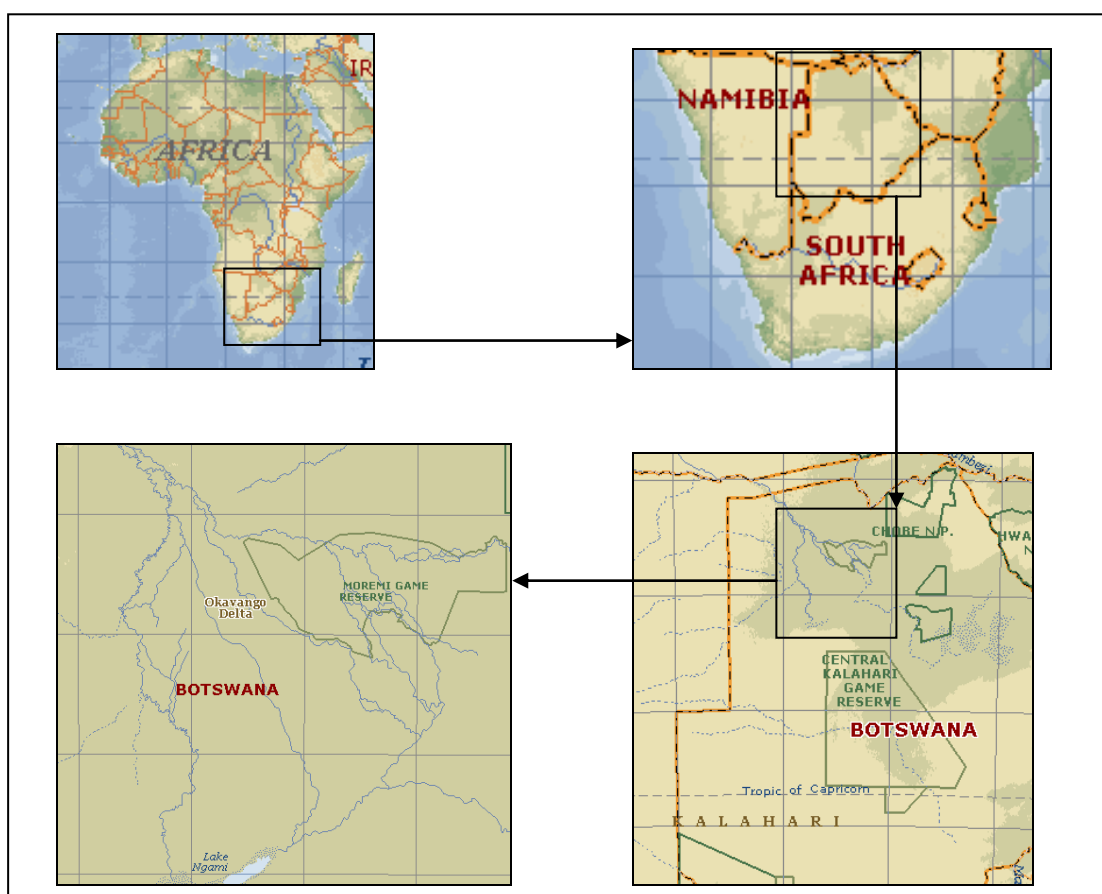


Figure 3.1: The study locality – The Okavango Delta and Panhandle situated in northeastern Botswana in southern Africa (Adapted from Microsoft Encarta, 2006)

3.1 Formation of the Okavango Basin

Between 135 and 200 million years ago (Jurassic and Cretaceous periods) Gondwanaland, consisting of all the continents today in the southern hemisphere, began to break up (Stuart & Stuart 1995; Bailey 1998). As the fragments of Gondwanaland split off Africa, its southern edges lifted, and in combination with sporadic but ongoing tectonic volatility, created enormous drainage basins (Bailey 1998). Over time the prominent landforms became eroded by winds and rain, depositing debris in the low-lying areas on the continent, one of which was the Kalahari Basin.

According to McCarthy (2005) Africa's southern interior is fundamentally a closed basin known as the Kalahari Basin, stretching some 2 200 km from South Africa through Botswana and Angola up to the Democratic Republic of the Congo (Haddon & McCarthy 2005). These sediments have been collecting since the end of the Cretaceous around 65 million years ago. In the early Cenozoic, immense lakes occupied the central area of the basin. During the Cenozoic, global cooling, changes in the ocean currents and the uplift of the southern African region, caused the interior of the continent to become more arid (McCarthy 2005).

The East African Rift System started to influence southern Africa approximately 20 million years ago (during the Miocene), due to its various branches extending southwards. The Zambezi River has been particularly affected. The Zambezi River was originally a small coastal river, which drained the eastern escarpment, while the lower Zambezi cut back into the interior, its progress assisted by uplift along the Kalahari-Zimbabwe Axis. Its progress was later assisted by faults related to the East African Rift along the Luangwa Valley (McCarthy & Rubidge 2005).

Geological instability during the Pliocene (around 3 million years ago) forced the great river to change its flow to the east. Still crossing the Kalahari, it now joined the Limpopo River and terminated in the Indian Ocean. A distortion of the earth's crust dammed up the river into Lake Makgadikgadi in what is north-eastern Botswana today. This lake survived several humid and arid periods, but started losing its water

to rivers finding their way to the sea when Africa's subcontinent tilted again (Bailey 1998).

The glacial periods during the Pleistocene (between 1.6-0.01 million years ago) caused an extreme drought in southern Africa, establishing a large sand sea in the African interior, stretching from South Africa nearly to the equator (McCarthy 2005). In this sand sea the Delta re-established itself during the Holocene (10 000 years ago to the present), when the global climate was milder and there was heightened rainfall in the interior. According to McCarthy (2005) the fact that sand underlies the whole Delta is important, because this means that the river never carries mud or silt, but only sand.

The highest part of this basin is the Benguela Plateau in Angola. This is where (between 4 and 5 million years ago, during the Pliocene) the first run-off from the rainfall began to collect and form the Cubango River, known as the Okavango downstream. Over time and down the gradient into the savanna, the water gained momentum, and was joined by the Chobe and the Cuito to swell its volume. Further down the Upper Zambezi also joined the Okavango River, and then this swollen river crossed 400 km of the dry Kalahari. At that time (approximately 5 million years ago) the Okavango joined the Orange River in flowing westwards and ended its journey in the Atlantic Ocean (Bailey 1998).

The formation of three parallel faults, Thamalakane, Kuyene and Gomare extended the great East African Rift System, a succession of loosely linked valleys that stretch for 6 400 km down the African continent (Bailey 1998). The sinking of the area between these fault lines, created a depression disrupting the flow of the river. This channel filled with the sediment carried down by the river and in arid times the debris blown in by the wind. The accumulating material formed a gently sloping fan (Bailey 1998). As the fault along the Luangwa Valley extended southeastwards, the lower Zambezi captured rivers arising from the north, the Kafue, the upper Zambezi and lastly the Kwando (McCarthy & Rubidge 2005). It is anticipated that the Okavango River will subsequently be diverted into the Zambezi River as well (McCarthy & Rubidge 2005), but in the interim the Okavango Delta was formed.

The Delta was formed as the Okavango River kept on flowing into the fan created by the geological faults mentioned above, depositing more and more sediment. Some of the channels became clogged-up and changed their course. The remaining momentum of the river was sapped by evaporation. Over time less and less water flowed from the Okavango into Lake Makgadikgadi, causing the lake to dry up, but the inland Delta persevered. In addition, the diversion of the Kafue, Upper Zambezi and Kwando Rivers by the Lower Zambezi also deprived Lake Makgadikgadi of water. At the same time, the Kalahari became more arid as a result of the cooling of the Benguela current along the west coast and the rise of the eastern escarpment (McCarthy & Rubidge 2005).

Lake Makgadikgadi gradually shrank in size and today all that is left are the salt pans. However, it is possible that the link between the Kwando/Upper Zambezi River System and the Makgadikgadi Pans was periodically re-established during the last 50 000 years as a result of fault movement causing extensive but sporadic flooding of the pans (McCarthy & Rubidge 2005). Today, this Delta itself consists of countless channels bordered by papyrus, forested islands and quiet anoxic lagoons.

In 2009, for the first time since 1984, the extensive rainfall resulted in the re-establishment of the link between the Okavango Delta and the Chobe River and the Caprivi was also flooded. Figure 3.2 shows a satellite view of part of the Zambezi Catchment on 30 April 2009 and Figure 3.3 a graph of the various years of the floods of the Okavango Delta.



Figure 3.2: Satellite view of the Caprivi, 30 April 2009 [Provided by www.aliboats.co.za]

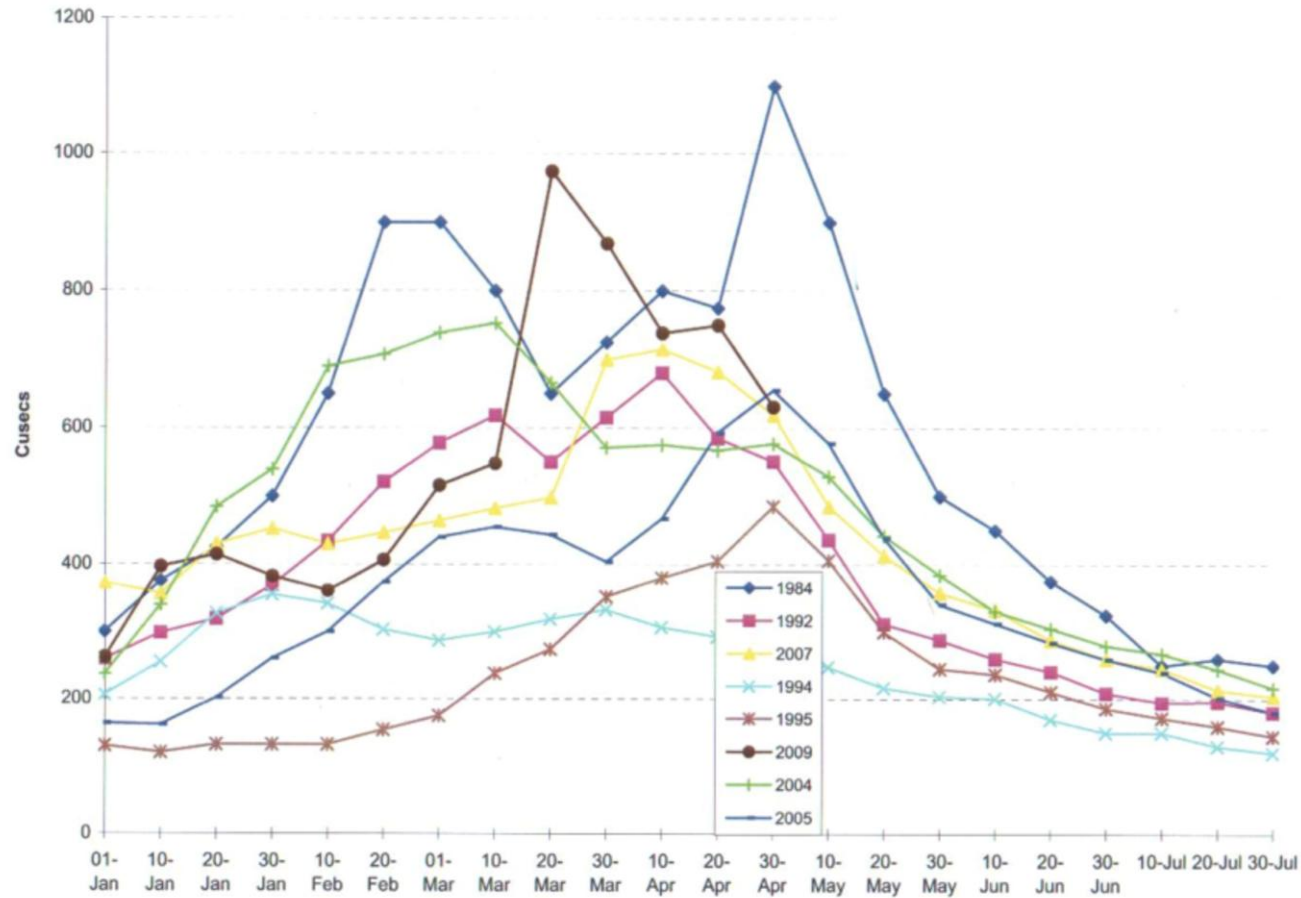


Figure 3.3: Graph of the flood in the Okavango Delta in selected years from 1984 to 2009, measurements are in cubic m/s and were taken every 10 days from Mohembo [Provided by www.aliboats.co.za]

3.2 Present structure of the Okavango Delta

Today, the river, as in the beginning, originates in the Angolan Highlands, flows through the Caprivi Strip and enters Botswana. The river can be divided into three distinct regions (Bailey 1998):

- **The Panhandle,**

The Panhandle is a broad and winding run of river and floodplain heading south-east into the Delta proper. The Panhandle is trapped between two faults that lie at right angles with the faults that dam the Delta in the end. This Panhandle is around 100 km long and 90 m wide, dividing briefly at Nxamaseri, and reuniting at Sepupa downstream. When the water crosses the Gomare Fault the land falls away and the river starts looking like a Delta, or rather the alluvial fan that it truly is (Stuart & Stuart 1995).

Every year approximately 11 billion m³ of water flows through the Panhandle, the speed of the flow peaking in late summer, February/March (Figure 3.3). The floodwaters are restricted between the relatively narrow shoulders of the faults bordering the Panhandle, and the water level can rise by as much as 2 m. In the Delta the rainfall is moderate and the area is semi-arid. However, the rainfall in the Delta itself adds another 5 billion cubic metres of water to the system (Bailey 1998). This amount is minimal in comparison to the amounts of water lost by evaporation from the open floodplains, as the annual evaporation from the Delta is four times the annual rainfall (Gumbricht *et al.* 2004).

- **The permanently flooded area,**

The second part of Delta is the permanently flooded area. Here the Delta retains water all year round and the vegetation is lush, consisting of reeds, grasses, shrubs and trees. This lush vegetation traps suspended sediments, which cause a flow decline down the fan (McCarthy *et al.* 1997). McCarthy *et al.* (1997) added that most of the suspended sediments are set down in the upper fan, while the solute is deposited in the seasonal fan.

Beyond the village of Seronga lies the northwestern most boundary of the depression. This 6 000 km² permanent swamp consists of a multitude of channels, floodplains, lagoons and islands (Bailey 1998). This is the last area where the Okavango is a river, the convex nature of the land causing the river to split into several channels that fan out over a large area. The gradient is slight, only 65 m over the distance of 250 km. In the past there were larger channels with stronger flow, but with time the sediment carried down by the river raised the beds of the channels and flow became slower. More and more water escaped into the surrounding swamps.

According to Bailey (1998) one of the three distributaries, the Thaoge flowed strongly to the south, filling lake Ngami. Even in the 1970's this lake used to fill in times of exceptional rainfall, but today there is nothing but bushveld where the lake used to be. However, since 2003 and 2006-2008 the lake once again contained some water all year round. At first it was thought that the flow could be re-established, but in time it became obvious that the channels in the Delta have a restricted lifespan. From establishment to failure, a channel seems to have an existence of approximately 200 years. It may be expected that the salinity of water leaving the Delta would be high. However, this is not the case, in fact, the salinity barely doubles over the distance of the Delta. The reason according to Bailey (1998) being that the islands act as purifiers of the water flowing through the Delta. The purification is a result of the plants on the islands. In the Delta most of the water is lost through transpiration of the plants and not direct evaporation from the water surface.

- **Seasonally flooded area.**

The third part is the seasonally flooded area, which is grassland most of the year until the floods come every winter to transform it into a wetland. Rainwater pools dot the forests around the Delta during the summer; when winter comes it dries out into clay husks.

3.3 A short overview of major river basins in Africa

Most rivers in Africa are seasonal. The Zambezi is the largest river in southern Africa and geological evidence points to hydrological changes over time (Jubb 1967). This river flows into the Indian Ocean. The Okavango River that arises in Angola loses itself in the Okavango swamp. However, according to Jubb (1967) the Zambezi and the Okavango were connected by the Chobe swamp, during the heavy flood season.

The topography of the land through which the river flows, influences the environments and niches available for colonisation of different species of fish (Jubb 1967). The natural run of the river may include areas of rapids, deep rocky-bottomed pools or sandy-bottomed pools. Several habitats are included in every area; these habitats would be changing over time. In areas where human populations are large enough to impact on the environment, the rivers are constantly changing (Jubb, 1967). The impacts include soil erosion causing silting up, and the effects of pollution are diverse. In addition the building of dams and reservoirs changes the entire system. The formation of layers (epilimnion, thermocline and hypolimnion) of water in large reservoirs will also create a completely new habitat. At first the productiveness of the system increases, but then the productiveness decreases dramatically.

According to Stewart (2001) there is palaeontological evidence of a homogenous Pan-African fish fauna, mostly founded on the present general dispersal of a number of taxa. There was a time in the history of Africa where the waterways were for all intents and purposes interconnected. This permitted the wide dispersal of the ancestral fauna of today's freshwater populations. The break-up of this early system isolated biotas, leading to differentiation (taxonomically and phylogenetically) within new boundaries (Stewart 2001).

According to Shahin (2002) approximately seven major rivers drain the major part of the surface of the African continent, and the remainder is occupied by inland drainage basins that do not reach the sea (Figure 3.4).



Figure 3.4 Map of Africa indicating the major rivers of the continent (courtesy of the Aquatic Parasitology Research Group)

Shahin (2002) arbitrarily identified river basins in Africa according to their size. Only the major river basins in Africa as described by Shahin (2002) are discussed below. In addition the Okavango Delta is also discussed, as it is the focus area of this study.

- **The Orange River,**

The Orange River (Figure 3.5) is approximately 2 200 km long and is the longest river in southern Africa (south of the Zambezi). The Orange River drains parts of four countries, and flows through various climatic regions. The river has three sources, all in the Lesotho Highlands, the Senqu, the Seati and the Malibamatso Headwater. Once the river enters South Africa, it runs south and west over sandstone, mudstone and shales and forms part of the border between South Africa and Lesotho. Due to the low resistance of some of the Karoo sediments, the Orange River has carved out a wide valley and is joined by several tributaries from both sides on its way to the coast. The Gariep Dam was completed in 1971 and it is situated in the Northern Cape. Two of the most

important tributaries of the Orange River are the Vaal and the Caledon Rivers. The Caledon River joins the Orange River first then turns northeast where the P.K. le Roux Dam is located. Leaving the dam it flows towards the confluence with the Vaal River then turns west. This part of the river is the Middle Orange and it contains the Boegeberg Dam, which was built in 1931. After being joined by the Sak River it flows over the Augrabies Falls. This is the Lower Orange, which flows to the west defining the lower border of the Kalahari Desert and flowing through the Namib Desert to Alexander Bay where it empties into the Atlantic Ocean. The last tributary is the Fish River, which joins the Orange River approximately 100 km before the river mouth.

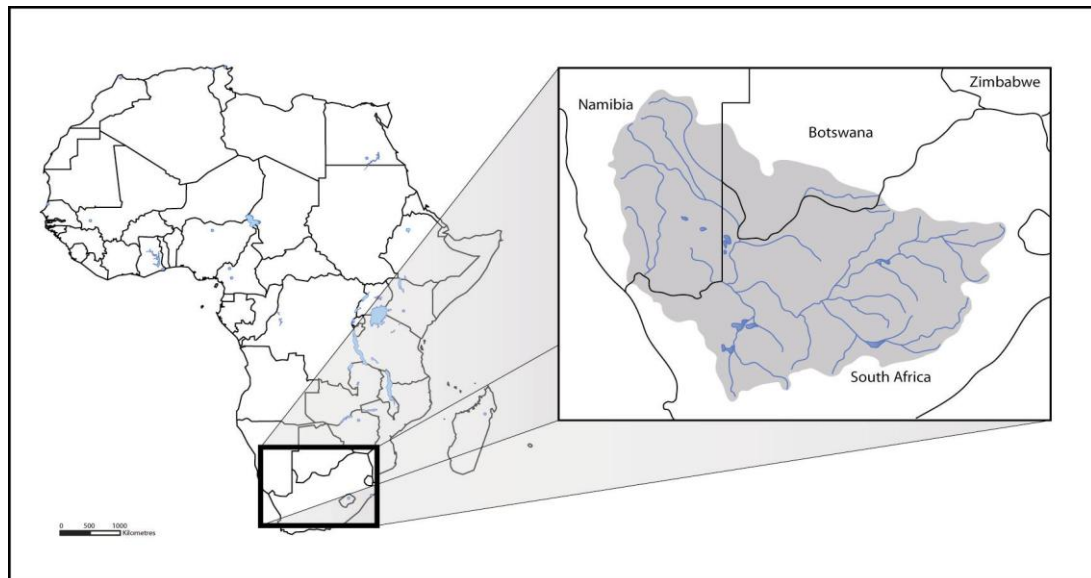


Figure 3.5 Map of the Orange River Basin (courtesy of the Aquatic Parasitology Research Group)

- **The Zambezi River,**

The course of the Zambezi River (Figure 3.6) runs for 2 740 km, draining parts of eight countries, making the Zambezi-Basin the fourth largest drainage basin in Africa. In addition, the Zambezi is the largest African river that empties into the Indian Ocean. The river lies between latitude 10° and 20°S and longitudes 20° and 37°E. The Zambezi has three distinct regions:

- The plateau course, which lies in Angola and partly in Zambia. This part of the river also receives intermittent streams from the Okavango Swamps. The Lungue-Bungo, Kabompo, Luanginga and the Chobe Rivers join the upper part of the Zambezi. The Okavango-Chobe River System is connected to the Zambezi through the Okavango-Chobe Lake.
- The Middle Zambezi, also known as the Gwembe Trough. This is the part below the Victoria Falls. The successive fault lines present in this area produce a narrow gorge of crushed rock through which the river flows. In this area the river is characterised by many waterfalls and rapids. The Kafue River flows into the Zambezi below the Kariba Reservoir, which is formed by the Kariba Dam. The Luangwa River also joins the Zambezi in this area. The lower part of the Middle Zambezi cuts through the rocky barrier of the Kariba Gorge.
- The third part of the Zambezi flows through the Mozambique Plain. The Cabora Bassa Reservoir is present in this part of the river, and before the Zambezi discharges into the Indian Ocean, the Shire River flows into it.

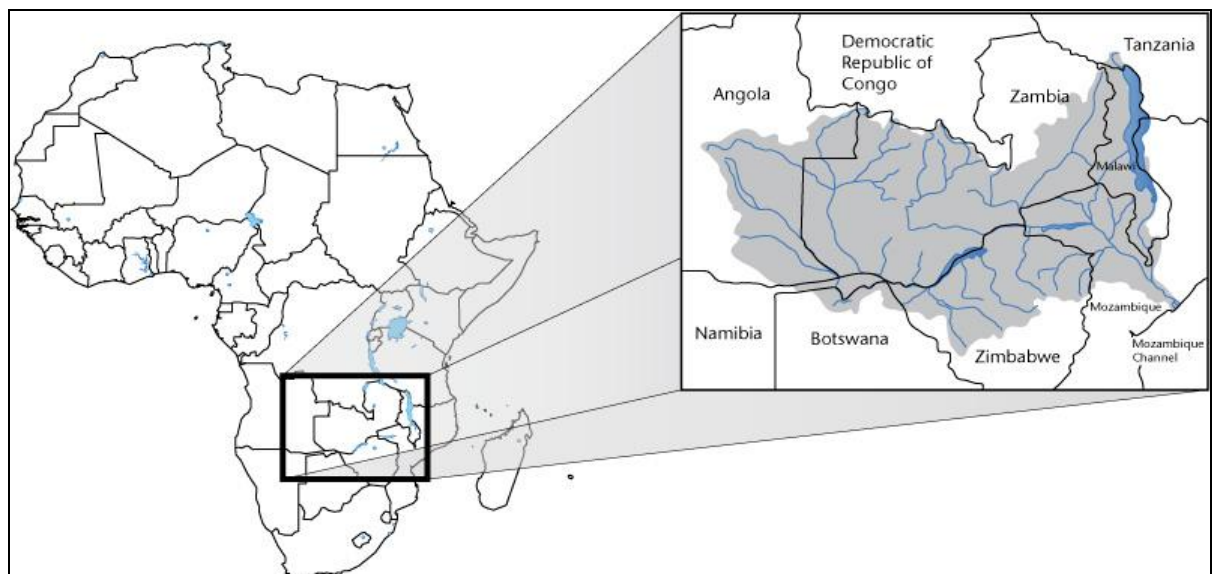


Figure 3.6 Map of the Zambezi River Basin (courtesy of the Aquatic Parasitology Research Group)

- **The Congo River**

The Congo River is between 4 700 and 5 100 km long. It contains many rapids and cataracts, and navigation is impossible.

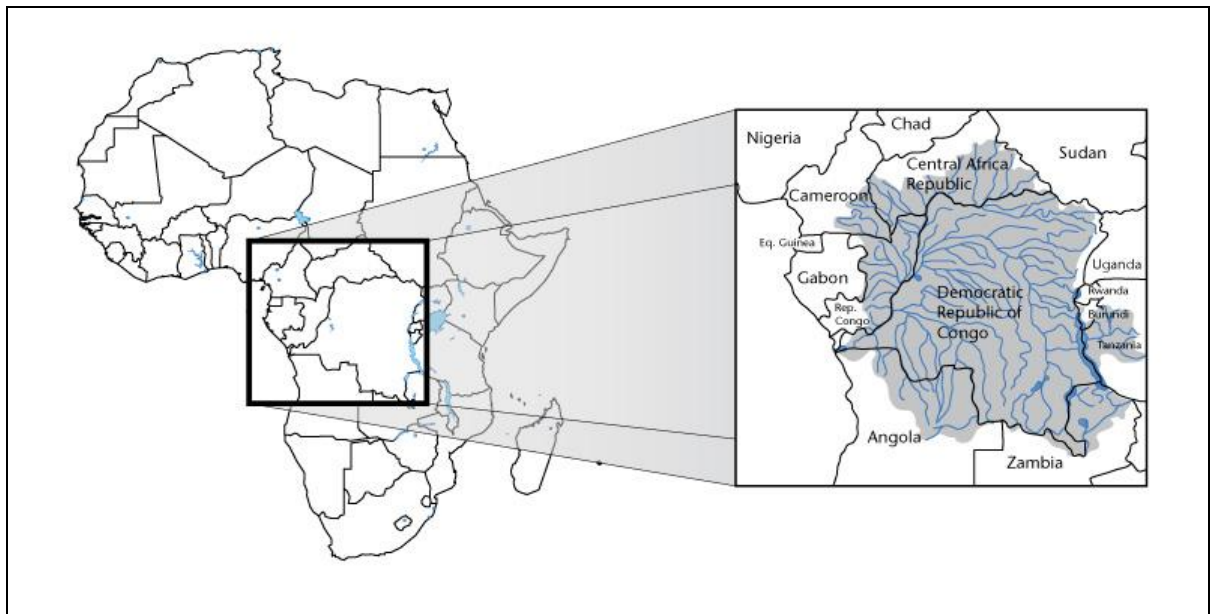


Figure 3.7 Map of the Congo River Basin (courtesy of the Aquatic Parasitology Research Group)

The Congo basin extends from latitude 09°15' N in the Central African Republic to 13°28' S in Angola and Zambia, and longitude 31°10'E through the Great Lakes in the Rift Valley to 11°18'E on the Atlantic Ocean (Figure 3.7). The Congo Basin is a shallow depression situated on the Great African Plateau. The Congo River drains nine countries. A large section of the basin enjoys a tropical climate with no dry season. The climatic conditions of the basin ensure that the total area has a water surplus. The source of the river is indicated to be the Chambeshi River, which rises south of Lake Tanganyika and is transferred by the Bangweulu Lake into the Luapula River. Several tributaries flow into the Luapula River before it discharges into Lake Mweru. The Luapula River joins the Lualaba River below Lake Mweru. The principal tributaries of the Congo (other than the Lualaba) are the Kasai and the Ubangi Rivers.

- **The Niger River**

The Niger River has a length of between 4 000 and 4 200 km, and is the third longest river in Africa and the longest and largest watercourse in West Africa. The Niger and its tributaries drain parts of 10 countries.

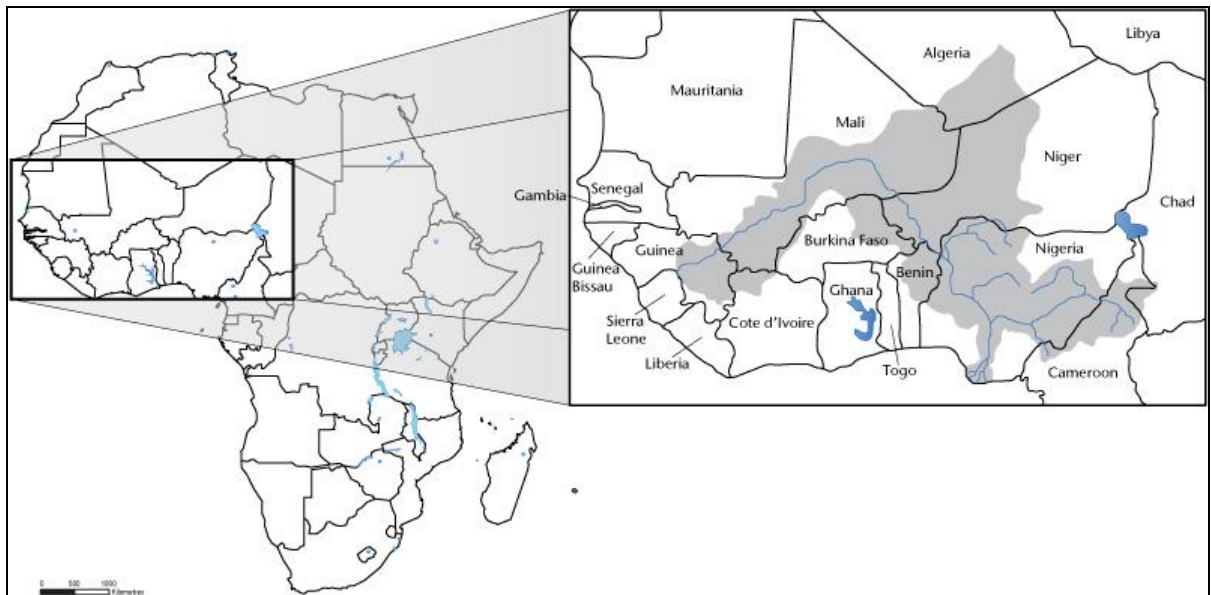


Figure 3.8 Map of Niger River Basin (courtesy of the Aquatic Parasitology Research Group)

The source of the Niger River is located at 9°5'N and 10°47'W, whilst the northernmost part of its course is located at 17°N and its mouth lies at 4°30'N and 6°E (Figure 3.8). The river passes through various climates. The Tembi River is often considered the main source of the Niger River, while the Upper Niger is formed by the confluence of four tributaries: the Niger, the Niandan, the Milo and the Tinkisso Rivers. The river then flows across the interior plateau to the north-east towards the Inland Delta in Mali. The Niger River crosses the border into Guinea and is joined by the Fie and the Sangarani Rivers near Kangare in Mali. As part of the Inland Delta (450 km in length and 200 km in width) the Bani River joins the Niger River. The Inland Delta is basically a swamp consisting of sandy soil. Between Ségou and Timbuktu the Niger River approaches close to the Sahara Desert, causing high rates of evaporation. Below Diré, the Niger River flows into Lake Téli, which in turn supplies Lake

Faguibine. Near Timbuktu the floodplains become smaller and the lacustrine zone terminates.

Downstream from the Internal Delta in Mali, the river turns in a north-easterly direction, before turning south-easterly, forming a great bend. Here the river flows through an arid area before crossing the boundary into the Republic of Niger. Another three tributaries (the Faroul, Dargol and Sirba Rivers) join the Niger from Burkina Faso. South of Niamey another two tributaries join the Niger (the Garoubi and Tapoa Rivers). The river forms the boundary between Niger and Benin for around 80 km and is then joined by the Mekrou River. Subsequently, the Niger River flows through Nigeria where it is joined by several streams. However, the most influential tributary of the Niger River is the Benue River, which joins it from the east, doubling the discharge. Below the confluence of the Niger and the Benue Rivers, the river turns south where some small tributaries including the Anambra River flow into it. The river finally discharges into the Atlantic Ocean in the Gulf of Guinea by means of the Delta of the Niger River.

- **The Nile**

Presently the Nile Basin covers one tenth of the surface area of Africa ($2.9 \times 10^6 \text{ km}^2$) situated between 4°S and 31°N latitude and from $21^\circ 30'\text{E}$ to $40^\circ 30'\text{E}$ longitude (Figure 3.9). The Nile is one of the longest rivers in the world, 6 500 km from the head near Lake Tanganyika to the mouth of the Nile in the Mediterranean Sea and traverses 10 countries. The length of the Nile means that the river passes through areas with diverse climates.

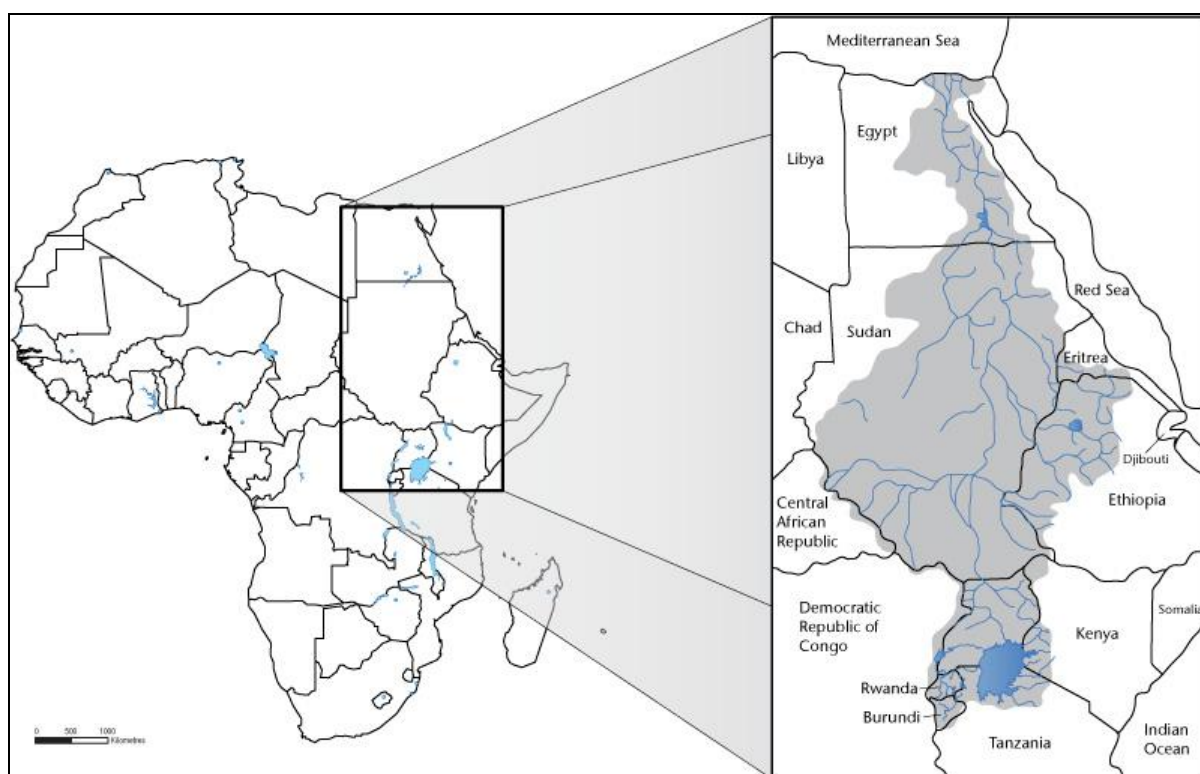


Figure 3.9 Map of the Nile River Basin (courtesy of the Aquatic Parasitology Research Group)

The Kagera Basin comprises a complex of streams of different sizes as well as lakes and swamps. The Kagera River is the last upstream tributary of the Nile, and feeds Lake Victoria. Water from Lake Victoria flows into the upper Victoria Nile, which is the only outlet of Lake Victoria and connects this lake to Lake Kyoga. Lake Kyoga is a submerged river valley covered with swamps. The outflow continues into the Kyoga Nile (sometimes referred to as the Lower Victoria Nile), mostly a sluggish, swampy river that enters Lake Albert through a swampy Delta. Lake Albert also receives water from the Semliki River, which also connects Lake Edward with Lake Albert.

The Nile River, here known as Bahr el-Jebel or the White Nile, leaves Lake Albert at the extreme northern corner of the lake. The White Nile is generally a broad, sluggish stream associated with swamps and lagoons. Many small streams join the river from both sides. North of Bor, the valley widens, becoming less defined and including extensive swamps on both sides of the

river as it continues to Lake No, a shallow lagoon. Walls of papyrus and tall swampy grasses cover the riverbanks. In this area the Bahr el–Chazal, Bahr el-Zaraf and river Sobat join the White Nile.

Below the confluence of the Sobat the White Nile flows north and is joined by the Blue Nile just upstream of Khartoum. Here the river, though sluggish, has a well-defined channel and is mostly free of swamps. Approximately 40 km upstream a dam was built in 1937. However, the rapid silting up of this dam and the construction of the Aswan High Dam in 1965 eliminated its function.

The Blue Nile rises in the Ethiopian Plateau and contains Lake Tana, a fresh water body in north central Ethiopia and the Tissisat Falls, the Damazin Rapids as well as the Roseires Dam, which was completed in 1966. The Dinder and the Rahad Rivers are important tributaries of the Blue Nile.

The Atbara River is the last tributary in the Nile System and joins the Nile River 320 km downstream from the confluence of the Blue and White Niles. Subsequent to the confluence, the Main Nile runs from Khartoum to Aswan as placid reaches of mild slope interrupted with cataracts. The Old Aswan Dam was completed in 1900, and the storage capacity increased in 1912 and 1937. The Aswan High Dam (completed in 1965) was constructed to cope with the water storage needs of the Sudan and Egypt. The lake formed by this dam is called Lake Nasser in Egypt and Lake Nubia in the Sudan. The water assigned to Egypt then flows below Aswan to supply the Nile Valley with its water needs and then on to the Delta. The Esna, Nag-Hammandi and Assuit Barrages were built across the river here to control the water level for irrigation and navigation.

Around 23 km north of Cairo the river splits into the Rosetta and Dameitta branches, and the Delta Barrage, present at the bifurcation point was completed in the latter half of the 19th century. From here the Delta flows into the Mediterranean Sea.

3.4 The unique nature of the Okavango Delta

The Okavango Swamp (Figure 3.10) is located at approximately 20°S latitude and 23°E and varies seasonally in area between 6 000 and 12 000 km (Shahin 2002).

According to Ramberg *et al.* (2007), the Okavango River originates in the Angolan Highlands, flowing through the Caprivi Strip of Namibia and ends in the Okavango Delta or in wet periods in the Makgadikgadi Saltpans of the Kalahari.

The River annually carries 660 000 t of sediment. Despite the large amounts of sediment the salinity at the outlets of the swamp is only 120 ppm (Shahin 2002). According to Ramberg, *et al.* (2007) the Okavango Delta, in its present state is almost completely isolated from other wetlands.

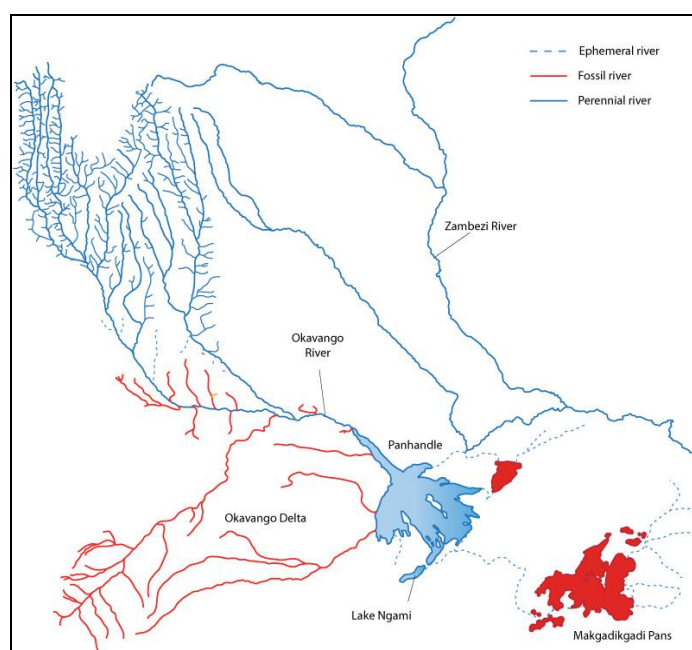


Figure 3.10: A graphic representation of the Okavango Delta (courtesy of the Aquatic Parasitology Research Group)

Several characteristics of the Okavango that makes it a unique system (Ramberg *et al.* 2007):

- It terminates in the Kalahari, unlike the other major rivers in Africa that all terminate in the ocean,

- The extreme spatial and temporal variations in the flooding pattern. The flooding pattern changes over at least four time scales,
- Its hydrology is unique, as flooding occurs in the cold season. Usually (in other rivers) the cold season is also the dry season and flooding occurs in the warm season. This is also the case in the Okavango Delta so consequently the system has two wet periods with high biological production,
- The pronounced continental climate and the elevation of 1 000 m above sea level causes the shallow water to freeze in the cold season,
- The aquatic and wetland species present in the Delta originate from the tropical regions to the north of the Delta and are probably less well adapted to the specific Delta environment. Consequently, the aquatic and wetland communities are likely under pressure to adapt to the Delta, and
- Habitat density is high resulting in an “edge effect” favouring species using more than one habitat.

3.5 Factors affecting fish distribution in Africa

The distribution of fish species is affected by several factors one of which is the fact that freshwater systems are typically limited and precise in scope (Skelton 1993; 2001). In the past this was not necessarily the case, because often water bodies that are now no longer connected, were connected in the past. Consequently, the distribution of freshwater fish is generally more marked and limited than that of amphibians and terrestrial animals.

According to Skelton (1993; 2001) the most obvious of these obstacles are physical barriers, physico-chemical tolerance, ecological barriers, as well as biological and behavioural factors. However, rivers change over time, because of geological factors such as mountain building, rifting and erosion. On the one hand the retreat of sea levels during cooler periods of the earth's history may cause river basins to be

connected. On the other hand the rising of sea levels may separate river basins. Rock falls may cause the formation of lakes; changes in the earth surface may redirect or block a river's course. It is possible that waterfalls may form over time creating a barrier restricting the movement of the fish fauna, i.e. causing restriction of movement upstream or downstream. The gradient of a river can be an obstacle for fish species that are not strong enough swimmers. Thermal tolerance may also limit the distribution of fish.

At the present time the drainage pattern of Africa consists of a small number of large systems draining the interior, each of these in turn broken up by a series of shorter river systems that drain the coastal plain. The Okavango River flows south and east from the Angolan Highlands to the Delta in Botswana. In the past, years of high rainfall caused the Delta to overflow into the Linyanti-Chobe and therefore this river is usually considered to be part of the Zambezi system. However, in the recent past the two systems have not been in contact. Most recently, the high rainfall in 2009 made a tentative connection between the Linyanti-Chobe and the Delta again. This is most likely not a permanent connection, but it does underline the dynamic nature of the Okavango System.

Southern Africa has a small number of natural freshwater lakes, however, as a result of human activities many dams have been created. Other standing water habitats in Africa include wetlands like the Okavango Delta (Skelton 1993; 2001).

According to Skelton (1993; 2001) in recent history the actions of man and the consequences thereof, like pollution, and water transfer schemes that link water basins may allow the movement of fish fauna from one basin to another. Another effect of human influence is the introduction of alien species, which also caused the decline of several indigenous species.

3.6 Biodiversity of the Okavango Delta

- **Plants**

Ramberg *et al.* (2007) state the flora of the Okavango Delta comprises around 1 260 taxa on species level, belonging to 530 genera and 134 families. The most diverse families are the grasses Poaceae, sedges, Cyperaceae, followed by the Asteraceae and Fabaceae. However, most of the genera are represented by one or two species only.

- **Invertebrates**

Ramberg *et al.* (2007) indicate that the known data of invertebrate species present in the Delta are far from complete. However, information on Odonata and Lepidoptera is available, as well as on the Mollusca and zooplankton.

- **Amphibians**

Currently a total of 33 amphibians have been recorded from the Okavango Delta (Ramberg *et al.* 2007). All of the noted amphibian species occur close to water, and only three to five species are more terrestrial. Of the 33 species in Botswana, twelve are restricted to the Okavango and Chobe, whilst eight of the species are restricted to the Okavango Delta only. These species are tropical, and therefore the Okavango Delta is at the southern end of their distribution.

- **Reptiles**

A total of 64 reptiles have been recorded from the Okavango Delta (Ramberg *et al.* 2007). Six of the species recorded are confined to the water, four species of terrapins (Pelomedusidae), the Nile crocodile and one snake. The python and four other snake species (members of the Colubridae) mainly occur in swamp habitats. However, most of the reptile species (52 out of 64) are terrestrial. Most of the reptiles present have a wide distribution in the southern and central parts of Africa. In comparison, ten of the species recorded

have a distribution that is restricted to the Okavango and the Chobe in Botswana.

- **Birds**

The bird fauna of the Okavango Delta is well documented (Ramberg *et al.* 2007), and 444 confirmed bird species occur in the Delta. Most are species with a wide distribution, belonging to 74 families of which the most important in terms of numbers are Accipitridae (eagles, hawks, buzzards and kites) of which 28 species have been recorded, Sylviidae (warblers, apalises, cisticolas, *etc.*) of which 31 species have been recorded, Ploceidae (sparrows, weavers, bishops, widows queleas, *etc.*) of which 25 species have been recorded and Ardeidae (herons, egrits and bitterns) of which 18 species have been recorded. The majority of the birds present in the Delta are breeding residents. However, there are a number of palaeartic migrants, which are all waders. These migrants visit the Okavango specifically as a result of the available wetland habitats.

- **Mammals**

A large variety of large mammals occur in the Delta and occur locally in high numbers. There are an ever-larger variety of smaller mammals present. According to Ramberg *et al.* (2007) approximately 122 mammal species represented by 12 orders and 34 families are present in the Okavango Delta, and all the larger species have a widespread distribution across the African savanna. Almost half of the mammal species recorded in the Delta are bats or rodents.

3.7 Fish fauna of the Okavango Delta

In the late Pleistocene, the average rainfall was higher, and the Okavango River was a tributary of the Zambezi River (Leveque 1997). Even today, the Okavango spills over into the Zambezi via the Selida Spillway in times of heavy rainfall. This explains why the fauna found in the Zambezi and in the Okavango Rivers are similar in some

respects. Therefore the fish fauna of the Okavango forms part of the Zambezi ichthyofauna. Table 3.1 lists the dominant fish families, genera and species in the Okavango System (Ramberg *et al.* 2007).

Table 3.1: The dominant fish families, number of genera and species in the Okavango System (Ramberg *et al.* 2007)

Family	Number of genera	Species number in the Okavango System	Species number in the Okavango below Popa Falls (Botswana only)
Mormyridae	5	6	6
Kneriidae	2	2	0
Cyprinidae	5	25	17
Distichodontidae	2	3	3
Characidae	4	4	4
Hepsetidae	1	1	1
Claroteidae	1	1	1
Amphillidae	2	3	2
Shilbeidae	1	1	1
Clariidae	2	7	6
Mochokidae	2	8	6
Poeciliidae	1	3	3
Cichlidae	7	18	18
Anabantidae	2	2	2
Mastacembelidae	1	2	1
Total	38	86	71

Ramberg *et al.* (2007) state that as a result of the geographic features and the climate variability of the Delta, there are no confirmed endemic species in the Okavango. The long-term climatic variations are unknown, but evidence exists of very dry spells, such as fossil sand dunes and wetter spells as shown by alluvial

exertion from the Delta into the Zambezi. The conditions have probably alternated between wet and dry several times in the last 100 000 years.

Consequently, there were times that the Okavango Delta has been isolated from the Zambezi in the dry periods. In contrast, it was probably connected to the Zambezi and formed part of a wetland complex present in the central and southern part of western Zambia, south-western Angola, the Caprivi Strip of Namibia and northern Botswana. Present similarities in the fish fauna of the Zambezi and the Okavango attest to the connection in the past. Subsequently, an irregular link has been established between the Zambezi and the Okavango. In contrast, several endemic species occur in the larger Okavango-Zambezi wetland complex.

According to Skelton (1993; 2001) specialist-feeding types occur in the larger river systems such as the Okavango with complex fish communities. These specialisations include: large and small piscivores, molluscivores, scavengers, insectivores, herbivores, algal grazers and detritivores. Add to this that the fish feed at different strata in the river, at different times and in different ways. In addition, the body form of an organism gives an indication of the biology and habitat requirements of that creature. A long, eel-like body enables the fish to live in holes, crevices or to move through dense vegetation (Skelton 1993; 2001), while active swimmers have a body that is generally the broadest in the middle and tapered towards either end. This aids in their movement that is done with a wave-like thrust that moves through the body. Fish adapted to living in habitats with fast-flowing water or fish that swim very fast have slender bodies with curved fins and a deeply forked or crescent shaped tail fin (Skelton 1993; 2001). Cichlids are deep-bodied fishes that swim by beating their tail and are capable of delicate manoeuvring or hovering by paddling with the fins. Ramberg *et al.* (2007) divided the fish fauna of the Okavango into five groups according to habitat utilisation in more detail and these are discussed below:

- **Open and midwater fast swimmers**

Fishes in this category have a body tapering at both ends, forked or lunate caudal fins and large eyes laterally placed. The dorsal and anal fins are short acting as a rudder during high speed manoeuvring. These fish are predators

that occur mostly in the upper layer of open water of flowing rivers. The fish live in roving shoals and a typical example of these fish is the tigerfish *Hydrocynus vittatus* Castelnau, 1861 (Characidae) occurring in the Panhandle and the upper part of the Delta. Other representatives of this category are members of the Cyprinidae, and *Hepsetus odoe* (Bloch, 1794). However, *Hepsetus odoe* differs from the rest of the category in preferring quiet waters of lagoons and channels.

- **Midwater slow swimmers**

Fishes in this group have a deep and laterally compressed body. The caudal fin is shortened and deep, and the dorsal fin is long and extends the length of the body; from behind the head to the base of the tail. The dorsal fin is supported by spines in the anterior part and with flexible soft rays in the posterior part. Having broad fins make the fish agile, and enables them to hover in open water and the vegetation of lagoons. This category includes representatives of the genera *Oreochromis* Günther, 1889, *Tilapia* A. Smith, 1840 and *Sargochromis* Regan, 1920 of the family Cichlidae. Fish in this category have relatively small mouths feeding on plants, insects and detritus while others are omnivores. The agility of movement of this group, resulted in a group of species within this category specialising as predators i.e. the genus *Serranochromis*.

- **Surface feeders**

Fishes in this group have rounded abdomens and flat dorsal surfaces. This allows them to feed at the surface of the water. The mouths of these fish face upward, and near the top of the heads are exceptionally large eyes. This is an adaptation for locating prey (insect larvae and plankton) and avoiding predators. This category includes the genus *Aplocheilichthys* Bleeker, 1863 (Poeciliidae), occurring in small shoals throughout the Okavango River and Delta. Members of this category serve as food source for many birds and other fish.

- **Bottom dwellers**

Fishes in this group have dorso-ventrally compressed bodies and mouths that face downward. Eyes are placed on the dorsal side of the heads. These are the only generic characteristics of this group, as the benthic habitat offers many diverse niches. Bottom dwellers' food sources range from detritus, benthic algae, adult and larval insects, oligochetes, snails and other fishes. This category includes the Clariidae, of which *Clarias gariepinus* (Burchell, 1822) at least is a keystone species. Other representatives are from the families Mochokidae and Amphiliidae.

- **Dense vegetation and rocky habitats**

Fish that live in the underwater jungle created by the papyrus beds need specific adaptations to avoid predators and find food in the unique, dark habitat. Successful designs for survival in this habitat are diverse. Some successful inhabitants are the spiny eel *Aethiomastacembelus frenatus* (Boulenger, 1901), and members of the endemic African fish family Mormyridae.

Chapter 4: Morphological study – Material and Methods

The Aquatic Parasitology Group of the Department of Zoology and Entomology at the University of the Free State (UFS) has been collecting fish from various sites in the Okavango River since 1997. Collections have been conducted in the Delta and in the Panhandle. As a member of the group from 2004 to 2006 I assisted in the collection of fish, as well as utilising specimens that were collected prior to my involvement in the group. In Figure 4.1 the sites from where the hosts were collected are highlighted. The map and the information included in the Tables in paragraph four were obtained from the databases of the Aquatic Parasitology Group at the University of the Free State.

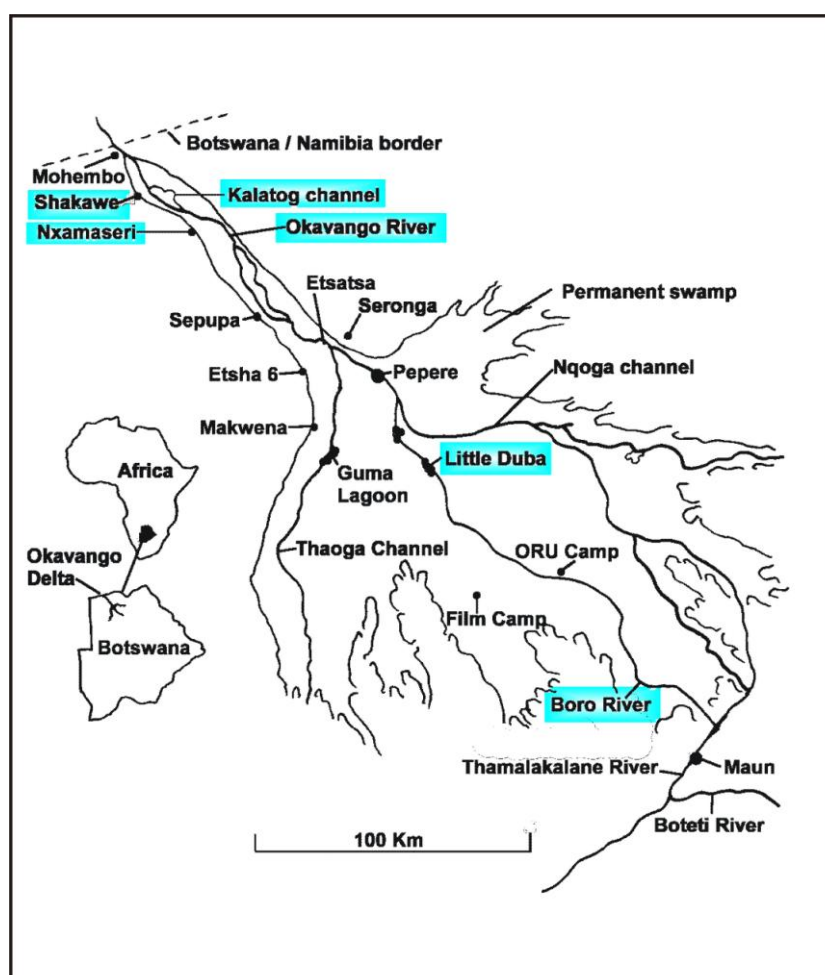


Figure 4.1: Map of the Okavango Delta with the collection sites highlighted (map courtesy of the Aquatic Parasitology Group, UFS)

During the sampling, fish were caught indiscriminately using either gill nets or cast nets. The caught fish were kept in aerated containers with sufficient oxygen on the boat, until examination at the field laboratory. Once at the field laboratory, the fish were examined for branchiurans in the gill chambers, mouth and on the skin surface. According to Van As & Van As (2001) it is important that the collection be done from freshly caught fish, to ensure that the parasites were collected from the actual hosts. The identification of the fish hosts was done according to Skelton (1993, 2001).

The fish were anaesthetised using MS 222, except in the case of *Clarias* species. Due to the hardness of *Clarias* species it was necessary to incapacitate the fish by shocking them with copper rods attached to a portable power source, and subsequently severing the neural cord behind the head.

During 2002-2004 the archival material of *D. ranarum* (previously collected over a period of 10 years also from the waters of the Okavango Delta and Panhandle) was examined and the most common hosts were identified. The hosts from which fewer branchiurans were collected were actively pursued, to make the population studied more representative. The hosts that were identified as potential hosts of *D. ranarum*, were collected using methods specific to the host species. *Clarias* Scopoli, 1777 was collected by using cast nets, especially during the barbel runs in September. The local children were employed in catching *Synodontis* Cuvier, 1816 species and the sports fishermen also contributed to the catch of *Serranochromis* Regan, 1920 species and *Oreochromis* Günther, 1889 species. *Hepsetus odoe* (Bloch 1794) was collected by line fishing. Where other projects were not being run on a certain host species, the fish were examined for branchiurans and then released into the river. Archival specimens (stored in 70% to 90% ethanol) were removed from their containers and studied under the Nikon microscope to study the morphology of the specimens.

Branchiurans are not affected by MS 222 (Van As & Van As 2001), and the skin and the fins of collected fish were examined with the naked eye or with a hand lens or dissection microscope subsequent to anaesthetisation. The

location of the parasites was assisted by their movement (if they are moving they are easier to see), and the parasites were removed from the fish host by using a scalpel or a fine brush. As specimens may also be present in the gill chambers, the fish were dissected, and the gills examined using a dissection microscope. Collected parasites were fixed in 70% ethanol, with, some specimens fixed in 100% ethanol. In addition some specimens were stored in CTAB-buffer to utilise during extractions using this (CTAB) method. Further analyses were done in the laboratory.

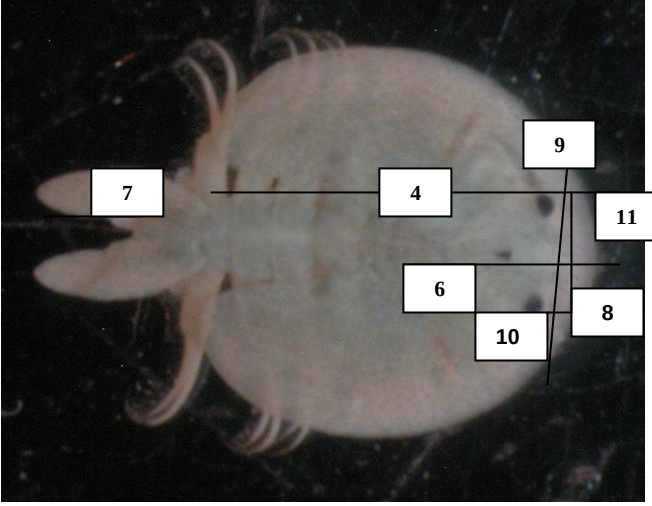
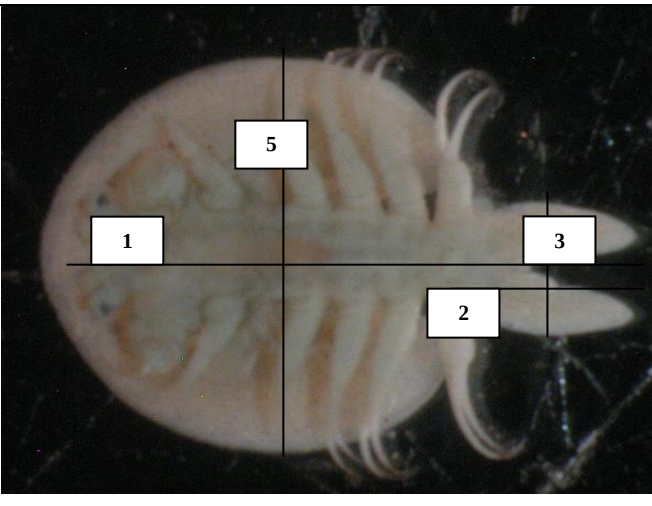
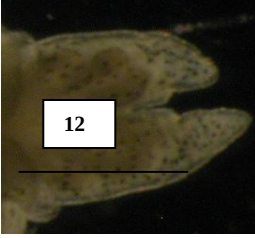
Measurements were obtained from the photographs taken using a Nikon Coolpix 990 attached to the Wild microscope. Photographs were taken of the ventral and the dorsal surface of *D. ranarum* to facilitate the comparison of morphological features of the specimens.

One by one the specimens were placed on a microscope slide moistened with 70% ethanol and covered with a cover slip. The larger specimens were placed on a cavity slide.

The size of the specimens was calibrated using a scale that was photographed prior to taking photographs of the specimens themselves. The photographs were converted to bitmap images using XnView 1.82.4 (Copyright Gougelet 2006), for measuring in Scion Image for Windows, (Copyright Scion Corporation 2000).

Several body measurements of all individuals from different populations (collected from each of the host species) were taken and percentages and relationships between the measurements were calculated using Microsoft Excel 2000 (Copyright Microsoft corporation). Statistical data were obtained using Microsoft Excel 2000. Relevant calculations are given in Appendix B. The measurements chosen in the morphological study was partially sourced from Van As & Van As (1999) and is shown in Table 4.1. All the measurements mentioned in Van As & Van As (1999) were utilised and subsequently other possibly significant measurements were also taken. The measurements were taken in μm .

Table 4.1: Body measurements of *Dolops ranarum* (Stuhlman, 1891) taken with Scion Image

Nr	Measurements taken		
1	Total length		
2	Abdomen length		
3	Abdomen width		
4	Carapace length		
5	Carapace width at the widest point		
6	Anterior carapace length		
7	Length of furcal rami		
8	Distance between eyes		
9	Width of carapace in line with eyes		
10	Length of eyes		
11	Width of eyes		
12	Length of testes or		
13	Length of ova		

Data from the measurements were illustrated in graphs and compared with each other (see Appendix B). To determine whether there were any significant relationships between the different measurements, percentages were calculated as shown in Table 4.2. The standard deviation of the measurements was calculated to get an idea of the variation in the total

population and the variation in the populations found on the separate host species groups.

Table 4.2: The calculations of proportions of *Dolops ranarum* (Stuhlman, 1891) specimens collected from all hosts

Nr	Calculation
1	Abdomen as percentage of the total length
2	Carapace as percentage of the total length
3	Anterior carapace length as a percentage of the total carapace length
4	Length of furcal rami as percentage of abdomen length
5	Length of testes or ova as a percentage of abdomen length
6	Percentage of distance between the eyes to the width of the carapace at that point

The measurements were sorted according to the sex of the specimen. Adults were identified according to the visibility of mature reproductive organs in the case of *D. ranarum*. In the other genera of the family Argulidae, the juveniles hatch with maxillulae formed into hooks, which progressively develop into suckers during the ontogeny of the organism. Adults can therefore be identified by the presence of maxillular suckers and the absence of hooks.

Copulatory structures are also present on the legs of the adult representatives of *Argulus* and *Chonopeltis*, which is not the case in *Dolops* species. Thus making it easier to identify adult specimens of *Argulus* and *Chonopeltis* species. During the ontogeny of *Dolops* the juveniles have the same features as the adults, but are just smaller.

Chapter 5: Morphology - Results

Appendix A includes dorsal and ventral photos of each of the specimens studied, as well the date the specimen was collected, location from which it was collected and host species. The fish hosts examined and the incidence of *D. ranarum* found during the course of the research in the Okavango by the Aquatic Parasitology Research group is presented in Table 5.1, as is the percentage of infestation. It was found that several of the hosts infested with the parasite were found to have more than one individual of *Dolops ranarum* associated with it.

Table 5.1: The number of fish hosts examined, and the number of fish infected with *Dolops ranarum* (Stuhlman 1891) (information from the Aquatic Parasitology Group, University of the Free State)

Host Species	Number examined	Incidence	Percentage of Infection
<i>Clarias gariepinus</i>	55	7	12.73
<i>Clarias ngamensis</i>	16	0	0
<i>Clarias stappersii</i>	4	1	25
<i>Hepsetus odoe</i>	107	1	0.93
<i>Serranochromis macrocephalus</i>	28	4	14.29
<i>Serranochromis robustus</i>	5	1	20
<i>Serranochromis angusticeps</i>	27	2	7.41
<i>Oreochromis andersonii</i>	78	4	5.13
<i>Oreochromis macrochir</i>	63	5	7.94
<i>Synodontis nigromaculatus</i>	99	2	2.02
<i>Shilbe intermedius</i>	98	1	1.02
<i>Tilapia rendalli</i>	72	1	1.39
All host species	652	29	4.45

Tables illustrating the exact measurements of the collected specimens can be found in Appendix B. In Figure 5.1 the incidence of *D. ranarum* on the collected hosts can be clearly seen. It is obvious that when comparing the number of host specimens

examined to the incidence of *D. ranarum* on the hosts, that the infestation was higher on the clariids and the serranos, as is shown in Table 5.1.

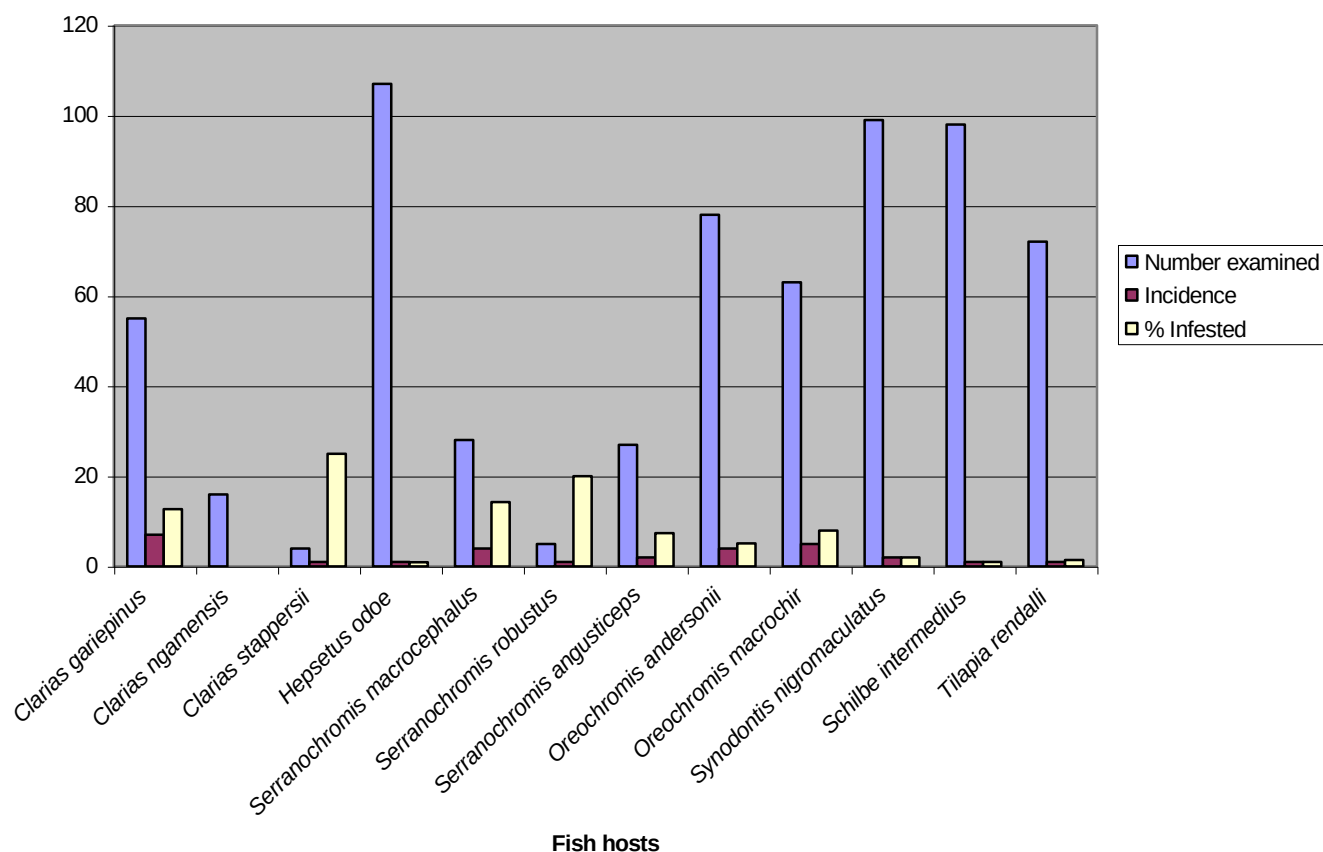


Figure 5.1: Graph of the occurrence of *Dolops ranarum* (Stuhlman, 1891) on collected fish hosts from 1997 – 2006

In Figures 5.2, 5.3 and 5.4 the standard deviation of the total population in relation to the separate populations found on clariids and cichlids are illustrated. The clariids and the cichlids were selected, as these were the most dominant hosts found to be infected. The graph is split into three graphs for clarity.

In the case of the measurement of the total length of the body of specimens, the standard deviation was lower for the total population than it was for the population of *D. ranarum* present on clariid hosts (Figure 5.2). This was also true for the length of the abdomen of *D. ranarum* specimens collected from all hosts when compared to specimens collected from representatives of Clariidae hosts.

Comparing the width of the abdomen of *D. ranarum* specimens collected showed that the standard deviation was higher for the total population (*D. ranarum* collected from all hosts) than for either of the single host groups (*D. ranarum* specimens collected from clariid or cichlid hosts). The length and the width of the carapace showed that *D. ranarum* specimens collected from clariid hosts showed greater variation in this population than the total population (*D. ranarum* collected from all hosts), but by a very small margin (Figure 5.2).

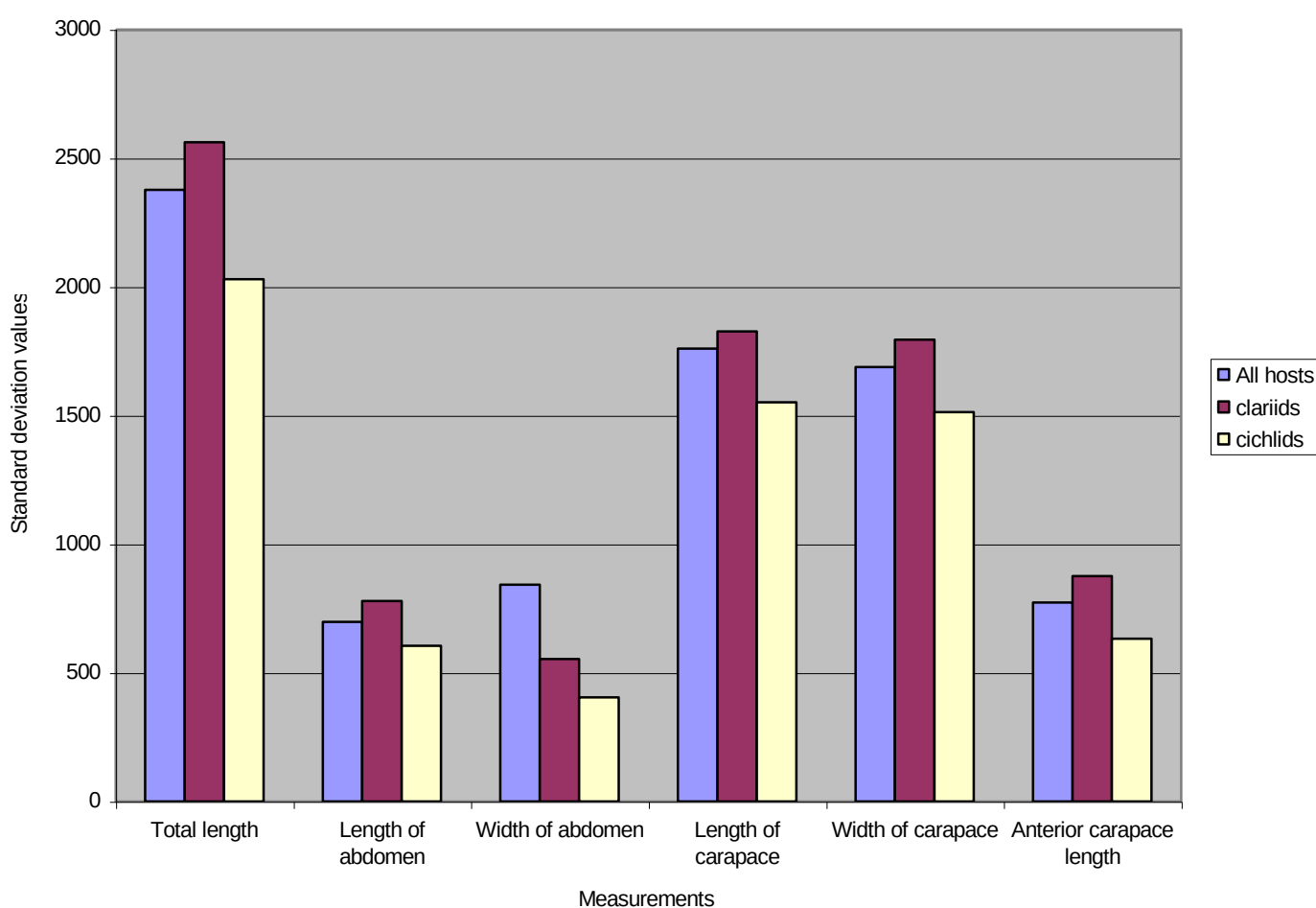


Figure 5.2: Graph showing the standard deviation of measurements of *Dolops ranarum* (Stuhlman, 1891) individuals from all host populations, as well as collected from clariid hosts and cichlid hosts separately

There was a higher standard deviation for the *D. ranarum* specimens collected from clariid hosts than for the total population collected from all hosts for the length of the

carapace, the width of the carapace over the eyes taken exactly touching the edge of eye, the width of the carapace over eye in straight line, the distance between the eyes, the distance from right eye to anterior carapace edge and the distance from left eye to anterior carapace edge (see Figure 5.3).

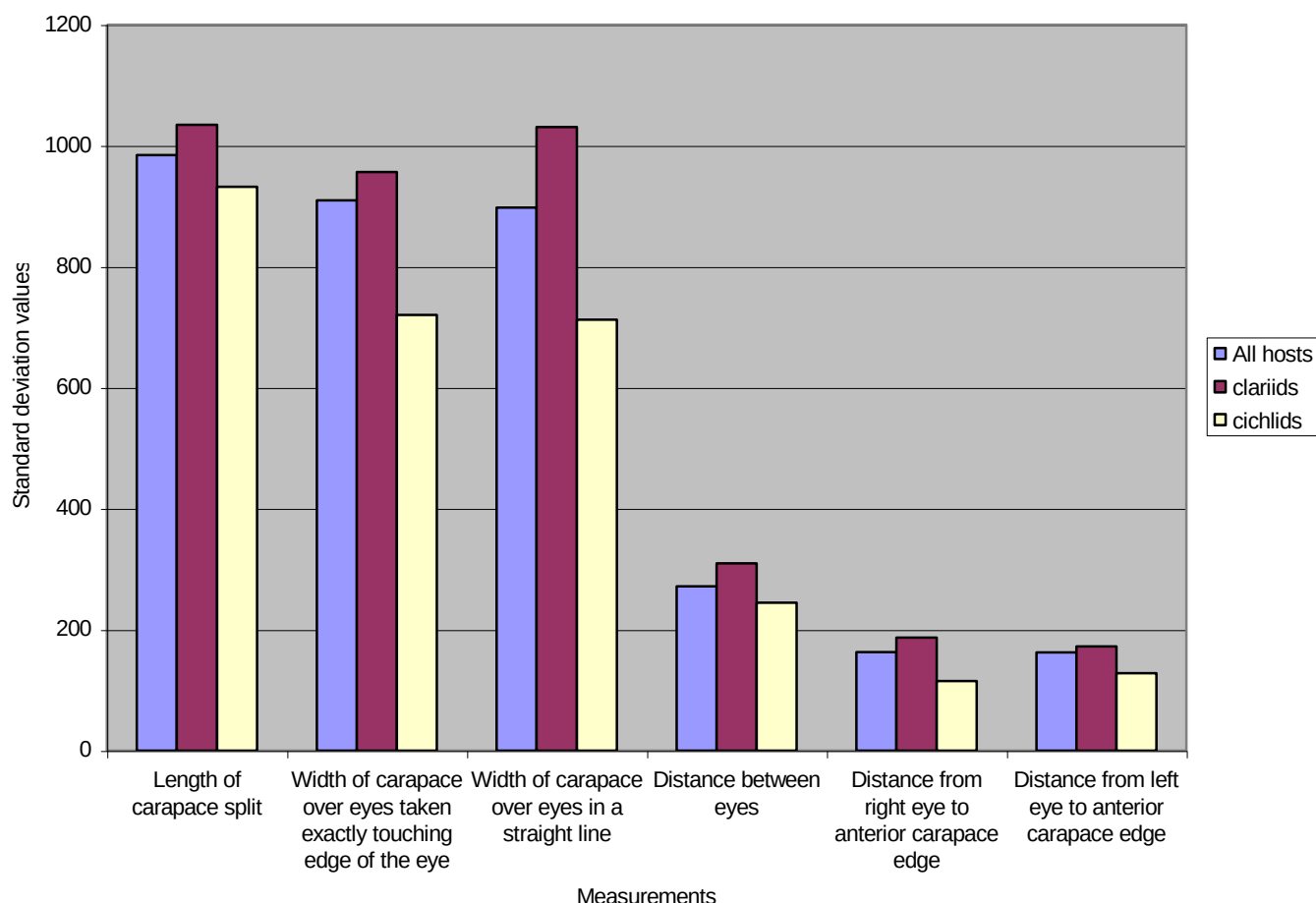


Figure 5.3: Graph showing the standard deviation measurements taken of *Dolops ranarum* (Stuhlman, 1891) individuals from all host populations, as well as collected from Clariid hosts and Cichlid hosts separately

The standard deviation of the various measurements taken of *D. ranarum* individuals from firstly the entire population and second and thirdly the populations collected from clariid hosts and cichlid hosts, is illustrated in Figure 5.4. In this graph the measurements of the length of the eye, the width of the eye, the length and width of the furcal rami and the length of the testes and ova are indicated.

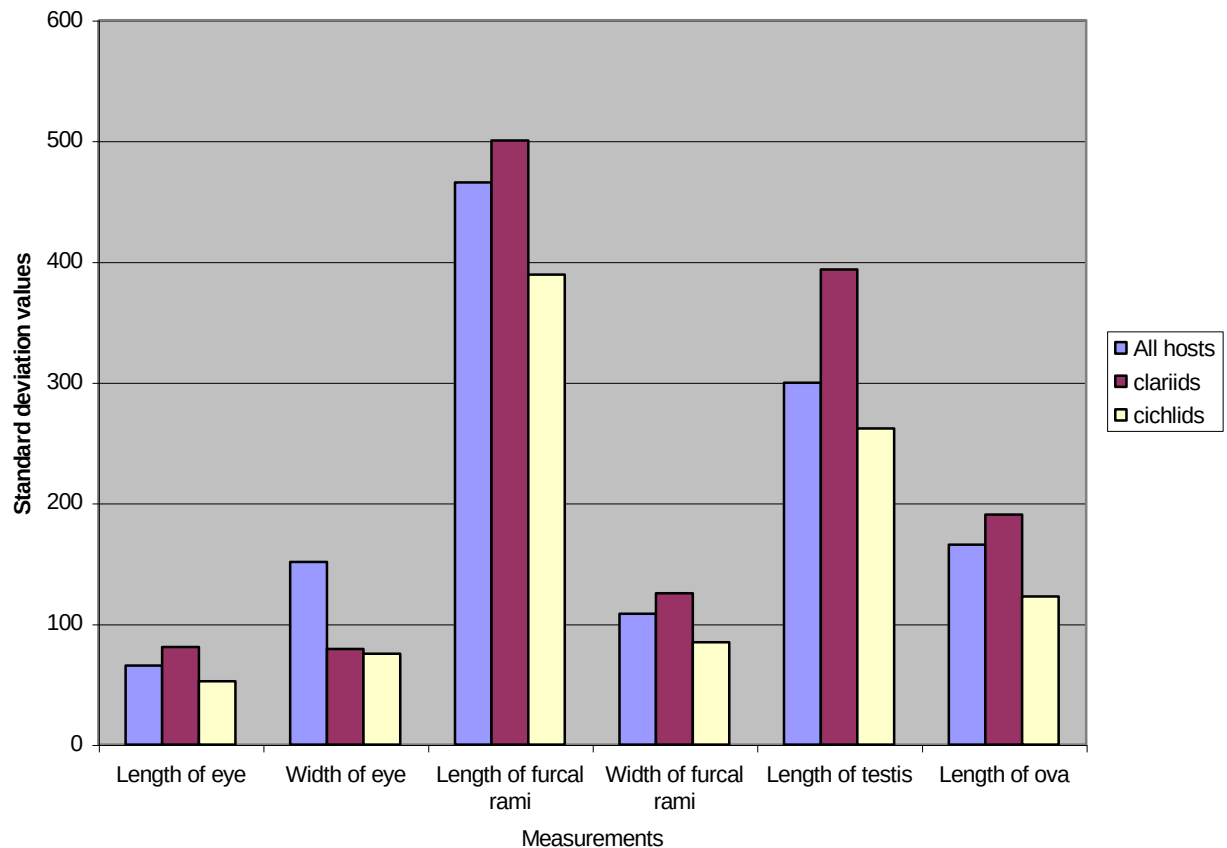


Figure 5.4: Graph showing the standard deviation of measurements taken of *Dolops ranarum* (Stuhlman, 1891) individuals from all host populations, as well as collected from clariid hosts and cichlid hosts separately

The attachment sites of branchiurans collected from several host species in the Okavango between 1997 and 2006 are illustrated in Figure 5.5. In this graph the branchiurans collected are indicated according to the position on the host *D. ranarum* from the skin or the gills and the presence of *Argulus* species and *Chonopeltis lisikili*.

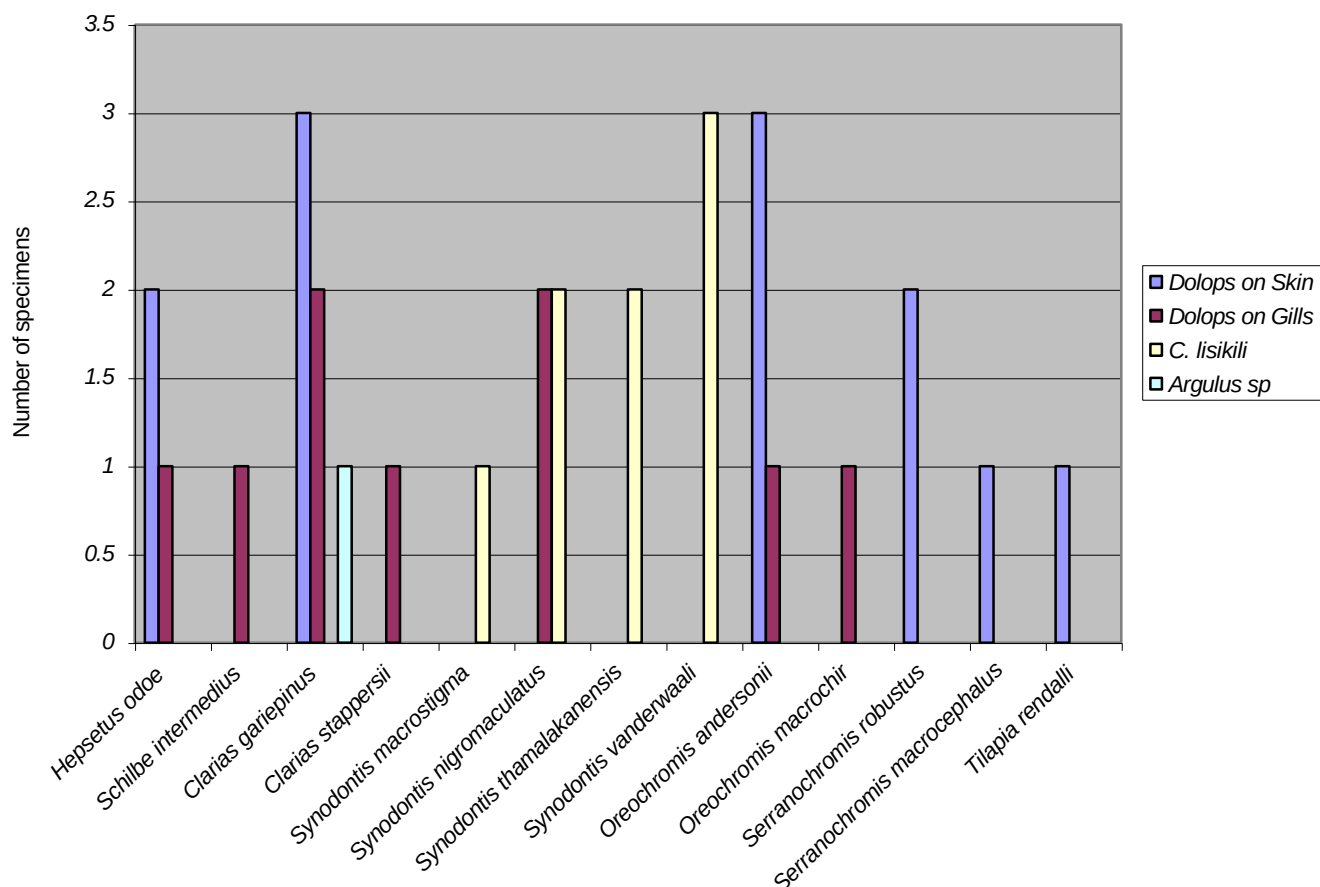


Figure 5.5: Graph showing the attachment sites of *Dolops ranarum* (Stuhlman, 1891) and other representatives of the Branchiura collected in the Okavango Delta between 1997 and 2006

Chapter 6: Comparison of Morphological and Molecular taxonomy

The classification of species of plants and animals has been developing from the earliest times. However, the organisation found in taxonomy in the present day originated around 300 years ago with Carl von Linné (Linnaeus). As the science of taxonomy gained adherents, the methods utilised to classify organisms became more sophisticated. As the science evolved deficiencies were identified and new methods were added to the process of classifying species. This was done to ensure that the most correct conclusions were drawn about the taxonomic position of a species relative to other species.

Knowledge of the biodiversity of the earth continues to increase with new species being found even today. The latest development in the evolution of the science of taxonomy is the inclusion of molecular information in the classification and description of new species. An example of a group where molecular methods have been utilised to elucidate species diversity is the freshwater crustaceans (including branchiurans). Proudlove & Wood (2003) use the example of an amphipod, *Niphargus* species, where a population, even in a very small geographical distribution and with a hydrological connection, proved to contain two different species despite the proximity of the populations. This is comparable to the conditions in which *D. ranarum* occur in the Okavango Delta.

With the development of molecular methods that are relatively reliable, the question arises; does it matter whether morphological or molecular methods are used to describe new species? The concept of molecular taxonomy has already gained ground amongst taxonomic groups who study species that are the least suitable for morphological methods for example viruses, bacteria and protists (Herbert *et al.* 2003).

One method of molecular taxonomy that is being investigated is DNA barcoding. DNA barcoding refers to the use of a short section of DNA sequence to identify a particular species (Dasmahapatra & Mallet 2006). However, the use of a single gene or a portion of a single gene for the

description of species from a wide taxonomic range, including birds, fish and insects is highly controversial (Herbert *et al.* 2003).

Lipscomb *et al.* (2003) protested against using “an infestimally tiny fragment” to identify a species, and then show only a small percentage of difference. They claimed that the promoters of molecular taxonomy did not grasp the importance or intellectual content of taxonomy based on all possible information. Lipscomb *et al.* (2003) warned that to change taxonomy from a field “rich in theory and knowledge” to a “high tech service industry” would be detrimental to science.

Tautz *et al.* (2003) however did not suggest that the other attributes of species should be ignored. They said that DNA sequencing should be used as the backbone around which the other information should be structured. The taxonomic DNA databases should include all possible information, even deeming existing DNA databases which are currently inadequate for taxonomy because of the lack of extra information.

6.1 Molecular taxonomy

In the last part of the 20th century, molecular techniques became more generally used in taxonomy. According to Proudlove and Wood (2003) the inclusion of molecular techniques in analysis of groups where other methods still leave cryptic taxa can be invaluable.

In the pursuit of the most practical and accurate methods of molecular taxonomy, Herbert *et al.* (2003) noted that mitochondrial DNA is better suited for certain aspects of molecular analysis than genomic DNA. This was because mitochondrial DNA lacks introns, is limited in its exposure to recombination, it has a haploid nature of inheritance and robust primers are available for the sequencing. There is no real reason why research should focus on the COI gene, but it does have two important advantages. Firstly, the universal primers for the COI gene are stout, and facilitates recovery of its 5' end from representatives of most, perhaps all animal phyla. Secondly, the

gene seems to have a greater range of phylogenetic signal than any other mitochondrial gene, precisely because of its rate of high base substitutions at third-position nucleotides that seem to be three times greater than that of 12S or 16S rDNA (Herbert *et al.* 2003). The evolution of this gene is fast enough to permit the differentiation between not only closely related species, but perhaps even phylogeographic groups within a species (Herbert *et al.* 2003).

The COI gene, has also received some attention in forensic entomology. In a non-forensic context it has been used to identify species of fish, birds, insects and primates (Dawney *et al.* 2006). The anticipated growth in COI data has recently led one leading journal to form a dedicated barcoding section for COI sequence publication, smoothing the way for the COI gene to becoming a key taxonomic identification tool. Herbert *et al.* (2003) achieved considerable success with this gene, and concluded that a COI database could serve as the basis for a global bio-identification system for animals; in essence a DNA barcoding system that would (with the creation of an exhaustive database) facilitate in species identification.

Consequently, for the reasons mentioned above it was decided to sequence the COI gene to determine whether more than one species could be distinguished in the population of *D. ranarum* present on different hosts present in the Okavango Delta.

6.2 Taxonomic collections and databases

Taxonomic collections (kept at museums, for instance) are used to store specimens of described species. The published descriptions and keys are utilised to identify specimens collected during fieldwork.

Currently DNA databases are being developed. The information gained from these databases will be used for the identification of species. This development has raised questions such as: should only one small specimen be available, should the specimen be stored as the type specimen or be utilised to obtain a DNA sequence? Other type specimens would have been

rendered unsuitable for DNA extraction due to the method of preservation, especially older specimens. One of the logistical problems with DNA extraction regarding smaller specimens, is that type specimens would be lost if DNA extraction was performed. Larger type specimens (as well as plants and fungi) would not be lost if a small tissue sample was used for DNA extraction. In the case of *D. ranarum* specimens are small. Consequently, once the specimens are utilised for DNA extraction, nothing of the original specimen can be saved for the collection. This makes it imperative that the specimens are photographed and measured before being utilised for DNA extraction.

According to Tautz *et al.* (2003) the accuracy of DNA taxonomy is such that the molecular part of species descriptions should be central to the science of taxonomy, while morphology should be an auxiliary method. The suggestion entails that the first specimen collected should be sequenced and kept for reference, while its DNA sequence is seen as an approximation until more specimens can be collected and compared. This sequence is then released to appropriate databases including the species description and other known and relevant information.

Once sufficient species descriptions are entered into the database, new specimens can be checked against the existing data and using the sequences that are known, a decision can be made whether a new species description is warranted (Tautz *et al.* 2003). DNA sequences are ubiquitous, meaning that regardless of the life stage of the collected specimen, the species will be accurately identified.

Tautz *et al.* (2003), emphasized that the process would be difficult in the beginning, and an intense effort should be made to create a concentrated overview of all known taxa. Once the database is sufficiently complete, all new sequences can be quickly and accurately placed. This information would be applicable to all fields of study in taxonomy and biodiversity, and would be able to place any sequence from a new specimen into context even if no exact match can be found.

There are databases in existence, for instance the National Centre for Biotechnology Information (NCBI) and the European Bioinformatics Institute (EBI), but these are not suitable for taxonomic purposes (Tautz *et al.* 2003).

To bring all existing species into a DNA species database could be a problem. Experienced morphological taxonomists would have to be employed to identify the newly collected specimens as the same species as the type specimen. The type specimens would generally be impossible to use as sources for DNA to sequence, because it would destroy the type specimen in the case of small organisms (Tautz *et al.* 2003).

6.3 Problems encountered in taxonomy – species boundaries

One area where DNA taxonomy fails the test for ease in identifying species, is when newly isolated gene pools of sister species are encountered (Tautz *et al.* 2003). The closely related species will still share alleles either because of continued gene flow between the sister species or because of their recent phylogenetic divergence, causing confusion in distinguishing accurately between these species. The organelles (like mitochondria) would then give a different result for sequencing of the genomic DNA and mitochondrial DNA (mtDNA). This situation is not much different from the problems encountered in morphological taxonomy in species that are morphologically very similar.

Will & Rubinoff (2004) suggested that the same problems that constrain the accuracy of species boundaries in morphological taxonomy are also present when working on a molecular level. There is one aspect of morphological taxonomy that makes it possibly more accurate than molecular work: the morphologically knowledgeable taxonomists have a complement of complex morphological characters to use when making their conclusions. In the case of the molecular work, the researcher depends on a small part of a single gene (Will & Rubinoff 2004). However, in the case of molecular work the sequence data is unbiased, while the taxonomist assigns weight to characters

of their choice. Lipscomb *et al.* (2003) argued that it is not true that the molecular approach is less biased than the morphological one, as the choice of gene to be amplified, the alignment and identification of paralogues and orthologues complicate matters.

It boils down to a question of ranges. There is variability in a population regarding phenotype and genotype, these continuous ranges become disjunct only at species boundaries, and this is equally true for molecular as for as morphological characters. Similar problems apply to molecular methods of defining a species as those that apply to morphological traits. When a species variation is not discrete and when species do not meet in nature, how can one possibly determine species boundaries? In the case of molecular work, the difference is quantified as the number of changes present in base pairs.

Unfortunately the same problems apply to molecular methods of defining a species as those that apply to morphological traits, albeit on a smaller scale. Molecular methods create a situation where even related species that are isolated in their natural distribution can be compared on a genetic level. The difference is quantified as the number of changes present in base pairs, as opposed to the comparison of morphological characteristics in a morphological comparison. The question arises: when is the number of base pairs that are different significant? When is it just variation within a species and when is it enough in order for a new species to be declared?

While parameters may be established, these will have to be specific to the taxon and the geographical distribution. The fact that speciation rate is the highest at the equator and diminishes towards the poles and that the rate at which this happens is not linear or even gradual; makes it impossible to designate a constant to indicate the rate of speciation. However, developing a set of parameters for each taxon at a given geographical point is also not an effective solution. It is therefore imperative that each species be dealt with on an individual basis.

In the case of *D. ranarum* in Africa it would be interesting to see what the genetic difference is between the populations of *D. ranarum* present on different host species and whether this distance is significant. In the study conducted by Herbert *et al.* (2003) difficulties were experienced with the species assignment of four congeneric species pairs, which were genetically distinct, but had low divergences suggesting recent evolutionary origin. It is possible that this may also occur at the different populations of *D. ranarum*, because morphological results indicate no obvious differences?

According to Tautz *et al.* (2003), the combined rates for mutation and fixing of a mutation are between 0.1% and 2% per million years for naturally evolving sites. This gives an idea of how long it takes for newly evolved species to become distinct from one another on a molecular level. Although different for every case, it seems that the minimum time needed for complete isolation to occur is 100 000 years. There are exceptions, for instance the fish species of Lake Victoria where the morphological differences have built up more quickly than the molecular differences; in these exceptional cases the morphological evidence carries more weight than the molecular data (Tautz *et al.* 2003). Even in this case there is some use for the molecular data, giving information on the origin of the pioneer population, and a timeframe for the divergence of the sympatric species. Very recent divergences will still be apparent only from molecular data (Tautz *et al.* 2003).

6.4 Comparison of traditional taxonomy and molecular taxonomy

Will & Rubinoff (2004) seemed quite indignant at the suggestion that the identification of new species is the responsibility of taxonomists. However, the question arises: Who else could be responsible? Taxonomists are highly trained individuals, often expert in fields where very few other specialists can be found. According to Will & Rubinoff (2004) the role of taxonomists is to create identification tools not to do the identification. This would be practical in an ideal world, but due to the complexity of many taxonomic groups, tools can be fashioned for identification up to a certain taxonomic level. If the

specimen were to be assigned to species or subspecies level, an expert would be necessary.

However, Herbert *et al.* (2003) stated that there are four major constraints to the use of traditional taxonomy to clear the backlog of unidentified species and to ensure that all species presently recognised are valid.

- Phenotypic flexibility and genetic changeability in characters utilized for species descriptions can cause incorrect identifications,
- This method neglects morphologically cryptic taxa found in many groups,
- Morphological keys are often efficient only for a specific life stage or gender therefore many specimens cannot be identified, and
- Contemporary interactive versions signify a major improvement, but the use of keys often requires such a high level of proficiency that misdiagnoses are frequent.

An advantage that molecular work has over morphological work is that it is immaterial in which life stage the specimen is collected, the molecular information will be identical in all life stages Tautz *et al.* (2003). Another advantage is that keys would not be necessary. The problem is that a foolproof DNA extraction and sequencing protocol must be found for the particular taxon before results can be guaranteed.

Molecular taxonomy as a method could perhaps be rendered less repugnant to its detractors by using more than one sequence to determine the boundaries of species. As mentioned previously, a point in favour of molecular taxonomy would have to be that very little expertise on a specific organism is necessary, just general skills involved in DNA extraction, PCR and sequencing (Herbert *et al.* 2003).

According to Lipscomb *et al.* (2003) there is no reason to give gene sequences greater weight than any other character. Lipscomb *et al.* (2003) also states that when species boundaries were decided by a range of characters (morphological), the data give added information on distribution of characters among organisms, and they argue that a sequence does not. Perhaps here it is not the method that is at fault, but merely the timing. With added research into the genomes of several different organisms, the coding regions of the DNA strand may become as revealing to us as the morphological characters given providing information about the entire organism, throughout its life cycle.

Lipscomb *et al.* (2003) call the sequence similarities revealed tedious in comparison with the detailed picture obtained from the morphological and ecological data about an organism, and do not anticipate that it is possible that sufficient information could be obtained from genotypical data alone. Currently, species descriptions are based on a broad range of data. Subsequently phylogenies are reconstructed to explain patterns of organismic uncertainties within a taxon, but when nothing is known about organisms except the sequence of their DNA, there are no evolutionarily interesting patterns to explain – just patterns of sequence similarity.

Unfortunately proponents of morphological taxonomy such as Lipscomb *et al.* (2003) wilfully deny that exactly the same weaknesses ascribed to molecular taxonomy are just as present in morphological taxonomy. There are even local examples, like the blue and black wildebeest, and the blesbok and bontebok. While each of these have their own species name (or subspecies), members from the two populations can mate and have fertile offspring, really making them one species according to Mayr's popular definition of species (Mayr 1969). Here there is also a percentage of difference only between either two populations, as is the case in domestic and South African wild cats to mention but a few examples that occur locally.

In addition, there is currently still a general perception that DNA technologies are more expensive than traditional taxonomy (Tautz *et al.* 2003). This may

have been true in the past, but today the cost of labour is constantly increasing while the cost of automation is decreasing. Taxonomists are expensive and time-consuming to train, and it would be fair to say that their valuable time and expertise were wasted in routine identification.

In my opinion, all of these methods, and all of the species definitions have their strong and weak points and I agree with Giribet *et al.* (2005) who are of the opinion that a combined approach of molecular and morphological evidence is the best way to approach such taxonomic tangles. One way of clarifying taxonomy would be to take into account that reductionalist thinking might not be the most effective approach when dealing with biodiversity. Perhaps it would make the classification of the different phyla more specific (precise), if we adapted the criteria to the case at hand. For instance, while sexual reproduction is common in larger complex life forms, it is in reality a very small fraction of life on earth that utilizes this strategy, therefore Mayr's definition is extremely limited in application, but where it can be applied, it encompasses most of the cases encountered, with the exceptions shown earlier of course. The greatest doubt with regard to this approach is that perhaps one is only moving the blurred boundaries between taxa up the taxonomic hierarchy.

In the end, all this speculation is useless without a species concept that taxonomists can measure their taxonomic units by. Therefore it is most important that a workable species concept be agreed upon, before the methods of establishing species can be considered.

Chapter 7: Molecular Section – Material and Methods

7.1 Collection and storage

The collection of the specimens of *Dolops ranarum* was described in the material and methods of the morphological section of this dissertation. Fresh specimens were stored in cetyltrimethylammonium bromide (CTAB) buffer, because ethanol may have a negative impact on the quality of the DNA. Mandrioli *et al.* (2006) found a significant increase in the quality and quantity of DNA extracted by preserving moths in 100% ethanol, particularly with specimens conserved at 4°C. However, preservation in 75% ethanol gave poor results regardless of the temperature the specimens were stored at. This indicated that only 100% ethanol offers reasonable DNA preservation.

Previously collected specimens utilised in this particular study had been stored in 70% to 90% ethanol at room temperature for several years.

7.2 Extraction

Several extraction methods were tested to obtain high quality DNA from *D. ranarum*. Modern extraction kits, such as the Promega Wizard kit and the DNeasy Blood and tissue kit from Qiagen were used as well as the phenol-chloroform extraction method and the CTAB extraction method. Each of the techniques used included at least three duplicated specimens. The specimens were stored in ethanol (70-90%), except for the specimens utilised during the CTAB extraction

- **Promega Wizard Kit: Animal tissue extraction**

The protocol as described in the Wizard Genomic DNA Purification Kit Technical Manual, #TM050 was followed. Modifications were made to the protocol after no success was achieved with the DNA extractions. To avoid contamination from the host, the alimentary canal was removed using a dissection needle. The volumes of the chemicals were halved and the RNase step was included.

- **CTAB extraction**

The CTAB extraction was performed according to Murray & Thomson (1980). Doyle & Doyle (1987) utilised this extraction method for DNA extraction from plant tissues, but this protocol has been utilised for DNA extraction from animal tissues (Braband *et al.* 2002; Remerie *et al.* 2004), in particular crustacean tissues. It was ensured that the entire specimen was dissolved during the incubation period (at 65°C) before completing the protocol.

- **DNeasy Blood and Tissue kit from QIAgen**

The extraction was performed according to the instructions of QIAamp DNA Mini and Blood Mini Handbook 11/2007 Isolation of Genomic DNA from tissues, and Micro Dissected Tissue. The QIAgen Mini Kit is designed to extract DNA from small samples or from specimens with degraded DNA. This kit includes QIAgen Mini Spin Columns to make the extraction process easier. Optimised buffers and enzymes are designed to lyse samples, stabilise nucleic acids, and enhance selective DNA adsorption to the QIAamp membrane present in the QIAgen Mini Spin Columns. Subsequently alcohol is added and lysates loaded onto the QIAamp Mini Spin Column. Wash buffers were used to remove impurities. Pure, ready-to-use DNA is then eluted in water.

- **Phenol–Chloroform DNA extraction**

This, it is a method that has been successfully utilised for DNA extraction from a range of organisms. This extraction was performed according to standard methods as discussed in Sambrook *et al.* (1994).

7.3 Ascertaining the presence of DNA

The success of each of these extraction methods was evaluated by agarose gel electrophoresis (Sugden *et al.* 1975). A 0.8% gel was prepared, 3µl of DNA was

mixed with 2µl loading buffer (BFB) and loaded into the wells in the gel. The power source was set to 100V for 20min. Ethidium bromide (EtBr) was used to stain the gel as EtBr fluoresces under UV light when intercalated in DNA.

The quality and quantity of the DNA was also tested using the Nanodrop, according to the protocol set out in the NANODROP ND-1000 Spectrophotometer V3.2 User's Manual. This method gives a more accurate reading of the amount of DNA present in specimens extracted. Nanodrop readings were taken in triplicate to ensure that the readings were accurate. Using the Nanodrop instead of agarose gel electrophoresis means that smaller amounts of extracted DNA can be utilised to ascertain the presence of DNA. In addition the Nanodrop provides rapid results.

7.4 Polymerase Chain Reaction (PCR)

After DNA was extracted, a PCR reaction was done using universal primers (HCO, LCO) (Table 7.1) to invertebrates of the Cytochrome Oxidase I (COI) gene from the mitochondrial genome (Folmer *et al.* 1994) using the Perkin-Elmer Applied Biosystems Geneamp PCR system 9600

Table 7.1: The sequences of the primers used for the amplification of the Cytochrome Oxidase I gene from the mitochondrial genome by polymerase chain reaction (Folmer *et al.* 1994) of *Dolops ranarum* (Stuhlman 1891)

Primer	Sequence
LCO1490	5'-GGTCAACAAATCATAAAGATATTGG-3'
HCO2198	5'-TAAACTTCAGGGTGACCAAAAAATCA-3'

PCR conditions from several publications were considered including Stillman & Reeb (2001), Bucklin & Allen (2003), Lörz & Held (2003), Zhao & Murphy (2003), Remerie *et al.* (2004), Lavery *et al.* (2003) and Davolos & McLean (2005). PCR conditions tested are listed in Tables 7.4 and 7.6.

Table 7.2: Ingredients of the 10 μ l polymerase chain reaction used in the DNA extraction of *Dolops ranarum* (Stuhlman, 1891) to amplify the Cytochrome Oxidase I gene from the mitochondrial genome

Ingredients	Volumes (in μ l)
5 x Buffer (including dNTPs)	2
Taq polymerase	0.1
MgCl	0.6
Primers	1 (0.5 of each)
Water	1
DNA	5.3
<i>Total reaction</i>	<i>10</i>

Table 7.3: The polymerase chain reaction programme used to amplify the Cytochrome Oxidase I gene extracted mitochondrial DNA of *Dolops ranarum* (Stuhlman, 1891)

Programme 1		
Temperature ($^{\circ}$ C)	Time	Cycles
94	1 min	1
94,45,72	Each 30 s	5
94,53,72	Each 30 s	35
72	10 min	1
4	30 min	1

The PCR reactions were done in triplicate. In Table 7.4 a new suggested PCR reaction, where the total reaction comprised 12,5 μ l is shown and in Table 7.5 the PCR programme used.

Table 7.4: The ingredients of the 12,5µl polymerase chain reaction to amplify the Cytochrome Oxidase I gene from the mitochondrial genome first attempted on the extracted DNA of *Dolops ranarum* (Stuhlman, 1891)

Ingredients	Volumes (in µl)
10 x Buffer	3.75
DNTPs	0.125
Taq polymerase	0.125
Primers	0.25 of each
Water	4
DNA	4
<i>Total reaction</i>	<i>12,5</i>

Table 7.5: The polymerase chain reaction programme used to amplify the Cytochrome Oxidase I gene from the mitochondrial genome of *Dolops ranarum* (Stuhlman, 1891)

Programme 2		
Temperature (°C)	Time	Cycles
94	1	1
94,45,72	40s,40s,1min	5
94,51,72	40s,40s,1min	35
72	5min	1
4		1

A Taguchi optimisation PCR reaction was done based on Cobb & Clarkson (1994) to optimise the PCR conditions for the COI gene. Taguchi methods are utilised to obtain the range of optimal conditions for any given PCR assay in a fraction of the number of reactions required (Viljoen *et al.* 2005). In this method the number of reactions is calculated using the following equation: $E=2k+1$, where E is the number of reactions and k being the number of variables to be tested. For instance, if there were 8 variables, 6561 reactions would have to be tested in block titration. In

comparison only 17 reactions are needed if the Taguchi method is used (Viljoen *et al.* 2005).

Each of the variable components was added to the reaction at one of the three different concentrations selected. These concentration values were chosen to fall within the range of concentrations that would be the most influential on the reaction. These values must also be sufficiently separated to allow for the determination of their effects on the reaction (Viljoen *et al.* 2005).

Table 7.6: Calculations for optimisation of polymerase chain reaction to amplify the COI gene from extracted DNA of *Dolops ranarum* (Stuhlman, 1891)

Constituents	Original	Doubled	A		B		C	
Primer	0.5	1	2	2	4	4	8	8
DNA	1	2	0.5	1	1	2	2	4
Buffer	3.75	7.5	0.5	3.75	1	7.5	1.5	11.25
dNTP	0.125	0.25	0.05	0.013	0.2	0.05	0.4	0.1
Taq	0.125	0.25		0.25		0.25		0.25
Water	7	14		17.99		11.2		1.4
<i>Total</i>	<i>12.5</i>	<i>25</i>		<i>25</i>		<i>25</i>		<i>25</i>

The PCR reaction was done using the Perkin-Elmer Geneamp system, and the same PCR protocol as indicated in Table 7.5.

As in the case of the DNA extractions, the presence of amplified DNA was evaluated by using agarose gel electrophoresis. The Nanodrop was used in later specimens. The NanoDrop is a cuvette free spectrophotometer. It uses 1 µl to measure the presence of DNA from 5 ng/µl to 3,000 ng/µl.

Chapter 8: Molecular Results

8.1 DNA extractions from *Dolops ranarum*

- Promega Wizard Kit

No positive results were obtained during the utilisation of this extraction kit.

- DNeasy Blood and Tissue kit from QIAgen: Isolation of DNA from tissues

During the use of two different protocols obtained from the Qiagen handbook, no results were obtained.

- CTAB extraction method

No results were obtained using this extraction method.

- Phenol-Chloroform Extraction

Results were obtained utilising the extraction method set out in Sambrook *et al.* (1994). Figure 8.1 is the agarose gel on which the DNA extractions (using the phenol-chloroform extraction) were run using gel electrophoresis, in order to ascertain the presence of DNA after the extraction. In each of the six lanes DNA extracted from different specimens of *D. ranarum* was loaded. DNA is present in specimens of *D. ranarum* in lanes 1, 2 and 6.



Figure 8.1: Gel electrophoresis of DNA extracted from *Dolops ranarum* (Stuhlman, 1891) using the phenol-chloroform extraction method adapted from Sambrook *et al.* (1991)

The Nanodrop was used to test for DNA in later specimens. The results of the concentration of DNA in the specimens extracted can be seen in Table 8.1.

Table 8.1: Concentration of DNA in the extracted samples measured using the Nanodrop (specs) in ng/μl. The averages are rounded to all have two decimals

Nr	Concentration 1 (ng/μl)	Concentration 2 (ng/μl)	Concentration 3 (ng/μl)	Average (ng/μl)
1	42.74	186.95	45.93	91.87
3	34.25	32.3	33.1	33.22
4	71.77	72.96	71.9	72.21
5	64.1	67.22	63.67	64.99
6	22.97	23.48	23.41	23.29
7	140.88	140.28	140.36	140.51
10	206.2	208.39	206.8	207.13
12	545.82	553.86	548.32	549.33
17	27.11	31.79	30.67	29.86
19	15.1	15.93	15.88	15.64
20	232.72	228.04	231.9	230.89
23a	96.51	88.37	93.11	92.66
23b	152.85	153.3	152.9	153.02
24	10.61	14.13	13.11	12.62
29	18.37	22.17	21.32	20.62
32	1825.97	1708.84	1715.33	1750.05

Table 8.1 continued				
Nr	Concentration 1 (ng/μl)	Concentration 2 (ng/μl)	Concentration 3 (ng/μl)	Average (ng/μl)
34	1646.23	1661.22	1692	1666.48
36	31.75	34.19	29.51	31.82
37	52.35	51.59	52.3	52.08
43	258.24	259.39	259.22	258.95
46	145.04	147.08	151.18	147.77

As indicated in Table 8.1, DNA has been extracted from all of the specimens. However, the quality of the DNA was unclear.

The amplification of the Cytochrome Oxidase subunit I gene with the LCO and HCO primers were unsuccessful as described in the molecular materials and methods section of this thesis. The specimens with the highest concentration of DNA according to the Nanodrop results were chosen for amplification. None of the PCR programs used yielded any positive results, since no DNA fragments could be observed on the yield gel.

Consequently no results were obtained for the amplification of the Cytochrome Oxidase I gene of the mitochondrial genome of *D. ranarum* and sequencing process could thus not continue.

Chapter 9: General Discussion

9.1 Morphological Study

The results from the measurements done during the course of the morphological study indicate that it is unlikely that the specimens of *Dolops ranarum* found on different host species are from genetically isolated groups that may amount to new species (or subspecies). However, this may not necessarily be true. These parasites are not densely populated enough to collect large numbers of specimens. Considering the numbers of specimens collected one may surmise that the study was done on too small a number of specimens. Table 5.1 (Chapter 5) shows the incidence of *D. ranarum* on the different fish hosts. From this Table it is clear that the incidence of *D. ranarum* on fish hosts is comparatively low, as all the specimens utilised in this study were collected over a period of 10 years. Consequently, it would appear that the population of *D. ranarum* is relatively small. The specimens studied would therefore be representative of the population.

The results obtained from the host species collected and the incidence of the parasite on these different hosts, show that in a pristine environment such as the Okavango Delta there is very little danger of violent reaction of the host to the parasite living on it and also a relatively low incidence of parasite infestation on the hosts. There appears to be a trend to the occurrence of more than one individual of *D. ranarum* per host. This may be to facilitate procreation, as such small creatures living on their fish hosts, may be likened to certain species of animals living on islands. In the case of animals living on islands the opportunity rarely arises to have contact with individuals from another island. The immature organisms must also hatch where the likelihood of attachment to a host is high. Contact with others of their species therefore does not occur except by endeavour, i.e. the animal leaves its host and moves to another host.

- **Aspects of the biology and ecology of fish hosts important to *Dolops ranarum***

Table 9.1 provides general information on host fishes from which *Dolops ranarum* was collected during the course of this study in the Okavango Delta. It is important to note that one representative of each of the genera of *Clarias*, *Serranochromis*, *Oreochromis*, *Tilapia* and *Synodontis* were chosen to illustrate the general biology and ecology of the genus. In the case of *Hepsetus odoe* and *Schilbe intermedius* these are the only species of these particular genera present in the Okavango Delta and are therefore discussed not as general representatives, but as the only representatives relevant to the study.

Table 9.1: General information on hosts of *Dolops ranarum* (Stuhlman, 1891) found in the Okavango Delta (Compiled using Skelton, 1993)

General representative of fish family	General information on fish family (Skelton 1993)	Biology and Ecology of general representative (Skelton 1993)
Clariidae		
<i>Clarias gariepinus</i> (Burchell, 1822)	Catfish are known for their hardiness and ability to breathe air and survive desiccation. Catfish are easily recognised by helmet-like heads, elongated bodies and long dorsal and anal fins. Clariid bones date back to the Miocene in Africa. The Clariidae include 12 African genera and 74 species with three genera and eight species present in southern Africa.	This hardy sharptooth catfish (babar) occurs in most habitats, but favours floodplains, large, sluggish rivers lakes and dams. <i>C. gariepinus</i> is omnivorous (preys, scavenges and grubs) and may hunt in packs, herding and trapping smaller fishes. In turn sharptooth catfish are hunted by a variety of predators, such as man, crocodiles, leopards and birds. <i>Clarias gariepinus</i> breeds in summer after the rains. Large groups of adult sharptooth catfish migrate to shallow, grassy verges of rivers and lakes. The eggs are laid on vegetation and subsequently hatch in 25-40 hours. The larvae are free-swimming and are able to feed within two-three days. The larvae remain within the vegetation cover and grow rapidly. However, the rate of growth is dependant on local conditions. Maturity may be reached after a year, but most take two or more years. The lifespan is approximately eight years.

Table 9.1 continued

General representative of fish family	General information on fish family (Skelton 1993)	Biology and Ecology of general representative (Skelton 1993)
Hepsetidae		
<i>Hepsetus odoe</i> (Bloch, 1794)	African pikes are easily recognisable by their pointed heads and crocodile-like jaws. Only one representative is present in Africa, and thus constitutes a unique feature of the African fish fauna.	African pikes generally occur in quiet, deep water. Juveniles and fry are found in well-vegetated marginal habitats. African pikes are predators that attack with a swift rush. The juveniles prey on small fish and invertebrates, while the adults prey on fish up to 30-40% of their own size. Growth is rapid and these fish mature in the second year. African pikes are multiple spawners, that breed over the summer months. Breeding pairs construct a floating foam nest in dense marginal vegetation, and eggs are incubated in the nest. The nest is guarded and tended by both parents. When the fry hatch, they drop to the water surface and remain hidden under the foam nest until it disintegrates. The life span is short, four to five years.
Cichlidae		
<i>Serranochromis robustus</i> , (Günther, 1864)	Cichlids are a large family of freshwater fish in Africa. The serranos, or largemouth Breams are a distinct assemblage within this group. They are large, predatory and occur in tropical southern and central Africa. Serranos have large mouths and conical teeth, a high number of scale rows on the cheeks, a large number of soft rays in the dorsal fin and often numerous bright egg-spots on the anal fin. All are mouthbrooders. Ten species are known of which seven are present in southern Africa.	Larger specimens of the nembwe are present in deep main channels and permanent lagoons, whilst smaller specimens occur in lagoons and secondary channels. <i>Serranochromis robustus</i> is a predator on fish - minnows as juveniles and squeakers as adults. Breeding takes place in summer with nests in the vegetated fringes of mainstreams.
<i>Oreochromis andersonii</i> , (Castelnau, 1861)	Representatives occur in Africa and the Levant. Members of <i>Oreochromis</i> are large, deep-bodied and mouthbrooding cichlids. Generally tolerant of wide temperature and salinity ranges, they have fine teeth in several rows on the jaws, fine pharyngeal teeth, a high number of gill rakers and long intestines. Feeds on diatoms, algae and detritus. The genus includes 33 known species and six indigenous and two introduced species occur in southern Africa.	Threespot tilapias are hardy fish that can tolerate fresh and brackish water, but prefers slow-flowing or standing water. Adults occur in deep open waters, while juveniles are present inshore among vegetation. Feeds on detritus, diatoms and zooplankton. The males excavate a large saucer-shaped nest, while the females mouthbrood the eggs, larvae and fry. Multiple broods are raised during the warmer months. Has a lifespan of seven to eight years.

Table 9.1 continued

General representative of fish family	General information on fish family (Skelton 1993)	Biology and Ecology of general representative (Skelton 1993)
<i>Tilapia rendalli</i> , (Boulenger, 1896)	Tilapines currently include all the substrate spawning species. Tilapines do not show sexual dimorphism, but exhibit a strong pair bond relationship. Both parents tend and guard the brood. <i>Tilapia</i> species are smaller than <i>Oreochromis</i> species and generally feed on coarser foods. Adults retain the tilapia spot. Thirty species are known, with four present in southern Africa.	<i>T. rendalli rendalli</i> , the northern redbreast tilapia is able to tolerate a wide range of temperatures and salinity. These fish prefer quiet well-vegetated water along river littoral or backwaters, floodplains and swamp. They generally feed on water plants and algae, but may take aquatic invertebrates and even small fish. Breeding pairs clear vegetation in shallow waters to shape a nest of 0.5-1.2 m across and excavate tunnel-like brood chambers in which eggs and larvae are protected. Juveniles remain within the brood chambers and several broods are raised every summer. Lifespan of approximately seven years.
Mochokidae		
<i>Synodontis nigromaculatus</i> Boulenger, 1905	This African squeaker family has 10 genera and 170 species. Mochokid catfish have complex mouths and strong spines in the dorsal and pectoral fins. Members of the genus <i>Synodontis</i> in particular have bony skulls and large sharp dorsal and well-barbed pectoral fin spines. <i>Synodontis</i> is a large genus with more than 100 species of which nine are present in southern Africa.	Spotted squeakers occur in pools and slow flowing reaches of rivers, preferring riverine habitats to floodplains. Squeakers hide in crevices or holes on the undersides of logs, often in an upside down position, and are active at night. Members of the genus <i>Synodontis</i> are generally bottom feeders on detritus as well as small benthic invertebrates, scavenging often. Breeds in summer during the rainy season.
Schilbeidae		
<i>Schilbe intermedius</i> Rüppel, 1832	Butterbarbels swim in midwater, and their body form is unusual – a depressed head with a large mouth, short deep abdomen and compressed tapered body with a long anal fin. The dorsal and pectoral spines are sharp. Schilbeids are present in Asia and Africa. There are 5 genera and 34 species of schilbeids. A single genus occurs in southern Africa.	Schilbeids shoal in standing or slow-flowing open water associated with emergent or submerged vegetation. These butterbarbels are more active at night or in subdued light, feeding in mid and surface waters on a wide variety of prey, such as fish and invertebrates and also plant seeds and fruit. Schilbeids breed during the rainy season and may be a single or multiple spawner, depending on the locality. The eggs are laid on vegetation, and adults reach a size of approximately 160 cm. Schilbeids live for six to seven years.

According to Skelton (1993) varied ranges of life cycles are found amongst fish, and indeed amongst fish hosts of *D. ranarum* (Table 9.1). Below, the breeding behaviour and selected habitat is discussed as incentives for host utilisation by *D. ranarum*. As mentioned previously, *D. ranarum* does not breed throughout the year like most tropical species (Van As & Van As 2001) also many of the host species breed during the summer months (Table 9.1). It may be possible that the breeding cycle of *D. ranarum* is synchronised with suitable hosts.

➤ Clariidae

Clarias gariepinus lays its eggs on vegetation (Skelton 1993; 2001) and *D. ranarum* also breeds by laying eggs on substrate (Avenant *et al.* 1989b). Should *D. ranarum* females detach from hosts close to the site where the eggs are laid by the fish host, and also lay their eggs in the close vicinity, it will allow the offspring of *D. ranarum* to parasitise the offspring of *C. gariepinus* juveniles after they both hatch. Providing hosts for a new generation of *D. ranarum*.

In addition, the niche occupied by *C. gariepinus*, as an organism, which often lives in the oxygen-poor layer of water at the bottom, makes it an ideal host for *D. ranarum*, as it is also adapted to living in habitats with low oxygen content, due to the presence of haemoglobin (Fox 1957). This may be the reason why there is a higher incidence of *D. ranarum* on *Clarias* species than on other hosts.

➤ Hepsetidae

The breeding habits of the African pike also provides an opportunity for *D. ranarum* to parasitise the offspring, as *D. ranarum* may also lay eggs on the submerged parts of marginal vegetation where the foam nest of the pike is constructed.

As mentioned previously, pikes occur in quiet deep water (Skelton 1993; 2001), which may also be low in dissolved oxygen. These conditions are suitable for *D. ranarum* equipped with haemoglobin.

➤ Cichlidae

The cichlids on which *D. ranarum* were found are representatives that are able to tolerate conditions of high salinity and variable temperatures. These are also harsh environments where high tolerance to lack of oxygen is advantageous. Nests in vegetation once again provide opportunities for juvenile *D. ranarum* to parasitise new hosts. Mouthbrooders seem to be more problematic for the utilisation by offspring of *D. ranarum*. However, it is possible that young *D. ranarum* specimens may parasitise the juveniles of the host mouthbrooders while in the mouth, as *D. ranarum* occurs in the mouth as well as the gill chambers of fish hosts. The uninfected mouthbrooder can now be colonised by free-swimming representatives of *D. ranarum*.

➤ Mochokidae

Representatives of the genus *Synodontis* are generally bottomfeeders, where the adaptation of *D. ranarum* to habitats with low dissolved oxygen content is once again advantageous to the parasite. Information on the breeding behaviour of *Synodontis* is not readily available.

➤ Schilbeidae

The butterbarbels lay their eggs on vegetation. This gives the fish lice the opportunity to parasitise young when they hatch, as *D. ranarum* also lays eggs on a substrate. If the eggs are laid in close proximity to where the hosts lay their eggs, infestation is possible.

It is important to note that butterbarbels do not fall into the category of bottomfeeders, or of fishes that occur readily in harsh conditions (high salinity, temperature fluctuations). This increases the possibility of it being an incidental rather than a preferred host.. When the presence of other fish lice (*Argulus*, *Chonopeltis*) is low, *Dolops* may be able to parasitise this fish species readily, but may be out-competed by its evolutionary more advanced relatives. However, more research is needed in this regard to be certain.

9.2 Remarks on fish as hosts to ectoparasites

- **Sites on the host where *Dolops ranarum* attaches**

Dolops ranarum is generally collected from the skin, mouth or gill chambers of the fish. Figure 5.5 in Chapter 5 indicates the position on the host of *D. ranarum* collected in the Okavango Delta, where it was noted for specimens collected from specific hosts. Characteristics of the attachment sites are discussed below:

- **Skin**

The function of skin in fish is much the same as that in other organisms (physical protection and impenetrable barrier between the internal- and the external medium) but adapted to the needs in the water. The skin secretes mucus to reduce friction and gives protection against parasites and diseases and for the primary healing of wounds. In some cases this secretion if it is toxic, also protects against predators (Skelton 1993; 2001). *Dolops ranarum* generally attaches to the areas around the fins, where there are fewer scales. This is not a factor in the case of the catfishes, butterbarbels and squeakers, as these fish do not have scales, but a slimy, smooth body. However, *D. ranarum* mostly occurs in the mouth and gill chambers of hosts.

➤ Gill chambers and mouth

The gill chambers, situated in the back of the mouth, house the gills, feather-like filaments arranged in rows along four to five pairs of gill arches. Healthy fish have bright red gills. On the inside of the gill arches are gill rakers forming an intermeshing sieve to strain the water passing between the arches and over the gills.

From Figure 5.5 (in Chapter 5) it can be seen that the number of *D. ranarum* collected from fish hosts was more than double that of the other representatives of the Branchiura collected during sampling. Slightly more specimens of *D. ranarum* were collected from the skin of hosts than from the gill chambers and mouth. It was also noted that *D. ranarum* more often occurred in the gill chambers of the catfish, butterbarbels and squeakers (except in the case of *C. gariepinus*), and more often on the skin of the serranos and tilapines.

It is possible that *D. ranarum* more often occurred within the gill chambers of the bottomfeeders as the parasites might be rubbed off on surrounding obstacles while fishes are feeding. The serranos and tilapines are not bottomfeeders (and neither is the pike), this might be the reason that *D. ranarum* was more often collected from the skin than the gills of these hosts.

In the case of *D. ranarum*, this parasite has developed haemoglobin (Fox 1957), which gives it a clear advantage over other fish parasites in a certain niche. This niche is where hosts are bottom-dwellers that live in areas with low dissolved oxygen percentages in the water. Other members of the Branchiura have not developed haemoglobin, and can generally be found on hosts that frequent areas with relatively (in comparison to the bottom dwellers' habitats) high oxygen content.

Another possible difference between the parasites could be the position on the host. This is apparent in *Chonopeltis*, where some species will only be found in the gill chambers and mouth and others will prefer to live on the skin of the host. These species have pigment spots over the ovaria to protect them from sunlight. In Table

2.5 (Chapter 2) the position of the parasite on the host can be seen and from this table it is apparent that *D. ranarum* was mainly found in the gill chambers of the host.

Looking at the small number of parasites present in the study, it is possible to think that this small group cannot be representative of the species. However, fishes that have been considered in this study have been collected from the Okavango for almost 10 years (1998 – 2006). During the run of this project, and on all the fish caught, these are the only specimens of *D. ranarum* that have been collected.

It stands to reason that the selection pressures that come to bear on parasites utilising diverse hosts, must be different. However, the pressures cannot be quantified due to the lack of information on the behaviour of the host.

The fact that the parasites were also collected from *Synodontis*, *Hepsetus* and *Schilbe* species seem to be because of chance contamination rather than because of natural infestation. It is possible that when the fish were kept together in the tanks the parasites fell off their original hosts and tried to attach to an unsuitable host body. In addition it is also a possibility that *D. ranarum* is an opportunistic parasite that may utilise any host that comes into its orbit.

The morphological comparison of *D. ranarum* has not given any fresh insights into the possible speciation of the organism over time, due to parasitising various hosts. Although there has been no speciation of *D. ranarum* as a result of utilisation of different hosts in the Okavango Delta, it is possible that there may have been a speciation event due to geographical isolation.

9.3 Molecular study: Extraction, Amplification and Sequencing

Herbert *et al.* (2003) made a case for DNA barcoding based on the following facts relating to the current method of species identification:

- Phenotypic plasticity and genetic variability of the characters employed for species recognition,
- The current method overlooks morphologically cryptic taxa,

- Keys are often effective only for a particular life stage or gender, many individuals cannot be identified, and
- The use of keys demands a high level of expertise, leading to misdiagnosis by inexperienced persons.

In contrast to the multiple morphological characters used for identification by traditional taxonomists, the DNA barcoding identification system is based on what can be described as a single complex character (a part of a gene, comprising 650 base pairs from the first half of the mitochondrial Cytochrome Oxidase Subunit I gene (COI). Therefore, barcoding results are often perceived as being unreliable and susceptible to errors in identification (Dasmahapatra & Mallet 2006).

Due to the lack of success during the course of this study, it is suggested that further study utilising different PCR conditions or even a different sequence such as 16S, 18S or 28S such as used by Møller *et al.* (2008) (see Table 9.3) is necessary. However, after submission of this dissertation, such a study was done in Europe and can be used as reference for further work in future.

Table 9.2: Sequence length of 18S and 28S in Branchiuran genera (adapted from Møller *et al.* (2008))

Argulidae Leach, 1819	Collection details	Sequence length (base pairs)		
		18S	28S	16S
<i>Argulus japonicus</i> Thiele, 1900	9/11-2004 Boskop Dam, North West Province, RSA	1833	620	539
<i>Argulus foliaceus</i> (L)	11/6-2006 Utterslev Mose, Denmark	1796	709	576
<i>Argulus mobilis</i> Thiele, 1904		1843		
<i>Argulus</i> sp 1		529		
<i>Argulus</i> sp 2			764	
<i>Chonopeltis</i> Thiele 1900			3790	
<i>Chonopeltis australis</i> Fryer, 1977	30/11-2004 Maselspoort Dam, Free State, RSA	1741	713	582
<i>Dolops ranarum</i> Stuhlmann, 1891	17/11-2004 Tzaneen Dam, Limpopo Province, RSA	1822	662	461

Dasmahapatra & Mallet (2006) remarked on the success achieved by Herbert and co-workers using the COI gene, and suggested that the success may be partially due to biased tests of the method. Firstly because of underestimated intraspecific variation; too few individuals per species were analysed, or because sampling was carried out within a limited geographic area. These factors should have increased the likelihood of good results during the course of this study on *D. ranarum*. Secondly because of overestimation of interspecific variation: for most species, sister taxa (their closest relatives) were

not included in the analyses, because they do not necessarily occur in the sampled areas. These factors lead to inflated assignment accuracy rates.

Another objection to the utilisation of the barcoding region to identify species is that it is located in the mitochondrial genome, rather than the nuclear genome. This is because the nuclear genome contains the majority of genes and that this genetic material is inherited through both parents. Mitochondrial DNA (mtDNA) is only inherited through females. Interspecific hybridisation and infection by maternally transmitted endosymbionts may cause mitochondrial genes to flow between biological species. This may result in species groupings created using mtDNA differing from the true species groupings (Dasmahapatra & Mallet 2006).

Due to the fact that the amplification of this DNA was not successful, it is possible to conjecture that the DNA recovered from the stored material was of high quantity, but of low quality, i.e. the DNA extracted was fragmented due to the storage process. Possible reasons for the lack of PCR results are any of the following factors:

- Poor quality of the DNA,
 - Insufficient quantities of DNA,
 - The presence of a PCR inhibitor,
 - The DNA may not have been clean enough, or
 - The PCR conditions were not optimal.
-
- **Poor quality of the DNA**

Due to the storage of the specimens, it is very possible that the quality of the DNA might have been poor. Mandrioli *et al.* 2006 investigated the quality of DNA obtained from cabbage moths, *Mamestra brassicae* (L.) (Lepidoptera: Noctuidae), specimens that had been stored in ethanol (amongst other). It was noted that 100% ethanol (in particular with specimens conserved at 4°C) gave reasonable results, whereas preservation in 75% ethanol gave poor results regardless of whether the specimens were stored at room temperature or at 4°C. This indicates that only 100% ethanol offers reasonable DNA

preservation. However, Mandrioli *et al.* (2006) stated that the amplification yield was still reduced in comparison to that of fresh specimens, indicating that DNA degradation still occurs in ethanol preserved specimens. According to Mandrioli *et al.* (2006) the best DNA results (for cabbage moths) were obtained from specimens stored in acetone. Depending on the efficacy of acetone in the storage of crustaceans, this may be employed in future studies.

- **Insufficient quantities of DNA**

The Phenol-Chloroform extraction method yielded high quantities of DNA as can be seen in the Nanodrop results. DNA was even extracted from the stored material. The failure of the PCR reaction is thus not caused by insufficient quantities of DNA.

- **The presence of a PCR inhibitor**

According to Viljoen *et al.* (2005) the mechanisms of PCR inhibition may be divided in three categories based on when the inhibition occurs during the PCR process. These are: i) Failure of lysis or incomplete lysis, ii) degradation of the template nucleic acid or iii) polymerase inhibition. During PCR some common inhibitors in the samples included organic and phenolic compounds, heavy metals, lipids, certain cations, haemoglobin, or urea. It has also been noted that the powder from gloves and nylon as well as nitrocellulose filters, used in molecular techniques can be inhibitory.

It is possible that the presence of haemoglobin might have acted as a PCR inhibitor, as *D. ranarum* contains haemoglobin.

- **The DNA might not have been clean enough**

This is a very common inhibitor of polymerase in PCR (Viljoen *et al.* 2005). In future a washing step should be included in the DNA extraction method.

- **The PCR conditions were not optimal**

It is possible that the PCR conditions that were utilised during the course of this study were not as well adapted to the amplification of the DNA specimens as was thought.

Other possible reasons for the failure of PCR in this study are listed below. The failure of the PCR reaction might be as a result of one of or a combination of the following factors (Viljoen *et al.* 2005).

- **Operator failure**

This may result from failure to add a reaction component, or miscalculation or miss-measurement, or the selection of the incorrect PCR programme. Due to the number of repeated PCRs done, it is unlikely that there were operator failures during every repeat. As mentioned previously, several different PCR programs were used, and none yielded results.

- **Template issues**

The template may often be responsible for PCR failure where crude extracts are utilised. This is because the template may contain inhibitors, the template may be poorly quantified and not diluted correctly. Otherwise the template may be degraded or not present in the extract or the template and the primers are mismatched. It may be possible that the template was degraded due to the storage method of the specimens.

- **Temperature errors**

The annealing and denaturation temperatures are the most important temperatures during the PCR process. If the denaturation temperature is too high it could inactivate the enzyme, but if the temperature is too low, denaturation will not take place. If the annealing temperature is too high or too low, the primer will not anneal itself to the template DNA and amplification will

not take place. Various PCR programs were tested in this study with different annealing temperatures, but none of these programs gave any positive results.

- **Primers**

Primers may be degraded or there were problems with the primer synthesis. It is possible that the primers are not of the correct sequence or quality or may be impure or diluted. However, the primers utilised in the course of this study were commercial, and kept at the correct temperature, therefore it is unlikely that this was the factor that caused failure. The sequence of the primers was obtained from Folmer *et al.* (1994).

- **dNTPs**

The dNTPs used in this study were aliquoted and frozen. The aliquotes were not subjected to freeze-thaw cycles which could have contributed to the failure of the PCR reactions.

- **Enzyme**

It is not very likely that commercial enzyme could have been defective, since the enzyme is stable. Good quality control generally applies to commercial supplies.

From Table 9.3 it can be seen that the 18S and 28S, may have given better results than the COI gene that was used in this study. The Qiagen DNeasy Tissue Kit (Protocol for animal tissues) was also used in the study done by Møller *et al.* (2008) which gave them suitable DNA for further analysis. However, during the course of this study it was not successful. Since no success was achieved with the amplification of the COI gene, no DNA sequencing could be done.

During the course of this study the Wizard Kit was also utilised to extract DNA from *D. ranarum* specimens, with no success. This kit has been used successfully to extract genomic DNA from individual copepods from *Neocalanus* species,

(Sawabe & Ikeda 2005). The Wizard Genomic DNA Purification Kit animal tissue protocols were used (as described in the Wizard Genomic DNA Purification Kit Technical Manual, #TM050, with minor modifications. According to Sawabe & Ikeda (2005) longer incubation periods of the copepod homogenates are needed to obtain efficient amounts of genomic DNA from a single copepod. In addition, dehydration of specimens stored in 100% ethanol must be done for 10-11 hours. However, Sawabe & Ikeda (2005) found that nuclear 18S rRNA gene amplification products from the genomic DNA template extracted by using the phenol/chloroform method did not yield results. In contrast, the genomic DNA extracted using the Wizard Genomic DNA Purification Kit resulted in reproducible amplification of the nuclear 18S rRNA gene.

9.4 Concluding remarks

The aim of this study was to investigate whether there has been a speciation event in *Dolops ranarum* (Stuhlman 1891) in Africa due to the utilisation of different hosts by the fish louse. Specimens of *D. ranarum* were examined for morphological characters and DNA. The morphological characters revealed no significant differences between *D. ranarum* specimens obtained from different hosts.

The migration patterns observed of the fish hosts down the river systems of Africa reveal that the genus *Chonopeltis* Thiele, 1900 species gradually evolved into several distinctive species associated with each particular river system. It is possible that there may have been speciation in the case of *D. ranarum* as a result of geographical isolation, therefore; further study on *D. ranarum* populations from different river systems in Africa should be undertaken. The different river systems also offer diverse habitats to fish hosts that may have acted as drivers of evolution in the associated parasites. However, not enough material was available from different river systems to study geographical isolation of *D. ranarum* during the course of this study.

The evolution of branchiurans and fish since the rise of ancestral forms of crustaceans (in the Triassic) and fish (in the Silurian) has provided ample time for co-evolution. The geological changes that have taken place, and the changes in the

nature of the available habitats in Africa have shaped the relationships between *D.ranarum* and its fish hosts as it can be observed at present.

During the molecular section of this study no amplification of the COI gene was obtained for the purpose of sequencing. Amplification of the 16S, 18S and 28S may yield better results and should be included in future studies. Dasmahapatra & Mallet (2006) noted that as the advantages and limitations of barcoding were becoming apparent, it was clear that taxonomic approaches combining DNA sequencing, morphology and ecological studies would achieve maximum efficiency at species identification.

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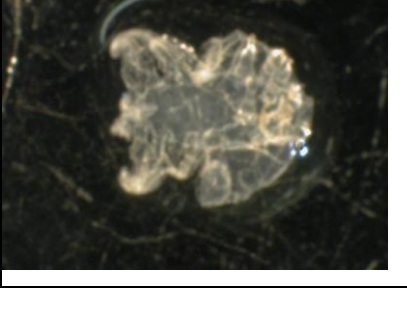
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Appendix A: Dorsal and ventral photos of each of the specimens studied, as well the date the specimen was collected, location collected and host species					
Nr	Collection nr	Locality	Host	Ventral	Dorsal
1	2000/8/12-10	Boro R	<i>C. gariepinus</i>		
2	2000/8/19-1	Okavango R	<i>C. gariepinus</i>		
3	2000/8/6-2	Okavango R	<i>C. gariepinus</i>		
4	2000/8/4-4	Okavango R	<i>C. gariepinus</i>		

Nr	Collection nr	Locality	Host	Ventral	Dorsal
7	2000/7/7-26	Okavango R	<i>C. gariepinus</i>		
7. 1					
7. 2					
7. 3					
13	2000/8/12-4	Bao R	<i>C. gariepinus</i>		
14	2000/8/14-7	Okavango R	<i>C. gariepinus</i>		

Nr	Collection nr	Locality	Host	Ventral	Dorsal
20	2003/01/10-7	Shakawe	<i>C. gariepinus</i>		
20 .1					
20 .2					
23	2001/08/28-1	Shakawe	<i>C. gariepinus</i>		
23 .1					
23 .2					

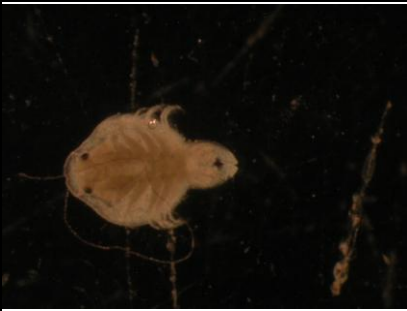
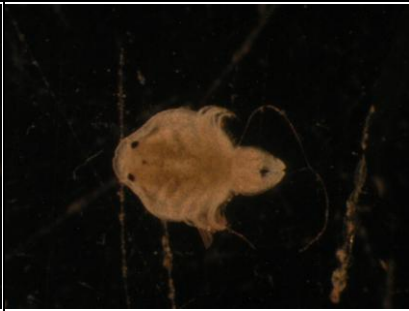
Nr	Collection nr	Locality	Host	Ventral	Dorsal
23 .3					
23 .4					
23 .5					
23 .6					

Nr	Collection nr	Locality	Host	Ventral	Dorsal
23.7					
23.8					
24	2001/08/18-1	Shakawe	<i>C. gariepinus</i>		
24.1					
24.2					
29	2001/10/04-13	Kalatog	<i>C. gariepinus</i>		

Nr	Collection nr	Locality	Host	Ventral	Dorsal
34	2003/01/08-3	Shakawe	<i>C. gariepinus</i>		
49	2001/08/20-1	Shakawe	<i>C. stapersi</i>		
5	2000/7/1-1	Okavango R	<i>S. robustus</i>		
5.1					
5.2					

Nr	Collection nr	Locality	Host	Ventral	Dorsal
6	2000/7/7-12	Shakawe	<i>S. robustus</i>		
6. 1					
6. 2					
6. 3					
10	2000/7/11-1	Okavango R	<i>S. robustus</i>		
10. 1					

Nr	Collection nr	Locality	Host	Ventral	Dorsal
10.2					
10.3					
51	2004/06/27-3	Shakawe	<i>S.robustus</i>		
32	2003/01/04-4	Shakawe	<i>S. macrocephalus</i>		
32.1					

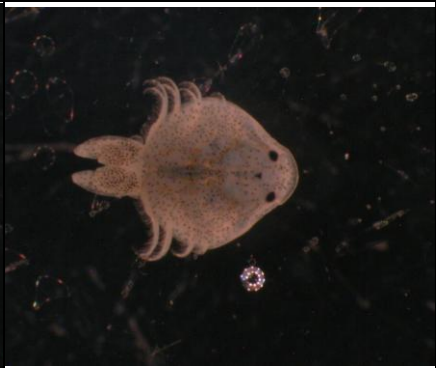
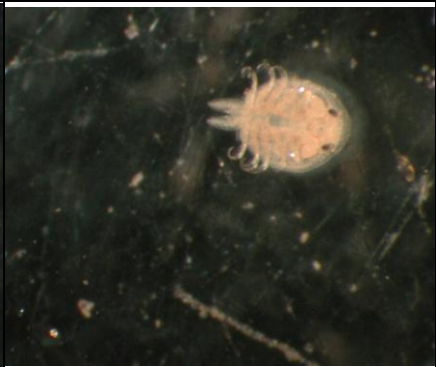
Nr	Collection nr	Locality	Host	Ventral	Dorsal
32 .2					
32 .3					
32 .4					
33	2003/12/10-1	Shakawe	<i>S. macrocephalus</i>		
33 .1					
33 .2					

Nr	Collection nr	Locality	Host	Ventral	Dorsal
33 .3					
36	2003/01/05-1	Shakawe	<i>S. macrocephalus</i>		
36 .1					
36 .2					
43	2003/01/05-6	Shakawe	<i>S. macrocephalus</i>		

Nr	Collection nr	Locality	Host	Ventral	Dorsal
28	2001/08/18-2	Shakawe	<i>S. intermedius</i>		
31	98/08/05-1	Kalatog	<i>S. greenwoodi</i>		
25	2003/01/06-3	Shakawe	<i>S. vanderwalii</i>		
21	2003/01/06-2	Shakawe	<i>S. nigromaculatus</i>		
21 .1					

Nr	Collection nr	Locality	Host	Ventral	Dorsal
21 .2					
35	98/6/16-11	Okavango	<i>S. nigromaculatus</i>		
37	2003/12/9-5	Shakawe	<i>S. angusticeps</i>		
40	2000/08/22-11	Okavango R	<i>S. angusticeps</i>		
45	2003/01/05-7	Shakawe	<i>S. angusticeps</i>		

Nr	Collection nr	Locality	Host	Ventral	Dorsal
12	99/07/03-09	Duba lagoon	<i>O. andersoni</i>		
19	2002/11/22-7	Shakawe	<i>O. andersoni</i>		
38	2001/10/25-6	Shakawe	<i>O. andersoni</i>		
17	2000/11/29-2	Shakawe	<i>O. macrochir</i>		

Nr	Collection nr	Locality	Host	Ventral	Dorsal
30	2003/12/7-5	Shakawe	<i>O. macrochir</i>		
39	2002/12/3-8	Shakawe	<i>O. macrochir</i>		
41	2003/12/14-4	Shakawe	<i>O. macrochir</i>		
44	2003/01/05-8	Shakawe	<i>O. macrochir</i>		
42	2002/11/21-4	Shakawe	<i>Oreochromis sp</i>		

Nr	Collection nr	Locality	Host	Ventral	Dorsal
9	2000/7/27-18	Nkamaseri	<i>H. odoe</i>		
27	2001/08/31-3	Kalatog	<i>H. odoe</i>		
47	2000/7/13-3	Okavango R	<i>H. odoe</i>		
46	2000/6/28-2	Shakawe	<i>Kurper</i>		
46 .1					

Nr	Collection nr	Locality	Host	Ventral	Dorsal
46 .2					
48	2000/8/22-2	Okavango R	<i>Kurper</i>		
48 .1					
48 .2					
48 .3					

Nr	Collection nr	Locality	Host	Ventral	Dorsal
50	2004/06/24-2	Okavango	<i>Nembwe</i>		
26	2002/12/3-7	Shakawe	<i>T. rendali</i>		

Appendix B: Measurements, calculations of measurements and statistical analysis of *Dolops ranarum* specimens collected from all hosts

Table 1 includes all measurements taken of *D. ranarum* specimens collected from clariid hosts. Table 2 includes the measurements of *D. ranarum* specimens collected from *Hepsetus odoe* hosts. Table 3 shows the measurements of *D. ranarum* specimens collected from *Serranochromis* hosts, while Table 4 shows the specimens collected from *Oreochromis* hosts. Table 5 includes the measurements of specimens of *D. ranarum* collected from *Synodontis* hosts.

Table 6 shows the calculations of the percentages of certain attributes of *D. ranarum* from specimens collected from clariid hosts. Table 7 shows the same calculations for cichlid hosts (*Serranochromis* and *Oreochromis* spp).

In addition Table 8 shows the statistical analysis for the specimens collected from all hosts, Table 9 shows the statistical analysis from all clariid hosts and Table 10 the statistical analysis of all cichlid hosts.

Table 1: Measurements of *Dolops ranarum* specimens collected from Clariid hosts

Measurements	Sp 1	Sp 2	Sp 3	Sp 4.1	Sp 4.2	Sp 7.1	Sp 7.2	Sp 7.3	Sp 13
Total length	5218.76	9882.49	6242.19	3125.01	4765.63	5069.84	7981.42	8744.19	5617.21
Length of abdomen	1507.89	2671.19	2187.5	992.19	1734.39	1562.82	2381.41	2725.6	1523.44
Width of abdomen	1171.9	2180.34	1351.56	656.25	1195.34	1181.4	1730.63	1907.07	1359.38
Length of carapace	3539.07	7430.6	3890.63	2000	3101.56	3562.84	5562.79	5795.35	4359.38
Width of carapace	3445.32	6940.43	4265.63	2140.63	3164.1	3479.08	5981.4	5962.82	4054.69
Anterior carapace length	1828.14	3113.38	2085.94	984.38	1570.31	1860.49	2679.13	2827.91	2000
Length of carapace split	1703.13	4010.99	1859.39	1078.13	1539.08	1674.42	3023.27	2911.63	2289.06
Width of carapace over the eyes taken exactly touching the edge of the eye	1936.65	2593.21	2416.2	1363.16	1883.59	2032.62	2680.05	2995.09	2083.99
Width of carapace over the eyes in a straight line	2023.44	2708	2578.13	1460.94	2062.51	1833.41	3026.85	3134.88	2171.88
Distance between the eyes	656.25	1124.97	781.25	335.94	601.61	716.28	921.12	967.44	750.04
Distance from the right eye to the anterior carapace edge	367.19	581.23	406.25	148.44	335.94	390.7	493.02	539.53	398.44
Distance from the left eye to the anterior carapace edge	414.06	383.54	398.44	140.63	351.56	344.31	381.4	511.63	359.38
Length of eye	257.81	303.72	195.31	132.81	171.88	176.74	241.86	251.16	203.13
Width of eye	265.63	283.38	226.56	140.63	172.05	158.14	204.65	241.86	187.5
Length of furcral rami	960.94	1860.14	1132.81	632.81	828.13	911.63	1395.35	1627.91	1085.94
Width of furcral rami		341.28	164.06		156.25	260.47	381.4	297.67	
Length of testis	890.63		1156.25		1179.71				
Length of ova		624.37		203.13		362.79	437.21	465.12	
Male/Female	m	f	m	f	m	f	f	f	unknown

Table 1 continued									
Measurements	Sp 20.1	Sp 20.2	Sp 23.1	Sp 23.2	Sp 23.3	Sp 23.4	Sp 23.5	Sp 23.6	Sp 23.7
Total length	7505.96	6519.69	3771.66	3795.28	4496.06	4496.06	6212.6	6275.59	7086.63
Length of abdomen	2184.29	1850.39	1110.24	1133.86	1598.43	1598.43	1992.13	2196.86	2322.83
Width of abdomen	1429.71	1307.09	771.65	866.14	984.25	984.25	1212.6	1370.1	1503.94
Length of carapace	5258.14	4716.54	2551.18	2614.17	2779.53	2779.53	4047.27	4307.09	4685.04
Width of carapace	5035.75	4417.32	2637.81	2598.44	3070.87	3070.87	4377.95	4228.35	5078.74
Anterior carapace length	2287.53	2125.98	1322.83	146	1440.94	1440.94	2007.87	1984.25	2330.71
Length of carapace split	2740.28	2566.93	1291.34	168	1338.58	1338.58	2102.36	2346.46	2440.94
Width of carapace over the eyes taken exactly touching the edge of the eye	2025.48	1709.59	1797.36	204.36	1960.14	1960.14	2818.91	2591.41	3120.43
Width of carapace over the eyes in a straight line	2081.02	1826.77	1881.89	230	2078.74	2078.74	2874.02	2740.17	3488.19
Distance between the eyes	746.62	771.65	456.69	53	551.18	551.18	732.28	692.91	818.9
Distance from the right eye to the anterior carapace edge	301.83	401.57	354.33	40.05	464.57	464.57	551.18	464.57	551.18
Distance from the left eye to the anterior carapace edge	325.66	385.83	378.03	42.05	346.46	346.46	496.06	527.56	503.94
Length of eye	142.97	165.35	141.95	19	165.35	165.35	188.98	173.23	251.97
Width of eye	214.46	196.85	141.73	20	173.23	173.23	188.98	188.98	251.97
Length of furcral rami	1278.82	1000	732.28	732.28	889.76	889.76	1275.59	1425.2	1582.7
Width of furcral rami	158.86	220.47	110.24	188.98	125.98	125.98	244.09	181.1	252.09
Length of testis					716.54				
Length of ova	571.88	299.21	259.84	29			322.83	370.08	433.07
Male/Female	f	f	f		m	unknown	f	f	f

Table 1 continued						
Measurements	Sp 23.8	Sp 24.1	Sp 24.2	Sp 29	Sp 34	Sp 49
Total length	7818.9	774.1	1011.51	5078.74	742.45	572.66
Length of abdomen	2543.31	256.12	315.11	1708.66	194.24	202.88
Width of abdomen	1559.06	156.83	179.86	1377.95	139.57	122.3
Length of carapace	5196.86	500.72	631.65	3519.69	533.81	346.76
Width of carapace	5425.2	503.6	751.08	3472.44	597.13	392.81
Anterior carapace length	2275.59	210.07	292.09	1669.29	243.17	174.1
Length of carapace split	2968.5	274.82	382.74	1866.14	315.11	178.42
Width of carapace over the eyes taken exactly touching the edge of the eye	3271.9	281.97	362.18	1672.93	259.19	208.65
Width of carapace over the eyes in a straight line	3559.06	271.98	375.54	1826.77	274.82	217.27
Distance between the eyes	858.27	84.89	129.5	700.79	94.96	69.06
Distance from the right eye to the anterior carapace edge	685.04	58.99		314.96	43.17	33.12
Distance from the left eye to the anterior carapace edge	692.96	50.36		307.09	38.85	36
Length of eye	228.35	21.63		220.47	15.83	27.34
Width of eye	236.22	18.71		165.35	23.02	18.71
Length of furcral rami	1700.79	165.47	194.24	913.39	109.35	105.04
Width of furcral rami	519.69	31.65	31.65	212.6	41.75	12.95
Length of testis				858.27		102.16
Length of ova	440.94	50.36	48.92		46.04	
Male/Female	f	f	f	m	f	m

Table 2: Measurements of *Dolops ranarum* specimens collected from *Hepsetus odoe* hosts

Measurements	Sp 9	Sp 27	Sp 47
Total length	4157.49	6196.86	5956.85
Length of abdomen	1338.58	1842.52	1741.07
Width of abdomen	818.9	1433.07	1338.13
Length of carapace	2811.02	4086.61	4388.49
Width of carapace	2551.18	4338.59	4043.17
Anterior carapace length	1322.86	2283.52	
Length of carapace split	1535.43	1834.66	
Width of carapace over the eyes taken exactly touching the edge of the eye	1544.27	2620.29	2259.73
Width of carapace over the eyes in a straight line	1606.3	2456.74	2460.47
Distance between the eyes	527.62	653.54	820.14
Distance from the right eye to the anterior carapace edge	330.71	527.62	446.28
Distance from the left eye to the anterior carapace edge	314.96	692.91	417.27
Length of eye	157.48	204.72	201.44
Width of eye	165.35	220.47	230.22
Length of furcral rami	803.15	1267.74	1050.36
Width of furcral rami	78.74	267.72	100.72
Length of testis	771.65		
Length of ova		291.34	273.76
Male/Female	m	f	f

Table 3: Measurements of *Dolops ranarum* specimens collected from *Serranochromis* species hosts

Measurements	Sp 5.1	Sp 5.2	Sp 6.1	Sp 6.2	Sp 6.3	Sp 10.1	Sp 10.2	Sp 10.3	Sp 10.4
Total length	5700.79	6692.91	3614.21	3606.31	unknown	4094.5	2598.44	2897.73	879
Length of abdomen	1858.27	2283.46	1118.11	1039.37		1236.25	700.79	889.76	280
Width of abdomen	1125.98	1346.46	834.65	905.65	1767.44	976.41	574.8	677.21	189
Length of carapace	3622.05	4330.71	2496.06	2598.43	5655.81	2858.31	1779.54	1929.13	690
Width of carapace	3661.42	4370.08	2401.59	2629.93	5655.82	2661.42	1708.66	1952.82	626
Anterior carapace length		2385.83	1425.28	1464.57	2716.28	1732.28	1102.39	1070.87	326
Length of carapace split		1755.91	937.01	1094.49	2920.99	1070.87	637.8	811.18	375
Width of carapace over the eyes taken exactly touching the edge of the eye	2130.38	2403.01	1473.47	1354.42	2840.32	1590.55	973.9	1097.91	343.37
Width of carapace over the eyes in a straight line	2149.61	2590.55	1551.18	1362.2	2697.67	1590.55	1086.61	1212.6	336
Distance between the eyes	755.91	858.27	519.69	535.49	995.35	574.8	354.33	417.32	113
Distance from the right eye to the anterior carapace edge	338.67	307.09	291.34	228.35	493.02	283.46	181.1	212.6	77
Distance from the left eye to the anterior carapace edge	291.34	425.2	283.46	220.47	539.53	259.84	157.48	173.23	61
Length of eye	188.98	236.22	181.1	157.48	241.86	157.68	141.73	141.95	33
Width of eye	196.85	204.72	125.98	102.36	279.07	165.35	102.36	110.24	36
Length of furcral rami	1220.47	1181.1	622.05	598.43		724.41	314.96	535.43	172
Width of furcral rami	275.59	220.47	157.48	196.85		275.59	94.82	133.86	28
Length of testis		1346.46							
Length of ova			213.18	189.14	372.09	307.09	125.98	181.1	66
Male/Female	unknown	m	f	f	f	f	f	f	f

Table 3 continued									
Measurements	Sp 32.1	Sp 32.2	Sp 32.3	Sp 33.1	Sp33.2	Sp 33.3	Sp 36.1	Sp 36.2	Sp 37
Total length	2392.6	2923.08	3352.79	2328.91	4700.27	5204.25	4074.27	4196.29	2429.71
Length of abdomen	832.89	965.52	1108.75	758.62	1538.46	1374.01	1363.4	1251.99	716.2
Width of abdomen	647.21	748.01	832.89	679.05	1055.7	992.04	970.82	917.77	763.93
Length of carapace	1538.46	1803.71	2217.51	1602.12	3352.79	3761.28	2610.08	3045.1	1718.83
Width of carapace	1639.26	1989.39	2339.52	1522.55	3262.6	3570.29	2811.67	3076.93	1708.22
Anterior carapace length	896.55	1066.31	1278.51		1729.44	2058.36	1347.48	1549.08	864.74
Length of carapace split	700.27	742.71	901.86		1644.56	1740.05	1289.12	1389.93	811.69
Width of carapace over the eyes taken exactly touching the edge of the eye	808.11	923.63	1141.37	671.98	1445.16	1562.94	1317.74		756
Width of carapace over the eyes in a straight line	880.65	1082.23	1188.33	726.79	1501.33	1522.56	1358.1	1909.82	795.76
Distance between the eyes	376.66	419.1	477.45	350.13	625.99	641.91	530.5	557.03	360.74
Distance from the right eye to the anterior carapace edge	90.34	106.1	190.98	180.37	350.13	403.18	201.59	244.03	137.93
Distance from the left eye to the anterior carapace edge	106.1	111.41	190.98	143.33	328.91	360.74	249.34	297.08	148.64
Length of eye	100.8	122.02	143.24	111.41	212.2	190.98	148.92	164.46	90.19
Width of eye	90.19	137.93	148.54	116.71	212.2	206.9	137.93	143.63	116.71
Length of furcral rami	392.57	477.45	604.77	419.1	1039.8	732.1	583.55	753.32	419.1
Width of furcral rami	116.71	111.41	74.27	106.1	143.24	148.54	334.22	259.95	95.49
Length of testis	519.89	615.38	668.44		960.21		907.16		
Length of ova						514.59		270.56	
Male/Female	m	m	m	unknown	m	f	m		unknown

Table 4: Measurements of *Dolops ranarum* specimens collected from *Oreochromis* species hosts

Measurements	Sp 12	Sp 17.2	Sp 19.1	Sp 30	Sp 41	Sp 42	Sp 50
Total length	10612.7	2732.29	3598.46	3818.93	1026.32	1740.13	5333.35
Length of abdomen	2843.93	826.77	1039.37	1220.47	282.89	467.11	1956.52
Width of abdomen	1965.32	606.3	826.77	1055.15	243.42	427.64	1304.35
Length of carapace	7826.59	1874.02	2480.31	2677.17	750	1184.21	3492.87
Width of carapace	7502.89	1811.02	2401.57	2582.68	680.92	1223.68	3681.19
Anterior carapace length	2901.76	1015.75	1448.84	1283.46		654.61	1695.71
Length of carapace split	4635.84	834.65	1094.49	1346.46		523.03	1826.32
Width of carapace over the eyes taken exactly touching the edge of the eye	3179.53	979.55	1443.55	1089.46	465.17	674.6	2063.68
Width of carapace over the eyes in a straight line	3213.89	1094.49	1566.93	1094.49	453.95	694.08	2304.35
Distance between the eyes	1248.55	417.32	496.06	511.81	200.66	273.03	652.17
Distance from the right eye to the anterior carapace edge	473.99	141.73	244.09	284.45	105.26	131.58	347.83
Distance from the left eye to the anterior carapace edge	473.99	149.61	236.22	267.72	82.24	118.42	434.78
Length of eye	196.53	118.11	165.35	181.1	59.3	98.68	217.39
Width of eye	346.82	110.24	149.61	212.6	42.76	85.53	0
Length of furcral rami	1930.64	472.44	637.8	685.04	180.92	292.76	1000
Width of furcral rami	312.14	165.35	157.48	189.14	32.89	65.79	217.39
Length of testis				716.54			927.99
Length of ova		228.35	196.85				
Male/Female	unknown	f	f	m	unknown	unknown	m

Table 5: Measurements of *Dolops ranarum* specimens collected from *Synodontis* species hosts

Measurements	Sp 21.1	Sp 21.2	Sp 25	Sp 35
Total length	7776.77	2353.49	8273.39	2705.58
Length of abdomen	2065.14	1413.95	2215.83	923.08
Width of abdomen	1507.01	6232.56	1553.96	806.37
Length of carapace	5702.33	6381.4	5985.61	1904.52
Width of carapace	6009.33	2883.72	5697.84	1830.24
Anterior carapace length	2790.71	2632.62		1045.09
Length of carapace split	2809.3	2694.56		859.43
Width of carapace over the eyes taken exactly touching the edge of the eye	3008	3562.79	2884.17	843.77
Width of carapace over the eyes in a straight line	2874.42	1041.86	2661.87	896.57
Distance between the eyes	948.84	520.93	906.47	387.27
Distance from the right eye to the anterior carapace edge	520.93	344.19	446.04	127.43
Distance from the left eye to the anterior carapace edge	474.42	223.26	518.19	169.76
Length of eye	241.86	223.26	215.83	122.02
Width of eye	232.56	1134.88	215.83	111.41
Length of furcral rami	1255.81		1525.18	376.66
Width of furcral rami	251.16		417.51	
Length of testis				
Length of ova	530.23		489.21	
Male/Female	f	unknown	f	unknown

Table 6: Calculations of the percentages of certain attributes of *D. ranarum* from specimens collected from clariid hosts

Percentages	Sp 1	Sp 2	Sp 3	Sp 4.1	Sp 4.2	Sp 7.1	Sp 7.2	Sp 7.3	Sp 13
Length of abdomen as a % of total length	0.288936	0.270295	0.350438	0.3175	0.363937	0.308258	0.298369	0.311704	0.271209
Length of carapace as a% of total length	0.678144	0.751896	0.62328	0.639998	0.650818	0.702752	0.696967	0.662766	0.776076
Anterior carapace length as % of carapace	0.516559	0.418994	0.536145	0.49219	0.506297	0.522193	0.481616	0.487962	0.458781
Width of carapace as % of Length of carapace	0.97351	0.934034	1.096385	1.070315	1.020164	0.976491	1.075252	1.028897	0.930107
Carapace split as % of carapace	0.481237	0.539794	0.477915	0.539065	0.496228	0.469968	0.543481	0.502408	0.525088
Length of furcal rami as % of abdomen length	0.637275	0.696371	0.517856	0.637791	0.477476	0.583324	0.585934	0.597267	0.712821
Length of testis as % of abdomen	0.590647	0	0.528571	0	0.680187	0	0	0	0
Length of ova as % of abdomen									
Width of abdomen as % of length	0.777179	0.816243	0.617856	0.661416	0.689199	0.755941	0.726725	0.699688	0.89231
	Sp 20.1	Sp 20.2	Sp 23.1	Sp 23.2	Sp 23.3	Sp 23.4	Sp 23.5	Sp 23.6	Sp 23.7
Length of abdomen as a % of total length	0.291007	0.283816	0.294364	0.298755	0.355518	0.355518	0.32066	0.350064	0.327776
Length of carapace as a% of total length	0.700529	0.72343	0.676408	0.688795	0.618215	0.618215	0.651462	0.686324	0.66111
Anterior carapace length as % of carapace	0.435045	0.45075	0.518517	0.055849	0.518411	0.518411	0.496105	0.460694	0.497479
Width of carapace as % of Length of carapace	0.957706	0.936559	1.033957	0.993983	1.104816	1.104816	1.081704	0.981719	1.084033
Carapace split as % of carapace	0.52115	0.54424	0.506174	0.064265	0.481585	0.481585	0.519451	0.54479	0.521007
Length of furcal rami as % of abdomen length	0.585463	0.540427	0.659569	0.645829	0.556646	0.556646	0.640315	0.648744	0.681367
Length of testis as % of abdomen	0	0	0	0	0.448277	0	0	0	0
Length of ova as % of abdomen									
Width of abdomen as % of length	0.654542	0.706386	0.69503	0.763886	0.61576	0.61576	0.608695	0.623663	0.64746

Table 6 continued						
Percentages	Sp 23.8	Sp 24.1	Sp 24.2	Sp 29	Sp 34	Sp 49
Length of abdomen as a % of total length	0.325277	0.330862	0.311524	0.336434	0.26162	0.354277
Length of carapace as a% of total length	0.664654	0.646841	0.624462	0.693024	0.718984	0.605525
Anterior carapace length as % of carapace	0.437878	0.419536	0.462424	0.474272	0.455537	0.502076
Width of carapace as % of Length of carapace	1.043938	1.005752	1.189076	0.986576	1.118619	1.132801
Carapace split as % of carapace	0.57121	0.54885	0.605937	0.5302	0.590304	0.514535
Length of furcal rami as % of abdomen length	0.668731	0.646064	0.61642	0.534565	0.562963	0.517744
Length of testis as % of abdomen	0	0	0	0.502306	0	0.503549
Length of ova as % of abdomen						
Width of abdomen as % of length	0.613004	0.61233	0.570785	0.806451	0.718544	0.602819

Table 7: Calculations of the percentages of certain attributes of *D. ranarum* from specimens collected from cichlid hosts

Percentages	Sp 5.1	Sp 5.2	Sp 6.1	Sp 6.2	Sp 6.3	Sp 10.1	Sp 10.2	Sp 10.3	Sp 10.4
Length of abdomen as a % of total length	0.3259671	0.341176	0.309365	0.2882087		0.3019294	0.2696964	0.3070541	0.318544
Length of carapace as a% of total length	0.6353593	0.6470594	0.690624	0.7205232		0.6980852	0.6848494	0.6657384	0.784983
Anterior carapace length as % of carapace	0	0.5509097	0.5710119	0.5636365	0.4802637	0.6060504	0.6194803	0.5551052	0.472464
Width of carapace as % of Length of carapace	1.0108695	1.0090909	0.9621524	1.0121227	1.0000018	0.9311166	0.9601695	1.0122801	0.907246
Carapace split as % of carapace	0	0.4054555	0.3753956	0.421212	0.5164583	0.3746515	0.3584072	0.4204901	0.543478
Length of furcal rami as % of abdomen length	0.6567775	0.5172414	0.5563406	0.5757622		0.5859737	0.4494356	0.601769	0.614286
Length of testis as % of abdomen	0	0.5896578	0	0		0	0	0	0
Length of ova as % of abdomen									
Width of abdomen as % of length	0.6059292	0.5896578	0.7464829	0.8713451		0.789816	0.8202172	0.7611154	0.675

Table 7 continued									
Percentages	Sp 32.1	Sp 32.2	Sp 32.3	Sp 33.1	Sp33.2	Sp 33.3	Sp 36.1	Sp 36.2	Sp 37
Length of abdomen as a % of total length	0.348111	0.330309	0.330695	0.32574	0.327313	0.264017	0.334637	0.298356	0.294768
Length of carapace as a% of total length	0.643008	0.617058	0.661392	0.687927	0.713319	0.722732	0.640625	0.725665	0.707422
Anterior carapace length as % of carapace	0.582758	0.591176	0.576552	0	0.515821	0.54725	0.51626	0.508712	0.503098
Width of carapace as % of Length of carapace	1.06552	1.102943	1.055021	0.950335	0.9731	0.949222	1.077235	1.010453	0.993827
Carapace split as % of carapace	0.455176	0.411768	0.406699	0	0.490505	0.462622	0.493901	0.456448	0.472234
Length of furcal rami as % of abdomen length	0.471335	0.4945	0.545452	0.552451	0.675871	0.53282	0.428011	0.601698	0.585172
Length of testis as % of abdomen	0.6242	0.637356	0.602877	0	0.624137	0	0.665366	0	0
Length of ova as % of abdomen									
Width of abdomen as % of length	0.777065	0.774722	0.751197	0.895112	0.686206	0.722003	0.712058	0.733049	1.066643
	Sp 12	Sp 17.2	Sp 19.1	Sp 30	Sp 41	Sp 42	Sp 50		
Length of abdomen as a % of total length	0.267974	0.302592	0.288837	0.319584	0.275635	0.268434	0.366846		
Length of carapace as a% of total length	0.737473	0.685879	0.68927	0.701026	0.730766	0.68053	0.654911		
Anterior carapace length as % of carapace	0.370757	0.542017	0.584137	0.479409	0	0.552782	0.485478		
Width of carapace as % of Length of carapace	0.958641	0.966382	0.968254	0.964705	0.907893	1.03333	1.053916		
Carapace split as % of carapace	0.592319	0.445379	0.441271	0.502942	0	0.44167	0.522871		
Length of furcal rami as % of abdomen length	0.678863	0.571429	0.613641	0.561292	0.639542	0.626747	0.511112		
Length of testis as % of abdomen	0	0	0	0.587102	0	0	0.474306		
Length of ova as % of abdomen									
Width of abdomen as % of length	0.691058	0.733336	0.795453	0.864544	0.860476	0.915502	0.666668		

Table 8: Statistical analysis for *Dolops ranarum* specimens collected from all hosts

Total length		Length of abdomen		Width of abdomen	
Mean	4559.514909	Mean	1417.950727	Mean	1121.841786
Standard Error	320.6015875	Standard Error	94.16807433	Standard Error	112.5896134
Median	4196.29	Median	1374.01	Median	988.145
Mode	4496.06	Mode	1598.43	Mode	984.25
Standard Deviation	2377.645008	Standard Deviation	698.3691304	Standard Deviation	842.543517
Sample Variance	5653195.785	Sample Variance	487719.4423	Sample Variance	709879.578
Kurtosis	-0.211590002	Kurtosis	-0.712075121	Kurtosis	24.61253791
Skewness	0.394039326	Skewness	0.070686677	Skewness	4.103657091
Range	10040.06	Range	2649.69	Range	6110.26
Minimum	572.66	Minimum	194.24	Minimum	122.3
Maximum	10612.72	Maximum	2843.93	Maximum	6232.56
Sum	250773.32	Sum	77987.29	Sum	62823.14
Count	55	Count	55	Count	56
Confidence Level (95.0%)	642.7680396	Confidence Level (95.0%)	188.7957855	Confidence Level (95.0%)	225.6345718
Length of carapace		Width of carapace		Anterior carapace length	
Mean	3265.45125	Mean	3212.833036	Mean	1588.038039
Standard Error	235.1591229	Standard Error	225.5627485	Standard Error	108.2179954
Median	2951.705	Median	3070.87	Median	1549.08
Mode	2779.53	Mode	3070.87	Mode	1440.94
Standard Deviation	1759.769738	Standard Deviation	1687.957048	Standard Deviation	772.8310691
Sample Variance	3096789.532	Sample Variance	2849198.996	Sample Variance	597267.8614
Kurtosis	-0.105755563	Kurtosis	-0.215461146	Kurtosis	-0.587529196
Skewness	0.489665345	Skewness	0.43995559	Skewness	-0.080332438
Range	7479.83	Range	7110.08	Range	2967.38
Minimum	346.76	Minimum	392.81	Minimum	146
Maximum	7826.59	Maximum	7502.89	Maximum	3113.38
Sum	182865.27	Sum	179918.65	Sum	80989.94
Count	56	Count	56	Count	51
Confidence Level (95.0%)	471.2692976	Confidence Level (95.0%)	452.0377383	Confidence Level (95.0%)	217.3623295
Length of carapace split		Width of carapace over the eyes taken exactly touching the edge of the eye		Width of carapace over the eyes in a straight line	
Mean	1592.664902	Mean	1703.309455	Mean	1727.999464
Standard Error	137.8948311	Standard Error	122.6393083	Standard Error	119.9724056
Median	1389.93	Median	1672.93	Median	1716.535
Mode	1338.58	Mode	1960.14	Mode	1826.77
Standard Deviation	984.7660671	Standard Deviation	909.5174525	Standard Deviation	897.791275
Sample Variance	969764.207	Sample Variance	827221.9963	Sample Variance	806029.1734
Kurtosis	0.764275883	Kurtosis	-0.927339668	Kurtosis	-0.818610003
Skewness	0.861737896	Skewness	0.093259191	Skewness	0.079303106
Range	4467.84	Range	3358.43	Range	3341.79
Minimum	168	Minimum	204.36	Minimum	217.27
Maximum	4635.84	Maximum	3562.79	Maximum	3559.06
Sum	81225.91	Sum	93682.02	Sum	96767.97
Count	51	Count	55	Count	56
Confidence Level (95.0%)	276.9700326	Confidence Level (95.0%)	245.8772222	Confidence Level (95.0%)	240.4300144

Table 8 continued					
Distance between the eyes		Distance from the right eye to the anterior carapace edge		Distance from the left eye to the anterior carapace edge	
Mean	574.9082143	Mean	312.3505455	Mean	303.3470909
Standard Error	36.26887121	Standard Error	21.976555	Standard Error	21.90985925
Median	554.105	Median	330.71	Median	314.96
Mode	551.18	Mode	464.57	Mode	346.46
Standard Deviation	271.4113798	Standard Deviation	162.982494	Standard Deviation	162.487865
Sample Variance	73664.13707	Sample Variance	26563.29334	Sample Variance	26402.30627
Kurtosis	-0.16345349	Kurtosis	-0.854076638	Kurtosis	-0.38317278
Skewness	0.013998241	Skewness	0.03627752	Skewness	0.237665003
Range	1195.55	Range	651.92	Range	656.96
Minimum	53	Minimum	33.12	Minimum	36
Maximum	1248.55	Maximum	685.04	Maximum	692.96
Sum	32194.86	Sum	17179.28	Sum	16684.09
Count	56	Count	55	Count	55
Confidence Level (95.0%)	72.68442428	Confidence Level(95.0%)	44.06037814	Confidence Level (95.0%)	43.92666108
Length of eye		Width of eye		Length of furcal rami	
Mean	164.1723636	Mean	177.8870909	Mean	846.2859259
Standard Error	8.820825961	Standard Error	20.39585168	Standard Error	63.37519281
Median	165.35	Median	165.35	Median	778.235
Mode	165.35	Mode	165.35	Mode	1000
Standard Deviation	65.41699615	Standard Deviation	151.2596844	Standard Deviation	465.7106542
Sample Variance	4279.383385	Sample Variance	22879.49212	Sample Variance	216886.4134
Kurtosis	0.18573929	Kurtosis	30.2124208	Kurtosis	-0.471504455
Skewness	-0.590880977	Skewness	4.747514384	Skewness	0.397910756
Range	287.89	Range	1134.88	Range	1825.6
Minimum	15.83	Minimum	0	Minimum	105.04
Maximum	303.72	Maximum	1134.88	Maximum	1930.64
Sum	9029.48	Sum	9783.79	Sum	45699.44
Count	55	Count	55	Count	54
Confidence Level (95.0%)	17.6847066	Confidence Level (95.0%)	40.89125604	Confidence Level (95.0%)	127.114479
Width of furcal rami		Length of testis		Length of ova	
Mean	181.7566	Mean	822.4853333	Mean	297.2341935
Standard Error	15.32609102	Standard Error	77.42398072	Standard Error	29.74335956
Median	161.46	Median	858.27	Median	291.34
Mode	220.47	Mode	716.54	Mode	#N/A
Standard Deviation	108.3718289	Standard Deviation	299.8617879	Standard Deviation	165.6040174
Sample Variance	11744.4533	Sample Variance	89917.09186	Sample Variance	27424.69057
Kurtosis	0.771927511	Kurtosis	1.462214207	Kurtosis	-0.798170758
Skewness	0.774717435	Skewness	-0.565892158	Skewness	0.087873089
Range	506.74	Range	1244.3	Range	595.37
Minimum	12.95	Minimum	102.16	Minimum	29
Maximum	519.69	Maximum	1346.46	Maximum	624.37
Sum	9087.83	Sum	12337.28	Sum	9214.26
Count	50	Count	15	Count	31
Confidence Level (95.0%)	30.79891431	Confidence Level (95.0%)	166.0580709	Confidence Level (95.0%)	60.74398142

Table 9: Statistical analysis for *Dolops ranarum* specimens collected from all clariid hosts

Total length		Length of abdomen		Width of abdomen	
Mean	5116.859583	Mean	1603.925417	Mean	1112.465417
Standard Error	522.8851438	Standard Error	158.9906589	Standard Error	113.036638
Median	5148.75	Median	1653.545	Median	1203.97
Mode	4496.06	Mode	1598.43	Mode	984.25
Standard Deviation	2561.603593	Standard Deviation	778.8919763	Standard Deviation	553.7641708
Sample Variance	6561812.967	Sample Variance	606672.7107	Sample Variance	306654.7568
Kurtosis	-0.391749812	Kurtosis	-0.553749435	Kurtosis	-0.165501873
Skewness	-0.322100551	Skewness	-0.563946513	Skewness	-0.424137572
Range	9309.83	Range	2531.36	Range	2058.04
Minimum	572.66	Minimum	194.24	Minimum	122.3
Maximum	9882.49	Maximum	2725.6	Maximum	2180.34
Sum	122804.63	Sum	38494.21	Sum	26699.17
Count	24	Count	24	Count	24
Confidence Level (95.0%)	1081.66886	Confidence Level (95.0%)	328.8967887	Confidence Level (95.0%)	233.8337832
Length of carapace		Width of carapace		Anterior carapace length	
Mean	3487.925	Mean	3545.519167	Mean	1620.876667
Standard Error	372.9256608	Standard Error	366.4095432	Standard Error	178.88961
Median	3550.955	Median	3475.76	Median	1844.315
Mode	2779.53	Mode	3070.87	Mode	1440.94
Standard Deviation	1826.955162	Standard Deviation	1795.032835	Standard Deviation	876.3765295
Sample Variance	3337765.164	Sample Variance	3222142.88	Sample Variance	768035.8214
Kurtosis	-0.197232989	Kurtosis	-0.426363979	Kurtosis	-0.647534946
Skewness	-0.110588665	Skewness	-0.231689753	Skewness	-0.471169503
Range	7083.84	Range	6547.62	Range	2967.38
Minimum	346.76	Minimum	392.81	Minimum	146
Maximum	7430.6	Maximum	6940.43	Maximum	3113.38
Sum	83710.2	Sum	85092.46	Sum	38901.04
Count	24	Count	24	Count	24
Confidence Level (95.0%)	771.4544561	Confidence Level (95.0%)	757.9748581	Confidence Level (95.0%)	370.0608493
Length of carapace split		Width of carapace over the eyes taken exactly touching the edge of the eye		Width of carapace over the eyes in a straight line	
Mean	1767.0125	Mean	1842.883333	Mean	1950.209167
Standard Error	211.1546186	Standard Error	195.2516905	Standard Error	210.3868834
Median	1781.26	Median	1960.14	Median	2070.625
Mode	1338.58	Mode	1960.14	Mode	1826.77
Standard Deviation	1034.442145	Standard Deviation	956.5340262	Standard Deviation	1030.681026
Sample Variance	1070070.551	Sample Variance	914957.3432	Sample Variance	1062303.377
Kurtosis	-0.491816214	Kurtosis	-0.590792034	Kurtosis	-0.582650292
Skewness	0.038319631	Skewness	-0.584881194	Skewness	-0.478899845
Range	3842.99	Range	3067.54	Range	3341.79
Minimum	168	Minimum	204.36	Minimum	217.27
Maximum	4010.99	Maximum	3271.9	Maximum	3559.06
Sum	42408.3	Sum	44229.2	Sum	46805.02
Count	24	Count	24	Count	24
Confidence Level (95.0%)	436.8060141	Confidence Level (95.0%)	403.9083456	Confidence Level (95.0%)	435.2178349

Table 9 continued					
Distance between the eyes		Distance from the right eye to the anterior carapace edge		Distance from the left eye to the anterior carapace edge	
Mean	590.2825	Mean	364.7769565	Mean	337.4895652
Standard Error	63.17808627	Standard Error	38.93912399	Standard Error	35.94517559
Median	696.85	Median	398.44	Median	359.38
Mode	551.18	Mode	464.57	Mode	346.46
Standard Deviation	309.5081486	Standard Deviation	186.7454783	Standard Deviation	172.3870062
Sample Variance	95795.29404	Sample Variance	34873.87367	Sample Variance	29717.2799
Kurtosis	-0.613284703	Kurtosis	-0.43572618	Kurtosis	0.046330174
Skewness	-0.578739007	Skewness	-0.585207536	Skewness	-0.431873311
Range	1071.97	Range	651.92	Range	656.96
Minimum	53	Minimum	33.12	Minimum	36
Maximum	1124.97	Maximum	685.04	Maximum	692.96
Sum	14166.78	Sum	8389.87	Sum	7762.26
Count	24	Count	23	Count	23
Confidence Level (95.0%)	130.693651	Confidence Level(95.0%)	80.7548872	Confidence Level (95.0%)	74.54581158
Length of eye		Width of eye		Length of furcal rami	
Mean	167.9213043	Mean	169.2104348	Mean	976.26375
Standard Error	16.86801932	Standard Error	16.52088159	Standard Error	102.1135807
Median	173.23	Median	187.5	Median	937.165
Mode	165.35	Mode	173.23	Mode	732.28
Standard Deviation	80.89617877	Standard Deviation	79.23136474	Standard Deviation	500.252337
Sample Variance	6544.191739	Sample Variance	6277.609159	Sample Variance	250252.4007
Kurtosis	-0.061443018	Kurtosis	0.103173896	Kurtosis	-0.496841887
Skewness	-0.71100722	Skewness	-0.931372838	Skewness	-0.22896563
Range	287.89	Range	264.67	Range	1755.1
Minimum	15.83	Minimum	18.71	Minimum	105.04
Maximum	303.72	Maximum	283.38	Maximum	1860.14
Sum	3862.19	Sum	3891.84	Sum	23430.33
Count	23	Count	23	Count	24
Confidence Level (95.0%)	34.98216851	Confidence Level (95.0%)	34.26224817	Confidence Level (95.0%)	211.2377482
Width of furcal rami		Length of testis		Length of ova	
Mean	193.2957143	Mean	817.26	Mean	310.299375
Standard Error	27.3278512	Standard Error	160.7131739	Standard Error	47.64322827
Median	181.1	Median	874.45	Median	342.81
Mode	125.98	Mode	#N/A	Mode	#N/A
Standard Deviation	125.2319467	Standard Deviation	393.665271	Standard Deviation	190.5729131
Sample Variance	15683.04048	Sample Variance	154972.3456	Sample Variance	36318.03519
Kurtosis	0.962708577	Kurtosis	2.26361916	Kurtosis	-0.967228952
Skewness	0.770272906	Skewness	-1.37524973	Skewness	-0.202780165
Range	506.74	Range	1077.55	Range	595.37
Minimum	12.95	Minimum	102.16	Minimum	29
Maximum	519.69	Maximum	1179.71	Maximum	624.37
Sum	4059.21	Sum	4903.56	Sum	4964.79
Count	21	Count	6	Count	16
Confidence Level (95.0%)	57.00487222	Confidence Level (95.0%)	413.1256905	Confidence Level (95.0%)	101.5491997

Table 10: Statistical analysis for *Dolops ranarum* specimens collected from all cichlid hosts

Total length		Length of abdomen		Width of abdomen	
Mean	3772.844167	Mean	1164.704583	Mean	897.3588
Standard Error	414.4163263	Standard Error	123.5257241	Standard Error	81.02397774
Median	3602.385	Median	1074.06	Median	834.65
Mode	N/A	Mode	1039.37	Mode	N/A
Standard Deviation	2030.217081	Standard Deviation	605.1499884	Standard Deviation	405.1198887
Sample Variance	4121781.396	Sample Variance	366206.5085	Sample Variance	164122.1242
Kurtosis	4.693603121	Kurtosis	1.519129149	Kurtosis	1.467117534
Skewness	1.667515464	Skewness	1.060113952	Skewness	0.828357873
Range	9733.72	Range	2563.93	Range	1776.32
Minimum	879	Minimum	280	Minimum	189
Maximum	10612.72	Maximum	2843.93	Maximum	1965.32
Sum	90548.26	Sum	27952.91	Sum	22433.97
Count	24	Count	24	Count	25
Confidence Level (95.0%)	857.2843201	Confidence Level (95.0%)	255.5320814	Confidence Level (95.0%)	167.2252367
Length of carapace		Width of carapace		Anterior carapace length	
Mean	2715.8036	Mean	2698.8848	Mean	1455.186364
Standard Error	310.3041646	Standard Error	302.5631081	Standard Error	134.8240736
Median	2496.06	Median	2401.59	Median	1386.38
Mode	N/A	Mode	N/A	Mode	N/A
Standard Deviation	1551.520823	Standard Deviation	1512.81554	Standard Deviation	632.3809594
Sample Variance	2407216.863	Sample Variance	2288610.859	Sample Variance	399905.6778
Kurtosis	4.069779331	Kurtosis	3.385207809	Kurtosis	0.546965183
Skewness	1.691027504	Skewness	1.536469541	Skewness	0.730228476
Range	7136.59	Range	6876.89	Range	2575.76
Minimum	690	Minimum	626	Minimum	326
Maximum	7826.59	Maximum	7502.89	Maximum	2901.76
Sum	67895.09	Sum	67472.12	Sum	32014.1
Count	25	Count	25	Count	22
Confidence Level (95.0%)	640.4361871	Confidence Level (95.0%)	624.4594351	Confidence Level (95.0%)	280.3820586
Length of carapace split		Width of carapace over the eyes taken exactly touching the edge of the eye		Width of carapace over the eyes in a straight line	
Mean	1322.010455	Mean	1363.741667	Mean	1438.5888
Standard Error	198.7059256	Standard Error	146.9632311	Standard Error	142.5293465
Median	1082.68	Median	1229.555	Median	1358.1
Mode	1094.49	Mode	#N/A	Mode	1094.49
Standard Deviation	932.0134049	Standard Deviation	719.9698542	Standard Deviation	712.6467323
Sample Variance	868648.987	Sample Variance	518356.591	Sample Variance	507865.3651
Kurtosis	7.377295949	Kurtosis	0.74658021	Kurtosis	0.401354302
Skewness	2.466944018	Skewness	1.023108544	Skewness	0.817704438
Range	4260.84	Range	2836.16	Range	2877.89
Minimum	375	Minimum	343.37	Minimum	336
Maximum	4635.84	Maximum	3179.53	Maximum	3213.89
Sum	29084.23	Sum	32729.8	Sum	35964.72
Count	22	Count	24	Count	25
Confidence Level (95.0%)	413.2316655	Confidence Level (95.0%)	304.0161925	Confidence Level (95.0%)	294.1660526

Table 10 continued					
Distance between the eyes		Distance from the right eye to the anterior carapace edge		Distance from the left eye to the anterior carapace edge	
Mean	530.5308	Mean	241.8484	Mean	244.4424
Standard Error	48.93688706	Standard Error	23.04017958	Standard Error	25.62009631
Median	511.81	Median	228.35	Median	236.22
Mode	417.32	Mode	N/A	Mode	N/A
Standard Deviation	244.6844353	Standard Deviation	115.2008979	Standard Deviation	128.1004816
Sample Variance	59870.47287	Sample Variance	13271.24688	Sample Variance	16409.73338
Kurtosis	2.223227014	Kurtosis	-0.285039119	Kurtosis	-0.213799965
Skewness	1.147878655	Skewness	0.572203861	Skewness	0.682909597
Range	1135.55	Range	416.02	Range	478.53
Minimum	113	Minimum	77	Minimum	61
Maximum	1248.55	Maximum	493.02	Maximum	539.53
Sum	13263.27	Sum	6046.21	Sum	6111.06
Count	25	Count	25	Count	25
Confidence Level (95.0%)	101.00075	Confidence Level (95.0%)	47.55258371	Confidence Level (95.0%)	52.87726905
Length of eye		Width of eye		Length of furcal rami	
Mean	152.0272	Mean	143.2492	Mean	666.25875
Standard Error	10.49236061	Standard Error	15.07019167	Standard Error	79.46369295
Median	157.48	Median	137.93	Median	601.6
Mode	181.1	Mode	102.36	Mode	419.1
Standard Deviation	52.46180305	Standard Deviation	75.35095835	Standard Deviation	389.2910016
Sample Variance	2752.240779	Sample Variance	5677.766924	Sample Variance	151547.4839
Kurtosis	-0.126131439	Kurtosis	1.277127556	Kurtosis	3.739721043
Skewness	-0.336207798	Skewness	0.685698002	Skewness	1.62381571
Range	208.86	Range	346.82	Range	1758.64
Minimum	33	Minimum	0	Minimum	172
Maximum	241.86	Maximum	346.82	Maximum	1930.64
Sum	3800.68	Sum	3581.23	Sum	15990.21
Count	25	Count	25	Count	24
Confidence Level (95.0%)	21.65516351	Confidence Level (95.0%)	31.10334051	Confidence Level (95.0%)	164.3829494
Width of furcal rami		Length of testis		Length of ova	
Mean	163.0320833	Mean	832.75875	Mean	242.2663636
Standard Error	17.32265695	Standard Error	92.61914696	Standard Error	36.96132928
Median	153.01	Median	811.85	Median	213.18
Mode	275.59	Mode	#N/A	Mode	#N/A
Standard Deviation	84.86334104	Standard Deviation	261.9665075	Standard Deviation	122.586861
Sample Variance	7201.786652	Sample Variance	68626.45107	Sample Variance	15027.53849
Kurtosis	-0.582537151	Kurtosis	1.123479418	Kurtosis	1.497687206
Skewness	0.398333541	Skewness	0.974764391	Skewness	1.003814315
Range	306.22	Range	826.57	Range	448.59
Minimum	28	Minimum	519.89	Minimum	66
Maximum	334.22	Maximum	1346.46	Maximum	514.59
Sum	3912.77	Sum	6662.07	Sum	2664.93
Count	24	Count	8	Count	11
Confidence Level (95.0%)	35.83459735	Confidence Level (95.0%)	219.0093244	Confidence Level (95.0%)	82.35498805

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