RISK ANALYSIS AND POTENTIAL TRANSMISSION AND IMPLICATIONS OF EXOTIC *GYRODACTYLUS* SPECIES ON CULTURED AND WILD CYPRINIDS IN THE WESTERN CAPE, SOUTH AFRICA

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Declaration

I declare that this is my own work, that **Risk analysis and potential implications of exotic *Gyrodactylus* species on cultured and wild cyprinids in the Western Cape, South Africa** has not been submitted for any degree or examination in any other university, and that all the sources I have used or quoted have been indicated and acknowledged by complete references.

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Abstract

The expansion of the South African aquaculture industry coupled with the lack of effective parasite management strategies may potentially have negative effects on both the freshwater biodiversity and economics of the aquaculture sector. Koi and goldfish are notorious for the propagation of parasites worldwide, some of which have already infected indigenous fish in South Africa. Koi and goldfish have been released into rivers in South Africa since the 1800's for food and sport fish and have since spread extensively. These fish are present in most of the river systems in South Africa and pose an additional threat the indigenous cyprinids in the Western Cape. Monogenean parasites of the genus *Gyrodactylus* are of particular concern, as their unique biology renders them a possible threat. *Gyrodactylus kherulensis* and *G. kobayashii* were identified from koi and goldfish respectively imported from Asia, Europe and locally bred fish. Morphometrics and the use of statistical classifiers, which includes univariate (ANOVA and Kruskal-Wallis), bivariate (Pearson's correlation) and multivariate (Principal Component Analysis) placed the two species within their respective groups. There was some intraspecific variation among the different populations collected from the various locations, especially in the hamulus and ventral bar features, but the marginal hooklets, however, remained static for both helminth species. This illustrates again the importance of the minor variations in the marginal hook features in gyrodactylid taxonomy. Infection trials conducted by co-habitation of infected koi and goldfish with two indigenous redfin minnow species, *Pseudobarbus burchelli* and *P. phlegethon* showed that both *G. kherulensis* and *G. kobayashii* could successfully transfer and establish themselves on *P. phlegethon*, where the infection increased rapidly initially, but remained relatively constant thereafter. *P. burchelli* appeared to be inherently resistant as the parasite population growth rate initially remained steady, until the infection died off. The wild-caught indigenous

fish were however not infected with any exotic *Gyrodactylus* species, but a new species, *G. burchelli* n. sp. described from the body surfaces of *P. burchelli.*

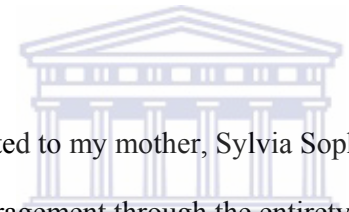

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Table of Contents

Chapter 1

Introduction and background

Aquaculture and its socio-economic and ecological implications

The worldwide aquaculture industry, for both edible and ornamental species, has increased exponentially over the past 30 years and is predicted to continue growing (Thoney and Hargis 1991) at about 10% per annum, primarily to provide for the needs of a growing human population (Hill 2005; Muir 2005). Aquaculture plays a pivotal role in society, by the creation of jobs within the fishing sector, which globally employs approximately 36 million people (Bartley and Subasinghe 1996; Naylor *et al*. 2000; Tidwell and Allan 2001; Muir 2005). Intensive fish farming techniques are becoming increasingly common but it has its drawbacks (Naylor *et al*. 2000). Intensive fish farming requires methodical management plans, as the increased density of fish may result in the potential cultivation and spread of fish parasites within enclosed facilities (Bartley and Subasinghe 1996; Naylor *et al*. 2000). The industry is thus compromised by diseases that result in considerable economic losses and major reductions in export trade, to an extent that the economy of a country may be negatively affected (Hill 2005). Aquaculture has therefore become one of the major vectors of fish diseases worldwide and if no effective disease control measures are implemented, pathogens will continue to propagate worldwide (Hill 2005; Murray and Peeler 2005).

The ornamental fish trade sector, a major branch of aquaculture, has experienced considerable growth in the last 50 years and fish-keeping has become increasingly common in many homes worldwide (Davenport 1996; Ponpornpisit *et al.* 2000). The increased demand to exhibit beautiful exotic fish supports the growth of the exotic fish trading sector (Arthington and McKenzie 1997). Ornamental fish trade is one of the prime contributors to the spread of alien fish species into natural environments (Andrews 1990). Alien species

introductions are perceived as one of the major threats to biodiversity (Koehn 2004). Modification of freshwater ecosystems by alien species is a global dilemma caused by the increased introductions of non-native fish (Moyle and Light 1996) and their parasites, and the associated lack of effective management strategies to control the influx of these alien species.

Freshwater ecosystems may be affected by both intentional and unintentional release of species (Arthington and McKenzie 1997; Koehn 2004; Lintermans 2004), and alien species management is predicted to be one of the major challenges faced by ecologists in the near future (Allendorf and Lundquist 2003). Homogenization, according to Rahel (2002) is defined as the loss of global differences in freshwater ecosystems, which are becoming more and more common, due to the loss of indigenous biodiversity and the positive establishment of alien fish in natural environments. Galli *et al*. (2005) deem alien introductions to be 'biogeographical pollution' and consider some freshwater ecosystems to have been irrevocably impaired as a result of this form of pollution.

Besides habitat degradation, competitive exclusion, niche displacement and alien species feeding off the spawn of indigenous fish species (Allan and Flecker 1993; Koehn 2004), one of the implications of alien fish establishment is the introduction of their parasites and other exotic diseases (Allan and Flecker 1993; Dove and Ernst 1998). At the current rate of species exchange via aquaculture, the spread of foreign diseases to wild indigenous fish stocks is unavoidable (Murray and Peeler 2005). The high fish densities within tanks and farms provide ideal environments for parasite proliferation (Thoney and Hargis 1991; Barker and Cone 2000).

Invasive alien cyprinids

Over 160 freshwater alien fish have become established in freshwater ecosystems of approximately 120 countries, as recorded by the Food and Agriculture Organisation (FAO) of the United Nations (Allan and Flecker 1993). Two representatives of the family Cyprinidae, namely *Cyprinus carpio koi* L.*,* the ornamental carp and *Carassius auratus* L.*,* the goldfish, are the most popular aquarium fish and are traded globally (Andrews 1990). The common carp (*Cyprinus carpio* L.) is widespread as a result of its edible properties and approximately 1.5 million metric tons are produced per annum for human consumption (Gilad *et al*. 2003). It is described as the earliest invasive fish that has its origins in Asia and has continued to be cultured in southern African waters since the 1700's (Allan and Flecker 1993). However, the koi carp, a subspecies of the common carp, is an attractive, colourful fish which is usually kept in outdoor pools or displayed in household aquaria and is also quite expensive as a result of its ornamental appeal (Gilad *et al*. 2003). The origin of koi keeping dates back to the first century A.D and has since become a worldwide attraction (Balon 1995). *Cyprinus carpio* as well as its ornamental subspecies *C. carpio koi* and *C. auratus* are widely-distributed species both in South Africa and globally (Skelton 2001; Kir and Tekin Ozan 2007) and are currently top sellers in the aquarium trade. The success of these cyprinids is attributable to their ability to withstand a wide range of environmental conditions (Andrews 1990; Kir and Tekin Ozan 2007; Tekin Ozan *et al.* 2008). These invasive cyprinids negatively impact native fish by competing for food, altering the habitat by making the water more turbid, and feeding off the spawn of indigenous fish (De Moor and Bruton 1988; Koehn 2004). The introduction of koi carp and goldfish fish has also led to the introduction of their parasites to various geographical localities, including South Africa (De Moor and Bruton 1988; Mouton *et al*. 2001). These fish are common carriers of the monogenean parasite genus *Gyrodactylus*.

Monogenean parasites and their aquacultural and ecological influences

Monogenean parasites have attained ubiquity in freshwater and marine ecosystems. These flatworms are largely ectoparasitic on both marine and freshwater fish; however endoparasitic forms do occur (Bush *et al.* 2001). Monogeneans generally range in size from about 0.2 mm - 2 mm, although some marine monogeneans like *Capsala martinieri* Bosc, 1811, may grow to 20 mm (Bush *et al*. 2001; Crespo and Crespo 2003). Monogeneans are characterized by their distinctive posterior attachment organ, the opisthaptor, which may consist of a suction disc, clamps or large hooks (hamuli) with additional marginal hooklets (Paperna 1996; Bush *et al.* 2001).

Monogeneans have simple, direct life cycles that lack an intermediate host. Apart from the viviparous Gyrodactylidae, they are predominantly oviparous. Development commences with the egg hatching releasing a larval ciliated, free-swimming form known as the oncomiracidium, which develops into the adult after attachment to a suitable host (Bush *et al.* 2001; Buchmann and Lindenstrøm 2002; Simkova *et al*. 2006). Monogeneans generally have reduced life cycles, as opposed to most other platyhelminths, and development under favourable conditions, generally lasts for only a few days (Simkova *et al*. 2006). The development time varies though, depending largely on environmental parameters (*e.g*. Cecchini *et al*. 1998; Jackson and Tinsley 1998; Ernst *et al*. 2005). Members from the Gyrodactylidae are generally viviparous and give birth to live young, thereby omitting the larval stage and producing reproductively-able adult offspring, although some oviparous forms exist like the oogyrodactylids (Paperna 1996; Bush *et al*. 2001; Peeler *et al*. 2004). This mode of reproduction is the primary reason for their ability to rapidly increase their populations size thereby resulting in fish mortalities in confined intensive culture conditions.

Monogeneans typically display high levels of host and site specificity (Whittington *et al.* 2000; Bush *et al.* 2001). Their attachment organs are primarily adapted to the site of attachment, and this has subsequently led to the specialization of these worms to specific sites on the fish. (Bush *et al.* 2001).

Monogeneans play a pivotal role in aquaculture health management (Ernst *et al*. 2005), by compromising both the integrity and economics of the fishing and aquaculture industries (Thoney and Hargis 1991). Under natural conditions, monogeneans are not known to regulate fish population size, but are able to increase epizootically in confined conditions particularly in extensive fish farming environments (Paperna 1996). In both confined and stressful conditions, monogenean populations can increase at alarming rates and result in almost uncontrollable disease outbreaks (Paperna 1996). Parasite disease management is expensive and the regulation of these parasites is imperative to the fishing industry, therefore integration of parasite biology and treatment strategies are vital to the economic growth of the aquaculture industry (Tubbs *et al.* 2005). Fish in confined conditions provide favourable conditions for the exponential growth of the parasite population (Barker and Cone 2000). The high host density within the tanks increases the parasitic transmission rate (Buchmann 1997). Because monogeneans lack an intermediate host, their turn-over rates are quite high and they result in high parasitic infection rates (Barker and Cone 2000). Monogenean parasites are therefore both highly invasive and result in localized epizootics and major stock loss, and consequently major economic loss in pisciculture where susceptible hosts are present (Barker and Cone 2000).

The genus *Gyrodactylus*

Members of the monogenean genus *Gyrodactylus* von Nordmann, 1832, are predominantly parasites of marine and freshwater fish (Cable *et al*. 2001). *Gyrodactylus* species are largely ectoparasitic and mainly parasitise the skin, fins and gills of their fish hosts (Buchmann and Bresciani 1997). They are relatively small monogenean parasites with a length range of approximately 0.2-0.8 mm, with relatively conserved morphology (Bakke *et al*. 2007; Gheorghiu *et al*. 2007). The genus *Gyrodactylus* has been described as a diverse group among the lower Monogenea and more than 400 species have been identified from about 400 fish hosts (Harris *et al.* 2004). Representatives of the genus are presumed to be underestimated in terms of numbers of species described, and they may be as diverse as the number of described fish species worldwide, which totals about 24 000 (Bakke *et al.* 2002) Twenty-four species of *Gyrodactylus* has been recorded from African freshwater fishes, and the African members of the genus are generally smaller than those from Europe (Christison *et al*. 2005; Přikrylová *et al*. 2009; Garcia-Vasquez *et al.* 2011).

Similar to many other monogeneans, in intensive fish culture conditions, members of the genus *Gyrodactylus* are among the most notorious of all fish parasites (Bakke *et al*. 1992; Xu *et al.* 2007). Gyrodactylids are unique among the monogeneans due to their viviparity which is their ability to give birth to live adult worms as well as displaying polyembryonism, the development of a juvenile worm inside the mother, with the juvenile carrying the next generation within its uterus, typically described as "Russian Doll" development (Cable and Harris 2002; Xu *et al.* 2007). The first-born daughter usually develops as result of asexual reproduction, thereafter the other daughters may be produced as a result of either sexual reproduction or parthenogenesis (Cable and Harris 2002). These worms then attach to the fish in close proximity to the mother (Cable *et al*. 2001). They are exceptional in terms of reproductive abilities, and this character has resulted in these parasites being among the most

successfully invasive and economically destructive parasites in aquaculture (Xu *et al.* 2007). These parasites are highly adaptable and capable of infecting a wide range of fish host (Cable and Harris 2002). The success of members of *Gyrodactylus* can also be seen in the ability of these parasites to infect fish ranging from tropical localities to fish in the Polar regions (Harris 1993).

One species of *Gyrodactylus* that has received the most recognition as a result of its highly invasive and negative economic implications is *Gyrodactylus salaris* Malmberg, 1957 which infects salmonids, particularly the susceptible Atlantic salmon, *Salmo salar* L., and has resulted in mass mortalities in fish hatcheries and rivers in Norway (Mo 1994). *Gyrodactylus salaris* is a highly invasive monogenean that has the potential to wipe out 98% of the Atlantic salmon over a five year period in uncontrolled conditions (Mo 1994). Since the introduction of the species into Norway in the 1970's with salmon parr imports, it has spread to 45 rivers and 37 fish hatcheries and has resulted in an approximate reduction of 520 t or 20% total catch per year (Mo 1994; Peeler *et al*. 2004). The primary reason for the huge epizootic effect of this parasite is that the Atlantic salmon have not evolved together with *G. salaris* and therefore lacks the immune defenses against the parasite as a result of it being a foreign pathogen to Norwegian rivers (Mo 1994; Peeler *et al.* 2004). The parasite is believed to originate from western Sweden, northern Finland and northern Russia (Mo 1994). *Gyrodactylus salaris* has attained immense proportions and control of the parasite has resulted in grave ecological implications. As a result, an attempt to remove the entire infected fish population with the chemical rotenone has been made (Peeler *et al*. 2004).

Dispersal of *Gyrodactylus* species according to Bakke *et al*. (1992), occurs via four modes of transmission: (1) host to host transmission, in the case of two hosts coming into contact with one another, the parasites are able to be conveyed; (2) by detached parasites on the bottom of

the tank or on the substrate (3) by detached parasites in the water column coming into contact with fish (4) by the parasites being transferred from dead infected fish to live fish.

The genus *Gyrodactylus* has been described as having the broadest host range among the monogeneans (Bakke *et al.* 1992). The largest percentage (59%) of species display strict host specificity, infecting and described from one host only, while the others have a broader range (Buchmann *et al*. 2004; Harris *et al.* 2004). However, examples referred to in Bakke *et al.* (1992) proposed that some *Gyrodactylus* species are able to successfully attach to foreign hosts, but lack the ability to feed off or reproduce on those fish. One such example is *G. errabundus* Malmberg, 1970 which lives and feeds on *Zoarces viviparus* (L.), but can be transported by other species. However, *G. macrochira* Hoffman & Putz, 1964, infects an array of hosts and uncertainty still remains to which is its principal host (Bakke *et al.* 1992). Harris *et al*. (2004) also states that host specificity should be appropriately defined because *G. gasterostei* Gläser, 1974, which has been shown to infect six hosts, is originally described from and restricted to *Gasterosteus aculeatus* L. yet during cold weather conditions, it will transfer to other hosts. Gyrodactylids have gained global attention due to their potential economic implications as well as their potential to transfer from host to host in the wild (Harris *et al.* 2004). Parasite host-switching within the group is regarded to be their means of speciation, particularly to unrelated hosts (Harris 1993; Bakke *et al*. 2002). Representatives of the genus *Gyrodactylus* are described by Bakke *et al.* (2002) as being narrowly host specific, however recent evidence suggesting that the genus is not as host-specific as previously assumed has come under light (King and Cable 2007). *Gyrodactylus turnbulli* Harris, 1986 previously regarded as a specialist, has the ability to infect a range of closely related fish, and use cyprinids as possible reservoir hosts (King and Cable 2007). *Gyrodactylus salaris* also has the ability to propagate to a broad range of fish species (Bakke

et al. 2002). There is not enough empirical evidence to assume the host specificity of members of the genus *Gyrodactylus* and is due to lack of research to determine their host ranges, and more such studies are encouraged. Cryptic species may also pose an additional taxomomic problem. The recent focus on cryptic species with the aid of molecular technology has also improved the understanding of this. An example is *G. ulinganisus* Garcia-Vasquez *et al*., 2011, from the Mozambique tilapia, *Oreochromis mossambicus* Peters which is morphologically indistinguishable from *G. cichlidarum* Paperna, 1968, but it is genetically different enough to be regarded as a separate yet cryptic species (Garcia-Vasquez *et al.*, 2011).

Attachment of gyrodactylids to their hosts is achieved by the use of the opisthaptor, which consists of 16 marginal hooklets and a pair of hamuli connected by a ventral and dorsal bar (Bakke *et al*. 2007). Morphological differences of the opisthaptoral armature of *Gyrodactylus* are primarily used as tools for species discrimination and the technological advancement of microscopy improves the use of morphology, yet dimensionality in the opisthaptoral organs has placed a major restriction on the use of morphology in taxonomy (Harris 1998; Zietara and Lumme 2003; Olstad *et al*. 2009). Morphological variations, particularly of the marginal hooklets of *Gyrodactylus,* are unique to each species, though it may be a reflection of the ecological conditions common to the species, particularly water temperature, locality and host (Zietara and Lumme 2003; Davidova *et al*. 2005; Olstad *et al*. 2009). Due to intraspecific variation and in some cases, similarity in *Gyrodactylus* species, some taxonomic confusion has arisen. The relative sizes of the opisthaptoral features, particularly the size of hamuli and the marginal hooklets change with annual fluctuations in water temperature (Davidova *et al.* 2005). This is particularly important, as the initial step in assessing the risk of an invasive species involves the positive identification of a potential hazard, especially if it

is known to have pathogenic effects in other parts of the world. Statistical methods using data acquired from the morphometric measurements have demonstrated some success in species separation (Shinn *et al.* 2001; Shinn *et al*. 2004). The method is also effective to determine the intraspecific variation and subtle differences among various populations.

South African freshwater biodiversity

South Africa is the third most biologically diverse country globally and boasts between 250 000 and 1 000 000 floral and faunal species, with a large portion of these endemic to the country (Wynberg 2002). The freshwater ecosystems accommodate 15 families, 29 genera and 94 indigenous freshwater fish species, the majority of which belong to the family Cyprinidae (Skelton *et al*. 1995; 2001). Approximately 61% of the freshwater fish species found in Southern Africa are endemics and the majority of these are confined to the Western Cape (Skelton 2001). The Cape Floristic Region is a globally renowned biodiversity hotspot characterized by high species diversity and high species endemism (Myers *et al.* 2000). The region previously accommodated 19 native fish species, now elevated to 23 species with the recent inclusion of the evolutionary significant units (ESU's) 15 of which are endemic, and six are near endemic (Impson 2007). Of the 23, 19 are cyprinid fish, and the majority of these are threatened, primarily by alien fish introduction in the area (Impson 2007). Even though few species exist within the region, the indigenous freshwater fish are considered the most endangered biotic element in the Western Cape (Impson *et al*. 2000).

The Animal Health Act, No.7 of 2002, which prohibits the entrance of foreign animal diseases into the country, restricts pathogens from entering and spreading, and essentially protects indigenous fish as well as cultured fish from infections by potentially pathogenic diseases. A permit issued by the Department of Agriculture together with a health certificate

issued by the country of import is necessary to declare the imported fish free of pathogens. The fish should also be held within a quarantine facility, examined and chemically treated if parasites are found. All precautions should be carried out by the holder of the permit to prevent and reduce negative impacts caused by these alien species on biodiversity. In the case of freshwater fish imports, it is imperative that effective quarantine methods be carried out to prevent alien parasites from establishing themselves on and in native fish. The responsibility therefore rests upon the importer of the fish to ensure that they are clear of parasites. These laws are not strictly enforced and the effective treatment of exotic fish is not carried out and few qualified personnel are available to inspect the imported fish (Mouton *et al*. 2001). Mitigation measures to reduce the risk of establishment of these potentially hazardous parasites into natural freshwater ecosystems should be initiated by assessing the potential risk that the exotic pathogen poses to native fish, and to propose an effective quarantine protocol based on the individual parasite biology. Training of government personnel as well informing importers and breeders about the consequent effects of establishment in the ecologically-sensitive Cape Floristic Region would be beneficial.

About 60% of all commercially traded fish in South Africa are non-indigenous and imported (Mouton *et al*. 2001). The aquaculture industry in South Africa has grown over the past 2 decades, in 1998, a total of 5301 t of fish were produced for aquaculture purposes, yielding a total of ZAR 228.986 m (US$ 38.167 m) (Hoffman *et al*. 2000). Koi carp was the major contributor to the total, and the sector was valued at ZAR 135 m, followed by goldfish, *Carassius auratus* which was worth ZAR 15.6 m in 1998 (Hoffman *et al*. 2000). The goldfish and koi industries have attained global accreditation for quality and disease control (Hoffman *et al*. 2000). However, koi carp and goldfish have been positively identified as transporters of exotic parasite species, such as *Argulus japonicus* Thiele, 1900; *Trichodina acuta* Lom, 1961; *Ichthyopthirius multifiliis* Fouquet, 1876 and *Bothriocephalus*

acheilognathi Yamaguti, 1934 into southern Africa (De Moor and Bruton 1988; Mouton *et al*. 2001). Carp have also been implicated as carriers of four dactylogyrid species onto the African continent, *Pseudocolpenteron pavlovski* Bychowsky and Gussev, 1955; *Dactylogyrus anchoratus* Dujardin, 1845; *D. minutus* Kulwiec, 1927 and *D. extensus* Mueller and Van Cleave, 1932 (De Moor and Bruton 1988). These parasites inadvertently enter the country's natural water ecosystems when infected fish are sold to the public with intact parasites and are released, or escape into natural water bodies, particularly if they are kept in outdoor pools (Arthington and McKenzie 1997).

Quarantine measures to control monogeneans in exotic fish trade are therefore imperative and a thorough examination of fish should be undertaken prior to importation and before introduction to a culture system (Thoney and Hargis 1991). The presence of monogeneans should be dealt with immediately and deemed as an extreme ecological and economic risk (Thoney and Hargis 1991). Aquaculture frequently has harmful effects on ecological conservation (Beveridge *et al.* 1994). There is always a huge risk when considering the potential of monogeneans to establish itself onto indigenous fish species (Dove and Ernst 1998). The severity of the effects of introduced parasites might be enhanced if the exotic and native fish are related (Bauer 1991; Dove 2000). The relatedness of the host fish to the indigenous fish aids transmission between them; however, the native fish host would not have evolved the immunological defence strategies to limit the parasite numbers (Dove 2000). The relatedness of koi carp and goldfish to the indigenous cyprinids could therefore potentially be a risk to the freshwater biodiversity of South Africa, and particularly to the Cape Floristic Region. A prime example of this the propagation of the fish louse, *Argulus japonicus* and the Asian tapeworm, *Bothriocephalus acheilognathi* presumed to have been transported into the country with common carp, and have already infected native cyprinid

fish in South Africa (De Moor and Bruton 1988). *B. acheilognathi* was found at 100% prevalences in the intestines of largemouth yellowfish in the Vaal Dam (Retief *et al*, 2007).

Little information exists about the influence of exotic monogeneans and their implications on biodiversity and economics in South Africa. Extensive conservation measures should be enforced to ensure that introduced parasite species are dealt with accordingly by effective treatment strategies.

This dissertation intends to morphologically identify different populations of members of the genus *Gyrodactylus* from imported koi carp and goldfish respectively introduced from various geographic localities. The study also aims to determine whether these exotic *Gyrodactylus* species can propagate to indigenous cyprinids in the Western Cape, and to assess the risk associated with their introduction.

References:

ALLAN, J.D., Flecker, A.S. 1993. Biodiversity conservation in running waters. *Bioscience* **43** (1), 32-43.

ALLENDORF, F. W., Lundquist, L. L. 2003. Population biology, evolution, and control of invasive species. *Conservation Biology* **17**(1), 24–30.

ANDREWS, C. 1990. The ornamental fish trade and fish conservation. *Journal of Fish Biology* **37**, (Suppl A) 53 –59.

ARTHINGTON, A.H. & McKenzie, F. 1997. Review of impacts of displaced/introduced fauna associated with inland waters, Australia: State of the Environment Technical Paper Series (Inland Waters), Department of the Environment, Canberra. 69 pp.

BAKKE, T.A., Cable, J., Harris, P.D. 2007. The biology of gyrodactylid monogeneans: The "Russian-doll killers" *Advances in Parasitology* **64**, 161-376.

BAKKE, T.A., Harris, P.D., Jansen, P.A., Hansen, L.P. 1992. Host specificity and dispersal strategy in gyrodactylid monogeneans, with particular reference to *Gyrodactylus salaris* (Platyhelminthes, Monogenea). *Diseases of Aquatic Organisms* **13**, 63-74.

BAKKE, T.A., Harris, P.D., Cable, J. 2002. Host specificity dynamics: observations on gyrodactylid monogeneans. *International Journal for Parasitology* **32**, 281–308.

BALON, E.K. 1995. Origin and domestication of the wild carp, *Cyprinus carpio*: from Roman gourmets to the swimming flowers. *Aquaculture* **129**, 3-48.

BARKER, D.E., Cone, D.K. 2000. Occurrence of *Ergasilus celestis* Copepoda and *Pseudodactylogyrus anguillae* Monogenea among wild eels *Anguilla rostrata* in relation to stream flow, pH and temperature and recommendations for controlling their transmission among captive eels. *Aquaculture* **187**, 261-274.

BARTLEY, D.M., Subasinghe, R.P. 1996. Historical aspects of international movement of living aquatic species. *Revue Scientifique et Technique* **15**(2), 387-400.

BAUER, O.N. 1991. Spread of parasites and diseases of aquatic organisms by acclimatization: a short review. *Journal of Fish Biology* **39**, 679 – 686.

BEVERIDGE M.C.M., Ross, L.G., Kelly, L.A. 1994. Aquaculture and biodiversity. *Ambio*, **23**(8), 497-502.

BUCHMANN, K. 1997. Infection biology of gill parasitic monogeneans with special reference to the congeners *Pseudodactylogyrus bini* and *P. anguillae* (Platyhelminthes: Monogenea) from European eel. Dissertation. Royal Veterinary and Agricultural University, Frederiksberg, Denmark, 208 pp.

BUCHMANN, K., Bresciani, J. 1997. Microenvironment of *Gyrodactylus derjavini* on rainbow trout *Oncorhynchus mykiss*: association between mucous cell density in skin and site selection. *Parasitology Research* **84**, 17-24.

BUCHMANN, K., Lindenstrøm, T. 2002. Interactions between monogenean parasites and their fish hosts. *International Journal for Parasitology* **32**, 309–319.

BUCHMANN, K., Madsen, K.K. Dalgaard, M.B. 2004. The homing of *Gyrodactylus salaris* and *G. derjavini* (Monogenea) on different host and response post-attachment. *Folia Parasitologica* **51**, 263-267.

BUSH, A.O., Fernandez, J.C., Esch, G.W., Seed, R. 2001. Parasitism: The Diversity and Ecology of Animal Parasites. Cambridge University Press, Cambridge, U.K. 566 pp.

CABLE, J., Harris, P.D. 2002. Gyrodactylid developmental biology: historical review, current status and future trends. *International Journal for Parasitology* **32**, 255–280.

CABLE, J., Tinsley, R.C., Harris, P.D. 2001. Survival, feeding and embryo development of *Gyrodactylus gasterostei* (Monogenea: Gyrodactylidae). *Parasitology* **124**, 53-68.

CECCHINI, S., Saroglia, M., Berni, P., Cognetti-Varriale, A.M. 1998. Influence of temperature on the life cycle of *Diplectanum aequans* (Monogenea, Diplectanidae), parasitic on sea bass, *Dicentrarchus labrax* (L.). *Journal of Fish Diseases* **21**, 73-75.

CHRISTISON, K.W., Shinn, A.P., van As, J. 2005. *Gyrodactylus thlapi* n. sp. (Monogenea) from *Pseudocrenilabrus philander philander* (Weber) (Cichlidae) in the Okavango Delta, Botswana. *Systematic Parasitology* **60**, 165–173.

CRESPO, J.F., Crespo, R.F. 2003. Monogenean parasites in Mexican fish: a recapitulation. *Técnica Pecuana en México* **41**(2), 175-192.

DAVENPORT, K. E. 1996. Characteristics of the current international trade in ornamental fish, with special reference to the European Union. *Revue Scientifique et Technique de l'Office International des Epizooties* **15**, 435–443.

DAVIDOVA, M., Jarkovsky, J., Matejusova, I., Gelnar, M. 2005. Seasonal occurrence and metrical variability of *Gyrodactylus rhodei* Zitnan 1964 (Monogenea, Gyrodactylidae). *Parasitology Research* **95**, 398–405.

DE MOOR, I.J., Bruton, M.N. 1988. Atlas of alien and translocated indigenous aquatic animals in southern Africa. South African National Scientific Programmes Report No. 144, 1-310 pp.

DOVE, A.D.M. 2000. Richness patterns in the parasite communities of exotic poeciliid fishes. *Parasitology* **120**, 609-623.

DOVE, A.D.M., Ernst, I. 1998. Concurrent invaders –four exotic species of Monogenea now established on exotic freshwater fishes in Australia. *International Journal for Parasitology* **28**, 1755-1764.

ERNST, I., Whittington, I.D., Corneillie, S., Talbot, C. 2005. Effects of temperature, salinity, desiccation, and chemical treatments on egg embryonation and hatching success of *Benedenia seriolae* (Monogenea: Capsalidae), a parasite farmed *Seriola* spp. *Journal of Fish Diseases* **28**, 157-164.

GALLI, P., Stefani, F., Benzoni, F., Zullini, A. 2005. Introduction of alien host–parasite complexes in a natural environment and the symbiota concept. *Hydrobiologia* **548**, 293–299.

GARCIA-VASQUEZ, A., Hansen, H., Christison, K.W., Bron, J.E., Shinn, A.P. 2011. Description of three new species of *Gyrodactylus* von Nordmann, 1832 (Monogenea) parasitising *Oreochromis niloticus niloticus* (L.) and *O. mossambicus* (Peters) (Cichlidae). *Acta Parasitologica* **56** (1): 20-33.

GHEORGHIU, C., Cable, J., Marcogliese, D.J. Scott, M.E. 2007. Effects of waterborne zinc on reproduction, survival and morphometrics of *Gyrodactylus turnbulli* (Monogenea) on guppies (*Poecilia reticulata*). *International Journal for Parasitology* **37**, 375–381.

GILAD, O., Yun, S., Adkison, M.A., Way, K., Willits, N.H., Bercovier, H., Hedrick, R.P. 2003. Molecular comparison of isolates of an emerging fish pathogen, koi herpesvirus, and the effect of water temperature on mortality of experimentally infected koi. *Journal of General Virology* **84**, 2661-266.

HARRIS, P.D. 1993. Les interactions entre la reproduction et la biologie des populations chez les Monogenes Gyrodactylidae: revue. *Bulletin Francais de la Peche et de la Pisciculture* **328**, 47–65.

HARRIS, P.D. 1998. Extreme morphological variation between related individuals of *Gyrodactylus pungitii* Malmberg, 1964 (Monogenea). *Systematic Parasitology* **39**, 137-140.

HARRIS, P.D., Shinn, A.P., Cable, J., Bakke, T.A. 2004. Nominal species of the genus *Gyrodactylus* von Nordmann 1832 (Monogenea: Gyrodactylidae), with a list of principal host species. *Systematic Parasitology* **59**, 1–27.

HILL, B.J. 2005. The need for effective disease control in international aquaculture. *Developmental Biology* **123**, 3-12.

HOFFMAN, L.C., Swart, J.J., Brink, D. 2000. The 1998 production and status of aquaculture in South Africa. *Water SA* **26** (1), 133-136.

IMPSON, N.D. 2007. State of Biodiversity: Western Cape Province. Chapter 3: Freshwater fishes, Western Cape State of Biodiversity 2007. Western Cape Conservation Board, Cape Town ISBN 978-0-620-39289-1.

IMPSON, N.D., Bills, I.R., Cambray, J.A. 2000. State of Biodiversity: Western Cape Province, South Africa.Freshwater Fishes. Western Cape State of Biodiversity 2002. Western Cape Nature Conservation Board, Cape Town. ISBN: 0-620-29893-6.

JACKSON, J.A., Tinsley, R.C. 1998. Effects of temperature on oviposition rate in *Protopolystoma xenopodis* (Monogenea: Polystomatidae). *International Journal for Parasitology* **28**, 309-315.

KING, T.A., Cable, J. 2007. Experimental infections of the monogenean *Gyrodactylus turnbulli* indicate that it is not a strict specialist. *International Journal for Parasitology* **37**, 663-672.

KIR, I., Tekin Ozan, S. 2007. Helminth Infections in common carp, *Cyprinus carpio* L., 1758 (Cyprinidae) from Kovada Lake (Turkey). *Türkiye Parazitoloji Dergisi,* **31** (3), 232-236.

KOEHN, J.D. 2004. Carp (*Cyprinus carpio*) as a powerful invader in Australian waterways. *Freshwater Biology* **49**, 882–894.

LINTERMANS, M. 2004. Human-assisted dispersal of alien freshwater fish in Australia. *New Zealand Journal of Marine and Freshwater Research* **38**, 481–501.

MO, T.A. 1994. Status of *Gyrodactylus salaris* problems and research in Norway. In: *Parasitic Diseases of Fish* (eds. Pike, A. W. & Lewis, J. W.), Samara Publishing Ltd, Dyfed, pp. 43-56.

MOUTON, A., Basson, L., Impson D. 2001. Health status of ornamental fishes imported to South Africa: a pilot study. *Aquarium Sciences and Conservation* **3**, 327-333.

MOYLE, P.B., Light, T. 1996. Biological invasions of freshwater: empirical rules and assembly theory. *Biological Conservation* **78**, 149-161.

MUIR, J. 2005. Managing to harvest? Perspectives on the potential of aquaculture. *Philosophical Transactions of the Royal Society* B: *Biological Sciences* **360** (1453), 191-218.

MURRAY, A.G., Peeler, E.J. 2005. A framework for understanding the potential for emerging diseases in aquaculture. *Preventative Veterinary Medicine* **67**, 223-235.

MYERS, N., Mittermeier, R.A., Mittermeier, C.G., da Fonseca, G.A.B., Kent, J. 2000. Biodiversity hotspots for conservation priorities. *Nature* **403**, 853–858.

NAYLOR, R.L., Goldburg, R.J., Primavera, J.H. Krautsky, N. Beveridge, M.C.M., Clay, J., Folke, C., Lubchenco, J., Mooney, H., Treoll, M. 2000. Effect of aquaculture on world fish supplies. *Nature* **405**, 1017-1024.

OLSTAD, K., Bachmann, L., Bakke, T.A. 2009. Phenotypic plasticity of taxonomic and diagnostic structures in gyrodactylosis-causing flatworms (Monogenea, Platyhelminthes). *Parasitology* **136**, 1305–1315.

PAPERNA, I. 1996. Parasites, infections and disease of fishes in Africa: An update. CIFA Technical Paper No. 31 Food and Agriculture Organization of the United Nations, Rome. 220 pp.

PEELER, E.J., Gardiner, R., Thrush, M.A. 2004. Qualitative risk assessment of routes of transmission of the exotic fish parasite *Gyrodactylus salaris* between river catchments in England and Wales. *Preventive Veterinary Medicine* **64**, 175–189.

PONPORNPISIT, A., Endo, M., Murata, H. 2000. Experimental infections of a ciliate *Tetrahymena pyriformis* on ornamental fish. *Fisheries Sciences* **66**, 1026-1031.

PŘIKRYLOVÁ, I., Matějusova, I., Musilová, N., Gelnar, M. 2009. *Gyrodactylus* species (Monogenea: Gyrodactylidae) on the cichlid fishes of Senegal, with the description of

Gyrodactylus ergensi n. sp. From the Mango tilapia, *Sarotherodon galilaeus* L. (Teleostei: Cichlidae). *Parasitology Research* **106** (1): 1-6.

RAHEL, F.J. 2002. Homogenization of freshwater faunas. *Annual Review of Ecological Systems* **33**, 291–315.

RETIEF, N., Avenant-Oldewage, A., du Preez, H.H. 2007. Ecological aspects of the occurrence of asian tapeworm, *Bothriocephalus acheilognathi* Yamaguti, 1934 infection in the largemouth yellowfish, *Labeobarbus kimberleyensis* (Gilchrist and Thompson, 1913) in the Vaal Dam, South Africa. *Physics and Chemistry of the Earth* **32**, 1384-1390.

SHINN, A.P., Gibson, D.I., Sommerville, C. 2001. Morphometric discrimination of *Gyrodactylus salaris* Malmberg (Monogenea) from species of *Gyrodactylus* parasitising British salmonids using novel parameters. *Journal of Fish Diseases* **24**, 83-97.

SHINN, A.P., Hansen, H., Olstad, K., Bachmann, L., Bakke, T.A. 2004. The use of morphometric characters to discriminate specimens of laboratory-reared and wild populations of *Gyrodactylus salaris* and *G. thymalli* (Monogenea). *Folia Parasitologica* **51**, 239-252.

SKELTON, P. 2001. A Complete Guide to the Freshwater Fishes of Southern Africa. Struik Publishers, Cape Town, South Africa, pp 1- 395.

SKELTON, P.H., Cambray, J.A., Lombard, A., Benn, G.A. 1995. Patterns of distribution and conservation status of freshwater fishes in South Africa. *South African Journal of Zoology* **30**, 71-81.

SIMKOVA, A.S., Verneau, O., Gelnar, M., Morand, S. 2006. Specificity and specialization of congeneric monogeneans parasitizing cyprinid fish. *Evolution* **60** (5), 1023–1037.

TEKIN OZAN, S., Kir, I., Barlas, M. 2008. Helminth parasites of common carp (*Cyprinus carpio* L., 1758) in Beyşehir Lake and population dynamics related to month and host Size. *Turkish Journal of Fisheries and Aquatic Sciences* **8**, 201-205.

THONEY, D.A., Hargis, W.J. 1991. Monogenea (Platyhelminthes) as hazards for fish in confinement. *Annual Review of Fish Diseases* **1**, 133-153.

TIDWELL, J.H., Allan, G.L.A. 2001. Fish as food: aquaculture's contribution: Ecological and economic impacts and contributions of fish farming and capture fisheries. *European Molecular Organisation Reports* **2** (11), 958–963.

TUBBS, L.A., Poortenaar, C.W., Sewell, M.A., Diggles, B.K. 2005. Effects of temperature on fecundity in *in vitro*, egg hatching and reproductive development of *Benedenia seriolae* (Monogenea) parasitic on yellowtail kingfish *Seriola lalandi*. *International Journal for Parasitology* **35**, 315-327.

WHITTINGTON, I.D., Cribb, B.W., Hamwood, T.E., Halliday, J.A. 2000. Host-specificity of monogenean (Platyhelminth) parasites: a role for anterior adhesive areas. *International Journal of Parasitology* **30** (3), 305-320.

WYNBERG**,** R. 2002. A decade of biodiversity conservation and use in South Africa: tracking progress from the Rio earth summit to the Johannesburg world summit on sustainable development. *South African Journal of Science* **98**, 233-243.

XU, D., Shoemaker, C.A., Klesius, P.H. 2007. Evaluation of the link between gyrodactylosis and streptococcosis of Nile tilapia, *Oreochromis niloticus* (L.). *Journal of Fish Diseases* **30**, 233–238.

ZIETARA, M.S., Lumme, J. 2003. The crossroads of molecular, typological and biological species concepts: two new species of *Gyrodactylus* Nordmann, 1832 (Monogenea: Gyrodactylidae). *Systematic Parasitology* **55**, 39–52.

Chapter 2

First records of *Gyrodactylus kherulensis* Ergens 1974 from *Cyprinus carpio koi* L. and of *Gyrodactylus kobayashii* Hukuda, 1940 from *Carassius auratus* L. in South Africa: A morphological comparison of populations of various geographic origins imported into South Africa

Abstract

Gyrodactylus kherulensis Ergens, 1974 and *G. kobayashii* Hukuda, 1940 are reported for the first time from *Cyprinus carpio koi* L. (koi carp) and *Carassius auratus* L. (goldfish) respectively imported to South Africa from Asia and Europe and were also present on South African bred fish. A comprehensive morphological analysis was undertaken to compare different populations of *G. kherulensis* and *G. kobayashii* from various localities. Intraspecific variation of the hamulus measurements from different locations were evident in both species. Univariate analysis (ANOVA and Kruskal-Wallis tests) showed that a German population of *G. kherulensis* had significantly larger overall dimensions, particularly having larger hamuli compared to collections from Asia and the other European populations. The *G. kobayashii* population collected from South African bred goldfish (Population 1) had significantly larger hamuli and ventral bar characters when compared to the remaining populations of *G. kobayashii* in this study, except Population 6 from Japan. The marginal hooklets are the most reliable characters to place the different populations of *G. kherulensis* and *G. kobayashii* within their respective groups.

Introduction

The cyprinids, *Cyprinus carpio* L. and *Carassius auratus* L. and their subspecies are widespread ornamental fish that have achieved cosmopolitan distribution, and have encroached upon a wide range of freshwater environments worldwide, including the natural aquatic habitats in South African river systems (Skelton 2001; Kir and Tekin Ozan 2007). Their worldwide propagation has led to the international spread of their parasites, including unidentified species of the notorious monogenean genus *Gyrodactylus* into South Africa (De Moor and Bruton 1988'; Mouton *et al.* 2001). Aquatic parasites with a direct life-cycle have a predisposition to colonise or invade new regions, since they do not require intermediate hosts (Hayward *et al*. 2001). Members of the genus *Gyrodactylus* have direct life-cycles, plus they exhibit unique reproductive and survival strategies, which have greatly contributed to the invasive success of some members of the genus (Cable and Harris 2002; Bakke *et al.* 2007). *Gyrodactylus kherulensis* Ergens, 1974 and *G. kobayashii* Hukuda, 1940 are common parasites of koi and goldfish respectively, and have been widely propagated along with their commonly-traded hosts.

Ergens (1974) described *Gyrodactylus kherulensis* from the skin, gills, fins and nasal cavities of *Cyprinus carpio haematopterus* L. in the River Kherulen, Mongolia. Based on a single specimen, *Gyrodactylus kherulensis* was initially thought to be a synonym of *G. osablahensis*. After numerous specimens of *G. kherulensis* were analysed morphologically, the two species were discriminated by the shapes of the ventral bar membrane, the hamuli and the dorsal bar (Ergens 1974). The shapes of the ventral bar, marginal hooklets and dorsal bar of *G. kherulensis* are, however, described as being similar to those of *G. stankovici* Ergens, 1970 also a parasite of carp (Ergens 1974). *Gyrodactylus kherulensis* is one of 17 *Gyrodactylus* species recorded from fish of the genus *Cyprinus* (Harris *et al.* 2004). The

primary hosts of *G. kherulensis* include common carp, *C. carpio* and its subspecies, and this parasite has not been documented from any other host genus (Harris *et al*. 2004).

Gyrodactylus kherulensis was documented on the fins and gills of cultured carp (*Cyprinus carpio*) in Japan, the first incidence of the parasite in that region (Ogawa and Egusa 1978). Subsequently, it was later recorded in Hokkaido, Japan from cultured carp (Ogawa 1994). *Gyrodactylus kherulensis* was listed as an introduced species in Iraq in 1988, where it was found on the gills of common carp (Ali *et al*. 1988). Published records indicate that *G. kherulensis* first entered Europe was in 1987 from *C. carpio* (Lux 1987; Lux 1990). The species was later reported from the Czech Republic and Slovakia, where it is listed as an alien parasite species (Šefrová and Laštůvka 2005). *Gyrodactylus kherulensis* is also documented as a parasite in Russia (Blanc 2001) and is reported from carp in North America (Hoffman 1998).

Gyrodactylus kobayashii was initially described in 1940 from the body surfaces and fins of cultured Korean goldfish (*Carassius auratus*) (Ergens and Ogawa 1978). The original description included measurements of the attachment organs and whole body measurements; but the holotype was destroyed in World War II. The measurements of the various attachment organs in the original description did not coincide with the measurements of the original drawings (Ergens and Ogawa 1978). *Gyrodactylus kobayashii* was then re-described by Ergens and Ogawa (1978) who collected additional specimens and measured them, and also re-measured Hukuda's drawings to standardize the measurements. The re-measured drawings closely resembled and were in the same range as the newly collected specimens (Ergens and Ogawa 1978). According to Ergens and Ogawa (1978), *G. kobayashii* resembles *G. elegans yamagutii* Yin et Sproston, 1948 morphologically, and measurements of the opisthaptoral characters were similar, therefore the authors suggested that they were the same

species. *Gyrodactylus elegans yamagutii* is now regarded junior synonym of *G. kobayashii* (Harris *et al.* 2004).

Gyrodactylus kobayashii is one of 9 *Gyrodactylus* species found on members of the genus *Carassius* and has also been found on the minnow, *Leuciscus walewskii* Dybowski. *Gyrodactylus kobayashii* is an alien species in Australia, and was the first exotic monogenean species to be recorded on that continent (Fletcher and Whittington 1998). These parasites were also introduced to the Czech Republic and Slovakia (Šefrová and Laštůvka 2005), the United Kingdom (Cable *et al*. 1999), North America (Hoffman 1998) and Iran (Jalali *et al.* 2005), transported on cultured goldfish.

The published distribution records of *G. kherulensis* and *G. kobayashii,* together with the geographical range of their hosts, are indicative of a broad distribution range from Northern Europe and North America to Australia.

Identification and taxonomy of *Gyrodactylus* species is primarily based on the morphology of the posterior attachment organs, namely the marginal hooklets, hamuli, and ventral and dorsal bars (Olstad *et al.* 2009). The opisthaptoral characters also vary quite considerably in size due to changes in water temperature, host, geographic locality, life history, microhabitat and age (Mo 1991; Appleby 1996). As a consequence of size variation, new species have been identified which only somewhat differ morphologically from the original species, leading to subsequent synonymy (Appleby 1996). Analyses including larger samples sizes of populations exposed to varying environmental conditions will contain specimens at the extremes of variation among the species (Dmitrieva and Dimitrov 2002). However, the inclusion of additional morphometric measurement parameters, together with statistical classification methods has proven useful to separate species (Shinn *et al*. 2001).

Generally, variations among members of the same species of *Gyrodactylus* tend to be very small and the basic shape and haptoral organ dimensions are usually similar (Harris 1998a). However, some gyrodactylids exhibit considerable morphological intraspecific differences, such as in the case of *G. pungitii* Malmberg, 1964 where major differences were illustrated in the shape of the hamuli, and the specimens was initially presumed to be a different species (Harris 1998a). Another gyrodactylid species that shows major phenotypic variations is *G. arcuatus* Bychowsky, 1933 from *Gasterosteus aculeatus* L., 1758 and different morphotypes of the same species from different hosts are presumed (Geets *et al.* 1999). Intraspecific variation of the opisthaptoral character dimensions are observed from a number of gyrodactylid species from the same population and the same exposure to environmental conditions (Harris 1998a).

Water temperature has been shown to affect the size of the opisthaptoral characters in *Gyrodactylus* species (Dmitrieva and Dimitrov 2002; Dávidova *et al*. 2005). Hamuli are generally larger when the water temperature is cooler, and reduced in size during warmer months. This is evident in *G. rhodei* Zitnan, 1964 populations sampled seasonally (Dávidova *et al.* 2005). The hamuli and ventral bars of gyrodactylids are most sensitive to macro- and micro-environmental change, while the marginal hooklets are more stable characters (Dmitrieva and Dimitrov 2002). The variation in size of the hamulus variables can be attributable to ontogenetic development and the differences in the commencement of organ development, with the marginal hooklets developing first and attaining their absolute size prior to birth (Dmitrieva and Dimitrov 2002). The shape and size of the marginal hooklets are thus the most taxonomically reliable or stable discriminatory characters separating and grouping members within the genus *Gyrodactylus* (Shinn *et al.* 2001).

The use of univariate and multivariate statistics to detect variation among closely-related species of *Gyrodactylus* has proven very useful (*e.g.* Geets *et al*. 1999; McHugh *et al*. 2000; Shinn *et al*. 2001; Shinn *et al*. 2004). Statistical classification is also useful to distinguish different populations of the same species, an example of this being the differences found among *G. cichlidarum* Paperna, 1968 infecting Nile tilapia, *Oreochromis niloticus niloticus* L. and Mozambique tilapia, *O. mossambicus* Peters, 1852 (Cichlidae) from various geographic origins and from different host species (Garcia-Vasquez *et al*. 2010). Based on similar rationale, this study aims to test whether multivariate analysis of opisthaptoral characters can be used to discriminate between populations of *G. kherulensis* and *G. kobayashii*.

The accurate identification of parasites is of particular importance in aquaculture, as the biology of the species sheds some light on species-specific parasite management protocols that are essential to effectively eradicate these parasites (Hayward *et al*. 2007). Parasite identification plays an even greater role in biosecurity, and the identification of potentially pathogenic parasites to both aquaculture and to local freshwater fish biodiversity, and their propagation into areas where they may have a pathogenic effect (Hayward *et al*. 2007).

The study therefore aims to provide the first species-level identification and detailed morphological description of the exotic *G. kherulensis* from koi carp and *G. kobayashii* from goldfish imported into South Africa. The study intends to test the hypothesis that both the hamuli and ventral bar characters show a greater degree of variation due to the effects of various environmental parameters expressed from different geographical localities to discriminate populations of the same species, however the marginal hooklet dimensions remain relatively constant between populations, serving as confirmation that the different

populations used in the study represent the same species. This hypothesis will be tested by morphologically comparing different populations of *G. kherulensis* and *G. kobayashii* from various geographic origins with the use of univariate and multivariate analyses of the measurement of the opisthaptoral characters.

Materials and Methods

Morphometric diagnoses

Koi carp and goldfish were purchased from local breeders, importers, and retailers in the Cape Town metropolitan area and the country of origin was established for each population. Fish were imported from various locations, but primarily from Asian countries. The fish were therefore exposed to the varying conditions of the different stores or breeders and some fish were held for a few days, while others were distributed for retail immediately. The fish were euthanized with a lethal dosage of the anaesthetic 2-phenoxyethanol solution, and the parasites on the skin were removed and quantified. The fins and the gills were dissected and examined for the presence of *Gyrodactylus* species. Whole worms were preserved in 70% ethanol. For identification purposes, a maximum of 20 whole specimens per population were mounted in ammonium picrate glycerine (APG) for gross morphological analysis. The opisthaptors of 20 additional worms were cut off with a clean, sharp scalpel and the corresponding bodies were placed in 0.5 ml tubes in absolute ethanol for further molecular analyses (not included in this study). The proteolytic enzyme, Proteinase K, was used to remove excess tissue from the opisthaptor, which was subsequently mounted in glycerine ammonium picrate (Harris *et al*. 1999). Gross morphological variables were viewed at 1000x magnification using oil immersion and the opisthaptoral characters, including the various measurement parameters of the hamuli, ventral bar, marginal hooklet and dorsal bar, were measured. A total of 25 point to point measurements of the opisthaptoral variables were

made per individual (Fig. 1), according to Shinn *et al*. (2004). Measurements of different populations of *G. kherulensis* and *G. kobayashii* imported from various Asian and European countries are shown in Tables 1 and 5. The various populations from the same country of origin were collected from different retailers at different occasions. The soft body parts, which include the body length and width, were excluded from this analysis, because these measurements showed considerable variation due to various levels of contraction due to fixation and cover slip pressure.

Statistical analyses

A total of 126 specimens of *G. kherulensis* and 122 specimens of *G. kobayashii* were used for morphological analyses. Statistical analyses of the morphometric measurements of the *G. kherulensis* and *G. kobayashii* were done according to methods illustrated in Shinn *et al*. (2001), where univariate and multivariate analyses were performed to compare different populations of the same *Gyrodactylus* species from different geographic origins. The data from the various populations of the same geographic origin were collected at different times, from different traders and were therefore grouped according to country of origin, but numbered according to the different population. Raw measurement data were used for all measurements and cosine transformation was applied to all angle measurements to express these as linear functions (Shinn *et al.* 2001). All measurement data were analysed using the statistical package STATISTICA 8.0 © (StatSoft, Inc., 2007). The data were tested for normality and homoscedacisity using Levene's test of homogeneity.

Univariate analysis

Parametric data were analysed using a one-way analysis of variance (ANOVA) *post hoc* Tukey's honest significant difference (HSD) test for unequal sample sizes, while non-

parametric data were analysed using Kruskal-Wallis *post hoc* test for multiple comparisons of independent variables.

Multivariate analysis

Multivariate analyses were performed using Principal Components Analysis (PCA) to group the different populations and compare their positions in morphological space. Specimens with missing data were removed from the analysis. Principal components analysis (PCA) was performed on certain variables, and those having a high CV value were not included in the multivariate anaysis and were excluded measurements due to lack of repeatability and accuracy which result in a greater CV value. Analyses were performed separately on the hamuli, ventral bar, and marginal hooklet measurement data from all populations of the two species studied. Dorsal bar measurements were not analysed.

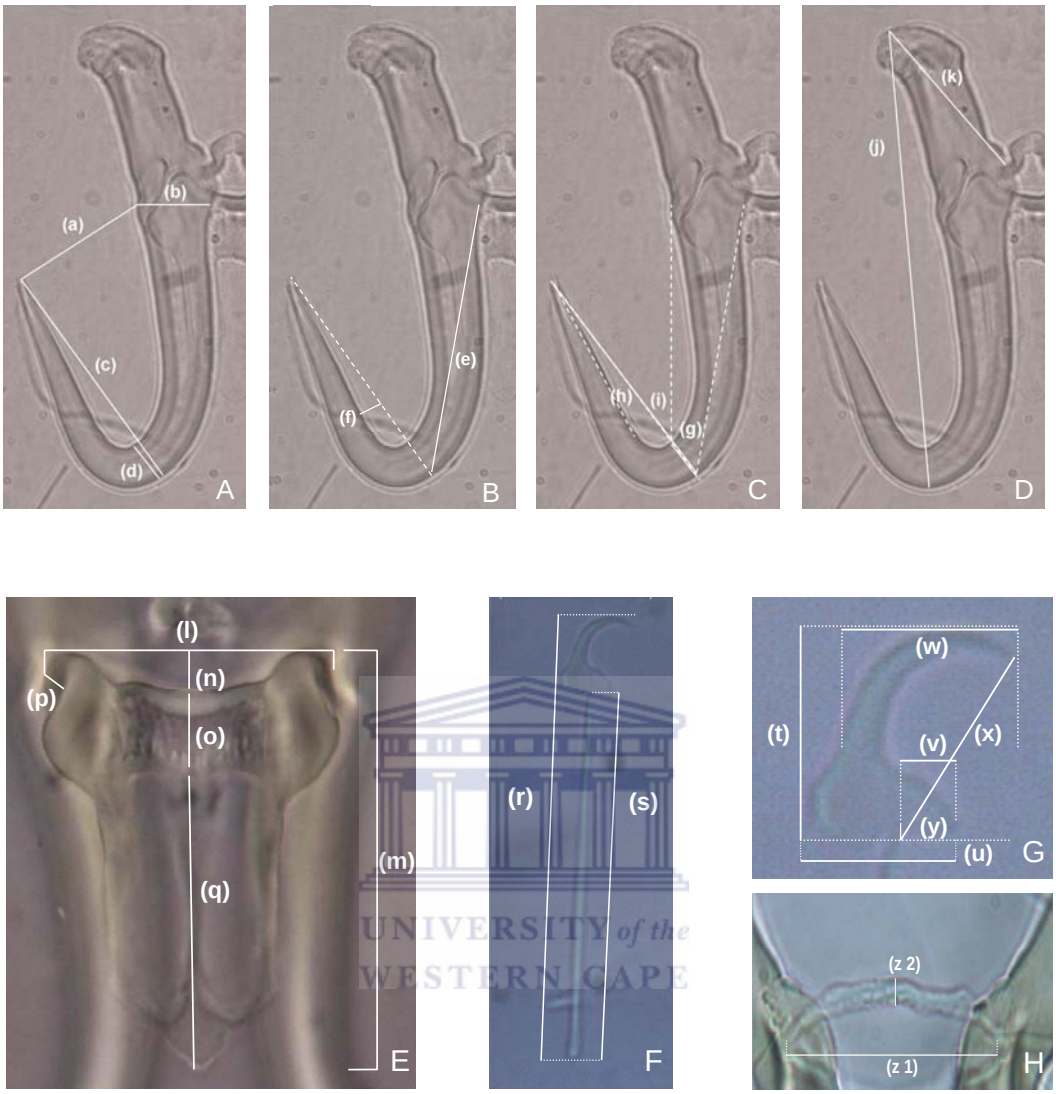


Figure 1 Morphometric measurements of the opisthaptoral characters of *Gyrodactylus* species used in the study. (A, B, C and D) Hamulus measurements: **a**- hamulus aperture, **b**- proximal shaft width, **c**- point length, **d**- distal shaft width, **e**- shaft length, **f**- inner curve length, **g**- aperture angle, **h**- inner curve angle, **i**- inner aperture angle **j**- root length, **k**- total length (E) Ventral bar measurements: **l**- total width, **m**- total length, **n**- process to mid length, **o**- median length, **p**- process, length, **q**- membrane length. (F and G) Marginal hooklet measurements **r**- total length, **s**- shaft length, **t**- sickle length, **u**- sickle proximal width, **v**- sickle toe length, **w**- sickle distal length, **x**- sickle aperture, **y**- sickle instep arch height and (H) Dorsal bar measurements: **z1**- dorsal bar length, **z2**- dorsal bar width

Results

The shape and measurements of the hamuli and marginal hooklets of *G. kherulensis* and *G. kobayashii* populations from different geographic locations were morphologically similar to published records and the majority of the measurements were well within the ranges reported for the original descriptions (Tables 1 and 4). Line drawings and photomicrographs of *G. kherulensis* are illustrated in Figure 2 and *G. kobayashii* are illustrated in Figure 4. The mean, standard deviation, range and CV (Coefficient of Variance) for the different *G. kherulensis* populations are shown in Table 1 and in Table 4 for *G. kobayashii*.

The CV value is the relative percentage of variation of a particular variable within a population (Shinn *et al.* 2001). High CV values could be due to inaccurate measurements, particularly variables which are inclined to distortion (Shinn *et al.* 2001). In order for relatively accurate measurements to be taken, the measurement must be repeatable and should not be left to the understanding of the researcher alone, such as in the case of the hamulus inner curve angle, where the researcher may use different areas as a point of reference (Du Preez and Maritz 2006). Measuring small structures using the software was limiting, thereby increasing the variance for each parameter measured. The greatest CV value for the ventral bar measurements were for the ventral bar process length and ventral bar process to mid length. The marginal hooklet toe length and sickle distal width also showed some degree of variability. This variability can primarily be as result of measurement error, particularly of the smallest measurements. The marginal hooklet instep / arch height has proven to be an unreliable discriminatory measurement and the CV values for these in all populations were very high. Accordingly, hamulus inner curve length, ventral bar process to mid length, ventral bar process length, marginal hooklet sickle distal width, marginal hooklet

toe length and the marginal hooklet arch height were not included in subsequent analysis due to their high variability, which may negatively skew the analysis.

Morphological diagnosis of populations of Gyrodactylus kherulensis

***Gyrodactylus kherulensis* Ergens, 1974**

Host: *Cyprinus carpio koi* L. (current study)

Type-host: *Cyprinus carpio haemopterus* L.

Site: Gills, skin, and fins

Locality: Kuilsriver, South Africa. $33^{0}54'06.04''S$ $18^{0}42'26.98''$ E

Voucher material field collection: K1AQT1GY1; K1AQT1GY2; K1AQT1GY3; K1AQT1GY4; DHKAQT1GY1; DHKAQT1GY2; DHKAQT1GY3; DHKAQT1GY4; DHKAQT1GY5; DHKAQT1GY5 (All specimens have been deposited in the collection of Dr. K.W. Christison at the University of the Western Cape).

Description:

Body length 364±36.1 (316-401) long and 82±18.7 (65-113) wide at the uterus, opisthaptor 86±12.8 (69-103) long and 84±18.6 (63-114) wide. Male copulatory organ (MCO) diameter is 13±5.0 (9-16); with one apical spine and 6 marginal spines. Hamulus aperture 23±1.5 (20-24) proximal shaft width 9±1.2 (6-11.3); hamulus point 34±4.7 (23-40) long; distal shaft width 6±0.6 (5-7); shaft 41±3.7 (34-46) long; inner curve length 5±1.1 (3-6); outer aperture angle 34±4.8 (23-43); point curve angle 15±5.3 (8-26); inner aperture angle 40±3.7 (36-50); root length 28±3.4 (21-33); hamulus total length 74±4.0 (67-82). Ventral bar total width 24±1.8 (21-27) and 35±3.6 (28-40) long; ventral bar process to mid length 3±0.6 (2-4); ventral bar median length 7±0.6 (6-8); ventral bar process 2±0.5 (1-3) long; ventral bar membrane 25±2.5 (20-28) long. Marginal hooklet total length 28±2.3 (24-31); marginal hook

shaft 23±2.5 (18-26) long; sickle 5.7±0.4 (5.1-6.4) long; sickle proximal width 4±0.4 (4-5); toe length 2±0.3 (1-2); sickle distal width 4±0.3 (3-4); sickle aperture 5±0.4 (4-6); instep / arch height 1±0.1 (1-1). Dorsal Bar 15±3.3 (11-20) long, and 2±0.3 (2-3) wide.

Remarks:

The hamuli of *Gyrodactylus kherulensis* were large and robust in form. The roots of the hamuli protruded outward. The ventral bars were long and tapered toward the tip, giving these characters a triangular shape at the base. It had two grooves in the mid sections of the membranes. The ventral bars had short processes which were inconspicuous in some cases. The dorsal bars had a median notch; however, this was only visible in proteinase K digested specimens and not in whole-mounted specimens. The marginal hooklets were small, the heels were circular, and the toes were triangular. All the populations from different suppliers were morphologically similar in the shape of the haptoral organs. Although the different populations of *G. kherulensis* were morphologically similar, there was some intraspecific variation in the sizes of the overall dimensions, and some populations appeared larger than others. The overall measurements of *G. kherulensis* from South African bred koi, and those from Asian origins, had similar morphometry with minimal mean size variations of the various characters of the opisthaptoral complex. The European populations of *G. kherulensis* from Scotland and Germany, however, were larger than the South African bred and Asian imported specimens. The German population of *G. kherulensis* had larger hamuli variables and the greatest hamulus total length, hamulus aperture, and hamulus point length, shaft length and root length. The ventral bar total width, ventral bar total length and membrane length were also larger in German populations.

Table 1: Morphometric measurements in µm (micrometers) of *Gyrodactylus kherulensis* on the skin and fins from koi imported into South Africa via live fish trade showing the mean the standard deviation and the range in parenthesis. The coefficient of variance expressed as a percentage for the hamuli, ventral bar and marginal hooklet measurements are shown

Country of Origin	**Population 1** (Current study) Kuilsriver, South Africa	**Population 2** (Current study Durban, South Africa	**Population 3** (Current study) Malaysia	**Population 4** (Current study) Malaysia	**Population 5** (Current study) Malaysia
Measurement	**Mean ± S.D.(range) n=10**	**Mean ± S.D.(range) n=20**	**Mean±S.D (range) n=10**	**Mean ± S.D.(range) n=20**	**Mean ± S.D.(range) n=6**
Hamulus					
Aperture (Hapert)	23±1.5 (20-24) 6.8%	21±2.4 (18-26) 11.5%	21± 1.7 (18-23) 8.1%	21±1.5 (18-23) 7.0%	19±2.4 (16-23) 12.8%
Proximal shaft width (HPrSW)	9±1.4 (6-11) 15.6%	9±1.2 (6-11) 13.2%	9± 0.4 (8-10) 5.0%	8± 0.7 (7-9) 8.1%	9±0.8 (8-10) 9.1%
Point length (HPL)	34±4.7 (23-40) 13.7%	33±4.8 (22-40) 14.9%	33± 1.9 (29-35) 5.8%	32±3.6 (24-37) 11.5%	31±5.3 (21-37) 14.3%
Distal shaft width (HDSW)	6.0±0.6 (5-7) 9.8%	6±0.7 (5-7) 11.6%	6± 0.5 (5-7) 7.8%	6±0.6 (5-7) 10.4%	7±0.7 (6-8) 10.2%
Shaft length (HSL)	4±3.7 (34-46) 9.0%	39±4.5 (29-45) 11.8%	38± 1.6 (35-40) 4.3%	39±3.9 (32-45) 9.9%	37±4.1 (26-42) 11.4%
Inner curve length (HICL)	5±1.1 (3-6) 24.5%	5±0.8 (3-6) 16.0%	4.0± 1.2 (2-6) 31.0%	4±0.7 (2-5) 18.2%	5±1.0 (4-6) 21.9%
Outer aperture angle (HAA)	34±4.8 (23-43) 14.1%	34±3.3 (30-43) 9.8%	33± 2.3 (29-37) 7.2%	35±2.3 (31-40) 6.6%	31±3.8 (24-36) 12.3%
Point curve angle (HPCA)	15±5.3 (8-26) 35.4%	17± 5.5 (9-27) 32.2%	12± 4.6 (8-23) 38.0%	14±6.3 (7-28) 43.7%	19±5.5 (13-27) 29.2%
Inner aperture angle (HICO)	40±3.7 (36-50) 9.1%	39±4.1 (34-53) 10.2%	38.3± 3.2 (33.0-43.5) 8.3%	39±2.7 (34-48) 6.9%	38±5.1 (30-45) 13.6%
Root length (HRL)	28±3.4 (21-33) 12.2%	28±4.9 (19-37) 17.8%	23.9± 2.5 (21.0-28.5) 10.3%	27±4.6 (18-33) 16.9%	26±4.0 (20-31) 15.4%
Total length (HTL)	74±4.0 (70-82) 5.4%	72±6.0 (60-82) 8.3%	64.0± 3.2 (60.1-68.5) 5.0%	67±5.6 (56-77) 8.3%	70±5.1 (64-80) 7.4%
Ventral bar					
Total width (VBTW)	24±1.8 (21-27) 7.6%	23±1.5 (20-26) 6.7%	22± 1.3 (20-24) 5.7%	24±2.2 (19-28) 9.3%	24±2.3 (22-28) 9.3%
Total length (VBTL)	35±3.6 (28-40) 9.6%	35±3.7 (29-41) 10.5%	33± 3.0 (29-37) 9.1%	35±3.7 (28-41) 10.4%	36±3.6 (31-42) 10.1%
Process to mid length (VBPML)	3±0.6 (2-4) 18.9%	3±0.5 (3-4) 15.0%	3± 0.6 (3-4) 18.6%	3±0.8 (2-6) 24.2%	3±0.5 (3-4) 16.4%
Median length (VBML)	7±0.6 (6-8) 8.3%	7±1.0 (5-9) 15.6%	7± 0.6 (6-8) 8.4%	7±0.9 (5-9) 14.2%	7±0.8 (6-8) 11.4%
Process length (VBProL)	2±0.5 (1-3) 30.5%	2±0.4 (1-3) 19.8%	2± 0.5 (1-3) 26.9%	2±0.4 (1-3) 26.0%	2±0.3 (1-2) 17.0%
Membrane length (VBMemL)	25±2.5 (20-28) 9.3%	26±2.8 (21-31) 10.7%	22± 2.8 (19-26) 12.6%	26±3.2 (19-30) 12.5%	25±2.9 (21-30) 11.5%
Marginal hooklets					
Total length (MHTL)	28±2.3 (24-31) 8.0%	27±1.6 (24-31) 6.5%	27±1.0 (25-28) 3.5%	28±1.2 (26-30) 3.5%	27±1.5 (24-28) 5.5%
Shaft length (MHSL)	23±2.5 (18-30) 10.7%	22±1.4 (19-25) 7.6%	22±1.3 (20-23) 5.7%	22±1.2 (21-24) 5.7%	22±1.7 (119-23) 7.6%

Sickle length (MHSickL)	6±0.5 (6-7) 7.6%	6±0.4 (5-6) 6.3%	6±0.4 (5-6) 6.7%	6±0.5 (5-6.) 6.7%	6±0.4 (5-6) 7.7%
Sickle proximal width (MHPW)	4±0.4 (4-5) 8.7%	4±0.7 (4-6) 19.5%	4±0.7 (3-5) 15.9%	4±0.4 (4-5) 8.5%	4±0.2 (3-4) 6.3%
Sickle toe length MHToeL)	2±0.3 (1-2) 17.3%	2±0.2 (1-2) 13.2%	2±0.3 (2-2) 15.6%	2±0.3 (1-2) 15.9%	2±0.3 (1-2) 16.8%
Sickle distal width (MHDW)	4±0.3 (3-4) 8.1%	4±0.4 (3-4) 9.9%	4±0.4 (3-5) 10.3%	4±0.3 (3-4) 8.3%	4±0.6 (3-5) 16.1%
Sickle aperture (MHAp)	5±0.4 (4-6) 8.6%	5±0.4 (4-6) 8.3%	5±0.3 (4-5) 6.7%	4±0.5 (4-5) 10.1%	5±0.3 (4-5) 6.6%
Sickle instep / arch height (MHIn)	1±0.1 (1-1) 18.9%	1±0.3 (0-2) 33.5%	1± 0.1 (1-1) 14.2%	1±0.1 (1-1) 14.9%	1±0.1 (1-1) 14.3%
Dorsal bar					
Length (DBL)	15±3.3 (11-20)	13±3.0 (7-20)	14± 2.4 (10-18)	14±3.0 (7-17)	15±3.3 (11-19)
Width (DBW)	2±0.3 (2-3)	2±0.4 (2-3)	3± 0.4 (2-4)	3±0.3 (2-3)	3±0.3 (2-3)

Table 1 continued

Country of Origin	**Population 6** (Current study) Japan	**Ogawa & Egusa (1978)** Japan	**Ergens (1974)** Mongolia	**Population 7** (Current study) Scotland	**Population 8** (Current study) Germany
Measurement	**Mean ± S.D.(range) n=20**			**Mean±S.D (range) n=30**	**Mean±S.D (range) n=10**
Hamulus					
Aperture	21±1.3 (19-24) 6.2%			23±1.5 (21-28) 6.6%	25±3.9 (20-32) 15.2%
Proximal shaft width	9±0.7 (8-10) 7.8%			10±1.0 (8-12) 10.5%	11±1.1 (9-12) 10.3%
Hamulus point length	34±1.5 (31-37) 4.4%	36 (31-40)	33 (29-33)	36±1.3 (32-38) 3.6%	40±2.8 (35-44) 6.9%
Distal shaft width	6±0.5 (5-7) 8.0%			5±0.3 (5-6) 6.4%	7±1.0 (5-8) 14.6%
Shaft length	40±1.5 (36-42) 3.9%	53 (46-59)	56 (45-57)	43±2.3 (37-47) 5.3%	47±4.0 (43-54) 8.5%
Inner curve length	4±0.4 (3-4) 11.0%			4±0.9 (2-5) 24.7%	5±3.0 (2-12) 62.4%
Outer aperture angle	32±1.4 (29-35) 4.5%			34±1.8 (30-37) 5.4%	32±3.8 (27-38) 11.7%
Point curve angle	10±2.2 (6-15) 22.4%			8±2.4 (4-14) 28.1%	10±2.6 (5-13) 26.7%
Inner aperture angle	37±1.4 (34-39) 3.8%			38±2.2 (34-43) 5.9%	36±11.3 (5-44) 31.9%
Root length	27±2.9 (21-32) 10.7%	31 (22-40)	30 (19-30)	26±2.1 (21-30) 8.2%	37±4.5 (31-44) 12.2%
Total length	66±3.6 (60-72) 5.4%	77 (64-91)	70 (61-77)	71±3.4 (65-78) 4.8%	86±5.3 (78-95) 6.2%
Ventral bar					
Total width	23±1.6 (21-26) 6.8%	24 (20-29)		24±1.9 (21-29) 7.9%	28±2.3 (24-31) 8.1%
Total Length	34±2.1 (30-37) 6.2%			34±2.5 (29-39) 7.4%	42±2.6 (37-46) 6.1%
Process to mid length	3±0.7 (2-5) 19.5%			3±0.9 (2-5) 25.2%	4±0.7 (2-5) 20.0%
Median length	7±0.9 (5-9) 13.1%	6.5-9		7±1.3 (5-10) 17.8%	8±1.2 (7-10) 14.7%
Process length	2±0.4 (1-2) 24.1%	0.5-1.5		1±0.5 (1-3) 38.0%	2±0.4 (1-3) 17.1%
Membrane length.	24±1.9 (20-28) 8.1%	18-33	19-26	24±1.8 (21-27) 7.5%	30±2.1 (27-33) 7.2%
Marginal hooklet					
Total length	27±0.9 (26-28) 3.9%	27-30	31 (26-31)	28±6.1 (26-32) 4.7%	29±2.0 (24-31) 6.9%
Shaft length	22±1.0 (21-24) 5.2%	22-25	7 (6-7)	24±4.5 (20-27) 4.5%	23±1.8 (20-26) 7.6%
Sickle length	6±0.3 (5-6) 5.8%	5.5-6		6±0.3 (5-6) 4.9%	6±0.7 (4-7) 11.5%
Sickle proximal width	4±0.5 (4-5) 10.1%	4-5		4±0.2 (4-5) 4.9%	5±0.6 (4-6) 12.2%
Sickle toe length	2±0.3 (1-3) 15.4%			2±0.2 (2-3) 9.2%	2±0.2 (1-2) 12..8%

Sickle distal width	4±0.4 (3-4) 11.7%	4.5-5.5		4±0.3 (4-5) 7.2%	4±0.7 (3-5) 17.9%
Sickle aperture	4±0.4 (4-5) 11.3%			5±0.5 (5-5) 3.9%	5±0.4 (4-5) 7.8%
Sickle instep / arch height	1±0.2 (0.8-1.3) 18.2%			1±0.1 (0-1) 21.0%	1±0.2 (1-1) 18.8%
Dorsal bar					
Length	13±2.4 (8-16)	18-31	17 (10-17)		15 ±3.6 (12-21)
Width	2±0.3 (2-3)	0.5	3 (3)		2±0.5 (2-4)

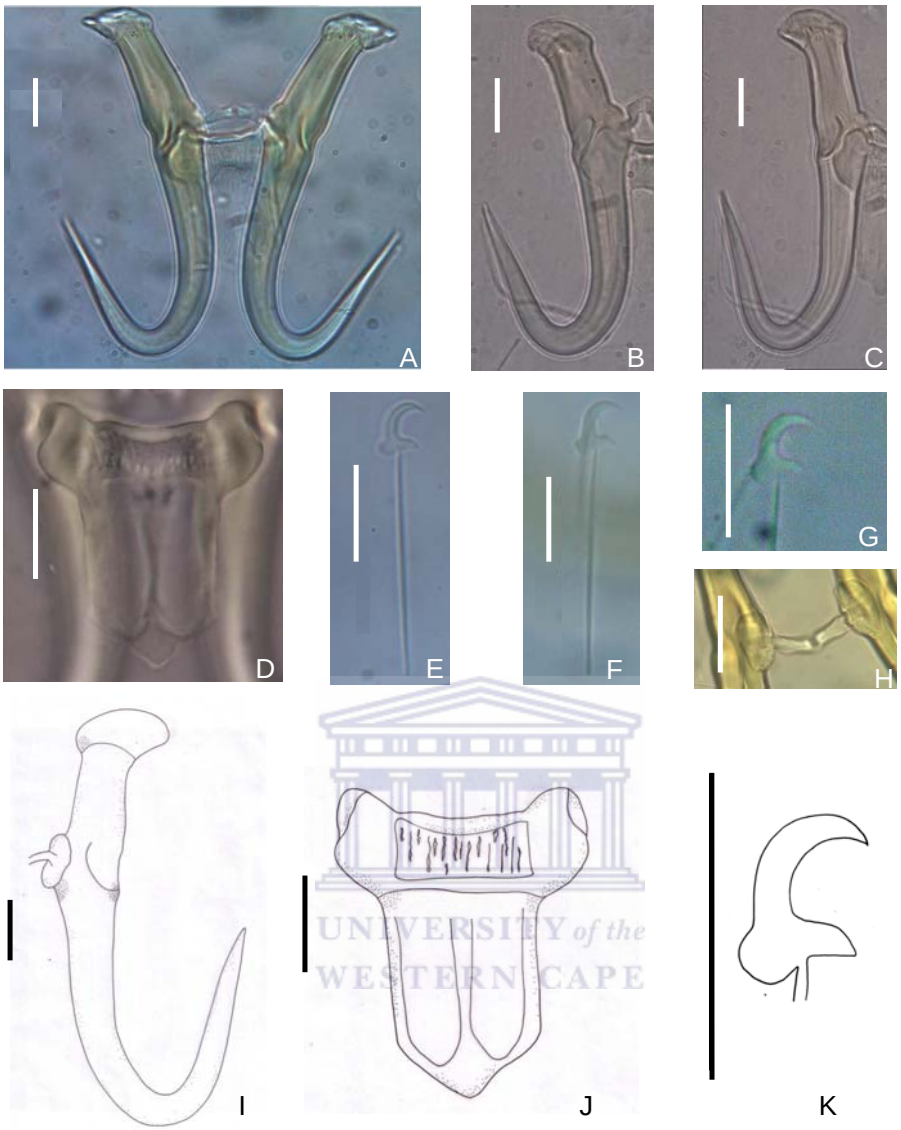


Figure 2 Photo-micrographs and line drawings of the opisthaptoral characters of *G. kherulensis* illustrating the central hook complex of Population 1 from South Africa (A), the hamulus (B) and (C) both from Population 4 from Japan. The ventral bar of Population 4 from Japan (D) and (E) is the marginal hooklet of Population 1 from South Africa, and the marginal hooklet (F), marginal hooklet sickle (G) and dorsal bar (H) all from the Population 2 from South Africa. The line drawing (I), (J) and (K) are the hamulus, ventral bar and marginal hooklet of Population 1, Population 2 and Population 1 respectively. Scale bar = 10 µm

Relatively low CV percentages were recorded for the hamuli measurements for *G. kherulensis* (Table 1). The CV percentages were greatest for hamulus inner curve length, ventral bar process to mid length, ventral bar process length, marginal hooklet sickle toe length and marginal hooklet instep /arch height across all populations. These variables were excluded from further analysis to avoid ambiguity.

Statistical analyses for Gyrodactylus kherulensis

Univariate statistics

Table 2.1 *Post hoc* test comparing the hamulus aperture lengths of the eight different populations of *G. kherulensis.* Significant p-values of are shown in bold

1							
1.000	**2**						
1.000	1.000	**3**					
1.000	1.000	1.000	**4**				
0.121	1.000	1.000	1.000	**5**			
0.503	1.000	1.000	1.000	1.000	**6**		
1.000	**0.007**	0.384	**0.034**	**0.001**	**0.001**	**7**	
1.000	**0.028**	0.319	0.092	**0.002**	**0.005**	1.000	**8**

Table 2.2 *Post hoc* test comparing the hamulus total lengths of the eight different populations of *Gyrodactylus kherulensis.* Significant p-values of are shown in bold.

1							
0.946	**2**						
0.018	0.171	**3**					
0.052	0.103	0.945	**4**				
0.578	0.989	0.578	0.989	**5**			
0.005	**0.004**	0.999	0.949	0.787	**6**		
0.669	0.989	0.389	0.515	0.999	0.050	**7**	
0.001	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**8**

Table 2.3 *Post hoc* test comparing the ventral bar total widths of the eight different populations of *Gyrodactylus kherulensis.* Significant p-values of are shown in bold.

1							
0.974	**2**						
0.803	0.995	**3**					
1.000	0.845	0.811	**4**				
0.999	0.890	0.571	0.999	**5**			
0.985	1.000	0.991	0.899	0.918	**6**		
1.000	0.685	0.728	0.999	0.999	0.763	**7**	
0.001	**0.000**	**0.000**	**0.000**	**0.022**	**0.000**	**0.001**	**8**

Table 2.4 *Post hoc* test comparing the ventral bar total lengths of the eight different populations of *Gyrodactylus kherulensis.* Significant p-values of are shown in bold.

1							
0.999	**2**						
0.877	0.979	**3**					
0.999	0.999	0.929	**4**				
1.000	0.999	0.859	0.999	**5**			
0.868	0.920	0.999	0.684	0.913	**6**		
0.993	0.999	0.995	0.988	0.995	0.991	**7**	
0.001	**0.000**	**0.000**	**0.000**	**0.005**	**0.000**	**0.000**	**8**

Table 2.5 *Post hoc* test comparing the marginal hooklet total lengths of the eight different populations of *Gyrodactylus kherulensis.* Significant p-values of are shown in bold.

1							
0.999	**2**						
0.997	0.999	**3**					
1.000	0.999	0.998	**4**				
0.993	0.999	1.000	0.996	**5**			
1.000	0.999	0.998	1.000	0.997	**6**		
1.000	0.999	0.998	1.000	0.997	1.000	**7**	
0.999	0.998	0.981	0.999	0.961	0.999	0.999	**8**

Table 2.6 *Post hoc* test comparing the marginal hooklet sickle lengths of the eight different populations of *Gyrodactylus kherulensis.* Significant p-values of are shown in bold.

1							
1.000	**2**						
0.110	1.000	**3**					
1.000	1.000	1.000	**4**				
1.000	1.000	1.000	1.000	**5**			
1.000	1.000	1.000	1.000	1.000	**6**		
1.000	1.000	0.628	1.000	1.000	1.000	**7**	
1.000	1.000	1.000	1.000	1.000	1.000	1.000	**8**

The various hamuli and ventral bar measurements were the most variable characters when comparing the eight populations of *G. kherulensis* (Tables 2.1-4). Population 8 from Germany appeared to have the greatest variation and had larger overall dimensions when compared to the populations from other geographic localities (Table 2.1). The hamulus total length, shaft length and root lengths of *G. kherulensis* from Germany were greatest, and because these variables are isometric, it is expected to be larger with a larger hamulus total length. These samples of *G. kherulensis* were obtained from a fish pathology laboratory in Switzerland screening a consignment of koi from Germany. The European populations, Population 7 from Scotland and Population 8 from Germany, differed significantly in the length of the hamulus aperture, when compared to the other populations (Table 2.1). There was a significant difference in the hamulus aperture length of Population 7 from Scotland compared to Population 2 from South Africa (p=0.006); Population 4 from Malaysia (p=0.0344); Population 5 from Japan (p=0.001) and Population 6 from Japan (p=0.000). The length of the hamulus aperture also varied significantly from Population 2 from South Africa (p=0.028), from Population 5 from Malaysia (p=0.002) and Population 6 from Japan (0.000). There were however no differences in the hamulus aperture length between the European populations of *G. kherulensis* and Population 1 from South Africa or Population 3 from Malaysia (Table 2.1).

The hamulus total length of the different populations of *G. kherulensis* was the variable showing clear differences in size differences between populations, particularly of the German population. Population 8 from Germany had a significantly larger mean hamulus total length when compared to the rest of the populations (p=0.000; p=0.000; p=0.000; p=0.000; p=0.000; p=0.000; p=0.000) (Table 2.2). The total hamulus lengths of the two South African populations (Population 1 and 2) were similar and no significant differences were evident. The hamulus total length of population 1 however differed significantly from Population 3 imported from Malaysia (p=0.0182) and from Population 6 imported from Japan (p=0.005). Population 2 from South Africa also differed from Population 6 imported into South Africa (p=0.004) (Table 2.2).

The ventral bar total width and ventral bar total length variables were statistically comparable between all the populations, except with Population 8, which varied significantly from all the other populations. The ventral bar total width of the German population was significantly larger than the rest of the populations (p=0.001; p=0.000; p=0.000; p=0.000; p=0.0218; p=0.000; p=0.001 consecutively) (Table 2.3). The ventral bar total length measurement for Population 8 from Germany were also significantly larger when compared to the rest of the populations (p=0.000; p=0.000; p=0.000; p=0.000; p=0.006; p=0.000; p=0.000 consecutively) (Table 2.4). The hamulus total length and ventral bar total length of the German population of *G. kherulensis* give an indication of the overall larger size of this population, when compared to the rest of the populations. The hamuli and ventral bar characters exhibited differences between the different populations of *G. kherulensis* from various geographical localities; the European populations could be distinguished from the rest, while the German population is the most distinct.

The marginal hooklet measurements showed minimal variation for the marginal hooklet total length, as well as the marginal hooklet sickle lengths (Tables 2.5-6). These measurements were similar in all populations and no significant differences were noted. The marginal hooklet variables are expected to be similar in all populations due to the stability of the character. The marginal hooklets showed no statistical differences between the populations from different geographical origins and confirm that the different populations are of the same species. The univariate comparisons of all measurements in the analysis are shown in Appendix 1.

Multivariate statistics

Table 3 Eigenvalues and variability percentages of all the opisthaptoral characters of *Gyrodactylus kherulensis*, and all of them separately for the first three principal components

All	**PC 1**	**PC 2**	**PC 3**
Eigenvalues	7.458	2.517	2.376
Total variance (%)	39.252	13.533	12.507
Cumulative variance (%)	39.252	52.785	65.292
Hamulus			
Eigenvalues	4.549	3.012	1.117
Total variance (%)	45.490	30.124	11.167
Cumulative variance (%)	45.490	75.614	86.784
Ventral bar			
Eigenvalues	2.831	0.790	0.329
Total variance (%)	70.773	19.752	8.215
Cumulative variance (%)	70.773	90.526	98.740
Marginal hooklets			
Eigenvalues	2.063	1.415	0.951
Total variance (%)	41.264	28.305	19.027
Cumulative variance (%)	41.264	69.569	88.596

The PCA factor score plots for all measured characters and the hamulus variables are shown in Fig. 3(a). Population 1 from Germany forms a separate cluster or all the measurements as

well as only the hamuli variables. The PCA score plots for the ventral bar and marginal hook characters are illustrated in Fig. 3b. The German population forms a discrete cluster when comparing the ventral bar variables, for the marginal hooklets however, all the populations form one tightly clustered group.

The first factor for all opisthaptoral variables accounts for the greatest amount of variance within the data. The first five factors all have eigenvalues greater than 1.0. Factor 1 account for 39.252 %, Factor 2 for 13.533 % and Factor 3 for 12.507 % of the variance. The majority of the hamulus and ventral bar variables have factor loading greater than 0.7 for the first factor, which is the most variable. These included the hamulus aperture, hamulus proximal shaft width, hamulus point length, hamulus shaft length, hamulus root length, hamulus total length, ventral bar total width, ventral bar total length, and ventral bar membrane length. The outer and inner hamulus angles had values greater than 0.7 for the second factor. On the sixth factor, marginal hooklet sickle proximal width was significant (> 0.7). For the *G. kherulensis* hamulus only, Factor 1, 2, and 3 accounted for 45.4 %, 30.1 % and 11.2 % respectively. The eigenvalues for the first three factors were greater than 1.00 and factor loadings above 0.7 included the hamulus aperture, hamulus proximal shaft width, hamulus point length, hamulus shaft length, hamulus root length and hamulus total length. The three angles were, however, greater in the second factor and hamulus shaft distal width exceeds 0.7 on the third factor. For the ventral bar, the first factor accounts for 70.773 % of the variation, while Factors 2 and 3 account for 19.752 and 8.215 respectively. Almost all the variables used in the analysis are varying on the first factor, except for ventral bar median length which was greater on the second factor. The marginal hooklets of *G. kherulensis* account for 41.264, 28.305 and 19.027 % of the variance explained for the first three principal components. The factor loadings illustrate the variances of the marginal hooklet total length and marginal hooklet

shaft length on factor 1, the marginal hooklet aperture on Factor 2 and the marginal hooklet sickle proximal width on the third factor. All this information is shown in Table 4 and the factor score plots of all the variables, the hamuli, the ventral bar and the marginal hooklet variables are illustrated in Fig. 3. All factor component loadings and factor score plots are shown in Appendix 2.

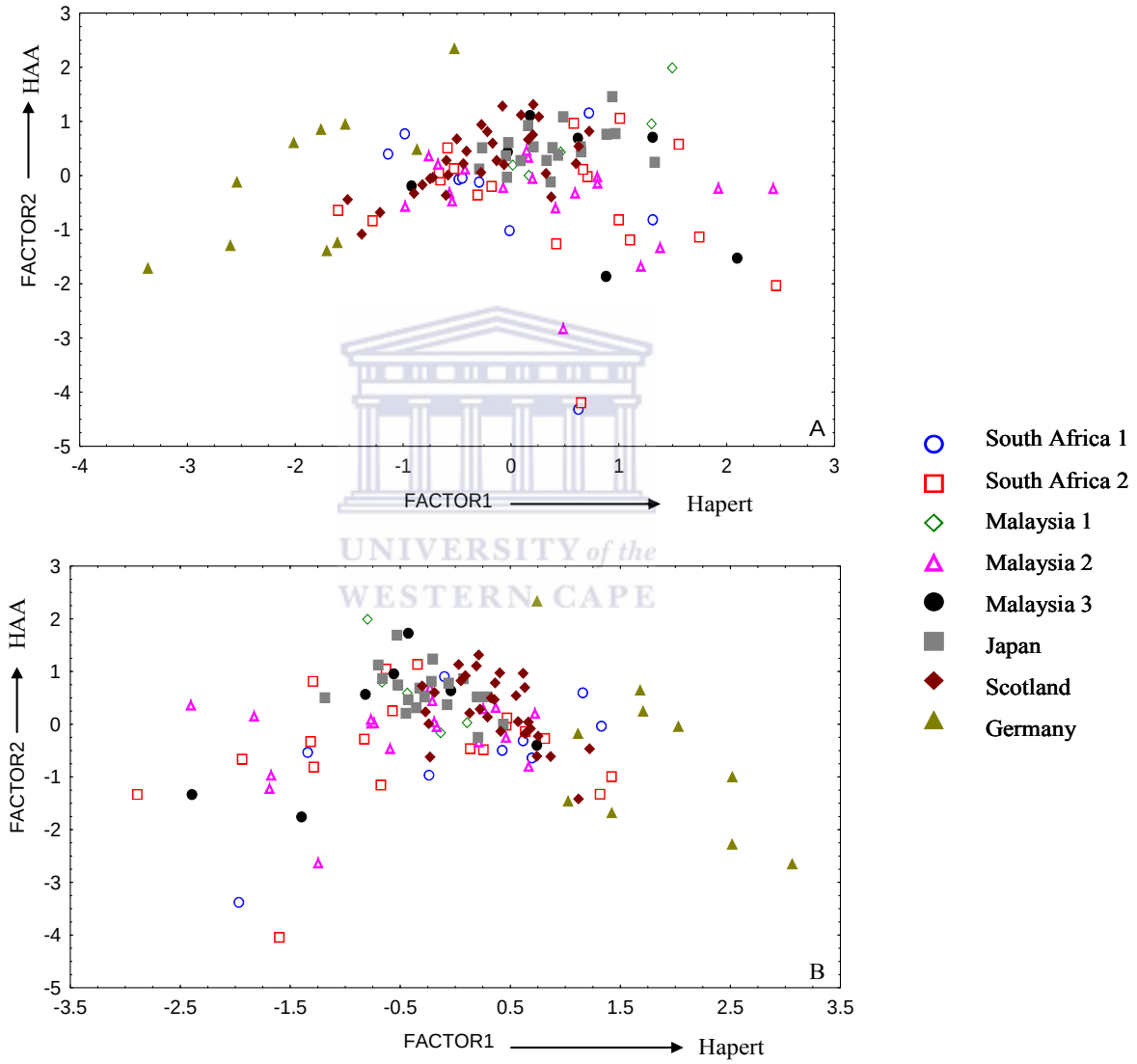


Figure 3 (a) Factor score plots of 118 specimens of *Gyrodactylus kherulensis* from eight populations for 19 variables which include all the parts of the central hook complex (A) and 10 variables of the hamuli only (B).

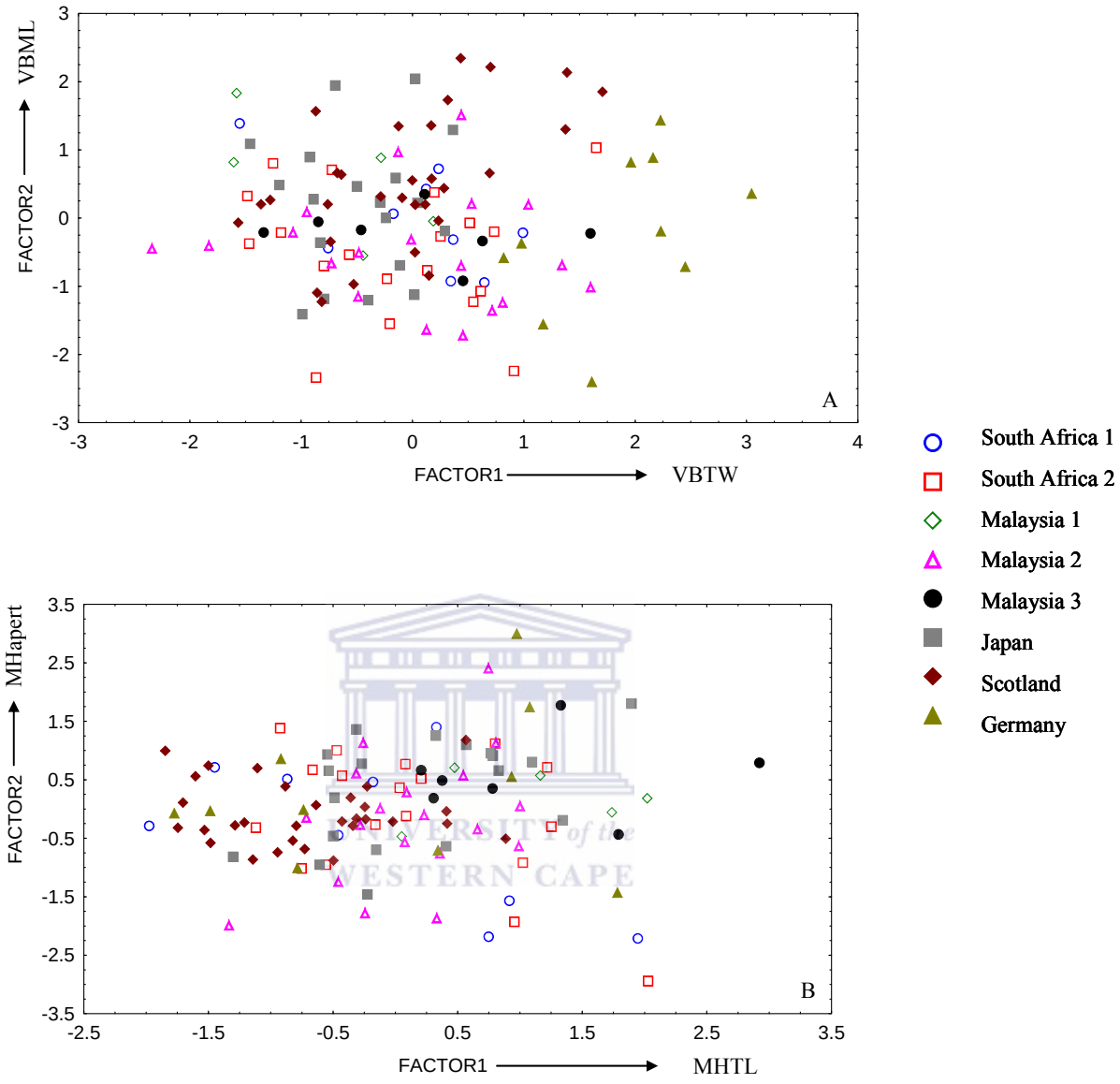


Figure 3 (b) PCA factor score plots of the morphometric measures of all the sclerite characters (A), the ventral bar variables (B) and the marginal hooklet variables of the different populations of *Gyrodactylus kherulensis* from various geographic origins.

Morphological diagnoses for Gyrodactylus kobayashii

***Gyrodactylus kobayashii* Hukuda, 1940**

Host: *Carassius auratus* L. (Current study)

Type-host: *Carassius auratus* L.

Site: Fins and skin

Locality: Kuilsriver, South Africa. $33^{0}54'06.04''S$ $18^{0}42'26.98''$ E

Voucher specimens field collections: G3AQTGY1; G3AQTGY6; G3AQTGY7; G3AQTGY8; G3AQTGY 12; G3AQTGY14; G3AQTGY16; G3AQTGY17; G3AQTGY18; G3AQTGY20; DHG2AQT1GY2; DHG2AQT1GY4; DHG2AQT1GY5; DHG2AQT1GY7; DHG2AQT1GY8; DHG2AQT1GY9; DHG2AQT1GY11; DHG2AQT1GY12; DHG2AQT1GY16; DHG2AQT1GY2 (All specimens have been deposited in the collection of Dr. K.W. Christison at the University of the Western Cape.)

Description:

Total body length 380±33.8 (338-429); total body width 90±7.6 (78-104) at the uterus; opisthaptor 86±8.1 (74-99) long and 93±11.8 (76-110) wide; anterior pharynx length 24.6±3.6 (20.3-29.6), anterior pharynx width 26.1±3.1 (23.7-31.3). Male copulatory organ diameter 11±1.7 (9-14) with one large apical spine and six smaller spines. Hamulus aperture 28±2.2 (23-37); proximal shaft 8±0.8 (6-9) wide; hamulus point 30±1.4 (22-33) long; distal shaft 5±0.6 (4-7) wide; shaft length 41±2.5 (36-45); inner curve 6±0.9 (4-8) long; outer aperture angle 50±3.8 (38-55); point curve angle 21±5.2 (12-31); inner aperture angle 51±3.8 (46-60); root length 21±1.9 (18-25). Total length of hamulus 70±2.4 (66-75). Ventral bar 26±1.4 (24-29) wide with a total length of 28±1.3 (26-31); ventral bar process to mid length 4±0.6 (2-5); ventral bar median length 6±0.9 (5-); ventral bar process 2±0.5 (2-4) long; ventral bar membrane 18±1.5 (16-21) long. Marginal hooklet total length 29±1.9 (25-31);

shaft 24±1.9 (20-26) long; sickle 6±0.4 (5-7) long; sickle proximal width 3.4±0.3 (3-4); sickle toe 1±0.2 (1-2) long; sickle distal width 4±0.6 (3-5); sickle aperture 5±0.5 (4-6) and the instep / arch height 1±0.2 (1-1). Dorsal bar 19.3±4.8 (12-26) long and 2±0.3 (2-3) wide.

Remarks

The hamuli of *G. kobayashii* had longer points and larger apertures than *G. kherulensis*; however, they were less robust, and smaller in size. The ventral bar attachments were small and situated almost directly opposite the dorsal bar attachment, which was relatively large compared to that of *G. kherulensis.* The ventral bars were simple and had fragile membranes, which were smoothly rounded at the base. The marginal hooklets were smaller than in *G. kherulensis* with a triangular toe and a rounded foot, extending dorsally toward the shaft. The dorsal bars were simple and no elaborate features were notable. There were some intraspecific variations between the various populations from the different localities. *G. kobayashii* from South African bred goldfish had the greatest mean total length and the associated larger shaft and root length. Population 4 imported from Malaysia had the smallest root length and hamulus aperture length, but has the same mean total length as Population 5 from Japan. The ventral bar measurements of all populations of *G. kobayashii*, however, Population 5 had the smallest ventral bar total length (Table 4).

Table 4: The mean ± the standard deviation, the range in parenthesis and the coefficient of variance percentage for the hamuli, ventral bar and marginal hooklets for populations of *Gyrodactylus kobayashii* imported to or bred in South Africa. The Coefficient of Variance expressed as a percentage for the hamuli, ventral bar and margi hooklet measurements are also shown.

Country of origin	**Population 1** (Current study) Kuilsriver, South Africa	**Population 2** (Current study) Malaysia	**Population 3** (Current study) Malaysia	**Population 4** (Current study) Malaysia
Measurement	**Mean ± S.D.(range) n=20**	**Mean ± S.D.(range) n=20**	**Mean ± S.D.(range) n=10**	**Mean ± S.D.(range) n=10**
Hamulus				
Aperture (Hapert)	28±2.2 (23-32) 13.4%	23±2.2 (19-27) 9.5%	24±2.0 (21-27) 8.3%	17±1.8 (15-20) 10.7%
Proximal shaft width (HPrSW)	8±0.8 (6-9) 6.4%	7±0.4 (7-8) 4.9%	7±0.5 (6-8) 7.9%	7±0.8 (5-8) 12.6%
Point length (HPL)	30±3.0 (22-33) 4.9%	29±1.5 (27-32) 5.1%	25±4.5 (18-30) 18.0%	22±3.0 (18-28) 13.8%
Distal shaft width (HDSW)	5±0.6 (4-7) 8.1%	4±0.3 (4-5) 5.9%	4±0.4 (4-5) 10.2%	5±0.6 (4-6) 11.8%
Shaft length (HSL)	41±2.5 (36-45) 4.3%	35±1.9 (31-39) 5.5%	33±2.7 (29-38) 8.2%	30±2.5 (25-35) 8.6%
Inner curve length (HICL)	6±0.9 (4-8) 13.3%	4±0.6 (3-5) 15.1%	4±0.7 (3-5.) 17.8%	5±1.2 (3-8) 25.6%
Outer aperture angle (HAA)	45±3.8 (38-55) 10.9%	40±2.8 (35-45) 6.9%	46±4.1 (40-51) 8.8%	38±3.1 (32-42) 8.3%
Point curve angle (HPCA)	21±5.2 (12-31) 25.7%	13±3.0 (8-20) 23.6%	21±8.8 (11-36) 41.4%	24±7.0 (11-38) 29.0%
Inner aperture angle (HICO)	51±3.8 (46-60) 9.9%	46±2.9 (40-52) 6.4%	53±5.1 (45-61) 9.6%	45±4.0 (40-54) 8.8%
Root length (HRL)	21±1.9 (18-25) 7.8%	18±1.4 (15-21) 7.9%	18±2.9 (12-21) 16.8%	14±1.7 (12-17) 12.1%
Total length (HTL)	70±2.4 (66-75) 2.9%	58±2.5 (53-63) 4.2%	57±1.6 (55-60) 2.8%	54±1.4 (52-56) 2.6%
Ventral bar				
Total width (VBTW)	26±1.4 (24-29) 5.3%	24±1.6 (21-28) 7.2%	25±1.4 (23-26) 5.8%	22±0.7 (21-23) 3.3%
Total length (VBTL)	28±1.3 (26-31) 7.4%	26±2.3 (22-29) 8.8%	24±1.3 (21-26) 5.5%	24±2.0 (21-27) 8.4%
Process to mid length (VBPML)	4±0.6 (2-5) 16.7%	3±0.5 (2.1-3.8) 24.0%	3±0.3 (2-3) 9.1%	3±0.6 (2-4) 19.8%
Median length (VBML)	6±0.9 (5-8) 10.5%	6±0.8 (4-7) 10.7%	6±0.8 (5-7) 12.7%	5±0.5 (5-6) 10.2%
Process length (VBProL)	2±0.5 (2-4) 17.6%	2±0.4 (1-3) 26.3%	2±0.3 (1-2) 17.1%	2±0.5 (1-3) 28.6%
Membrane length (VBMemL)	18±1.5 (16-21) 8.3%	17±2.3 (14-20) 12.0%	15±1.6 (12-17) 10.9%	16±1.6 (14-19) 10.2%
Marginal hooklet				
Total length (MHTL)	29±1.9 (25-31) 8.3%	27±1.1 (25-28) 3.0%	27±1.6 (24.4-28.8) 6.0%	27±0.9 (26-29) 3.3%
Shaft length (MHSL)	24±1.9 (20-26) 9.8%	22±1.1 (20-23) 3.1%	22±1.4 (20-24) 6.4%	23±1.2 (21-24) 5.4%

Sickle length (MHSickL)	6±0.4 (5-7) 8.0%	6±0.4 (5-6.) 5.2%	6±0.5 (5-7) 9.5%	5±0.2 (5-6) 4.1%
Sickle proximal width MHPrW)	3±0.3 (3-4) 13.9%	3±0.6 (2-4) 7.6%	4±0.3 (3-4) 9.2%	4±0.5 (3-4) 15.0%
Sickle toe length (MHToeL)	1±0.2 (1-2) 14.8%	2±0.3 (1-2) 18.4%	2±0.3 (1-2) 17.2%	1±0.3 (1-2) 22.3%
Sickle distal width (MHDW)	4±0.6 (3-5) 11.4%	3±0.6 (3-5) 10.5%	3±0.5 (3-4) 15.3%	3±0.3 (3-4) 9.9%
Sickle aperture (MHAp)	5±0.5 (4-6) 8.8%	5±0.2 (4-5) 7.0%	5±0.4 (4-6) 7.3%	4±0.4 (4-5) 9.8%
Sickle instep / arch height (MHIns)	1±0.2 (1-1) 19.9%	1±0.1 (1-1) 18.6%	1±0.2 (1-1) 26.1%	1±0.1 (1-1) 19.1%
Dorsal bar				
Length (DBL)	19±4.8 (12-26)	17±1.7 (14-20)	20±3.9 (14-25)	19±2.4 (16-22)
Width (DBW)	2±0.3 (2-3)	2±0.3 (2-3)	2±0.2 (2-2)	2±0.4 (2-3)

Table 4 continued

Country of origin	**Hukuda, 1940** Japan	**Population 5** (Current study Japan	**Population 6** (Current study Japan	**Population 7** (Current study China	**Population 8** (Current study Unknown
Measurement		**Mean ± S.D.(range) n=20**	**Mean ± S.D.(range) n=2**	**Mean ± S.D.(range) n=20**	**Mean ± S.D.(range) n=20**
Hamulus					
Aperture		21±2.1 (19-26) 9.9%	24±5 (20-28) 22.6%	22±2.9 (18-27) 7.8%	25±2.3 (19-27) 9.5%
Proximal shaft width		8±0.5 (7-9) 6.1%	7±0.2 (7-7) 2.4%	7±0.5 (6-8) 10.2%	8±0.4 (7-8) 4.8%
Hamulus point length	28-30	27±1.6 (24-30) 5.7%	26±4.9 (22-29) 19.1%	29±1.4 (26-31) 10.0%	29±2.0 (24-31) 7.0%
Distal shaft width		4±0.3 (4-5) 6.4%	5±0.1 (5-5) 2.9%	4±0.3 (4-5) 11.9%	4±0.3 (4-5) 6.4%
Shaft length	44-52	35±1.4 (33-38) 4.0%	35±1.3 (34-36) 3.7%	35±1.5 (31-38) 6.3%	37±1.9 (32-39) 5.2%
Inner curve length		4±0.5 (2-4) 14.1%	4±0.1 (4-4) 2.9%	4±0.5 (3-5) 15.3%	4±0.4 (3-5) 12.3%
Outer aperture angle		39±2.5 (34-45) 6.5%	39±5.7 (35-43) 14.5%	37±4.0 (32-44) 8.4%	43±3.5 (34-48) 8.1%
Point curve angle		11±3.9 (4-18) 37.6%	20±3.7 (17-22) 18.9%	13±3.2 (7-21) 24.9%	13±4.9 (8-29) 38.1%
Inner aperture angle		44±2.2 (41-50) 5.1%	51±14.9 (41-62) 29.1%	43±4.3 (36-50) 7.4%	47±3.5 (40-54) 7.3%
Root length	17-23	17±1.6 (15-21) 9.8%	18±0.2 (18-18) 0.9%	18±1.4 (16-21) 9.0%	18±1.3 (15-20) 7.6%
Total length	57-69	54±2.5 (51-60) 4.6%	62±6.9 (57-67) 11.1%	57±1.6 (54-60) 3.4%	58±2.4 (52-62) 4.2%
Ventral bar					
Total width	22-25	21±1.5 (19-25) 7.2%	23±0.9 (23-24) 4.0%	24±1.2 (22-26) 4.9%	24±1.3 (21-26) 5.6%
Total length		22±2.0 (17-25) 8.8%	27±1.1 (27-28) 4.2%	25±1.9 (22-29) 4.8%	24± 1.8 (21-28) 7.5%
Process to mid length		3±0.7 (2-5) 24.0%	4±0.9 (3-4.) 25.0%	3±0.5 (2-4) 16.4%	3±0.7 (2-4) 22.7%
Median length		5±0.5 (4-6) 10.7%	6±0.9 (5-6) 16.2%	6±0.6 (5-7) 13.8%	6±0.5 (5-7) 7.9%
Process length		2±0.6 (2-3) 26.3%	2±0.2 (2-3) 9.1%	2±0.4 (1-3) 22.8%	2±0.4 (1-3) 22.4%
Membrane length	14-16	14±1.6 (9-17) 12.0%	19±0.3 (18-19) 1.5%	16±1.4 (14-19) 7.7%	16±2.3 (12-22) 14.5%
Marginal hooklet					
Total length	25-28	28±0.7 (27-29) 3.0%	27±0.2 (27-27) 0.6%	26 ±2.7 (19-29) 6.5%	27±1.4 (25-29) 5.6%
Shaft length		23±0.7 (22-24) 3.1%	22±0.4 (22-23) 1.9%	21±2.4 (15-23) 8.2%	23±1.0 (21-25) 4.2%
Sickle length		6±0.3 (5-6) 5.2%	5±0.1 (5-5) 2.7%	5±0.6 (4-6) 6.3%	6±0.5 (5-6) 12.6%
Sickle proximal width		4±0.2 (4-4.) 7.6%	3±0.2 (3-4) 4.8%	4±0.7 (3-5) 7.7%	4±0.4 (3-4) 9.7%
Sickle toe length		2±0.2 (2-2) 18.4%	2±0.2 (2-2) 12.7%	2±0.3 (1-2) 18.7%	2±0.3 (1-2) 9.9%

Sickle distal width	3±0.3 (3-4) 10.5%	4±.01 (4-4) 2.4%	3±0.2 (2-4) 15.6%	4±0.4 (3-4) 69.7%
Sickle aperture	4±0.3 (4-5) 7.0%	4±0.6 (4.0-4.8) 12.7%	5±0.5 (4-5) 10.3%	4±1.3 (1-5) 23.0%
Sickle instep / arch height	1±0.2 (1-1) 18.6%	1±0.01 (1-1) 1.7%	1±0.2 (1-1) 21.5%	1±0.4 (1-2) 30.0%
Dorsal bar				
Length	19±3.4 (15-26)	15±0.9 (15-16)	15.8±2.1 (13-19)	14±2.2 (11-18)
Width	2±0.6 (2-3)	2±0.1 (2-2)	2.2±0.3 (2-3)	2±0.1 (2-2)

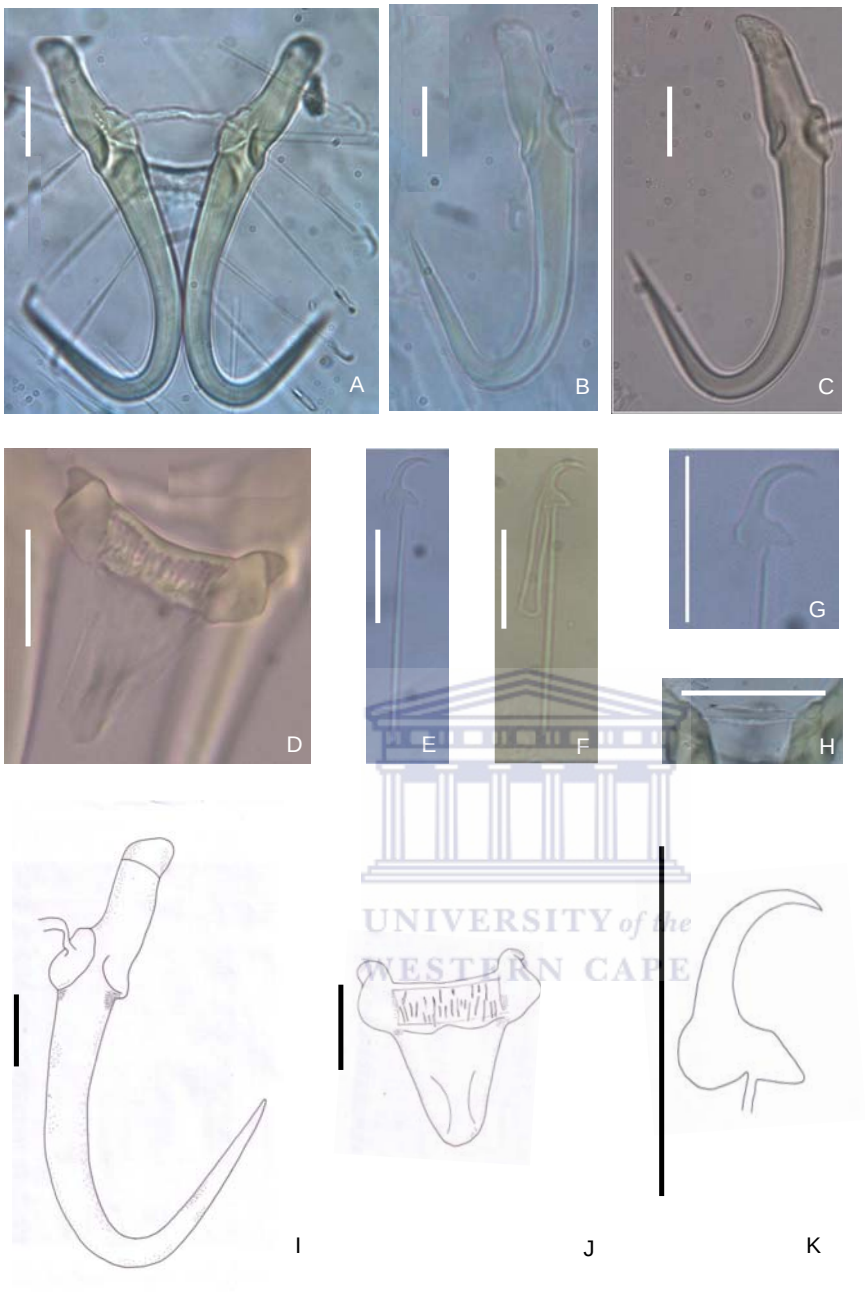


Figure 4 Photo-micrographs and line drawings of the opisthaptoral characters of *Gyrodactylus kobayashii* (A) central hook complex of Population 1 from South Africa (B), hamulus of Population 4 from Malaysia (D), hamulus of Population 3 from Malaysia (D) ventral bar of Population 7 from China (E) marginal hooklet of Population 1 (F) marginal hooklet of Population 2 from Malaysia (G) marginal hooklet sickle of Population 1 (H) and the dorsal bar from a specimen from Population 7 from China. Line drawings of the hamuli (I) of *G. kobayashii* from Population 1 from South Africa, ventral bar (J) of Population 7 and the marginal hooklet of Population 1.Scale bar = 10 µm

As in the case of *G. kherulensis*, the same variables which yielded high coefficients of variance in those populations showed similar results in *G. kobayashii*. The morphology of *G. kobayashii* is illustrated in Figure 4. Population 6 originally from Japan however, had larger CV values, and greater ranges, this was due to the small sample size of the population (n=2) (Table 4).

Statistical analyses for Gyrodactylus kobayashii

Univariate statistics

Table 5.1 *Post hoc* test comparing the hamulus aperture lengths of the eight different populations of *Gyrodactylus kobayashii.* Significant p-values of are shown in bold.

1							
0.001	**2**						
0.001	0.999	**3**					
0.000	**0.000**	**0.000**	**4**				
0.000	0.469	0.271	**0.010**	**5**			
0.704	0.999	1.000	0.0565	0.954	**6**		
0.000	0.757	0.524	**0.003**	0.999	0.984	**7**	
0.001	0.948	0.998	**0.000**	**0.003**	0.999	**0.012**	**8**

Table 5.2 *Post hoc* test comparing the hamulus total lengths of the eight different populations of *Gyrodactylus kobayashii.* Significant p-values of are shown in bold.

1							
0.153	**2**						
0.001	1.000	**3**					
0.000	0.114	1.000	**4**				
0.000	**0.001**	0.723	1.000	**5**			
1.000	1.000	1.000	1.000	1.000	**6**		
0.000	1.000	1.000	1.000	0.531	1.000	**7**	
0.001	1.000	1.000	0.339	**0.015**	1.000	1.000	**8**

Table 5.3 *Post hoc* test comparing the ventral bar total width of the eight different populations of *Gyrodactylus kobayashii.* Significant p-values of are shown in bold.

1							
0.036	**2**						
0.135	0.999	**3**					
0.000	**0.030**	**0.012**	**4**				
0.000	**0.000**	**0.000**	0.983	**5**			
0.336	0.988	0.968	0.987	0.865	**6**		
0.000	0.999	0.993	0.078	**0.000**	0.997	**7**	
0.000	0.915	0.774	0.312	**0.000**	0.999	0.963	**8**

Table 5.4 *Post hoc* test comparing the ventral bar total lengths of the eight different populations of *Gyrodactylus kobayashii.* Significant p-values of are shown in bold.

1							
0.0298	**2**						
0.000	0.292	**3**					
0.001	0.688	0.999	**4**				
0.000	**0.000**	0.335	0.407	**5**			
0.999	0.985	0.524	0.612	0.074	**6**		
0.000	0.988	0.809	0.973	**0.000**	0.905	**7**	
0.000	0.857	0.975	0.999	**0.001**	0.805	0.997	**8**

Table 5.5 *Post hoc* test comparing the marginal hooklet total lengths of the eight different populations of *Gyrodactylus kobayashii.* Significant p-values of are shown in bold.

1							
0.357	**2**						
0.345	1.000	**3**					
0.887	0.999	0.999	**4**				
0.468	0.999	0.996	1.000	**5**			
0.962	1.000	1.000	0.999	0.999	**6**		
0.012	0.999	0.999	0.989	0.828	1.000	**7**	
0.072	1.000	1.000	0.999	0.989	1.000	0.999	**8**

Table 5.6 *Post hoc* test comparing the marginal hooklet sickle lengths of the eight different populations of *Gyrodactylus kobayashii.* Significant p-values of are shown in bold.

1							
0.997	**2**						
0.999	0.999	**3**					
0.812	0.980	0.888	**4**				
0.624	0.996	0.945	0.999	**5**			
0.953	0.991	0.969	0.999	0.999	**6**		
0.033	0.627	0.386	0.999	0.872	1.000	**7**	
0.835	0.999	0.989	0.999	0.999	0.998	0.620	**8**

G. kobayashii populations showed a greater degree of intraspecific variability when compared to *G. kherulensis* populations. Differences were detected particularly for the hamuli and ventral bar characters. The hamulus aperture and hamulus total length are represented in Table 5.1 and Table 5.2. Population 1 from a South African bred source was the most variable population. It was, however, most similar to Population 6 from Japan. The variability is most evident in the hamulus aperture lengths of the eight different populations where Population 1 from South Africa differed significantly from the rest, except from the Japanese Population 6 (p=0.001; p=0.005; p=0.000; p=0.000; p=0.000; p=0.001). Population 4 from Malaysia also varied considerably compared to the rest for this measurement, and as with Population 1, was similar to Population 6 only (p=0.000; p=0.000; p=0.000; p=0.010; p=0.003; p=0.000 consecutively, and excluding Population 6) (Table 5.1). The hamulus total length was the most discriminatory variable. The hamulus total length is greatest for Population 1, which had larger overall dimensions compared to the rest of the populations, except for Population 2 from Malaysia and Population 6 from Japan. The remaining populations differed considerably for this character. Population 2 from Malaysia and Population 5 from Japan differ significantly for the total length of their hamuli (p=0.006), also between Population 5 from Japan and Population 8 from an unknown source (p=0.015) (Table 5.2). The hamulus total length again indicates that Population 1 is most different from

the rest and the greatest hamulus total length. The hamulus aperture and other hamulus variables are therefore also larger due isometric growth of the hamulus character.

The ventral bar total width measurement among the different populations were variable, again the most variability was noted for Population 1 (Table 5.3). Population 3 from Malaysia also shows some degree of variability of the ventral bar total bar total width variable when compared to some of the other populations.

The ventral bar total lengths of Population 1 from South Africa was significantly larger than the rest, except for Population 6 from Japan (p= 0.030; p=0.000; p=0.001; p=0.000; p=0.000; p=0.000). Population 6 has a broader variable size range due to the small population size (n=2). There were also significant differences between Populations 5 from Japan and Population 2 from Malaysia (p=0.000), and also between Population 5 and Population 8 from an unknown source country (p=0.001). Populations 5 from Japan and Population 7 from China also varied considerably in the total lengths of the ventral bars (p=0.000) (Table 5.4). The hamuli and ventral bar characters had the most differences and separated the various populations from different geographical origins and the differences may be due to the environmental conditions to which *G. kobayashii* were exposed.

The marginal hooklet total length measurements of the different populations were similar for most populations; however, there was a significant difference between Population 1 from South Africa and Population 7 from China (p=0.012). The same goes for the marginal hooklet sickle lengths of Population 1 and Population 7 (p=0.033) (Table 5.5 and 5.6). The marginal hooklets, which were the most taxonomically stable characters, show minimal

variation among the different populations and illustrated the similarities between the different populations of *G. kobayashii.*

Univariate statistics of all variables of *G. kobayashii* are shown in Appendix 3.

Multivariate statistics

Table 6 Eigenvalues and variability percentages of the opisthaptoral character measurements of *Gyrodactylus kobayashii* of the first three principal components

All	**PC 1**	**PC 2**	**PC 3**
Eigenvalues	6.719	3.086	1.957
Total variance (%)	35.364	16.239	10.301
Cumulative variance (%)	35.364	51.604	61.904
Hamulus			
Eigenvalues	4.418	2.173	1.184
Total variance (%)	44.180	21.729	11.843
Cumulative variance (%)	44.180	65.908	77.751
Ventral bar			
Eigenvalues	2.631	1.019	0.273
Total variance (%)	65.780	25.468	6.826
Cumulative variance (%)	65.780	91.248	98.074
Marginal hooklets			
Eigenvalues	1.975	1.385	0.970
Total variance (%)	39.502	27.700	19.400
Cumulative variance (%)	39.502	62.202	86.603

The multivariate analysis of the *G. kobayashii* populations, illustrated the variance of six of the 19 characters used for analysis for the first factor for some of the hamuli and ventral bar variables includes the hamulus aperture, hamulus shaft length, hamulus root length, hamulus total length, ventral bar total width and total length (Appendix 4). The second factor has component loading of >0.7 for the hamulus point curve angle. Factor 3 had significant loadings for marginal hooklet shaft length. The factor scores of all the measured variables

and the hamulus measurements are shown in Fig. 5a and the ventral bar measurement and the marginal hooklet measurements are illustrated in Fig. 5b. Population 1 from South Africa has the greatest variation and forms a discrete cluster, while Populations 3 and 4 also form separate clusters, but these populations overlap somewhat with the tightly grouped populations (Fig 5a). Similar to *G. kherulensis,* the hamuli are the most variable characters and Populations 1, 3 and 4 are clearly separated from each other (Fig 5a). The factor score plots comparing the ventral bar characters show a lesser degree of variation between the different populations (Fig. 5b). The factor score plots of marginal hooklets show minimal variation among the different populations (Fig. 5b). The eigenvalues for all principal components show that the first three are greater than 1.0. The first three factorial axes showed variances of 35.364, 16.239 and 10.301 % respectively. For the hamulus variables, the first factorial axis accounts for 44.2 % of the variance, while factors two and three accounts for 21.7 and 11.8%. The component loadings greater than 0.7 for factor one includes the hamulus aperture, hamulus point length, hamulus shaft length, hamulus root length and hamulus total length. The second factor's components include all the three hamulus angles and the hamulus distal shaft width for the third factor. The ventral bar variables has total percentage variation of 65.8, 25.5 and 6.8 % for the three factorial axes, and only have variable component loadings for factor one for the variables: ventral bar total width, ventral bar total length and ventral bar membrane length. The marginal hooklets have a total variance of 39.502, 27.700 and 19.400 % for the first three factorial axes which the first two have eigenvalues greater than 1.00. The significant component loadings for factor one characters include the marginal hooklet total length, marginal hooklet shaft length, and marginal hooklet sickle proximal width for factor three. All this is shown in Table 8 and the factor score plots are shown in Fig. 5. All the factor component loadings are tabulated and shown in Appendix 4.

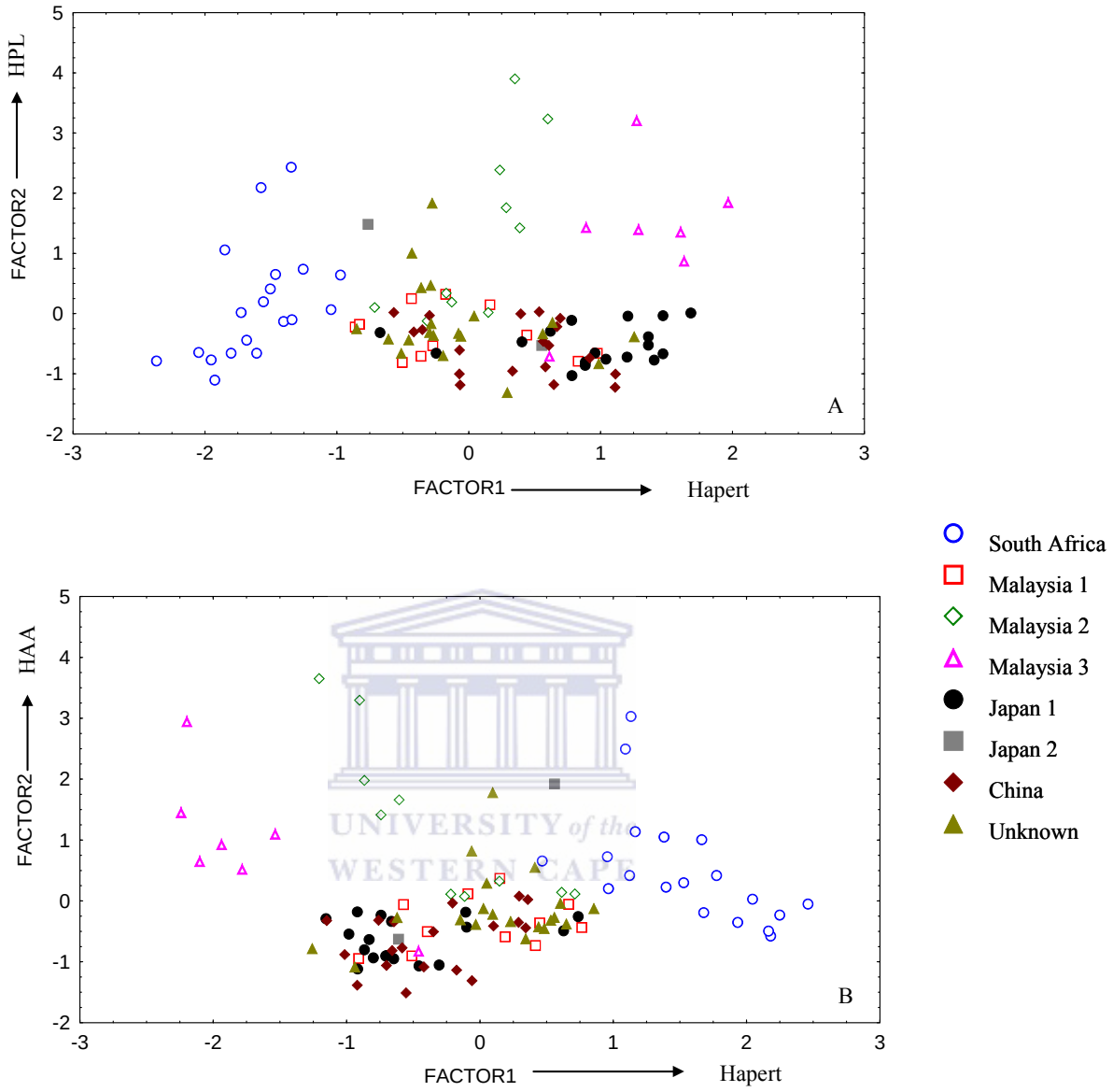


Figure 5 (a) Factor score plots of (A) all measured variables and (B) the hamuli measurements of *Gyrodactylus kobayashii.*

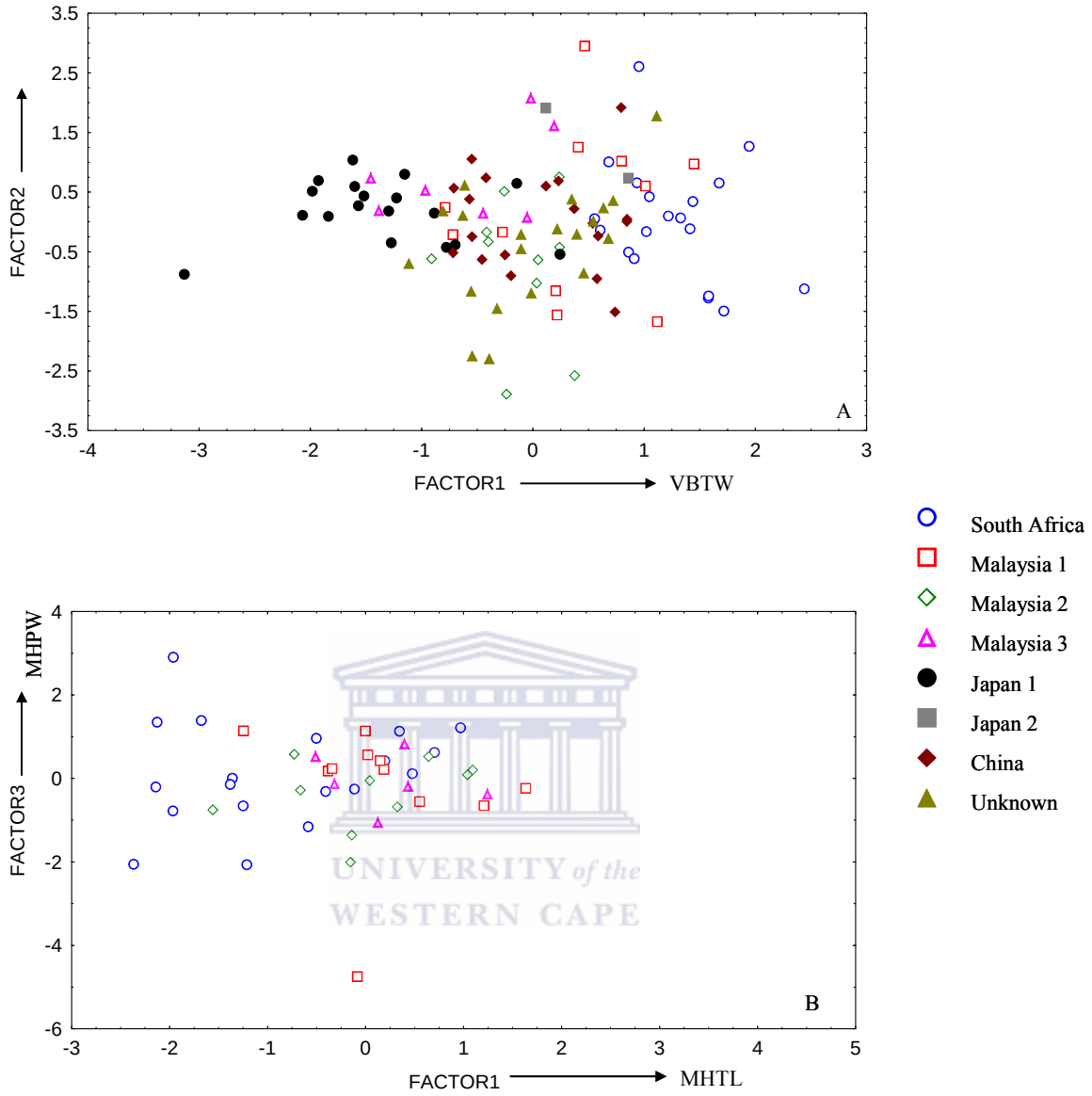


Figure 5(b) PCA factor score plots of the (A) ventral bar and (B) marginal hooklet variables of *Gyrodactylus kobayashii.*

Discussion

This is the first report of *Gyrodactylus kherulensis* on koi carp and *G. kobayashii* on goldfish in South Africa from the ornamental fish trade sector; however, neither of these species has yet been reported from feral populations of goldfish, koi carp or common carp in the Western Cape Province. Feral populations of *G. kherulensis* and *G. kobayashii* may exist, however none have been reported in South Africa. Members of the genus *Gyrodactylus* have been reported from goldfish in South Africa, but these samples have not been identified to species level (Mouton *et al.* 2001). No other *Gyrodactylus* species were found on the fish sampled, despite there being a number of species described from koi and goldfish. The eight populations of *G. kherulensis* and *G. kobayashii* analysed in this study showed inter- and intra-population differences in the sizes of the opisthaptoral characters. Intraspecific variation is common in *Gyrodactylus* species and may be attributable to phenotypic plasticity, due to the different environmental conditions to which the population was exposed and/or due the genotypic expression (Olstad *et al.* 2007).

The different populations of *G. kherulensis* could be readily distinguished from each other by variation in their hamulus and ventral bar characters. The European populations of *G. kherulensis,* particularly the German population, differed significantly from the rest of the populations. The remaining populations, however, showed relatively negligible variations. The univariate statistics comparing the different populations of *G. kherulensis* show a clear distinction of the German population. These differences are primarily due to the increased sizes of the central hook complex, particularly the larger mean hamulus total length and the associated larger sizes of the hamulus shaft length, hamulus point length and hamulus root length due to isometric growth of these variables.

The multivariate statistics comparing the populations supports the results suggested by the univariate statistics. The multivariate statistics clearly shows the German population of *G. kherulensis* are different in size but do overlap with the rest of the populations of G. *kherulensis*, while the rest form a tight group. Although the German population morphologically resembles the rest of the populations the size the hamuli and ventral bars of members of the populations are considerably larger than in the other populations. The populations of *G. kherulensis* found on the South African bred koi are morphologically similar to those from Asian origins.

The similarity of the South African populations and Asian populations of *G. kherulensis* may be due to these fish being imported in South Africa and the parasites may have acclimated to conditions in the region and their morphometrics may be a reflection of the environmental conditions to which the parasites have been exposed. The fish could also have been exposed to these parasites at the holding facility and infected by the same population, and hence little or no variation is seen between populations. The size of the opisthaptoral characters are reliant on a number of environmental factors, and water temperature is the primary factor determining haptoral organ size (Dmitrieva and Dimitrov 2002, Davidova *et al*. 2005). The variation in size may be due to genetic variability and the expression of larger sizes of the opisthaptoral organs. Maturation of *Gyrodactylus* species occurs within the uterus of the parent, which therefore potentially exposes both the mother and the daughter worm to the same external environment, due to both being present in the grandmother worm (Harris 1998b). Environmental factors therefore influence the size of the characters of the opisthaptor for both the mother and daughter worms to about the same degree, resulting in similarity of the character sizes (Harris 1998b).

Gyrodactylus kobayashii populations show a greater degree of intraspecific variation, particularly illustrated by the univariate statistics. When looking at the univariate analyses, the hamuli total length, shaft length and root length were the primary variables of major variation. The multivariate analyses also show that the hamuli variables have the greatest variation. The univariate statistics of the populations of *G. kobayashii* varies particularly for Populations 1, 3, 4 and 5. The ventral bar total width and length variables, hamulus aperture and hamulus total length variables are the most variable. Multivariate analyses of all the characters showed similar results as the univariate statistics. Populations 1, 3 and 4 were clearly separated from each other, forming 3 morphotypes, these populations did, however, overlap with the other tightly grouped populations.

Intraspecific variation in *G. kherulensis* and *G. kobayashii* is primarily attributable to the hamulus variables, particularly the hamulus total length, together with the hamulus shaft length, point length and root length due to the isometric growth of these variables. The ventral bar total width and length are also indicative of inter-population differences. Both the univariate and multivariate statistical analyses reflect the variability as result of these variables. Hamulus total length and ventral bar total length can therefore be used as the primary variables to group populations which appear to be dissimilar.

The multivariate analysis (PCA) has been the primary statistical classifier to distinguish *Gyrodactylus* species and this can even be used from only the marginal hooklet or the hamulus (Shinn *et al*. 2001) and a total of 25 point-to-point measurements were used to improve the reliability of the analysis (Shinn *et al.* 2004). Minor differences are usually expected between members of the same species. This method has, however, placed members of the same species with intraspecific or intra-populational variation into different groups,

and therefore has the potential to discriminate between populations of the same species of *Gyrodactylus* in this study. However, intraspecific variation should therefore be carefully considered when discriminating between similar species. *Gyrodactylus kherulensis* from Germany is morphologically identical to the other populations sampled, however, the size differences places them into different groups. Similarly, the South African bred populations of *G. kobayashii* have identical marginal hooklets and hamuli morphology when compared to the rest of the populations, but the size variation of the hamuli and ventral bar characters place this population within a different group. As the hamuli and ventral bar characters are the important characters in the classification and taxonomy of the genus, thus confusion may arise when variations of the hamuli among members of the same species exists. The marginal hooklet variables are, however, pivotal to the identification of *Gyrodactylus* species (Shinn *et al*. 2001). The marginal hooklets, although not unaffected by water temperature differences, are the most morphologically stable variables. In this case, as observed in both species studied, the marginal hooklets placed the two different species within their respective groups and the conservancy of the marginal hooklet makes this an excellent character for species identification, illustrating again the major role that the marginal hooklets play in the taxonomic classification of members of the genus *Gyrodactylus.*

The results support the initial hypothesis and the hamuli and ventral bars of all the populations of *G. kherulensis* and *G. kobayashii* from various geographic origins exposed to different environmental conditions play a great role in the sizes of these morphometric characters. More structure could be measured between populations of the same species from different parts of the world. This study also therefore highlights the increased need for genetic classifiers to confirm the identification of these species.

The identification of emerging disease into the ecologically-sensitive Western Cape Province, South Africa is of importance, as it is home to an array of indigenous fish, endemic to the Cape Floristic Region. Identification of these parasites is of particular importance for biosecurity purposes, to make sure hazardous or potentially hazardous pathogens are managed accordingly. The ornamental fish trade therefore poses a risk to indigenous fish as one of the important routes of infection for *Gyrodactylus* species into the Western Cape. The study also sheds some light on the need for increased knowledge on the phenotypic plasticity of *Gyrodactylus* species as a result of environmental influence and the effect that geographic distribution has on sclerite size on members of the genus.

References:

ALI, N. M., Mhaisen, F. T., Abul-Eis, E. S., Kadim, L. S. 1988. First occurrence of the monogenetic trematode *Gyrodactylus kherulensis* Ergens, 1974 in Iraq on the gills of the common carp *Cyprinus carpio*. *Journal of Biological Science Research*, **19** (3), 659-664.

APPLEBY, C. 1996. Variability of the opisthaptoral hard parts of *Gyrodactylus callariatis* Malmberg, 1957 (Monogenea: Gyrodactylidae) from Atlantic cod *Gadus morhua* L. in the Oslo Fjord, Norway. *Systematic Parasitology* **33** (3), 199-207.

BAKKE, T.A., Cable, J., Harris, P.D. 2007. The biology of gyrodactylid monogeneans: The "Russian-doll killers" *Advances in Parasitology* **64**, 161-376.

BLANC, G. 2001. Introduction of pathogens in European aquatic ecosystems: attempt of evaluation and realities. pp. 37–56. In: Uriate, A. & Basurco, B. (Eds) Environmental impact assessment of Mediterranean aquaculture farms. Zaragoza, CIHEAM-IMAZ.

CABLE, J., Harris, P.D., Tinsley, R.C., Lazarus, C.M. 1999. Phylogenetic analysis of *Gyrodactylus* spp. (Platyhelminthes: Monogenea) using ribosomal DNA sequences. *Canadian Journal of Zoology* **77**, 1439-1449.

CABLE, J., Harris, P.D. 2002. Gyrodactylid developmental biology: historical review, current status and future trends. *International Journal for Parasitology* **32**, 255-280.

DÁVIDOVA, M., Jarkovsky, J., Matêjusová, I., Gelnar, M. 2005. Seasonal occurrence and metric variability of *Gyrodactylus rhodei* Žitňan 1964 (Monogenea, Gyrodactylidae). *Parasitology Research* **95**, 398-405.

DE MOOR, I.J., Bruton, M.N. 1988. Atlas of alien and translocated indigenous aquatic animals in southern Africa. South African National Scientific Programmes Report No. 144: 310 pp.

DMITRIEVA, E., Dimitrov, G. 2002. Variability in the taxonomic characters of Black Sea gyrodactylids (Monogenea). *Systematic Parasitology* **51**, 199-206.

DU PREEZ, L., Maritz, M.F. 2006. Demonstrating morphometric protocols using polystome marginal hooklet measurements. *Systematic Parasitology* **63**, 1-15.

ERGENS, R. 1974. *Gyrodactylus kherulensis* sp. n. (Monogenoidea) from the carp. *Folia Parasitologica* **21**, 377-379.

ERGENS, R., Ogawa, K. 1978. Redescription of *Gyrodactylus kobayashii* Hukuda (Monogenoidea). *Vestnik Ceskslovenske Spolecnosti Zoologicke* **2**, 101-104.

FLETCHER, A.S., Whittington, I.D. 1998. A parasite-host checklist for Monogenea from freshwater fishes in Australia, with comments on biodiversity. *Systematic Parasitology* **41,** 159–168.

GARCIA-VASQUEZ, A., Hansen, H., Christison, K.W., Rubio-Godoy, M., Bron, J.E., Shinn, A.P. 2010. Gyrodactylids (Gyrodactylidae, Monogenea) infecting *Oreochromis niloticus niloticus* (L.) and *O. mossambicus* (Peters) (Cichlidae): A pan-global survey. *Acta Parasitologica* **55** (3), 215-229.

GEETS, A., Appleby, C., Ollevier, F. 1999. Host-dependent and seasonal variation in opisthaptoral hard parts of *Gyrodactylus* cf. *arcuatus* from three *Pomatoschistus* spp. and *G. arcuatus* from *Gasterosteus aculeatus*: a multivariate approach. *Parasitology* **119**, 27-40.

HARRIS, P.D. 1998(a). Extreme morphological variation between related individuals of *Gyrodactylus pungitii* Malmberg, 1964 (Monogenea). *Systematic Parasitology* **39**, 137-140.

HARRIS, P.D. 1998(b). Ecological and genetic evidence for clonal reproduction in *Gyrodactylus gasterostei* Glaser, 1974. *International Journal of Parasitology* **28**, 1595-1607.

HARRIS, P.D., Cable, J., Tinsley, R.C. 1999. Combined ribosomal DNA and morphological analysis of individual Gyrodactylid monogeneans. *The Journal of Parasitology* **85** (2), 188-191.

HARRIS, P.D., Shinn, A.P., Cable, J., Bakke, T.A. 2004. Nominal species of the genus *Gyrodactylus* von Nordmann 1832 (Monogenea: Gyrodactylidae), with a list of principal host species. *Systematic Parasitology* **59,** 1–27.

HAYWARD, C.J., Iwashita, M., Ogawa, K., Ernst, I. 2001. Global spread of the eel parasite *Gyrodactylus anguillae* (Monogenea). *Biological Invasions* **3**, 417–424.

HAYWARD, C.J., Bott, N.J., Itoh, N., Iwashita, M., Okihiro, M., Nowak, B.F. 2007. Three species of parasites emerging on the gills of mulloway, *Argyrosomus japonicus* (Temminck and Schegel, 1843), cultured in Australia. *Aquaculture* **265**, 27-40.

HOFFMAN, G.L. 1998. Parasites of North American Freshwater Fishes. Comstock Publishing Associates, Ithaca and London, 539 pp.

JALALI, B., Shamsi, S., Barzegar, M. 2005. Occurrence of *Gyrodactylus* spp. (Monogenea: Gyrodactylidae) from Iranian freshwater fish. *Iranian Journal of Fisheries Sciences* **4**, 19-30.

KIR, I., Tekin Ozan, S. 2007. Helminth Infections in common carp, *Cyprinus carpio* L., 1758 (Cyprinidae) from Kovada Lake (Turkey). *Türkiye Parazitoloji Dergisi,* **31** (3), 232-236.

LUX, E. 1987. Neues zum Artenbestand von *Gyrodactylus* bei Karpfen in der DDR. *Angewandte Parasitologie* **28**, 159-164.

LUX, E. 1990. Population dynamics and interrelationships of some *Dactylogyrus* and *Gyrodactylus* species on *Cyprinus carpio. Angewandte Parasitologie* **31** (3), 143-149.

MCHUGH, E.S., Shinn, A.P., Kay, J.W. 2000. Discrimination of the notifiable pathogen *Gyrodactylus salaris* from *G. thymalli* (Monogenea) using statistical classifiers applied to morphometric data. *Parasitology* **121**, 315-323.

MO, T.A. 1991. Seasonal variations of opisthaptoral hard parts of *Gyrodactylus salaris* Malmberg, 1957 (Monogenea: Gyrodactylidae) on parr of Atlantic salmon *Salmo salar* L. in the River Batnfjordselva, Norway. *Systematic Parasitology* **19** (3), 231-240.

MOUTON, A. Basson, L., Impson, D. 2001. Health status of ornamental freshwater fishes imported to South Africa: a pilot study. *Aquarium Sciences and Conservation* **3**, 327-333.

OGAWA, K. 1994. Monogenean parasites of freshwater fishes of Hokkaido, Japan. *Scientific Report of Hokkaido Fish Hatchery* **48**, 59-67.

OGAWA, K., Egusa, S. 1978. Seven species of *Gyrodactylus* (Monogenea: Gyrodactylidae) from *Plecoglossus altivelis* (Plecoglossidae), *Cyprinus carpio* (Cyprinidae) and *Anguilla* spp. (Anguillidae). *Bulletin of the Japanese Society of Scientific Fisheries* **44** (6), 613-618.

OLSTAD, K., Bachmann, L., Bakke, T.A. 2009. Phenotypic plasticity of taxonomic and diagnostic structures in gyrodactylosis-causing flatworms (Monogenea, Platyhelminthes). *Parasitology* **136**, 1305–1315.

OLSTAD, K., Shinn, A.P., Bachmann, L., Bakke, T.A. 2007. Host-based identification is not supported by morphometrics in natural populations of *Gyrodactylus salaris* and *G. thymalli* (Platyhelminthes, Monogenea). *Parasitology* **134**, 2041-2052.

ŠEFROVÁ, H., Laštůvka, Z. 2005. Catalogue of alien animal species in the Czech Republic. *Acta Universitatis Agriculturae et Slviculturae Mndelianae Brunensis Sbornik Mendelovy Zemedelske a Lesnicke Univerzity v Brne* **53**, 151-170.

SKELTON, P. 2001. A Complete Guide to the Freshwater Fishes of Southern Africa. Struik Publishers, Cape Town, South Africa, 395 pp.

SHINN, A.P., Gibson, D.I., Sommerville, C. 2001. Morphometric discrimination of *Gyrodactylus salaris* Malmberg (Monogenea) from species of *Gyrodactylus* parasitising British salmonids using novel parameters. *Journal of Fish Diseases* **24**, 83-97.

SHINN, A.P., Hansen, H., Olstad, K., Bachmann, L., Bakke, T.A. 2004. The use of morphometric characters to discriminate specimens of laboratory-reared and wild populations of *Gyrodactylus salaris* and *G. thymalli* (Monogenea). *Folia Parasitologica* **51**, 239-252.

Chapter 3

Challenge infections with the exotic monogeneans, *Gyrodactylus kherulensis* Ergens (1974) and *G. kobayashii* Hukuda (1940), on two indigenous redfin minnows in the Western Cape, South Africa: A preliminary study.

Abstract

Exotic fish parasites pose a potential risk to the freshwater fish biodiversity of the Cape Floristic Region (CFR). Parasites of the genus *Gyrodactylus* are described as the least host-specific group within the Class Monogenea and their unique reproductive strategies enhance their invasive potential. The biology of these parasites, together with the wide distribution of their exotic cyprinid hosts, koi carp and goldfish, present a potential infection pathway to the natural rivers within the CFR. The risk to the conservation status of indigenous cyprinids in the CFR may be increased due to their relatedness to the exotic cyprinid hosts. Cohabitation laboratory experiments were conducted to determine the susceptibility of the indigenous cyprinids, *Pseudobarbus burchelli* and *P. phlegethon* to *Gyrodactylus kherulensis* from koi and *Gyrodactylus kobayashii* from goldfish. These fish were held in tanks for 20 days with the endemic redfins to determine whether transfer of *G. kherulensis* and *G. kobayashii* to *P. burchelli* and *P. phlegethon* was possible. Preliminary results suggest that both *G. kherulensis* and *G. kobayashii* have the ability to at least transfer to *P. phlegethon*, however *P. burchelli* showed natural resistance to both parasite species.

Introduction

The Cape Floristic Region is home to an array of unique floral and faunal species, and is the smallest and most diverse global floral kingdom, constituting about 4% of the southern African landmass (Rebelo 1992). The CFR is a characteristically species rich region, globally

renowned for its status as a biodiversity hotspot, where both plants and animal species are facing threats of possible extinction (Myers *et al.* 2000; Pressey *et al*. 2003). The ichthyofauna of the CFR have been described as the most threatened organisms within the region, as well as the most endemic, with 86% of local fish being confined only to the CFR (Impson *et al*. 2000; Impson 2007). The high incidences of freshwater fish endemicity are largely attributable to the biogeographical history of the Cape Fold Mountains, which isolate the endemic species to the clear, slightly acidic, temperate waters of the Western Cape Province (Skelton 2001).

The ichthyofauna of the Western Cape Province until recently comprised 18 native described freshwater fish species, this being elevated to 23 with the recent inclusion of evolutionary significant units (ESU's) based on molecular data (Impson 2007). The family Cyprinidae make up the majority of the native freshwater fish species and are the most vulnerable group of freshwater fish in the CFR (Impson *et al*. 2000). These fish are largely threatened by factors brought about by anthropogenic influences, such as habitat destruction and alien fish introductions. As a result, 57% of the fish in the CFR are classified as Red Data Book species (Rebelo 1992; Cambray 2003). The documented accounts of alien species introductions highlight their ecological interactions with native fish species, which include predation, and competition for resources. The diseases introduced with the exotic fish have not generally been considered a threat to the indigenous fish in the area.

Koi carp (*Cyprinus carpio koi* L.) and common goldfish (*Carassius auratus* L.) are widely distributed alien invasive cyprinid fish which have established themselves in natural freshwater ecosystems worldwide, largely as result of ornamental trade (Koehn 2004). Their hardiness and highly adaptive abilities have made these cyprinids exceptionally successful invasive species (Andrews 1990; Mouton *et al*. 2001; Skelton 2001). Koi and goldfish are

renowned for their abilities to act as vectors for invasive monogenean parasites and other exotic pathogens, both locally and internationally (De Moor and Bruton 1988; Mouton *et al*. 2001). Monogenean parasites are among the most notorious parasites in aquaculture, and the biology of *Gyrodactylus* species makes these parasites exceptional invasive species (Bakke *et al*. 2002; King and Cable 2007). Generally, *Gyrodactylus* species are regarded as non-pathogenic in nature (with the exception of *G. salaris* Malmberg, 1957), however, in confined conditions these flatworms tend to increase at alarming rates, often resulting in gross pathology or mass mortality of infected fish (Harris *et al*. 2000; Olstad *et al*. 2006).

The genus *Gyrodactylus* is generally regarded as less host specific than other monogenean taxa and host-switching is recognised as the prominent force driving *Gyrodactylus* speciation (Bakke *et al*. 2002; King and Cable 2007). The host ranges of many *Gyrodactylus* species are primarily based on their original species descriptions and have not been revised since. Comprehensive geographical and host ranges have only been documented for a few *Gyrodactylus* species, resulting in potential underestimation of host ranges and overestimated host specificity. With the increasing scale of host movements and the associated potential disease risks, there is an increased need for experimental evidence to establish the possible host ranges of these monogeneans, and of the risks they pose to potential susceptible hosts (King and Cable 2007).

Host switching of *Gyrodactylus* species are also important in unrelated fish species, particularly in the case of the least specific gyrodactylid species (Bakke *et al*. 2002). An example of this is *Gyrodactylus turnbulli* Harris 1986, formerly presumed to be a specialist, now known to be capable of transferring to a wider range of hosts under artificial experimental conditions (King and Cable 2007). Comparing host and parasite phylogenies of

Gyrodactylus species and their hosts suggests host switching events occurred millions of years ago (Huyse and Volkaert 2005). Host-switching of *Gyrodactylus* species from goby to goby can be detected by genetic analyses, and is presumed to have taken place since the late Pleistocene (Huyse and Volkaert 2005). Parasite-host co-evolution also plays a major role in host specificity, as the parasites become accustomed to biology and behaviour of their host species (Bakke *et al*. 2002). The propagation of exotic parasites to different closely-related hosts is therefore probable and should be considered a significant threat to the vulnerable fish in the CFR; particularly where transmission pathways for pathogens from alien cyprinids to endemic cyprinids exist. *Gyrodactylus kherulensis* Ergens, 1974 from koi and *G. kobayashii* Hukuda, 1940 from goldfish have been identified as the most common external parasites on these exotic cyprinid during this study (see Chapter 2). The geographical ranges of these parasites have been broadened by the international propagation of ornamental fish via the aquaculture trade, which has resulted in widespread dispersal of pathogens. The potential for exotic parasites finding new susceptible hosts therefore increases. At the current rate of exchange, dispersal of foreign parasitic infections to wild and native populations worldwide is unavoidable (Murray and Peeler 2005; King and Cable 2007).

The inadvertent or deliberate release or escape of an infected fish represents a viable transmission pathway and may have ecologically devastating effects, particularly as the new host may not possess innate immunological defence strategies against the new pathogen (Dove 2000, Mouton *et al.* 2001). The potential for parasite transfer is enhanced when exotic and native species are related (Dove and Ernst 1998; Dove 2000). The infamous *G. salaris*, has had severe pathogenic effects on Atlantic salmon (*Salmo salar* L.), in Norway since its introduction in the 1970's, and has resulted in major ecological and economic damage to both cultured and wild stocks in Norway where it is capable of infecting a number of

salmonid fish species (Scholz 1999; Soleng and Bakke 2001; Dalgaard *et al*. 2003). *Gyrodactylus salaris* is an introduced parasite which has exploited its microhabitat primarily due to the host's susceptibility and inability to combat the foreign pathogen (Appleby and Mo 1997; Peeler *et al*. 2004).

The indigenous cyprinids, the Breede River redfin, *Pseudobarbus burchelli* Smith and the fiery redfin, *P. phlegethon* Barnard are respectively listed as near threatened and endangered by the IUCN, and are both endemic and geographically limited to rivers within the CFR. The risk of introduction and establishment of *G. kherulensis* from koi carp and *G. kobayashii* from goldfish in South African natural aquatic habitats are potentially great. This study intends to test the hypothesis that the exotic parasites, *G. kherulensis* and *G. kobayashii* from koi carp and goldfish respectively can infect local cyprinids in the CFR. The study therefore aims to determine whether *G. kherulensis* from koi carp and *G. kobayashii* from goldfish are able to transfer to the local cyprinids and produce a viable population. This is tested by experimentally infecting local cyprinids with these exotic parasites by cohabitation.

Materials and Methods

Fish collection/ host origins

Pseudobarbus burchelli and *P. phlegethon* were collected from the Hex River (S 33.52905^0; E 19.54032^0) and the Noordhoeks River (S 32.4316^0; E 19.0357^0) in the Western Cape by electrofishing respectively. The fish were collected by permit from Cape Nature and experiments were performed in accordance with the ethical standards of the University of the Western's Cape (UWC) ethics committee. The fish were transported to the laboratory in aerated local river water in plastic buckets. In the aquarium, the fish were placed in well-aerated tanks with local municipal dechlorinated water. Pre-existing *Gyrodactylus* infections were examined by anaesthetizing the fish in 2 ml of 2-phenoxyethanol per litre of water,

removal of parasites and identification of *Gyrodactylus* species. Identification of the *Gyrodactylus* species found was made to species level, following methods according to Shinn *et al.* (2004).

Infection experiments

The challenge trial experiment was undertaken in the aquarium at the Department of Biodiversity and Conservation Biology, University of the Western Cape. A number of infective tanks were set up in the laboratory prior to infection. The fish were held at a water temperature of 18^0C and a 12 h light: 12 h dark regime for the entire duration of the experiment. *Pseudobarbus burchelli* and *P. phlegethon* were infected with both *G. kherulensis* and *G. kobayashii*. Infection took place by co-habitation, as live fish to live fish transmission is regarded as the primary mode of transfer (Van Oosterhout *et al*. 2003). Infected koi carp and goldfish with <100 parasites per fish served as donor fish. A total of 60 *P. burchelli* and 10 *P. phlegethon,* were used for the experiment. Thirty *P. burchelli* were infected with *G. kherulensis*, while the remaining 30 were infected with *G. kobayashii*, however in the case of *P. phlegethon* only five per fish experiment were infected. *Pseudobarbus phlegethon* is endangered and very few of these fish were found while sampling, hence the low host numbers for the experiment. Equal amounts of infected fish were placed in the tanks with the naïve experimental fish. Stocking density in tanks was standardised to 2 g of fish biomass per litre by adjusting the water volume in all tanks for potential transmission.

The duration of the infection trial was 20 days, where each fish species was examined on days 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20. The number of *Gyrodactylus* specimens on the naïve fish was counted by sedating the fish in (0.3 ml/L) of 2-phenoxyethanol, and

immersion of the fish individually in the solution. The number of parasites on the sedated experimental fish was then counted under a stereomicroscope. Daily water changes were done, and fish were fed twice daily. The experiment was terminated on day 20.

Statistical analysis

The prevalence and incidence of infections were determined. Prevalence is the proportion of infected fish in the population (Margolis *et al.* 1982). Incidence is defined as the number of new cases of a parasitic infection within a population during a certain period / the number of uninfected members of the population at the start of the period (Margolis *et al*. 1982).

The abundances of parasites were used to compare the parasite loads on the two species of fish. The abundance of parasites is defined as the total number of parasites found, divided by the total number of the hosts within the population (Margolis *et al*. 1982).

The component parasite population growth rate (r) was determined using the equation $r = \ln (N_t+0.1) - \ln (N_{t-2}+0.1)$, where Nt is the total number of parasites in the component population, and N_{t-2} is the total number of parasites in the component population two days earlier. $N_t+0.1$ was used to avoid using the natural logarithms of zero (Van Oosterhout *et al*. 2003). A decline in the number counted the previous day yields a negative r-value, hence a reduction in growth rate and *vice versa*. The component population is defined as the total number of the infra-populations within a single host population (Esch *et al*. 2002), rather than the infra-population, which is the number of parasites found on a single host within a sample (Margolis *et al*. 1982).

The data were tested for normality and homoscedasticity using Levene's test of homogeneity. For parametric data, a two-way analysis of variance (ANOVA) was performed to analyze differences in susceptibility of the indigenous fish to the two parasite species and non-parametric data were tested using the Kruskal-Wallis test for multiple independent samples. Analyses were performed using STATISTICA 8.0 © (StatSoft, Inc., 2007).

Results

Statistical analysis of infections of G. kherulensis *and* G. kobayashii *on* P. burchelli

Table 1 Prevalence and incidence of *Gyrodactylus kherulensis* and *G. kobayashii* on *Pseudobarbus burchelli* (n=30).

Days	Prevalence (%)		Incidence (%)	
	G. kherulensis	*G. kobayashii*	*G. kherulensis*	*G. kobayashii*
0	0	0	0	0
2	0	0	0	0
4	16.67	16.67	16.67	16.67
6	23.08	16.67	4.00	0.00
8	15.38	6.67	-8.33	-12.00
10	12.5	23.33	-3.85	17.86
12	0	0	-11.11	-30.43
14	0	0	0	0
16	0	0	0	0
18	0	0	0	0
20	0	0	0	0

The incidence and prevalence of *G. kherulensis* and *G. kobayashii* on *P. burchelli* are shown in Table 1. Less than a quarter of the total fish in both experimental tanks were infected. The infection ceased on day 12 (Table 1). The infections of both *G. kherulensis* and *G. kobayashii* lasted for six days, from days 4-10. The extinction of the infection of both *G. kherulensis* and *G. kobayashii* is indicative of innate resistance of *P. burchelli* to both parasites.

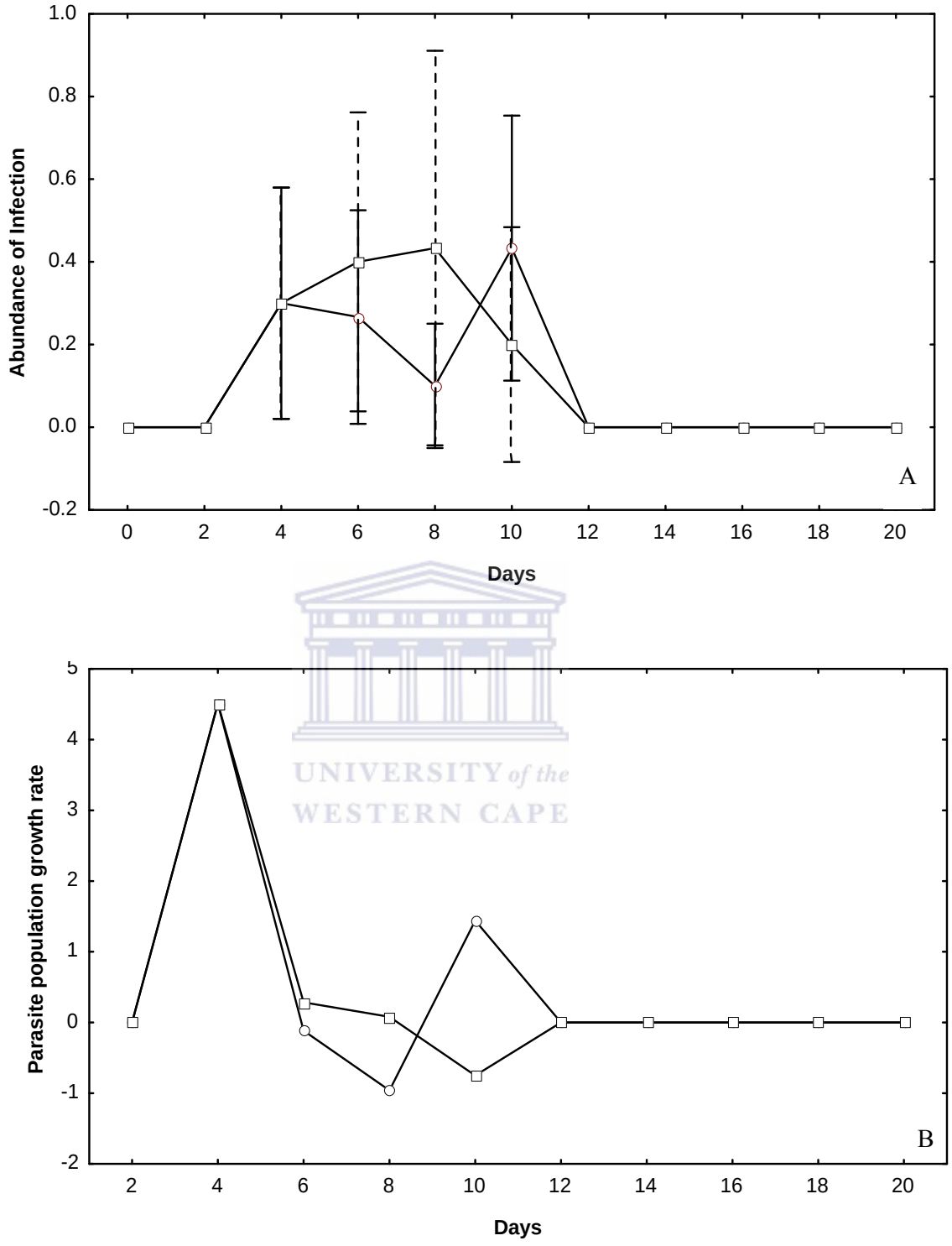


Figure 1A and B: Abundance (mean ± 95% CI) (A) and component parasite population growth rate of *Gyrodactylus kherulensis* and *G. kobayashii* on *Pseudobarbus burchelli* over a period of 20 days. (□= *G. kherulensis*, and dashed error bar; ○= *G. kobayashii*).

Recruitment of the parasites was observed on day 4 in all of the experimental tanks (Fig. 1A). Abundances of both *G. kherulensis* and *G. kobayashii* on *P. burchelli* are shown in Figure 1A. Infections on *P. burchelli* lasted for four days, from days 4 -10. On day 12, the infection had completely died off. *Gyrodactylus kherulensis* populations show a progressive increase until day 8. On day 10, *G. kherulensis* abundances on *P. burchelli* decreased, and the population died off on day 12. *Gyrodactylus kobayashii* abundances on *P. burchelli* decreased on day 6, and was further reduced on day 8. The abundance then slightly increases on day 10, and no parasites were observed on *P. burchelli* on day 12 or thereafter.

The total component population growth rates of both *G. kherulensis* and *G. kobayashii* on *P. burchelli* peaked on day 4, on the initial day of recruitment, and decreased thereafter (Fig. 1B). A reduction in the growth rate was observed on day 10. The growth rate peaked on day 4, and subsequently, decreased on day 8, resulting in a negative total growth rate. The growth rate of *G. kobayashii* on *P. burchelli* decreased to zero and no parasites were reported on these fish from day 12. The parasite population growth rate indicates again that *P. burchelli* are resistant to both *G. kherulensis* and *G. kobayashii*.

Statistical analysis of infections of G. kherulensis *and* G. kobayashii *on* P. phlegethon

Table 2 Prevalence and incidence of *Gyrodactylus kherulensis* and *G. kobayashii* on *Pseudobarbus phlegethon* (n=5).

Days	Prevalence (%)		Incidence (%)	
	G. kherulensis	*G. kobayashii*	*G. kherulensis*	*G. kobayashii*
0	0	0	0	0
2	0	0	0	0
4	40	40	6.67	13.33
6	40	80	0	0
8	40	80	0	3.85
10	40	100	0	0
12	20	100	-3.57	0
14	20	100	0	0
16	20	100	0	-4.00
18	40	100	3.45	0
20	20	75	-3.57	-3.85

The prevalence and incidence of *G. kherulensis* and *G. kobayashii* on *P. phlegethon* is shown in Table 2. The prevalence of *G. kobayashii* on *P. phlegethon* was the greatest, when compared to the other experimental tanks, and reached the maximum of 100% by day 10. This persisted until day 18, on day 20 the prevalence was reduced to 75%. Infections of both *G. kherulensis* and *G. kobayashii* lasted from day 4 to the end of the experiment. *Gyrodactylus kobayashii* infected all the fish, while *G. kherulensis* only infected a maximum of two fish.

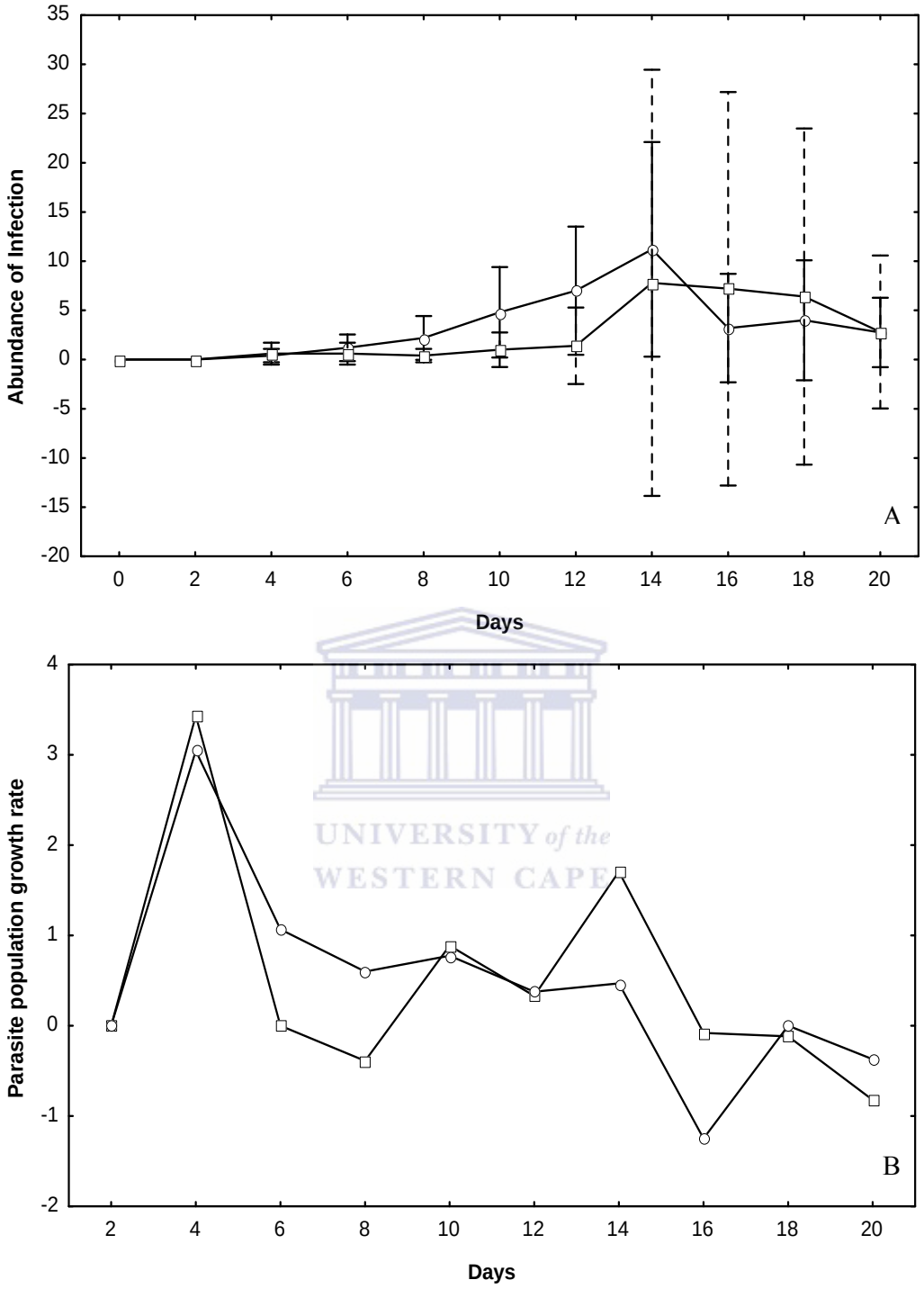


Figure 2A and B: Abundance (mean ± 95% CI) (A) and component parasite population growth rate of *Gyrodactylus kherulensis* and *G. kobayashii* on *Pseudobarbus phlegethon* over a period of 20 days. (□= *G. kherulensis,* and dashed error bar; ○= *G. kobayashii*).

The abundance of *G. kherulensis* on *P. phlegethon* remained relatively low until day 14, where a slight increase was observed. *Gyrodactylus kherulensis* populations on *P. phlegethon* then decreased thereafter. The abundance of *G. kobayashii* on *P. phlegethon* showed a steady increase from day 4, with the greatest abundance on day 14. On day 16, the population decreased and the abundances remained relatively constant till the end of the experiment (Fig. 2A).

The parasite population growth rate of *G. kherulensis* on *P. phlegethon* varied for the entire duration of the experiment, it peaked on day 4, when the parasites are initially reported on the body surface of the fish. There was a subsequent decline on days 6 and 8, and another increase on day 10. The growth rate decreased on day 12 followed by an increase on day 14. The growth then stabilized on days 16 and 18 (Fig. 2B). The parasite population growth rate of *G. kobayashii* on *P. phlegethon* peaks on day 4 and decreases progressively until day 16. There is a slight increase on day 18, and a reduction on day 20.

Statistical analysis of comparing infections of G. kherulensis *and* G. kobayashii *on* P. burchelli *and* P. phlegethon

Table 3 Comparison of the abundances of *Gyrodactylus kherulensis* and *G. kobayashii* on the indigenous *Pseudobarbus burchelli* and *P. phlegethon*. Significant values (p> 0.05) are shown in bold.

P. phlegethon/G. kherulensis			
1.000	***P .phlegethon/ G. kobayashii***		
0.034	**0.015**	***P. burchelli/ G. kherulensis***	1.000
0.027	**0.011**	1.000	***P. burchelli/G. kobayashii***

The abundance data were non-parametric, and a Kruskal-Wallis (comparing multiple independent samples) was used to compare the abundances of the four groups for the duration of the experiment. The results show significant differences between the infections in the two fish species (Table 3). There were no significant differences between *P. burchelli* infected with *G. kherulensis* and *G. kobayashii*; however, both these groups differed significantly from infections of both parasite species on *P. phlegethon* (Table 3). *Pseudobarbus phlegethon* were able maintain the infection till the end of the experiment, while the infection ceased on *P. burchelli* by day 10.

Table 4 Analysis of variance (ANOVA) test to compare the parasite growth rates of the four groups for 20 days.

P. phlegethon/G. kherulensis			
0.999	***P. phlegethon/ G. kobayashii***		
0.999	0.999	***P. burchelli/ G. kherulensis***	
0.999	0.999	0.999	***P. burchelli/G. kobayashii***

There were no significant differences in the parasite growth rates between the two parasites on the two species of fish (Table 4). There was, however, a significant difference in the days, with day 4 having a significantly greater growth rate in all groups (F=18.207; p= 0.000183). The exotic parasites were initially recorded to transfer onto the indigenous fish on day 4 in all experimental tanks. There was also no significant differences between the maximum parasite loads of the four groups (H= 12.446; p= 0.191) (Fig 4). *G. kherulensis* on *P. phlegethon* has the greatest maximum parasite load (Fig. 3).

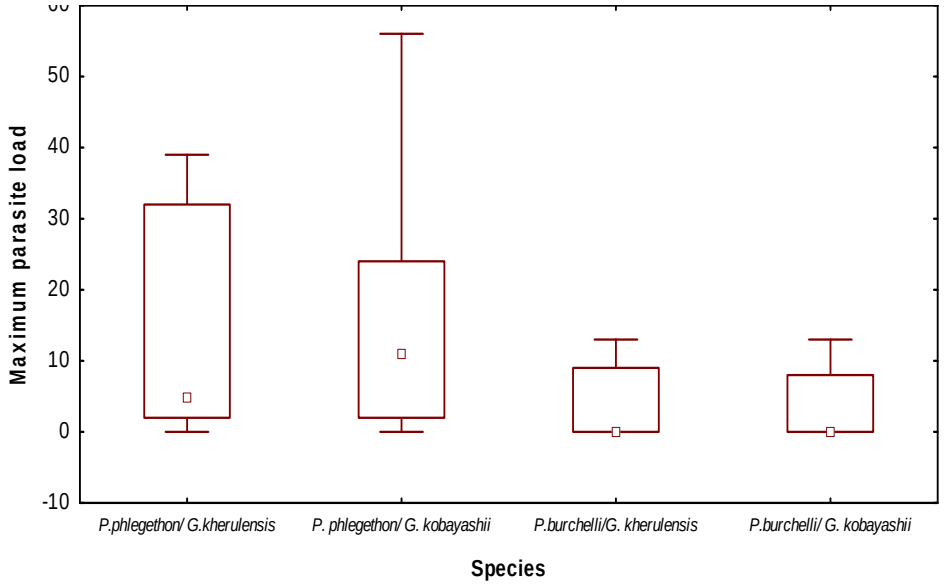


Figure 3 Boxplot of the maximum parasite load for *Gyrodactylus kherulensis* and *G. kobayashii* on *Pseudobarbus burchelli* and *P. phlegethon* showing the first and third quartile, the median is shown in the centre, and the error bars show the minimum and maximum values.

Discussion

Gyrodactylus kherulensis and *G. kobayashii* have been extensively spread worldwide, and their propagation is facilitated by the international commercial propagation of their hosts. These parasites have the potential to have detrimental effects on the biodiversity of indigenous fishes in the Western Cape, South Africa. There is a concern surrounding this and experimental infections give major insight to the potential consequences of these parasites.

This experimental study confirms that these species have the potential to establish themselves on indigenous cyprinid fish in the Western Cape, even in the presence of their natural hosts. No other challenge trials have been conducted ascertaining the host ranges of *G. kherulensis* and *G. kobayashii* and their potential host range is unknown.

The native cyprinids, *Pseudobarbus burchelli* and *P. phlegethon* were both successfully infected with *Gyrodactylus* species. however, the infection ceased to persist in *P. burchelli* and both parasite species continued on *P. phlegethon* for the entire duration of the experiment. *Gyrodactylus kherulensis* can be deemed a specialist (eg. Matejusova *et al*. 2000; Simkova *et al.* 2006), as it is listed as infecting only *Cyprinus carpio* and its subspecies (Harris *et al.* 2004), *G. kobayashii*, on the other hand, is capable of infecting two listed species, *Carassius auratus* and *Leuciscus walewskii* Dyboswski and can therefore be considered a species with a lower specificity (Harris *et al.* 2004). The preliminary results suggest that *G. kobayashii* poses a greater threat than *G. kherulensis*. *G. kobayashii* is a generalist (*e.g.* Harris *et al*. 2004), and shows an increased abundance and prevalence on *P. phlegethon*. Two of the five *P. phlegethon* were infected with *G. kherulensis* (40%) and seems to have established a sustained population on the infected fish. Similarly, *G. turnbulli* infecting *Poecilia reticulata*, regarded as a specialist, also showed an increased host range

empirically; transferring to unnatural hosts and this particular worm was also capable of using cyprinids as reservoir hosts (King and Cable 2007). Another example includes *G. bullatarudis* Turnbull, 1956 a tropical parasite capable of survival and reproduction on unrelated hosts from temperate environmental conditions (King *et al*. 2009). There is extensive experimental evidence showing the broad host range of the infamous monogenean parasite, *G. salaris* Malmberg, 1957, which is capable of infecting a wide range of fish within the family Salmonidae (eg. Soleng and Bakke 2001; Bakke *et al*. 2002; Olstad *et al.* 2006; Winger *et al*. 2008).

According to the three categories of infection proposed by Bakke *et al.* (2002), *P. burchelli* can be regarded as innately resistant to both parasite species, as parasite population growth rate remained constant and ceased thereafter. *Pseudobarbus phlegethon,* on the other hand, can be described as responding to both parasite species. In most experimental conditions, there is initial exponential parasite population growth followed by a period of parasite population reduction, to the point where the whole population reaches extinction, or only a few persist on the host (Harris *et al*. 2000).

The presence of both the indigenous fish and the exotic cyprinids in the same temperate habitat is of concern. The probability of interaction and therefore parasite transfer in the wild exists. Dislodged parasites in the water column may also be a means of transmission to a new host in the same habitat. The chances of acquiring a new host in the wild are low due to the reduced host density.

Although laboratory studies have explicitly illustrated the transfer of *Gyrodactylus* species to unnatural hosts, it can hardly be accepted as a successful host switch under natural conditions

(Zietara *et al.* 2008). The dynamics in river systems differ considerably from those under aquarium conditions and this therefore has implications in aquaculture in the mixing of species rather than naturally. A deterrent in natural systems is the possibility that the fishes' natural parasites might competitively exclude the foreign parasites (Poulin and Keeney 2008). Another limitation is the possibility that the fish might build up resistance against the parasites within a short period, preventing exponential growth rates (*e.g*. Van Oosterhout *et al*. 2003). Conversely, the parasite might evolve to the novel host over time, as some parasite genetic variation may occur, making the population capable of infecting and potentially exploiting the new host (Poulin and Keeney 2008; King *et al*. 2009).

Parasite transfer in the wild, although unlikely, should be considered a risk, especially in the presence of Red Data List species sharing the same temperate habitat. In this regard, it may have major implications for freshwater fish biodiversity in the Western Cape, South Africa, as virulent pathogens and highly fecund individuals may occur and results may be negative. Due to the majority of the fish being cyprinids and most of these already being threatened, alien parasite establishment poses an additional threat to these already vulnerable indigenous fish. Transmission routes therefore exist for *Gyrodactylus* species and these would therefore exist for other pathogens, such as fungi, bacteria and viruses, which may be less specific and more virulent. More infections trial studies are encouraged to determine the host ranges of exotic parasites and the effects they may have on indigenous fish in the CFR.

References:

ANDREWS, C. 1990. The ornamental fish trade and fish conservation. *Journal of Fish Biology* **37**, (Suppl A) 53 –59.

APPLEBY, C., Mo, T.A. 1997. Population dynamics of *Gyrodactylus salaris* (Monogenea) infecting Atlantic Salmon, *Salmo salar,* parr in the River Batnfjordselva, Norway. *The Journal of Parasitology* **83**, (1) 23-30.

BAKKE, T.A., Harris, P.D., Cable, J. 2002. Host specificity dynamics: observations on gyrodactylid monogeneans. *International Journal for Parasitology* **32**, 281–308.

CAMBRAY, J.A. 2003. Impact on indigenous species biodiversity caused by the globalisation of alien recreational freshwater fisheries. *Hydrobiologia* **500**, 217-230.

DALGAARD, M.B., Nielsen, C.V., Buchmann, K. 2003. Comparative susceptibility of two races of *Salmo salar* (Baltic Lule river and Atlantic Conon river strains) to infection with *Gyrodactylus salaris*. *Diseases of Aquatic Organisms* **53**, 173-176.

DE MOOR, I.J., Bruton, M.N. 1988. Atlas of alien and translocated indigenous aquatic animals in southern Africa. South African National Scientific Programmes Report No. 144: 310 pp.

DOVE, A.D.M. 2000. Richness patterns in the parasite communities of exotic poeciliid fishes. *Parasitology* **120**, 609-623.

DOVE, A., Ernst, I. 1998. Concurrent invaders of four exotic species of Monogenea now established on exotic freshwater fishes in Australia. *International Journal for Parasitology* **28,** 1755-1764.

ESCH, G.W., Barger, M., Fellis, K.J. 2002. The transmission of digenetic trematodes: style, elegance, complexity. *Integrative and Comparative Biology* **42**, 304-312.

HARRIS, P.D., Soleng, A., Bakke, T.A. 2000. Increased susceptibility of salmonids to the monogenean *Gyrodactylus salaris* following administration of hydrocortisone acetate. *Parasitology* **120**, 57-64.

HARRIS, P.D., Shinn, A.P., Cable, J., Bakke, T.A. 2004. Nominal species of the genus *Gyrodactylus* von Nordmann 1832 (Monogenea: Gyrodactylidae), with a list of principal host species. *Systematic Parasitology* **59,** 1–27.

HUYSE, T., Volkaert, F.A.M. 2005. Comparing host and parasite phylogenies: *Gyrodactylus* flatworms jumping from goby to goby. *Systematic Biology* **54**, 710-718.

IMPSON, N.D. 2007. State of Biodiversity: Western Cape Province. Chapter 3: Freshwater fishes, Western Cape State of Biodiversity 2007. Western Cape Conservation Board, Cape Town ISBN 978-0-620-39289-1.

IMPSON, N.D., Bills, I.R., Cambray, J.A. 2000. State of Biodiversity: Western Cape Province, South Africa Freshwater Fishes. Western Cape State of Biodiversity, pp 1-2.

KING, T.A., Cable, J. 2007. Experimental infections of the Monogenean *Gyrodactylus turnbulli* indicate that it is not a strict specialist. *International Journal for Parasitology* **37**, 663-672.

KING, T.A., van Oosterhout, C., Cable, J. 2009. Experimental infections with the tropical monogenean, *Gyrodactylus bullatarudis*: Potential invader or experimental fluke? *Parasitology International* **58,** 249-254.

KOEHN, J.D. 2004. Carp (*Cyprinus carpio*) as a powerful invader in Australian waterways. *Freshwater Biology* **49**, 882–894.

MARGOLIS, L., Esch, G.W., Holmes, J.C., Kuris, A.M., Schad, G.A. 1982. The use of ecological terms in parasitology (Report of an *ad hoc* committee of the American society of parasitologists). *Journal of Parasitology* **68**, 131-133.

MATEJUSOVA, I., Morand, S., Gelnar, M. 2000. Nestedness in assemblages of gyrodactylids (Monogenea: Gyrodactylidae) parasitizing two species of cyprinid- with reference to generalists and specialists. *International Journal of Parasitology* **30**, 1153-1158.

MOUTON, A. Basson, L., Impson, D. 2001. Health status of ornamental freshwater fishes imported to South Africa: a pilot study. *Aquarium Sciences and Conservation* **3**, 327-333.

MURRAY, A.G., Peeler, E.J. 2005. A framework for understanding the potential for emerging diseases in aquaculture. *Preventive Veterinary Medicine* **67**, 223–235.

MYERS, N., Mittermeier, R.A., Mittermeier, C.G., da Fonseca, G.A.B., Kent, J. 2000. Biodiversity hotspots for conservation priorities. *Nature* **403**, 853–858.

OLSTAD, K., Cable, J., Robertson, G., Bakke,T.A. 2006. Unpredicted transmission strategy of *Gyrodactylus salaris* (Monogenea: Gyrodactylidae): survival and infectivity of parasites on dead hosts. *Parasitology* **133**, 33-41.

PEELER, E.J., Gardiner, R., Thrush, M.A. 2004. Qualitative risk assessment of routes of transmission of the exotic fish parasite *Gyrodactylus salaris* between river catchments in England and Wales. *Preventive Veterinary Medicine* **64**, 175–189.

POULIN, R., Keeney, D.B. 2008. Host specificity under molecular and experimental scrutiny. *Trends in Parasitology* **24** (1), 24-28.

PRESSEY, R.L., Cowling, R.M., Rouget, M. 2003. Formulating conservation targets for biodiversity pattern and process in the Cape Floristic Region, South Africa. *Biological Conservation* **112**, 99-127.

REBELO, A.G. 1992. Red Data Book species in the Cape Floristic region: Threats, priorities and target species. *Transactions of the Royal Society of South Africa* **48** (1), 55-86.

SCHOLZ, T. 1999. Parasites in cultured and feral fish. *Veterinary Parasitology* **84**, 317–335.

SHINN, A.P., Hansen, H., Olstad, K., Bachmann, L., Bakke, T.A. 2004. The use of morphometric characters to discriminate specimens of laboratory-reared and wild populations of *Gyrodactylus salaris* and *G. thymalli* (Monogenea). *Folia Parasitologica* **51**, 239-252.

SIMKOVA, A.S., Verneau, O., Gelnar, M., Morand, S. 2006. Specificity and specialization of congeneric monogeneans parasitizing cyprinid fish. *Evolution* **60** (5), 1023–1037.

SKELTON, P. 2001. A Complete Guide to the Freshwater Fishes of Southern Africa. Struik Publishers, Cape Town, South Africa, 395 pp.

SOLENG, A, Bakke, T.A. 2001. The susceptibility of grayling (*Thymallus thymallus*) to experimental infections with the monogenean *Gyrodactylus salaris*. *International Journal for Parasitology* **31**, 793-797.

VAN OOSTERHOUT, C., Harris, P.D., Cable, J. 2003. Marked variation in parasite resistance between two wild populations in the Trinidadian guppy, *Poecilia reticulata* (Pisces: Poecilidae). *Biological Journal of the Linnean Society* **79**, 645-651.

WINGER, A.C., Primicerio, R., Kristoffersen, R., Siikavuopio, S.I., Knudsen, R. 2008. *Gyrodactylus salaris* infecting allopatric Arctic charr *Salvelinus alpinus* fry: an experimental study of host survival. *Journal of Fish Biology* **73**, 2198-2209.

ZIETARA, M.S., Kuusela, J., Veselov, Lumme, J. 2008. Molecular faunistics of accidental infections of *Gyrodactylus* Nordmann, 1832 (Monogenea) parasitic on salmon *Salmo salar* L. and brown trout *Salmo trutta* in NW Russia. *Systematic Parasitology* **69**, 123-135.

Chapter 4

Gyrodactylus burchelli n.sp. from the redfin minnow, *Pseudobarbus burchelli* Smith, 1841 (Pisces, Cyprinidae)

Abstract

A new species of *Gyrodactylus* von Nordmann, 1832 (Monogenea) is reported from the skin and fins of the endemic South African cyprinid, *Pseudobarbus burchelli* in the Western Cape Province, South Africa. *Gyrodactylus burchelli* n.sp. morphologically differs from the two species of *Gyrodactylus* known from African cyprinids, namely, *G. ivindoensis* Price & Gery, 1968 and *G. kyogae* Paperna, 1973. The differences are primarily illustrated by the slightly inward curved roots and the deep ventral bar articulation point which distinguish *G. burchelli* from *G. ivindoensis* and *G. kyogae*. The marginal hooklets of *G. burchelli*, however, somewhat resemble those of *G. ivindoensis*, but is G.burchelli has a shorter shaft length and a more rounded heel.

Introduction

The genus *Pseudobarbus* Smith, 1841, termed redfin minnows, consists of seven species endemic to the temperate regions of southern Africa (Swartz 2005). Redfins are primarily confined to the major rivers in the Cape Floristic Region, with the exception of one species, *Pseudobarbus quathlambae*, which is found in Lesotho (Swartz *et al*. 2009). *Pseudobarbus. burchelli*, more commonly known as the Breede River or Burchell's redfin, was the first species in the genus to be described in the 1800's (Skelton 1980). *Pseudobarbus burchelli* occur in the Breede River and adjoining tributaries, and their distribution is limited to the cool, temperate waters of the Western Cape, South Africa (Cambray and Stuart 1985; Skelton 2001). *Pseudobarbus burchelli* has been listed as "near threatened" by the IUCN red

data book list. The biology and ecology of the species has been extensively studied, and recently the taxonomy of some species within the genus, including *P. burchelli* have been re-examined with the aid of molecular data (Swartz et al. 2009).

Two *Gyrodactylus* species have been reported from the Cape Floristic Region, namely *G. cichlidarum* Paperna, 1968 and *G. ulinganisus* Garcia-Vasquez *et al*., 2011, both from the Mozambique tilapia, *Oreochromis mossambicus* Peters (Garcia-Vasquez *et al.*, 2011) and no monogeneans have been reported from freshwater fish endemic to the Cape Floristic region.

Members of the monogenean genus *Gyrodactylus* are a diverse and ubiquitous group of parasites (Harris *et al*. 2004). Over 400 species have been described, yet it is assumed that this group of parasites is at least as diverse as the number of described fish worldwide (Bakke *et al*. 2002; Harris *et al.* 2004). Currently, only 24 species of the genus *Gyrodactylus* have been described from Africa and of these, two, *G. tranvaalensis* Prudhoe and Hussey, 1977 from the sharptooth catfish, *Clarias gariepinus* Burchell and *G. ulinganisus* from *Oreochromis mossambicus* have been described from South Africa (Christison *et al*. 2005; Přikrylová *et al*. 2009; Garcia-Vasquez *et al.,* 2011; Shinn *et al*. unpublished). Only two species have been described from African cyprinids, these being *G. ivindoensis* Price & Gery, 1968, from *Barbus holotaenia* Boulenger in Gabon and *G. kyogae* Paperna, 1973 from *Barbus perince* Rüppell in Uganda (Christison *et al.* 2005). During a survey of monogenean parasites of local cyprinids in the Hex River in South Africa, a new species of *Gyrodactylus* was found on the body surfaces of the endemic cyprinid, *P. burchelli*.

The paper provides a detailed morphological description of *Gyrodactylus burchelli* n. sp. from the endemic Breede River redfin, *P. burchelli*.

Materials and Methods

Morphometric analysis

Pseudobarbus burchelli specimens were collected from the Hex River by electrofishing during the summer months of 2007. The fish were transported to the laboratory alive in local river water and held in buckets for 24 hours before processing. In order to assess parasite numbers, the fish were euthanised by an overdose (2 ml/L) of 2-phenoxyethanol. Parasites were removed from the skin and fins of the fish and the *Gyrodactylus* specimens were quantified and preserved in 70% ethanol. Whole specimens were mounted in glycerine ammonium picrate. The haptors of ten worms were cut off and the remaining bodies retained in absolute ethanol for subsequent molecular analysis, although the data are not presented here. The proteolytic enzymatic digestion was used to remove excess body tissue from the haptoral sclerites (Harris *et al*. 1999). The sclerites were viewed at 1000x magnification under a compound microscope and were measured according to Shinn *et al.* (2004). Photomicrographs of the haptoral sclerites were taken.

The indigenous redfin minnows were collected by a permit obtained from Cape Nature. The fish were collected and euthanised according to the ethical standards of the University of the Western's Cape (UWC) ethics committee.

Results

***Gyrodactylus burchelli* n.sp.**

Type-host: *Pseudobarbus burchelli* (Smith, 1841)

Site: Body surfaces, fins and gills of host fish

Type-locality: Hex River, De Doorns, South Africa (S 33.52905 ^{0}; E 19.54032 ^{0})

Etymology: The species was named for the type host

Type-material: Holotype: NHM (Natural History Museum)

: Paratypes: SAMCTA (South African Museum of Cape Town) 29478 (2 specimens)

:SAMCTA 29477 (2 specimens)

Description

Twenty coverslip flattened specimens 300.5±36.4 (227.9-336.1) long and 89.5±18.3 (66.1-113.8) wide; opisthaptor 65.9±4.3 (58.7-72.1) long and 67.1±10.8 (53.9-87.4) wide; anterior pharynx length 24.0±1.6 (22.2-26.9) and 23.9±2.2 (20.9-27.1) wide; posterior pharynx 26.6±2.9 (17.0-26.1) long and 22.0±3 (18.2-27.0) wide. Male copulatory organ (MCO) diameter 10.0±1.7 (8.0-12.0), with one large apical spine and seven smaller spines arranged posterior to the apical spine in a single row. Hamulus aperture 12.7±1.3 (9.5-14.9); proximal shaft width 6.0±0.6 (4.5-7.0); hamulus point 18.6±2.7 (12.0-23.2) long; distal shaft width 4.7±0.7 (3.2-6.0); Shaft 22.9±2.3 (17.7-27.7) long; inner curve length 4.8±0.6 (3.3-5.4); outer aperture angle 35.1^{0}±4.0 (27.3-44.2); point curve angle 26.9^{0}±7.2 (10.2-44.4), inner aperture angle 41.6^{0}±6.8 (27.7-55.2); hamulus root length 11.9±1.5 (9.7-15.5); total hamulus length 45.0±1.8 (41.1-47.7). Ventral bar total width 21.0±1.1 (19.1-22.8) and total length 20.8±1.8 (16.4-24.0); ventral bar process to mid length 3.2±0.6 (2.0-4.3); ventral bar median length

5.2±0.6 (3.9-6.0); ventral bar process length 2.2±0.4 (1.4-3.2); ventral bar membrane 12.5±1.9 (9.1-16.6) long. Marginal hooklets total length 26.4±1.7 (23.6-29.9); marginal hooklet shaft 21.2±0.9 (19.8-22.4) long; sickle 6.0±0.4 (5.4-6.5) long; sickle proximal width 3.6±0.2 (3.4-4.0); sickle toe length 1.6±0.3 (0.8-1.9); sickle distal width 4.0±0.4 (3.6-4.7); sickle aperture 4.8±0.4 (4.1-5.4); instep / arch height 1.0±0.3 (0.5-1.6). Dorsal bar 16.2±1.6 (14.3-18.9) long and 2.2±0.4 (1.6-3.1) wide.

Remarks

Marked differences between *G. burchelli* and the only two published records of *Gyrodactylus* species from African cyprinids, *G. ivindoensis* and *G. kyogae,* are evident. *Gyrodactylus burchelli* bears very little morphological similarity to either of these species. There is, however, a resemblance in the morphology of the marginal hooklets of *G. burchelli* and *G. ivindoensis*, however differences are noted by the longer shaft length of *G .ivindoensis.* The marginal hooklets of *G. burchelli* could be distinguished by the rounded heel of the marginal hook sickle, whereas *G. ivindoensis* has a more defined curve. *Gyrodactylus ivindoensis* also has a longer and more slender sickle blade. The marginal hooklets of *G. burchelli* and *G. kyogae* differ quite significantly in the shape and size of the marginal hooklets. The shape of the marginal hooklets and hamuli of *G. burchelli* are the most diagnostic characters for discrimination when comparing the three species. The hamulus roots and the deep ventral bar articulation point of *G. burchelli* is another distinguishing character when comparing the three species.

Table 1: Morphological measurements comparisons of *Gyrodactylus* species found on African cyprinids. All measurement are in micrometres (µm)

	***Gyrodactylus burchelli* n.sp- Type population**	***Gyrodactylus ivindoensis* (Price & Gery 1968)** (Shinn *et al*. unpublished)	***Gyrodactylus kyogae* (Paperna 1973)** (Shinn *et al*. unpublished)
Country of origin	**South Africa**	**Gabon**	**Uganda**
Host	***Pseudobarbus burchelli (Smith, 1815)***	***Barbus sp. (holotaenia aff)***	***Barbus neumayeri***
Holotype	**NHM**	**U.S Nat'l Parasite Coll. 62986**	**MRAC-M.T.35.925**
Measurement	**Mean±std dev (range) n=20**	**Mean (range)**	**Mean (range)**
Total body length	300.5±36.4 (227.9-336.1)	**292 (278-314)**	**(180-270)**
Total body width	89.5±18.3 (66.1-113.8)	**81 (79-92)**	\
Haptor length	65.9±4.3 (58.7-72.1)	\	**40-45**
Haptor width	67.1±10.8 (53.9-87.4)	\	**40-60**
Anterior pharynx length	24.0±1.6 (22.2-26.9)	\	\
Anterior pharynx width	23.9±2.2 (20.9-27.1)	\	\
Posterior pharynx length	22.6±2.9 (17.0-26.1)	\	\
Posterior pharynx width	22.0±3.0 (18.2-27.0)	\	\
MCO diameter	10.0±1.7 (8.0-12.0)	\	
Number of MCO spines	1:7	\	\
		****	****
Hamulus			
Aperture	12.7±1.3 (9.5-14.9)	16.5	14.9
Proximal shaft width	6.0±0.6 (4.5-7.0)	8.9	6.9
Point length	18.6±2.7 (12.0-23.2)	21.4	15.3
Distal shaft width	4.7±0.7 (3.2-6.0)	4.5	3.1
Shaft length	22.9±2.3 (17.7-27.7)	37.6	25.0
Inner curve length	4.8±0.6 (3.3-5.4)	1.2	1.5
Outer aperture angle	35.1±4.0 (27.3-44.2)	29.0	51.1
Point curve angle	26.9±7.2 (10.2-44.4)	4.4	6.9
Inner aperture angle	41.6±6.8 (27.7-55.2)	34.6	60.2

Root length	11.9±1.5 (9.7-15.5)	19.4	9.4
Total length	45.0±1.8 (41.1-47.7)	56.8	32.1 **(23-33)**
Ventral bar			
Total width	21.0±1.1 (19.1-22.8)	27.1	11.9
Total length	20.8±1.8 (16.4-24.0)	18.1 **(17-22)**	12.2 **(9-11)**
Process to mid length	3.2±0.6 (2.0-4.3)	2.5	1.6
Median length	5.2± 0.6 (3.9-6.0)	5.8	4.0
Process length	2.2±0.4 (1.4-3.2)	1.3	no process
Membrane length.	12.5±1.9 (9.1-16.6)	11.0	7.6
Marginal hooklets			
Total length	26.4±1.7 (23.6-29.9)	29.6 **(21-24)**	15.5 **(14-16)**
Shaft length	21.2±0.9 (19.8-22.4)	24.7	12.2
Sickle length	6.0±0.4 (5.4-6.5)	5.5	3.4
Sickle proximal width	3.6±0.2 (3.4-4.0)	3.3	2.9
Sickle toe length	1.6±0.3 (0.8-1.9)	1.9	1.7
Sickle distal width	4.0±0.4 (3.6-4.7)	2.5	2.1
Sickle aperture	4.8±0.4 (4.1-5.4)	5.3	3.1
Sickle instep / arch height	1.0±0.3 (0.5-1.6)	\	0.6
Dorsal bar			
Length	16.2±1.6 (14.3-18.9)	21.2 **(20-23)**	9.4
Width	2.2±0.4 (1.6-3.1)	2.6 **(14-17)**	0.7

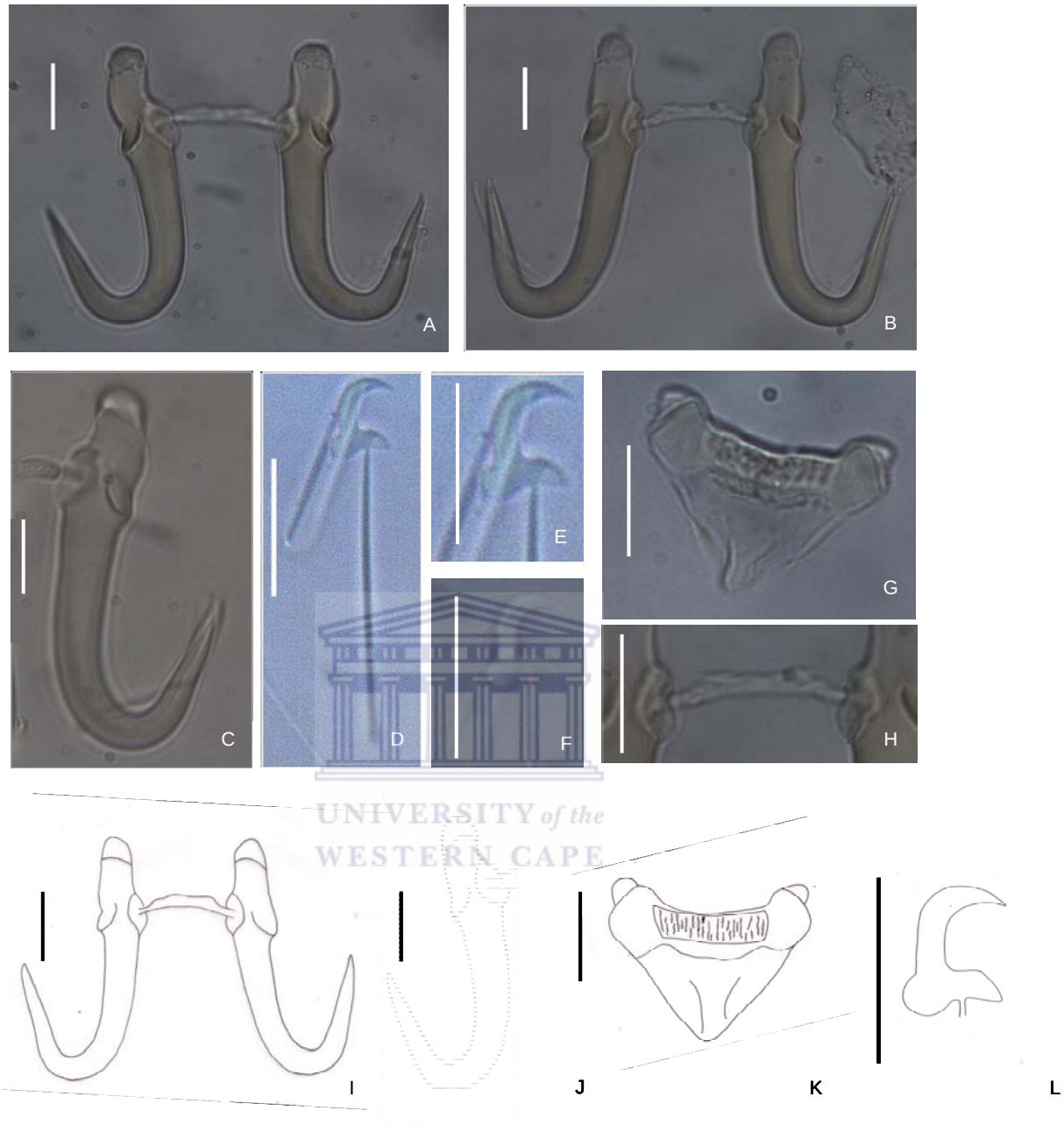


Figure 1 Micrographs and line drawing of the attachment organs of *Gyrodactylus burchelli* n. sp. A B and I– Hamulus complex, C and J- hamulus, D E F and L – marginal hooklets, G and K – ventral bar, H- dorsal bar. Scale bar = 10 µm.

Discussion

Pseudobarbus burchelli is one of the threatened native cyprinids of major ecological importance to the Cape Floristic Region. Population assessments show a decline in the numbers of *P. burchelli* in the wild and the reduction in population numbers has sparked major concern (Swartz and Impson 2007). Research directed towards conserving the indigenous fish species in the Western Cape has increased quite considerably with this increased pressure on population numbers. Taxonomic research and systematics of the threatened, endemic fish in the Western Cape is currently of key conservation importance (*e.g.* Swartz 2005; Swartz *et al*. 2009). However, the parasites of these fishes have not been examined, even though the indigenous fish are exposed to exotic fish and their potential to be invaded by alien parasites is therefore enhanced (Impson 2007; Swartz and Impson 2007). There is a lack of information regarding native African *Gyrodactylus* species, however, the unpublished manuscript of Shinn *et al.* intends to standardise the nomenclature of the species currently described from the African continent, thereby providing a stable platform for future taxonomic work on the African representatives of this genus. No *Gyrodactylus* species have been described from *P. burchelli* or any other endemic cyprinids in the Western Cape Province.

Gyrodactylus burchelli n. sp. is the third gyrodactylid species to be described from South Africa. The parasite is assumed to be a natural parasite of *P. burchelli* as it does not morphologically resemble any of the *Gyrodactylus* species known from the alien cyprinid fish that occur sympatrically with the native fish. *Gyrodactylus burchelli* was compared to *G. kyogae* and *G. ivindoensis*, the only two species known from African cyprinids, because monogenean parasites tend to be quite host specific, at least to the fish family. The morphometric measurements of the three African *Gyrodactylus* species from African cyprinids are shown in Table 1.

The morphology of the attachment organs of *G. burchelli* differs quite considerably from those of *G. ivindoensis* and *G. kyogae*. The hooks in all three species are markedly different from each other, with *G. ivindoensis* having the longest hamuli which have a total length of 55 µm (52 µm -58 µm) and *G. kyogae* having the smallest hamuli with a mean length of 32.1 µm ranging from 23 µm -33 µm. The marginal hooklets shaft and hamulus roots are most likely the most diagnostic features distinguishing the three species, *G. ivindoensis* has stout, straight roots; *G. kyogae* has short robust roots and *G. burchelli* has slightly twisted roots which curl inward in most specimens (Fig. 1A). *Gyrodactylus burchelli* however, has the smallest hamulus aperture and shaft lengths. Some intraspecific variations exist among specimens of *G. burchelli*, particularly in the hamulus roots. Generally, there is a slight inward curve in the majority of the specimens; however, some have relatively straight roots (Figs. 1A and B). The hamulus roots are the softest features of the hamuli and the twist might be due to movement of the parasite under the coverslip or mounting induced artefacts (Geets *et al.* 1999).

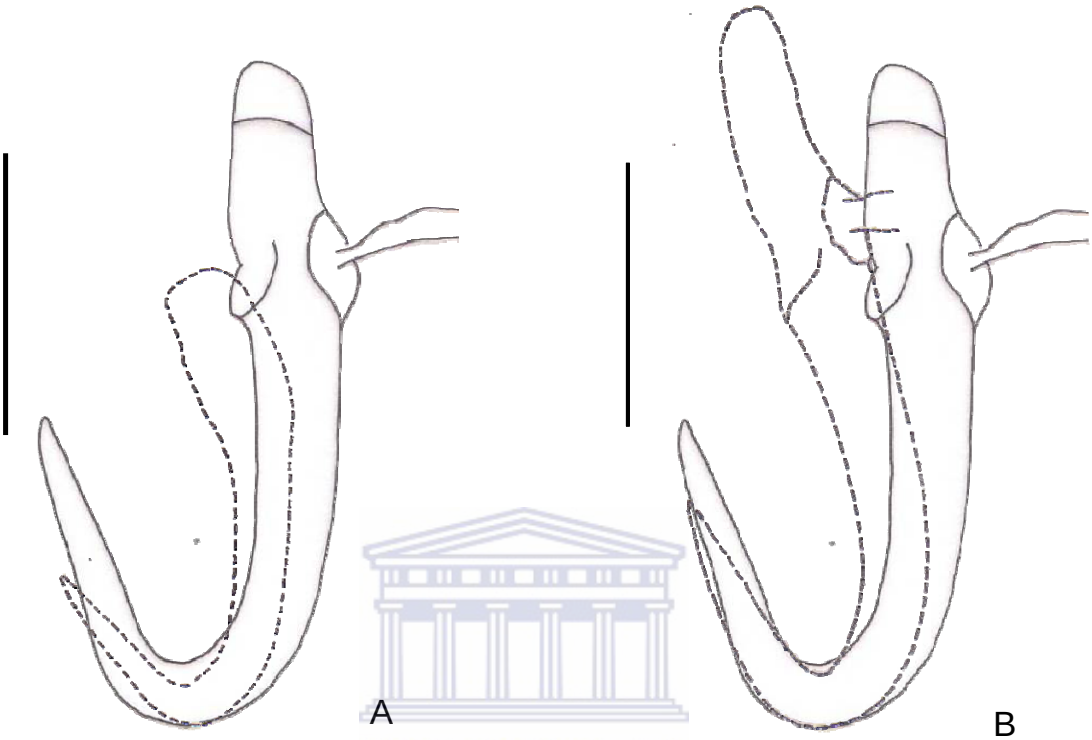


Figure 2 Morphological comparisons of the hamuli of *Gyrodactylus burchelli,* with A- *G. kyogae* and B- *G. ivindoensis.* The solid line represents *G. burchelli.* Scale bar = 10 µm.

The ventral bar of *G. burchelli* is shaped quite differently from *G. ivindoensis* and *G. kyogae*, with a sharply V-shape at the apex (Figs. 1G and 1K). The other two species have rounded ventral bar membrane bases, which have quite a simple form. The ventral bar processes in *G. burchelli* are quite conspicuous and large compared to *G. ivindoensis,* and no ventral bar processes can be seen in the original description of *G. kyogae*. The

ventral bar of *G. kyogae* is very small in comparison to the ventral bars of the other species, with a mean total length of 12.2 µm (Shinn *et al.* unpublished). This is particularly due to the short length of the membrane (7.6 µm). The shapes of the marginal hooklets are similar for *G. ivindoensis* and *G. burchelli*. The marginal hooklets of *G. ivindoensis* are larger. The sickle aperture area in *G. ivindoensis* is smaller and has a more slender and elongated sickle blade, whereas *G. burchelli* has a more robust sickle blade and a less attenuated rounded curve of the sickle heel (Figs. 3A and 3B).

Gyrodactylus burchelli morphologically also differs considerably from *G. kherulensis* and *G. kobayashii* found on koi and goldfish respectively, which have been identified as exotic species in the Western Cape (see Chapter 2). *Gyrodactylus burchelli* is much smaller in overall dimensions than the *Gyrodactylus* species from exotic cyprinids of Eurasian origin recorded in the area (Christison *et al.* 2005). No other alien *Gyrodactylus* species have been found on the native fish during the survey, but only a selected area was sampled and it is uncertain whether parasite transfer in the wild has already taken place.

Figure 3 Morphological comparisons of the marginal hooklets of A - *Gyrodactylus kyogae* and B- *G. ivindoensis.* The solid line represents *G. burchelli*. Scale bar=10 µm.

The data available on gyrodactylids in Africa are poor compared to those of Europe, Australia and Asia and research regarding this is lacking. Only two comparative *Gyrodactylus* species from cyprinid fish of North Africa are available, as these are the only two published records. Although *G. kyogae* occur on the same fish family as *G. burchelli*, this parasite might be distantly related to *G. burchelli* and it was assumed that they are phylogenetically similar and therefore compared. This emphasizes the need for additional surveys to identify the natural parasites of their endemic hosts. The presence of these endemic, threatened fish in an ecologically sensitive region requires research directed towards all aspects concerning the conservation of these fish. The conservation of these fish is largely dependant on the understanding of the biology and ecological interactions, so knowledge pertaining to their parasites and the potential transfer of exotic parasites is of paramount importance.

References

BAKKE, T.A., Harris, P.D., Cable, J. 2002. Host specificity dynamics: observations on gyrodactylid monogeneans. *International Journal for Parasitology* **32**, 281-308.

CAMBRAY, J.A., Stuart, C.T. 1985. Aspects of the biology of a rare redfin minnow, *Barbus burchelli* (Pisces, Cyprinidae), from South Africa. *South African Journal of Zoology* **20** (3), 155-165.

CHRISTISON, K.W., Shinn, A.P., van As, J. 2005. *Gyrodactylus thlapi* n. sp. (Monogenea) from *Pseudocrenilabrus philander philander* (Weber) (Cichlidae) in the Okavango Delta, Botswana. *Systematic Parasitology* **60**, 165–173.

GARCIA-VASQUEZ, A., Hansen, H., Christison, K.W., Bron, J.E., Shinn, A.P. 2011. Description of three new species of *Gyrodactylus* von Nordmann, 1832 (Monogenea) parasitising *Oreochromis niloticus niloticus* (L.) and *O. mossambicus* (Peters) (Cichlidae). *Acta Parasitologica*. **56** (1): 20-33.

GEETS, A., Appleby, C., Ollevier, F. 1999. Host-dependant and seasonal variation in the opisthaptoral hard parts of *Gyrodactylus* cf *arcuatus* from three *Pomatoschistus* spp. and *G. arcuatus* from *Gasterosteus aculeatus*: a multivariate approach. *Parasitology* **119**, 27-40.

HARRIS, P.D., Cable, J., Tinsley, R.C. 1999. Combined ribosomal DNA and morphological analysis of individual gyrodactylid monogeneans. *The Journal of Parasitology* **85** (2), 188-191.

HARRIS, P.D., Shinn, A.P., Cable, J., Bakke, T.A. 2004. Nominal species of the genus *Gyrodactylus* von Nordmann 1832 (Monogenea: Gyrodactylidae), with a list of principal host species. *Systematic Parasitology* **59**, 1–27.

IMPSON, N.D. 2007. State of Biodiversity: Western Cape Province. Chapter 3: Freshwater fishes, Western Cape State of Biodiversity 2007. Western Cape Conservation Board, Cape Town ISBN 978-0-620-39289-1.

PŘIKRYLOVÁ, I., Matějusova, I., Musilová, N., Gelnar, M. 2009. *Gyrodactylus* species (Monogenea: Gyrodactylidae) on the cichlid fishes of Senegal, with the description of *Gyrodactylus ergensi* n. sp. From the Mango tilapia, *Sarotherodon galilaeus* L. (Teleostei: Cichlidae). *Parasitology Research* **106** (1): 1-6.

SHINN, A.P., Christison K.W. & Garcia-Vasquez A. The *Gyrodactylus* von Nordmann, 1832 (Gyrodactylidae: Monogenea) fauna of Africa including a re-description of species of the genus. Personal communication.

SHINN, A.P., Hansen, H., Olstad, K., Bachmann, L., Bakke, T.A. 2004. The use of morphometric characters to discriminate specimens of laboratory-reared and wild populations of *Gyrodactylus salaris* and *G.thymalli* (Monogenea). *Folia Parasitologica* **51**, 239-252.

SKELTON, P.H. 1980. Systematics and biogeography of the redfin *Barbus* species (Pisces: Cyprinidae) from southern Africa. Ph.D thesis, Rhodes University, Grahamstown, South Africa.

SKELTON, P. 2001. A Complete Guide to the Freshwater Fishes of Southern Africa. Struik Publishers, Cape Town, South Africa, 395 pp.

SWARTZ, E.R. 2005. Phylogeography, phylogenetics and evolution of the redfins (Teleostei, Cyprinidae, *Pseudobarbus*) in southern Africa. Ph.D thesis, University of Pretoria, Pretoria, South Africa.

SWARTZ, E.R., Impson, D. 2007. *Pseudobarbus burchelli*. In: IUCN 2008. 2008 IUCN Red List of Threatened Species. <http://www.iucnredlist.org/>. Downloaded on 09 March 2009.

SWARTZ, E.R., Skelton, P.H., Bloomer, P. 2009. Phylogeny and biogeography of the genus *Pseudobarbus* (Cyprinidae): Shedding light on the drainage history of the rivers associated with the Cape Floristic Region. *Molecular Phylogenetics and Evolution* **51**, 75-84.

Chapter 5

Risk Analysis of *Gyrodactylus kherulensis* Ergens 1974 from *Cyprinus carpio koi* L. and *Gyrodactylus kobayashii* Hukuda, 1940 from *Carassius auratus* L. imported into South Africa: Discussion

Abstract

The ecological integrity of freshwater ecosystems in South Africa is compromised due to the continuous introduction of exotic species. The ornamental fish trade in South Africa is one of the continually growing aquaculture sectors, largely as result of an increased demand for fish keeping by hobbyist and breeders. Koi and goldfish are commonly traded fish known for the propagation of exotic parasites into South Africa. *Gyrodactylus kherulensis* Ergens, 1974 and *G. kobayashii* Hukuda, 1940 have been recorded from koi and goldfish respectively entering the area, but have however only be documented from the ornamental fish trade industry and none have been recorded in the wild. Exotic parasites from exotic cyprinids might be potentially threatening to indigenous cyprinids in the Cape Floristic Region (CFR). Experimental infections indicate that both *G. kherulensis* and *G. kobayashii* are able to infect the indigenous *Pseudobarbus phlegethon* Barnard, 1938, but *P. burchelli* Smith, 1841 was however innately resistant to both parasite species. These parasites therefore have the potential to propagate to feral carp and goldfish populations and ultimately to wild cyprinid populations which may respond to or be susceptible to the infection. This, coupled with the biology of these parasites, threatens the ecological health and biodiversity of the native fish in the CFR. The risk posed by these parasites to wild fish in the CFR are qualitatively evaluated and estimated as high.

Introduction

The global ornamental fish trade is unquestionably the largest ditributor of live animals worldwide (Ploeg 2007). The ornamental fish industry comprises an assemblage of various fish species from different geographical origins, particularly from tropical developing countries to Asian depots, where they are transported to their respective countries of destination (Whittington and Chong 2007). The live ornamental fish trade therefore serves as a conduit for one of the least discernible forms of invasion: the worldwide spread of fish pathogens, parasites and disease concurrently with their exotic hosts (Bright 1999; Murray and Peeler 2005; Whittington and Chong 2007). The overall risk estimation is exacerbated in areas with endangered species, as these pathogens could bring about local population declines, which could lead to extirpation and eventually extinction, thereby significantly increasing the consequences of introduction (Cleaveland *et al.* 2002). The CFR, of which the Western Cape Province constitutes the greatest portion, is habitat to a number of endemic and endangered fish species (Impson 2007). The region accommodates 23 indigenous fish species, with the majority (65%) of the freshwater fish in the region belonging to the family Cyprinidae (Impson 2007). The vast majority of these fish are threatened by extinction primarily due to the direct negative effects of alien fish introductions (Impson 2007).

The exotic cyprinids, *Cyprinus carpio* L. and *Carassius auratus* L. and their variants are among the most extensively distributed fish species; they are invasive on every habitable continent. Their invasive success is due to their ability to withstand and adapt to various environments and climatic conditions (Kir and Ozan 2007). *Cyprinus carpio* finds its origins in Asia. The common carp was initially introduced into southern Africa in the 18th century as a food and sport fish (Bruton and Van As 1986). *Cyprinus carpio* is the most

invasive fish in southern Africa, and is found in 10 of the 13 drainage basins within the region (Bruton and Van As 1986). *Cyprinus carpio* is widely distributed in the CFR and has a high level of impact in natural river systems, which it shares with indigenous fish (Impson *et al.* 2000). The ornamental variety of the carp, the koi carp, was introduced into South Africa in the 1970's where a local bought koi from Japanese fishermen, bred and sold the fish to local fish retailers in the area (Watt and De Kock 1996). Koi gained popularity in South Africa and were subsequently sold at pet shops (Watt and De Kock 1996). Importing then became more common and is currently still the most widely used means of obtaining these fish in bulk (Watt and De Kock 1996).

Goldfish are presumed have been introduced into southern Africa in 1726 (De Moor and Bruton 1988). These fish are originally from Eastern Asia (De Moor and Bruton 1988). Goldfish were distributed to different parts of the country from Jonkershoek in 1941, to control mosquito numbers in certain areas and are currently present in river systems on the Cape Flats (De Moor and Bruton 1988). Goldfish were also sold by Japanese sailors to locals in the 1970's along with koi, and by then it was already a popular ornamental fish in South Africa (Watt and De Kock 1996). Feral populations of goldfish have been recorded from rivers and dams in the Western Cape Province (De Moor and Bruton 1988; Skelton 2001). Goldfish usually compete for resources with local fish (Impson *et al.* 2000).

Both carp and goldfish have been identified as carriers of exotic parasites into South Africa and to various regions in the world (De Moor and Bruton 1988). The parasites, *Ichthyopthirius multifiliis* Fouquet, 1876; *Argulus japonicus* Thiele, 1900 and *Lerneae cyprinaceae* L., 1758 have been recorded on goldfish in southern Africa, although these

fish have been found to carry many other parasite species to different parts of the world. The common carp, on the other hand, were found to have introduced *Ichthyobodo necator* Hennegy, 1883; *Chilodonella cyprinid* Moroff, 1902; *C. hexasticha* Kiernik, 1909; *Apiosoma piscicola* Blanchard, 1885; *Trichodina acuta* Lom, 1961; *T. nigra* Lom, 1960; and *Trichodinella epizootica* Sramek-Husek, 1953 into South Africa. Four monogeneans have also been recorded from carp in Africa *Pseudacolpenteron pavlovski* Bychowsky and Gusev, 1955; *Dactylogyrus anchoratus* Dujardin, 1845; *D. minutus* Kulwiec, 1927 and *D. extensus* Mueller and Van Cleave, 1932, however, no documented evidence exists to support the introductions into South Africa and none of these have been recorded from feral populations of their natural hosts or from closely related hosts in natural water systems in southern Africa (De Moor and Bruton 1988). *Gyrodactylus cyprini* Diarova, 1964 has, however, been noted to have been translocated on their exotic carp hosts, but have however, not been recorded in southern Africa (De Moor and Bruton 1988). Gyrodactylosis is listed as an OIE (Office International des Épizooties) notifiable disease for *G. salaris* Malmberg, 1957 only but all *Gyrodactylus* species should be considered a potential risk, due to the similarity in the biology. The spread of the Koi Herpes Virus (KHV) worldwide is indicative of pathogen propagation and how its spread is facilitated by the ornamental fish trade. Koi Herpes Virus is an internationally recognised disease, which has resulted in the mass mortality of common carp and koi carp. This viral disease has spread worldwide, including to South Africa, and numerous deaths of imported koi have been reported (Hutoran *et al*. 2005; Pokorova *et al*. 2005).

The impacts associated with alien parasite species into South Africa, particularly the Western Cape Province, could be negative, as alien parasites from carp have already propagated to indigenous fish in South Africa, as in the case of *Bothriocephalus*

acheilognathi Yamaguti, 1934 (Brandt *et al*. 1981). This cestode was initially described from carp and has since spread worldwide with the translocation of its exotic natural host (Salgado-Maldonado and Pineda-Lopez 2003). This parasite has been discovered in the in the gut of the indigenous largemouth yellowfish, *Labeobarbus kimberleyensis* Gilchrist and Thomson, 1913 in the Vaal Dam, South Africa (Brandt *et al*. 1981). *Bothriocephalus acheilognathni* however had 100% prevalence in *L. kimberleyensis* sampled in the Vaal Dam and the greatest mean intensity was recorded in Autumn (Retief *et al*. 2007). The high numbers of the tapeworms within the intestines of *L. kimberleyensis* however had no effect on the fecundity of these fish (Retief *et al.* 2007). This study also indicated that the fish size and mean intensities of the parasites are not correlated, also that the tapeworms were predominately attached at the frontal end of the intestine of the fish (Retief *et al*. 2007). This particular parasite has also been reported from indigenous Australian fishes (Dove *et al.* 1997).

Cyprinid fish are the most likely to acquire the parasites of exotic carp and goldfish. The relatedness of indigenous cyprinids from the CFR to exotic cyprinids like *C. carpio* and *C. auratus* enhances the potential for host-switching of their pathogens (Dove 2000). This generally negatively affects the newly acquired host due to the lack of immunological defence against the exotic pathogen (Dove 2000).

The monogenean parasites, *Gyrodactylus kherulensis* from koi and *G. kobayashii* from goldfish have been recorded as foreign parasites entering the Western Cape Province on their exotic cyprinid hosts (see Chapter 2). Members of the genus *Gyrodactylus* are among the most invasive of fish parasites; this is due to the biology and reproductive mechanisms which include their single life cycle, their viviparity, polyembryonism and

parthenogenesis (Cable and Harris 2002). Due to their ability to rapidly proliferate in aquaculture conditions, these parasites pose a potential threat to native species if their hosts are released or escape into the wild. Where viable infection pathways exist, the spread of exotic pathogens to susceptible native fish is inevitable. The likelihood of these infections is improved by factors that enhance the infection pressure such as parasite fecundity and population growth rate and the rate of uninhibited introductions of new infected hosts. However, the spread of pathogens are largely dependant on the interactions between the host pathogen and the physical environment (Reno 1998). *Gyrodactylus kherulensis* and *G. kobayashii* imported into South Africa from various sources demonstrated prevalences ranging up to 100% and mean intensities from 2 - 342.3 were recoreded from the current study.

Aquaculture in South Africa is inconsequential compared to figures from the rest of the developed world (Hecht and Endemann 1998). The predicted expansion of the aquaculture sector in South Africa is imminent, as South Africa welcomes the sector for economic growth and has the appropriate infrastructure to encourage its growth. However, little work has been done on the parasitology and disease control of freshwater fish in southern Africa, although it is of cardinal importance in an aquaculture setting (Hecht and Endemann 1998). In terms of biomass produced, the majority of fish currently cultured in South Africa are freshwater fish, while the rest are marine (Botes *et al*. 2006). Koi, trout, and other ornamental fish are the principal freshwater fish produced in the country (Britz *et al.* 2009). No goldfish information was obtained from the respondents of the 2009 survey, however 12.5% of the respondents indicated that they produced goldfish in 2005 (Botes *et al.* 2006; Britz *et al.* 2009).

Indigenous fish are protected by the Animal Health Act (Act no. 7 of 2002) of South Africa, which obliges importers of exotic fish to obtain a permit prior to importation and that the necessary arrangements are made that these fish are sufficiently quarantined, held and examined for a certain period before they are distributed to local retailers. Also, a health certificate from the country of exportation is vital to the importation of these exotic fish. However, these laws are not always adhered to, and the lack of competent authority regulating fish importation facilitates the addition of the exotic parasites to the wild fish. Effective aquaculture and aquatic disease management strategies are necessary to mitigate the current situation.

The potential risks of the importation of representatives of the genus *Gyrodactylus* to freshwater biodiversity imported into the Western Cape are assessed in this chapter. It is imperative to identify and quantify risks prior to them causing major ecological damage, and to prevent potentially irrevocable damage to this unique biodiversity. Furthermore, this chapter identifies and illustrates the potential risks associated with the importation and culture of exotic cyprinid hosts for members of the genus *Gyrodactylus*.

Hazard identification

This is the first confirmed report of the monogenean species, *G. kherulensis* and *G. kobayashii* from the exotic cyprinid fish, koi and goldfish respectively, in the Western Cape Province, South Africa (see Chapter 2).

Despite a number of species of *Gyrodactylus* being described from koi and goldfish, only these 2 species were found entering the Western Cape during this study. *Gyrodactylus kherulensis* and *G. kobayashii* imported from various geographic origins and locally bred

populations showed intra-population variations in the morphometry of the attachment organs were evident among members of the species (see Chapter 2). Phenotypic plasticity of the opisthaptoral characters of both *G. kherulensis* and *G. kobayashii* populations, which may be attributable to a number of factors, of which water temperature is deemed to be the most influential factor (Mo 1991; Appleby 1996) (see Chapter 2). *Gyrodactylus kobayashii* populations showed intraspecific variation, and even though differences were evident in *G. kherulensis* populations, they were not as pronounced as seen with *G. kobayashii* (see Chapter 2). The phenotypic plasticity of the opisthaptoral characters is particularly due to environmental factors influencing the size of these characters (Olstad *et al.* 2007). Possible misidentification of similar *Gyrodactylus* species using only morphometrics is plausible due to phenotypic plasticity, therefore morphology and genetics are generally used to identify these parasites.

Gyrodactylids have unique reproductive mechanisms of which progenesis and viviparity are key strategies to enhance their invasive potential (Bakke *et al.* 2007). Progenesis is a process whereby the life cycle is accelerated by the animal having the ability to reproduce as a juvenile (Bakke *et al*. 2007). *Gyrodactylus* species bear a grown daughter *in utero*, and the daughter contains a developing juvenile within their uterus, which is fully grown when the daughter is released (Cable and Harris 2002; Bakke *et al*. 2007). Viviparity in *Gyrodactylus* species usually results in excessive population growth rates, particularly in aquaculture systems which provide ideal conditions for their proliferation (Cable and Harris 2002). The first daughter however, always develops by asexual reproduction, and the second daughter develops by parthenogenesis (Harris 1993; Bakke *et al*. 2007). Sexual reproduction is perceived to only occur from the third daughter onwards (Harris 1993). However, in unfavourable conditions or when the parasite population is low, these

parasites use asexual reproduction to ensure that viable offspring are produced (Harris 1993). Their diverse means of reproduction, coupled with the distribution of their hosts' species render these parasites a risk to the freshwater fish biodiversity in the Western Cape Province.

Release assessment

The primary mode of transfer of alien fish beyond their ranges is transport of live fish around the world (Whittington and Chong 2007). Koi carp and goldfish are commonly traded fish imported to South Africa, primarily from Indonesia, Malaysia, China and Japan, while the minority are locally bred. The primary source of ornamental fish is from developing countries and disease inspection in those areas are absent or very limited (Whittington and Chong 2007). Import risk assessments are largely dependant on knowledge of the distribution of the exotic pathogens and their pathogenicity, but this data is generally unavailable in both the developed and developing countries (Whittington and Chong 2007). During the current survey, it was established that the majority of the fish imported are transported directly from overseas suppliers, from major fish farms, to local retailers and local suppliers in South Africa. Infections are harboured within the holding facilities and are spread from the supplier to various parts of the world by transportation of large consignments of fish and the risk of these exotic parasites passing South African borders is high (Table 1 and Fig. 1). The infections are presumed to be passed on from major international wholesalers to international wholesalers and wholesale depots, and the risk of importation is presumed to be high. The fish from major international wholesalers were however (in this study) not examined and cannot be confirmed as the source of infection, however, it is presumed to find its origins there. Another possibility is that these parasites might have been acquired from local sources

harboured in the tanks of local breeders and retailers. Importation laws restrict the entry of exotic fish into the country without being quarantined, in order to limit or prevent pathogen introduction. These laws are however not strictly enforced and the importer generally neglects to effectively quarantine the infected fish (Mouton *et al*. 2001). An import health certificate from the country of export is mandatory, however, these certificates seldomly serve their purposes they were intended for as they generally only report on the presence or absence of internationally significant pathogens (*e.g.* OIE listed diseases), and metazoan parasites are however not considered. Furthermore, the diagnostic tests employed or the number of fish tested may not be sensitive enough to detect cryptic pathogens present in low prevalences in the imported fish population.

The invasiveness of koi carp and goldfish combined with the lack of effective quarantine measures make them exceptional transporters of the pathogens such as *Gyrodactylus* species into South Africa. The high host densities within the tanks in intensive farming practices result in the proliferation of these parasites in confined conditions and hence might result in the death of the infected fish (Thoney and Hargis 1991). Transporting the fish from country to country stresses the fish, resulting in the increased production of the stress hormone, cortisol in the blood. A study conducted by Harris *et al.* (2000), where various salmonid fish were injected with hydrocortisone acetate, resulting in immunosuppression and consequently an increased population growth of *G. salaris* on those fish. These fish then enter the import country with increased parasite intensities, with population numbers increasing rapidly in response to the fish's stress and concomitant immunosuppression. The aggregation of the fish during transport also enhances spread and parasite proliferation.

Exposure assessment

Koi carp and goldfish are both temperate species, and the temperate climates and river water of the CFR are conducive to their survival in natural freshwater ecosystems in South Africa (Mouton *et al*. 2001). These fish are however able to withstand varying environmental conditions and are able to survive high saline concentrations and temperature fluctuations. This attribute has contributed to the invasive success of these fish. Gyrodactylids are a group of diverse and ubiquitous parasites and their success can be seen in their abilities to infect fish from tropical regions to fish in the Polar regions (Harris 1993; Bakke *et al*. 2007). The success of both the parasite and its host increases the chances of exposure in natural river systems. Carp and goldfish have already been implicated for the propagation of disease and parasites in southern Africa (De Moor and Bruton 1988). Table 1 illustrates the various transmission pathways of introduction of both *G. kherulensis* and *G. kobayashii* into South Africa and the possibility of exposure of these parasites to local cyprinids.

Ornamental fish imported into South Africa are usually transported from the country of import to wholesalers, which are then sold to retailers and hobbyists. It is thought that ornamental fish held in aquaria are unlikely to be the source of the spread of diseases and pathogens to the wild. However, this is not always the case and both plants and animals imported for ornamental purposes end up in natural freshwater ecosystems. Infected fish entering the country initially end up at major suppliers and fish farms and the risk is considered high due to the high prevalence of *Gyrodactylus* species found on koi and goldfish in this study. These infected fish are then transported to local retailers and the risk of transfer is quite high, particularly since treatment of these parasites are often disregarded if the fish have no clinical external symptoms of infection. The water in

which the fish are transported may also contribute to infections (Table 1; Fig. 1). Koi and goldfish farms may be a direct source of infection of pathogens to river systems, where farms situated close to rivers may use recirculating systems pumping effluent containing pathogens from an infected source into river systems, the risk of this occurring is however considered low. Pre-exiting infections within the tanks and ponds of these retailers may exist. Fish are usually inadvertently released into the wild. Hobbyist with excess fish or those disposing of their fish will potentially do so by releasing their fish into rivers unaware of the dangers or legislative issues of exotic species introductions. The risk of infected fish sold to hobbyist is also quite high. Such an example is where someone introduced koi in Baviaanskloof, in the Western Cape, thinking it would aid their growth and reproduction. Escapism is another means of translocation of koi and goldfish into the wild. These fish can escape garden ponds and pond facilities during floods close to rivers but the chances of this happening are very low. This is however improbable and the risk of exotic parasites being introduced into local river systems is low, but both koi carp and goldfish have already been recorded in the natural rivers in South Africa (Fig. 1). The risk of the exotic parasites entering a local river system with local cyprinid fish is further reduced as the majority of these fish are endangered and are not found in all river systems, and the risk is therefore regarded as low. The overall risk of exotic *Gyrodactylus* species transferring to susceptible local cyprinids in the CFR is high, seeing that transfer to indigenous fish is plausible (Table 1).

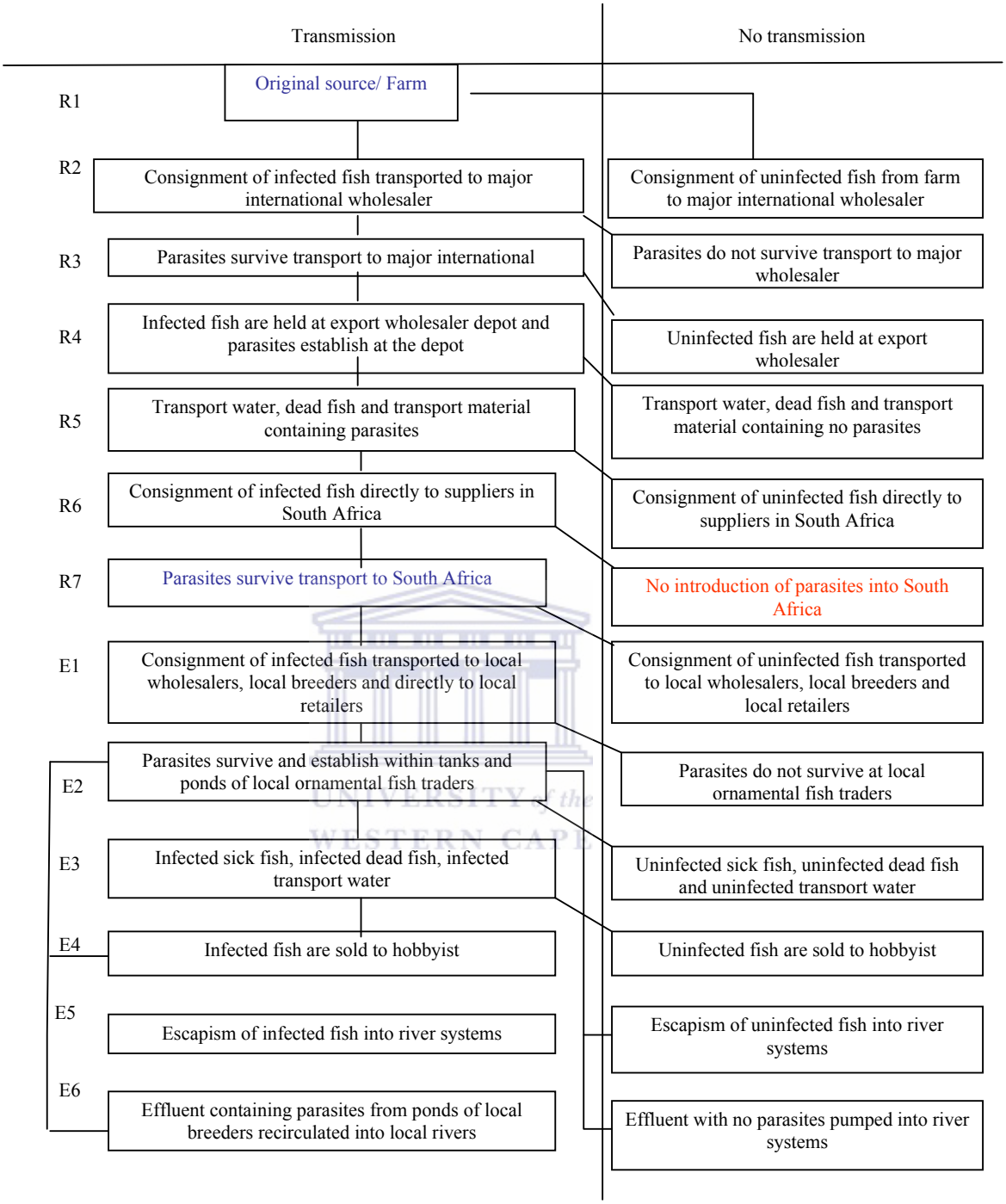
The introduction of koi and goldfish infected with *Gyrodactylus* species into natural river systems in the Western Cape is potentially ecologically damaging. *Gyrodactylus* species are highly pathogenic, particularly in confined conditions, and their enhanced population growth rates in confinement coupled with their biology is particularly hazardous in a case

of escapism, as these fish may harbour high parasite intensities as recorded from imported fish obtained from local pet traders. These parasites may detach from their hosts as result of abrasion, migration, and host-response, however these worms can remain unattached for a few hours before searching for a new potential host (Cable *et al*. 2001; Gheorghiu *et al*. 2007). Propagation of *Gyrodactylus* species occurs by means of four modes of transmission: (1) host to host transmission, in the case of two hosts coming into contact with one another, the parasites are able to be conveyed; (2) by detached parasites on the substrate (3) by detached parasites in the water column coming into contact with fish (4) by the parasites being spread from dead infected fish to live fish (Bakke *et al*. 1992). Transmission occurs primarily by direct host to host contact, by the parasites detaching from the host and re-infecting another fish in close proximity (Bakke *et al.* 1992; Cable *et al.* 2001) (Table 1) (also see Chapter 3). These parasites invest a lot of energy in their reproduction to ensure the survival of their offspring. The host specificity of gyrodactylids is based on their original species descriptions, although lack of sampling effort and experimental data may influence the host range assumptions of these parasites. Empirical evidence suggests that *G. turnbulli* Harris, 1986 and *G. bullatarudus* Turnbull, 1956 from the guppy, *Poecilia reticulata*, had increased host ranges, and were capable of infecting unrelated fish (King and Cable 2007). The tropical parasite, *G. bullatarudus* positively established itself on a temperate fish species (King *et al*. 2009).

Experimental infections of *G. kherulensis* and *G. kobayashii* on local cyprinids, *Pseudobarbus burchelli* and *P. phlegethon*, showed that these parasites have the ability to transfer to and establish themselves on *P. phlegethon*. An additional contributing factor to the potential exposure is that carp and its subspecies are however not migratory and are gregarious. The local cyprinids in the area are also gregarious and will shoal with other

fish species. Fish to fish transmission is enhanced if these fish have constant contact with each other. These pathways of potential infection of *Gyrodactylus* species, may simultaneously serve as pathways for other virulent pathogens into the Western Cape along with imported koi and goldfish.

Quarantine facilities responsible for inspecting live imported fish in the Western Cape are present but these institutions are deficient in personnel trained in parasite identification and treatment of pathogens. Also, the chance of a single specimen being detected during quarantine checks is highly improbable and a single monogenean has the potential ability to cause a clinical outbreak, particularly in conditions where fish are kept such as within tanks at wholesalers and retailers, which favours *Gyrodactylus* population growth (Cable and Harris 2002). Risk estimation of establishment and consequences are shown in Table 2.



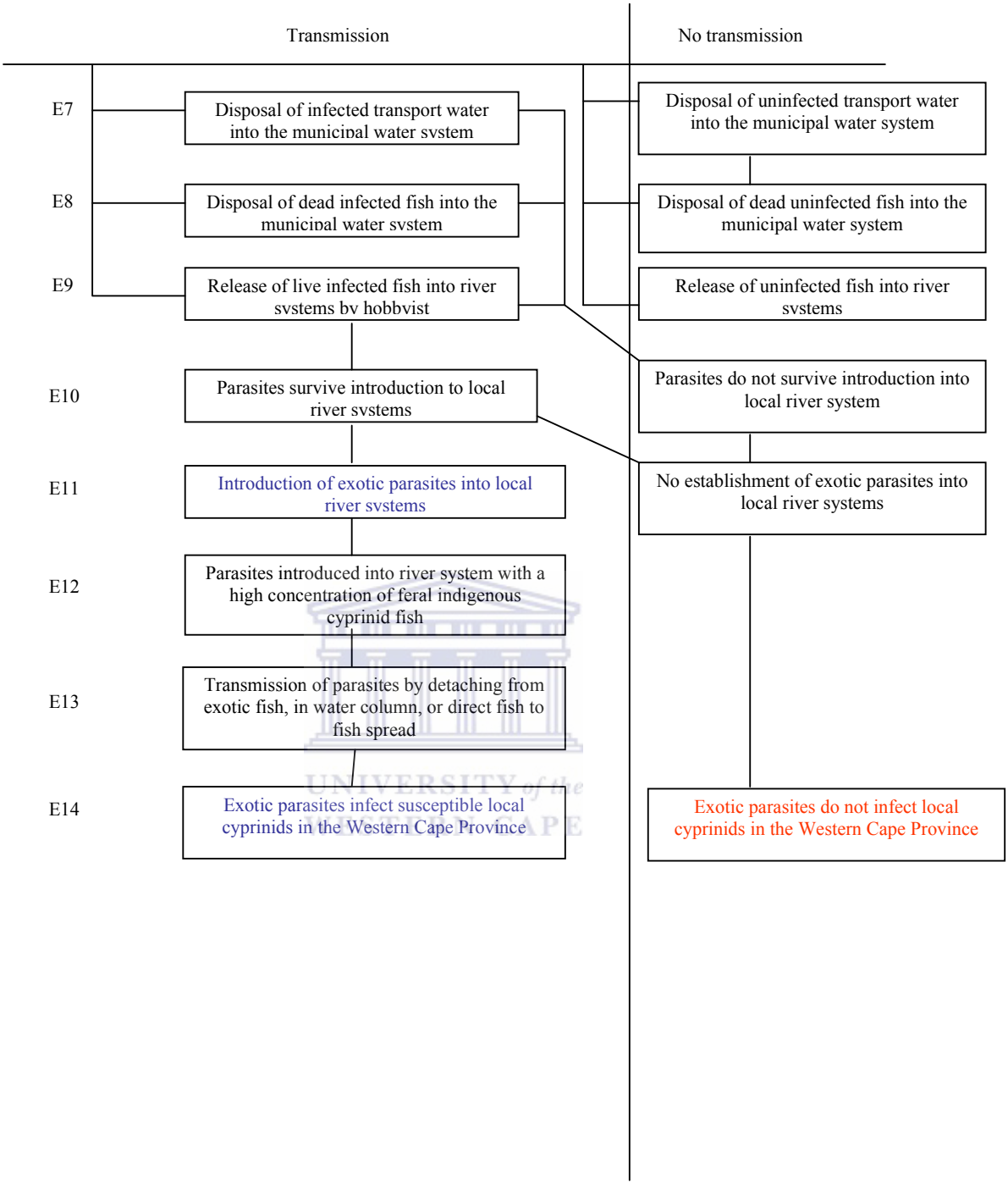


Figure 1 Release (R) and exposure (E) pathways of the introduction of *Gyrodactylus kherulensis* and *G. kobayashii* in the Western Cape numbered according the predicted subsequent events. Red indicates the discontinuation of the spread of *G. kherulensis* or *G. kobayashii* and the blue colour shows the probable pathways into South African river systems.

Table 1 Description of potential infection pathways of release (R) and exposure (E) of *Gyrodactylus kherulensis* and *G. kobayashii* exotic pathways into the river systems of the Western Cape and the potential risk of occurrence,

Pathway	Description of pathway	Risk
R 1	Original source of infection	
R 2	Consignment of infected fish transported to major international wholesaler	High
R 3	Parasites survive transport to international wholesaler	High
R 4	Infected fish are held at wholesale depot and parasites establish at depot	High
R 5	Transport water, dead fish, and transport material containing live parasites	High
R 6	Consignment of infected fish transported directly to suppliers in South Africa	High
R 7	Parasites survive transport to South Africa	High
E 1	Consignment of infected fish transported to local wholesalers, local breeders and to local retailers South Africa	High
E 2	Parasites survive and establish within tanks and ponds of local ornamental fish traders	High
E 3	Infected sick fish, infected dead fish and infected transport water	High
E 4	Infected fish are sold to hobbyist	High
E 5	Escapism of infected fish to local river systems	Low
E 6	Effluent containing parasites from ponds of local breeders recirculated into local river systems	Low
E 7	Disposal of infected fish into municipal water systems	Low
E 8	Disposal of infected dead fish into municipal water systems	Low
E 9	Release of infected fish into river systems by hobbyist	Medium
E 10	Parasites survive introduction into local river system	Low
E 11	Introduction of exotic parasites into local river systems	Low
E 12	Parasites introduced into river systems with a high concentration of indigenous cyprinid fish	Low
E 13	Transmission of parasites by detaching from exotic fish in water column or direct fish to fish contact	Low
E 14	Exotic parasites infect susceptible local cyprinids in the Western Cape Province	High

Consequence assessment

The consequences of alien parasite establishment in the Western Cape could have both economic and biological implications. The mass importation of infected koi carp and goldfish into the Western Cape, generally take place under conditions favouring parasite proliferation, and may result in mass mortality and therefore economic loss for the wholesalers and retailers. The potential consequences of establishment of *G. kherulensis* and *G. kobayashii* in river systems in the Western Cape may have negative results for susceptible indigenous cyprinid fish within the region. It has been demonstrated that these parasites are able to transfer onto indigenous fish tested, and may transfer to other indigenous cyprinids in the CFR (see Chapter 3). The implications of this transfer might be devastating in more susceptible hosts, and mortality of indigenous fish can be

considered the worst case scenario. The risk is estimated as high, medium and low, according to the pathways and the results shown by the current study (Table 2).

Transfer of exotic parasites to related indigenous fish might have grave implications for the native fish, because these fish have not evolved in unison with the parasites and lack the immunological defences to keep the parasites at bay (Dove 2000). Experimental evidence showed that the endangered Gila minnow, *Poeciliopsis o. occidentalis* infected with *G. turnbulli* has a reduced resistance to the parasite and appears partly susceptible to the exotic parasite (Hedrick *et al.* 2001). The resistance was further reduced in populations with a lower genetic variation (Hedrick *et al.* 2001). These fish had a lower resistance to the exotic *G. turnbulli* compared to any other related species of fish (Hedrick *et al.* 2001). Exposure to the exotic parasite therefore triggers a susceptibility response in the fish which then has a reduced resistance to this parasite (Hedrick *et al.* 2001). This is particularly threatening to vulnerable and rare species, as it may result in the extinction of these endangered fish with repeated exposure. *Gyrodactylus salaris* is the prime example of the implications of these parasites, and has been the result of mass mortalities of the susceptible Baltic strain of the Atlantic salmon, *Salmo salar* L. in Norway (Mo 1994).

Single populations of *P. burchelli* and *P. phlegethon* were tested in a preliminary experimental infection to *G. kherulensis* and *G. kobayashii*. No mortalities were noted due to infection with these exotic parasites as the experiment was too short and only a host response was measured by counting the amount of exotic parasites on the indigenous fish, however, neither of the fish was susceptible. However, other indigenous cyprinids in the CFR may be less resistant and succumb to the infection. Further studies and more

infection trials are therefore encouraged to determine exotic parasite host range and their consequences.

Even though the estimated consequences of the exposure of indigenous cyprinids to *Gyrodactylus* species may not be high, this study illustrates that the liklihood of potential infection pathways exist for these parasites and consequently for other far less host specific pathogens transmitted by these hosts. The recent outbreak of Epizootic Ulcerative Syndrome in the Zambezi Chobe watersheds is a good example of the dire consequences that these transmissions may hold, thereby significantly increasing the overall consequence of the release and exposure of these hosts and the need for improved aquatic biosecurity in this province country and broader southern African region.

Risk management

The management of exotic species which have established within natural river systems are among the major environmental problems faced by biologists. The identification of exotic pathogens are of cardinal importance, typically *G. kherulensis* and *G. kobayashii* are identified using morphological information, also to determine the pathogenicity (or the lack thereof) of the particular parasites under study, an experimental challenge trial is essential. This study has illustrated the significance of infection trial data as both *G. kherulensis* and *G. kobayashii* were able to survive on indigenous redfin minnows, endemic to the Western Cape. The data generated is indicative of a potential concern and management practices can be inferred from the information derived (see Chapter 3) (Murray and Peeler 2005). Importation directly from the main continent of export, which is Asia, should, by law, be accompanied with a health certification permit, certifying that the fish are disease free by appropriate sampling and testing of enough individuals to

ensure optimal sensitivity for detecting pathogens should they be present in a batch or consignment. However, this is not always the case, and the dearth of competent health officials, capable of identifying pathogens is one of the major challenges faced. To prevent the further influx of these potentially hazardous parasites, it is highly recommended that the government employ, train and promote aquatic animal health biologists and veterinarians and educate hobbyists and breeders about the potential consequences of the spread and implications of release of these parasites. The laws in the Western Cape are not that strictly enforced as these fish enter with substantial infections of *Gyrodactylus* species on the skin, as well as other external parasites of which the clinical symptoms are clear. The quarantine methods of monogenean parasites are generally methodical and are dependant on the life-cycle and infective stages of the parasites. The appropriate disposal of dead fish and the avoidance of the exchange of diseased fish and equipment between farms and breeding facilities would reduce the probability of spread (Murray and Peeler 2005). Control of parasites using chemical treatments within scientifically supported integrated parasites or pest management strategies are the favoured method of prevention of spread of these parasites, however parasite resistance and toxic build up of the chemicals may become problematic (Scholz 1999).

The promotion of high quality koi and goldfish breeding in South Africa is encouraged to minimise the importation of novel parasites as these fish have less pathogens and additionally promotes job creation in the sector.

Conclusion

The spread of disease is one of the undesirable consequences of the importation of ornamental fish. At the current rate of international live fish exchange, the probability of

disease establishment is anticipated. The responsibility of the preservation of the biodiversity of indigenous fish in the Western Cape rests largely on government officials. The ecological integrity of the immense species richness and endemism in South Africa is compromised by the perpetual spread of parasitic diseases resulting from live fish trade (Mouton *et al.* 2001), particularly the potential spread of *Gyrodactylus* species to the country's local cyprinids, the dominant southern African freshwater fish family (Skelton 2001).

References

APPLEBY, C. 1996. Variability of the opisthaptoral hard parts of *Gyrodactylus callariatis* Malmberg, 1957 (Monogenea: Gyrodactylidae) from Atlantic cod *Gadus morhua* L. in the Oslo Fjord, Norway. *Systematic Parasitology* **33** (3), 199-207.

BAKKE, T.A., Harris, P.D., Jansen, P.A., Hansen, L.P. 1992. Host specificity and dispersal strategy in gyrodactylid monogeneans, with particular reference to *Gyrodactylus salaris* (Platyhelminthes, Monogenea). *Diseases of Aquatic Organisms* **13**, 63-74.

BAKKE, T.A., Cable, J., Harris, P.D. 2007. The biology of gyrodactylid monogeneans: The "Russian-doll killers" *Advances in Parasitology* **64**, 161-376.

BOTES, L., Thompson, G., Louw, R. 2006. AISA 2006. Benchmarking survey of the South African Aquaculture (marine & freshwater) sector. AISA report. 94pp.

BRANDT, F. De W., Van As, J.G., Schoonbee, H.J., Hamilton-Attwell, V.L. 1981. The occurrence and treatment of bothriocephalosis in the common carp, *Cyprinus carpio* in fish ponds with notes on its presence in the largemouth yellowfish, *Barbus kimberleyensis* on the Vaal Dam, Transvaal. *Water SA* **7,** 35-42.

BRIGHT, C. 1999. Invasive Species: Pathogens of Globalization. Foreign Policy, No. 116, pp. 50-64.

BRITZ, P.J., Lee, B., Botes, L. 2009. AISA 2009. Aquaculture Benchmarking Survey: Primary Production and Markets. A Report for the Aquaculture Institute of South Africa and Swisscontact, Produced by Enviro-Fish Africa. (Pty.) Ltd. 130pp.

BRUTON, I.J., Van As, J.G. 1986. Faunal invasions. Faunal invasions of aquatic ecosystems in southern Africa, with suggestions for their management. In: I.A.W. Mcdonald, F.J Kruger & A.A Ferrar (Eds) The Ecology and Management of Biological Invasions in Southern Africa. Pp. 47-61. Oxford University Press, Cape Town.

CABLE, J., Harris, P.D. 2002. Gyrodactylid developmental biology: historical review, current status and future trends. *International Journal for Parasitology* **32**, 255-280.

CABLE, J., Tinsley, R.C., Harris, P.D. 2001. Survival, feeding and embryo development of *Gyrodactylus gasterostei* (Monogenea: Gyrodactylidae). *Parasitology* **124**, 53-68.

CLEAVELAND, S., Hess, G. R., Dobson, A., Laurenson, M. K., McCallum, H. I., Roberts, M. and Woodroffe, R. 2002. The role of pathogens in biological conservation. In: P.J. Hudson, A. Rizzoli, B. T. Grenfell, H. Heesterbeek, A. P. Dobson (Eds.), *The Ecology of Wildlife Diseases* (pp. 139-150) Oxford, U.K.: Oxford University Press.

DE MOOR, I.J., Bruton, M.N. 1988. Atlas of alien and translocated indigenous aquatic animals in southern Africa. South African National Scientific Programmes Report No. 144, 310 pp.

DOVE, A.D.M. 2000. Richness patterns in the parasite communities of exotic poeciliid fishes. *Parasitology* **120**, 609-623.

DOVE, A.D.M., Ernst, I. 1998. Concurrent invaders – four exotic species of Monogenea now established on exotic freshwater fishes in Australia. *International Journal for Parasitology* **28**, 1755-1764.

DOVE, A.D.M., Cribb, T.H., Mockler, S.P., Lintermans, S.P. 1997. The Asian tapeworm, *Bothriocephalus acheilognathii*, in Australian freshwater fishes. *Marine Freshwater Research* **48**, 181-183.

GHEORGHIU, C., Cable, J., Marcogliese, D.J. Scott, M.E. 2007. Effects of waterborne zinc on reproduction, survival and morphometrics of *Gyrodactylus turnbulli* (Monogenea) on guppies (*Poecilia reticulata*). *International Journal for Parasitology* **37**, 375–381.

HARRIS, P.D. 1993. Les interactions entre la reproduction et la biologie des populations chez les Monogenes Gyrodactylidae: revue. *Bulletin Français de la Pêche et de la Pisciculture* **328**, 47–65.

HARRIS, P.D., Soleng, A., Bakke, T.A. 2000. Increased susceptibility of salmonids of the monogenean *Gyrodactylus salaris* following administration of hydrocortisone acetate. *Parasitology* **120**, 57-64.

HECHT, T., Endemann, F. 1998. The impact of parasites, infections and diseases on the development of aquaculture in sub-Saharan Africa. *Journal of Applied Ichthyology* **14**, 213-221.

HEDRICK, P.W., Kim, T.J., Parker, K.M. 2001. Parasite resistance and genetic variation in the endangered Gila topminnow. *Animal Conservation* **4**, 103-109.

HUTORAN, M., Ronen, A., Perelberg, A., Ilouze, M., Dishon, A., Bejerano, I., Chen, N., Kotler, M, 2005. Description of an as yet unclassified DNA virus from diseased *Cyprinus carpio* species. *Journal of Virology* **79**, 1983-1991.

IMPSON, N.D. 2007. State of Biodiversity: Western Cape Province. Chapter 3: Freshwater fishes, Western Cape State of Biodiversity 2007. Western Cape Conservation Board, Cape Town ISBN 978-0-620-39289-1.

IMPSON, N.D., Bills, I.R., Cambray, J.A. 2000. State of Biodiversity: Western Cape Province, South Africa. Freshwater Fishes. Western Cape State of Biodiversity 2002. Western Cape Nature Conservation Board, CapeTown. ISBN: 0-620-29893-6.

KING, T.A., Cable, J. 2007. Experimental infections of the monogenean *Gyrodactylus turnbulli* indicate that it is not a strict specialist. *International Journal for Parasitology* **37**, 663-672.

KING, T.A., van Oosterhout, C., Cable, J. 2009. Experimental infections with the tropical monogenean, *Gyrodactylus bullatarudis*: Potential invader or experimental fluke? *Parasitology International* **58,** 249-254.

KIR, I., Tekin Ozan, S. 2007. Helminth Infections in common carp, *Cyprinus carpio* L., 1758 (Cyprinidae) from Kovada Lake (Turkey). *Türkiye Parazitoloji Dergisi*, **31** (3): 232-236.

MO, T.A. 1991. Seasonal variations of opisthaptoral hard parts of *Gyrodactylus salaris* Malmberg, 1957 (Monogenea: Gyrodactylidae) on parr of Atlantic salmon *Salmo salar* L. in the River Batnfjordselva, Norway. *Systematic Parasitology* **19** (3), 231-240.

MO, T.A. 1994. Status of *Gyrodactylus salaris* problems and research in Norway. In: Parasitic Diseases of Fish (eds. Pike, A. W. & Lewis, J. W.), Samara Publishing Ltd, Dyfed, 43-56 pp.

MOUTON, A. Basson, L., Impson, D. 2001. Health status of ornamental freshwater fishes imported to South Africa: a pilot study. *Aquarium Sciences and Conservation* **3**, 327-333.

MURRAY, A.G., Peeler, E.J. 2005. A framework for understanding the potential emerging diseases in aquaculture. *Preventive Veterinary Medicine* **67**, 223–235.

OLSTAD, K., Shinn, A.P, Bachmann, L., Bakke, T.A. 2007. Host-based identification is not supported by morphometrics in natural populations of *Gyrodactylus salaris* and *G. thymalli* (Platyhelminthes, Monogenea). *Parasitology* **134**, 2041-2052.

PEELER, E.J., Gardiner, R., Thrush, M.A. 2004. Qualitative risk assessment of routes of transmission of the exotic fish parasite *Gyrodactylus salaris* between river catchments in England and Wales. *Preventive Veterinary Medicine* **64**, 175–189.

PLOEG, A. 2007. The volume of the ornamental fish trade. In: S. Fosså, G.M.O. Bassleer, L.L. Chuan 1462 and A. Ploeg (Eds.) International Transport of Live Fish in the Ornamental Aquatic Industry. OFI 1463 Educational publication 2. OFI, Maarssen, The Netherlands, 48-64 pp.

POKOROVA, D., Vesely, T., Piackova, V., Reschova, S., Hulova, J. 2005. Current knowledge on koi herpervirus (KHV): a review. *Veterinary Medicine-Czech* **4**, 139-147.

RENO, P.W. 1998. Factors involved in the dissemination of disease in fish populations. *Journal of Aquatic Animal Health* **10**, 160-171.

RETIEF, N., Avenant-Oldewage, A., du Preez, H.H. 2007. Ecological aspects of the occurrence of asian tapeworm, *Bothriocephalus acheilognathi* Yamaguti, 1934 infection in the largemouth yellowfish, *Labeobarbus kimberleyensis* (Gilchrist and Thompson, 1913) in the Vaal Dam, South Africa. *Physics and Chemistry of the Earth* **32**, 1384-1390.

SALGARDO-MALDONADO, G., Pineda-Lopez, R.F. 2003. The Asian fish tapeworm *Bothriocephalus acheilognathi*: a potential threat to native freshwater fish species in Mexico. *Biological Invasions* **5**, 261–268.

SCHOLZ, T. 1999. Parasites in cultured and feral fish. *Veterinary Parasitology* **84**, 317–335.

SKELTON, P. 2001. A Complete Guide to the Freshwater Fishes of Southern Africa. Struik Publishers, Cape Town, South Africa, 395 pp.

THONEY, D.A., Hargis, W.J. 1991. Monogenea (Platyhelminthes) as hazards for fish in confinement. *Annual Review of Fish Diseases* **1**, 133-153.

WATT, R., De Kock, S. 1996. Living Jewels: Koi keeping in South Africa. Jonathan Ball Publishers, Jeppestown, South Africa, 159 pp.

WHITTINGTON, R.J., Chong, R. 2007. Global trade in ornamental fish from an Australian perspective: The case for revised import risk analysis and management strategies. *Preventive Veterinary Medicine* **81**, 92–116.

Chapter 6

References

ALI, N. M., Mhaisen, F. T., Abul-Eis, E. S., Kadim, L. S. 1988. First occurrence of the monogenetic trematode *Gyrodactylus kherulensis* Ergens, 1974 in Iraq on the gills of the common carp *Cyprinus carpio*. *Journal of Biological Science Research* **19** (3), 659-664.

ALLAN, J.D., Flecker, A.S. 1993. Biodiversity conservation in running waters. *Bioscience* **43** (1), 32-43.

ALLENDORF, F. W., Lundquist, L. L. 2003. Population biology, evolution, and control of invasive species. *Conservation Biology* **17**(1), 24–30.

ANDREWS, C. 1990. The ornamental fish trade and fish conservation. *Journal of Fish Biology* **37**, (Suppl A) 53 –59.

APPLEBY, C. 1996. Variability of the opisthaptoral hard parts of *Gyrodactylus callariatis* Malmberg, 1957 (Monogenea: Gyrodactylidae) from Atlantic cod *Gadus morhua* L. in the Oslo Fjord, Norway. *Systematic Parasitology* **33** (3), 199-207.

APPLEBY, C., Mo, T.A. 1997. Population dynamics of *Gyrodactylus salaris* (Monogenea) infecting Atlantic salmon, *Salmo salar*, parr in the River Batnfjordselva, Norway. *The Journal of Parasitology* **83**, (1) 23-30.

ARTHINGTON, A.H. & McKenzie, F. 1997. Review of impacts of displaced/introduced fauna associated with inland waters, Australia: State of the Environment Technical Paper Series (Inland Waters), Department of the Environment, Canberra. 69 pp.

BAKKE, T.A., Harris, P.D., Cable, J. 2002. Host specificity dynamics: observations on gyrodactylid monogeneans. *International Journal for Parasitology* **32**, 281-308.

BAKKE, T.A., Cable, J., Harris, P.D. 2007. The biology of gyrodactylid monogeneans: The "Russian-doll killers" *Advances in Parasitology* **64**, 161-376.

BAKKE, T.A., Harris, P.D., Jansen, P.A., Hansen, L.P. 1992. Host specificity and dispersal strategy in gyrodactylid monogeneans, with particular reference to *Gyrodactylus salaris* (Platyhelminthes, Monogenea). *Diseases of aquatic organisms* **13**, 63-74.

BALON, E.K. 1995. Origin and domestication of the wild carp, *Cyprinus carpio*: from Roman gourmets to the swimming flowers. *Aquaculture* **129**, 3-48.

BARKER, D.E., Cone, D.K. 2000. Occurrence of *Ergasilus celestis* Copepoda and *Pseudodactylogyrus anguillae* Monogenea among wild eels *Anguilla rostrata* in relation to stream flow, pH and temperature and recommendations for controlling their transmission among captive eels. *Aquaculture* **187**, 261-274.

BARTLEY, D.M., Subasinghe, R.P. 1996. Historical aspects of international movement of living aquatic species. *Revue Scientifique et Technique de l'office International des Epizootics* **15**(2): 387-400.

BAUER, O.N. 1991. Spread of parasites and diseases of aquatic organisms by acclimatization: a short review. *Journal of Fish Biology* **39**, 679 – 686.

BEVERIDGE M.C.M., Ross, L.G., Kelly, L.A. 1994. Aquaculture and biodiversity. *Ambio* **23**(8), 497-502.

BLANC, G. 2001. Introduction of pathogens in European aquatic ecosystems: attempt of evaluation and realities. pp. 37–56 in Uriate, A. & Basurco, B. (Eds) Environmental impact assessment of Mediterranean aquaculture farms. Zaragoza, CIHEAM-IMAZ.

BOTES, L., Thompson, G., Louw, R. 2006. AISA 2006. Benchmarking survey of the South African Aquaculture (marine &freshwater) sector. AISA report. 94 pp.

BRANDT, F. De W., Van As, J.G., Schoonbee, H.J., Hamilton-Attwell, V.L. 1981. The occurrence and treatment of bothriocephalosis in the common carp, *Cyprinus carpio* in fish ponds with notes on its presence in the Largemouth yellowfish, *Barbus kimberleyensis* on the Vaal Dam, Transvaal. *Water SA* **7,** 35-42.

BRIGHT, C. 1999. Invasive Species: Pathogens of Globalization. Foreign Policy, No. 116, pp. 50-64.

BRITZ, P.J., Lee, B., Botes, L. 2009. AISA 2009. Aquaculture Benchmarking Survey: Primary Production and Markets. A report for the Aquaculture Institute of South Africa and Swisscontact, produced by Enviro-Fish Africa. (Pty.) Ltd. 130 pp.

BRUTON, I.J., Van As, J.G. 1986. Faunal invasions. Faunal invasions of aquatic ecosystems in southern Africa, with suggestions for their management. In: I.A.W. Mcdonald, F.J Kruger & A.A Ferrar (Eds) The Ecology and Management of Biological Invasions in Southern Africa. Pp. 47-61. Oxford University Press, Cape Town.

BUCHMANN, K. 1997. Infection biology of gill parasitic monogeneans with special reference to the congeners *Pseudodactylogyrus bini* and *P. anguillae* (Platyhelminthes: Monogenea) from European eel. Dissertation. Royal Veterinary and Agricultural University, Frederiksberg, Denmark, 208 pp.

BUCHMANN, K., Bresciani, J. 1997. Microenvironment of *Gyrodactylus derjavini* on rainbow trout *Oncorhynchus mykiss*: association between mucous cell density in skin and site selection. *Parasitology Research* **84**, 17-24.

BUCHMANN, K., Lindenstrøm, T. 2002. Interactions between monogenean parasites and their fish hosts. *International Journal for Parasitology* **32**, 309–319.

BUCHMANN, K., Madsen, K.K. Dalgaard, M.B. 2004. The homing of *Gyrodactylus salaris* and *G. derjavini* (Monogenea) on different host and response post-attachment. *Folia Parasitologica* **51**, 263-267.

BUSH, A.O., Fernandez, J.C., Esch, G.W., Seed, R. 2001. Parasitism: The Diversity and Ecology of Animal Parasites. Cambridge University Press, Cambridge, U.K. 566 pp.

CABLE, J., Harris, P.D., Tinsley, R.C., Lazarus, C.M. 1999. Phylogenetic analysis of *Gyrodactylus* spp. (Platyhelminthes: Monogenea) using ribosomal DNA sequences. *Canadian Journal of Zoology* **77**, 1439-1449.

CABLE, J., Tinsley, R.C., Harris, P.D. 2001. Survival, feeding and embryo development of *Gyrodactylus gasterostei* (Monogenea: Gyrodactylidae). *Parasitology* **124**, 53-68.

CABLE, J., Harris, P.D. 2002. Gyrodactylid developmental biology: historical review, current status and future trends. *International Journal for Parasitology* **32**, 255–280.

CAMBRAY, J.A. 2003. Impact on indigenous species biodiversity caused by the globalisation of alien recreational freshwater fisheries. *Hydrobiologia* **500**, 217-230.

CAMBRAY, J.A., Stuart, C.T. 1985. Aspects of the biology of a rare redfin minnow, *Barbus burchelli* (Pisces, Cyprinidae), from South Africa. *South African Journal of Zoology* **20** (3), 155-165.

CECCHINI, S., Saroglia, M., Berni, P., Cognetti-Varriale, A.M. 1998. Influence of temperature on the life cycle of *Diplectanum aequans* (Monogenea, Diplectanidae), parasitic on sea bass, *Dicentrarchus labrax* (L.). *Journal of Fish Diseases* **21**, 73-75.

CHRISTISON, K.W., Shinn, A.P., van As, J. 2005. *Gyrodactylus thlapi* n. sp. (Monogenea) from *Pseudocrenilabrus philander philander* (Weber) (Cichlidae) in the Okavango Delta, Botswana. *Systematic Parasitology* **60**, 165–173.

CLEAVELAND, S., Hess, G. R., Dobson, A., Laurenson, M. K., McCallum, H. I., Roberts, M. and Woodroffe, R. 2002. The role of pathogens in biological conservation. In: P.J. Hudson, A. Rizzoli, B. T. Grenfell, H. Heesterbeek, and A. P. Dobson (Ed.), The Ecology of Wildlife Diseases (pp. 139-150) Oxford, U.K.: Oxford University Press.

CRESPO, J.F., Crespo, R.F. 2003. Monogenean parasites in Mexican fish: a recapitulation. *Técnica Pecuana en México* **41**(2), 175-192.

DALGAARD, M.B., Nielsen, C.V., Buchmann, K. 2003. Comparative susceptibility of two races of *Salmo salar* (Baltic Lule river and Atlantic Conon river strains) to infection with *Gyrodactylus salaris*. *Diseases of Aquatic Organisms* **53**, 173-176.

DAVENPORT, K. E. 1996. Characteristics of the current international trade in ornamental fish, with special reference to the European Union. *Revue Scientifique et Technique de l'Office International des Epizooties* **15**, 435–443.

DAVIDOVA, M., Jarkovsky, J., Matejusova, I., Gelnar, M. 2005. Seasonal occurrence and metrical variability of *Gyrodactylus rhodei* Zitnan 1964 (Monogenea, Gyrodactylidae). *Parasitology Research* **95**, 398–405.

DE MOOR, I.J., Bruton, M.N. 1988. Atlas of alien and translocated indigenous aquatic animals in southern Africa. South African National Scientific Programmes Report No. 144, 310 pp.

DMITRIEVA, E., Dimitrov, G. 2002. Variability in the taxonomic characters of Black Sea gyrodactylids (Monogenea). *Systematic Parasitology* **51**, 199-206.

DOVE, A.D.M., Cribb, T.H., Mockler, S.P., Lintermans, S.P. 1997. The Asian tapeworm, *Bothriocephalus acheilognathii*, in Australian freshwater fishes. *Marine Freshwater Research* **48**, 181-183.

DOVE, A., Ernst, I. 1998. Concurrent invaders of four exotic species of Monogenea now established on exotic freshwater fishes in Australia. *International Journal for Parasitology* **28,** 1755-1764.

DOVE, A.D.M. 2000. Richness patterns in the parasite communities of exotic poeciliid fishes. *Parasitology* **120**, 609-623.

DU PREEZ, L., Maritz, M.F. 2006. Demonstrating morphometric protocols using polystome marginal hooklet measurements. *Systematic Parasitology* **63**, 1-15.

ERGENS, R. 1974. *Gyrodactylus kherulensis* sp. n. (Monogenoidea) from the carp. *Folia Parasitologica* **21**, 377-379.

ERGENS, R., Ogawa, K. 1978. Redescription of *Gyrodactylus kobayashii* Hukuda (Monogenoidea). *Vestnik Ceskslovenske Spolecnosti Zoologicke* **2**, 101-104.

ERNST, I., Whittington, I.D., Corneillie, S., Talbot, C. 2005. Effects of temperature, salinity, desiccation, and chemical treatments on egg embryonation and hatching success of *Benedenia seriolae* (Monogenea: Capsalidae), a parasite farmed *Seriola* spp. *Journal of Fish Diseases* **28**, 157-164.

ESCH, G.W., Barger, M., Fellis, K.J. 2002. The transmission of digenetic trematodes: style, elegance, complexity. *Integrative and Comparative Biology* **42**, 304-312.

FLETCHER, A.S., Whittington, I.D. 1998. A parasite-host checklist for Monogenea from freshwater fishes in Australia, with comments on biodiversity. *Systematic Parasitology* **41,** 159–168.

GALLI, P., Stefani, F., Benzoni, F., Zullini, A. 2005. Introduction of alien host–parasite complexes in a natural environment and the symbiota concept. *Hydrobiologia* **548**, 293–299.

GARCIA-VASQUEZ, A., Hansen, H., Christison, K.W., Bron, J.E., Shinn, A.P. 2011. Description of three new species of *Gyrodactylus* von Nordmann, 1832 (Monogenea) parasitising *Oreochromis niloticus niloticus* (L.) and *O. mossambicus* (Peters) (Cichlidae). *Acta Parasitologica*. **56** (1): 20-33.

GARCIA-VASQUEZ, A., Hansen, H., Christison, K.W., Rubio-Godoy, M., Bron, J.E., Shinn, A.P. 2010. Gyrodactylids (Gyrodactylidae, Monogenea) infecting *Oreochromis niloticus niloticus* (L.) and *O. mossambicus* (Peters) (Cichlidae): A pan-global survey. *Acta Parasitologica* **55** (3), 215-229.

GEETS, A., Appleby, C., Ollevier, F. 1999. Host-dependent and seasonal variation in opisthaptoral hard parts of *Gyrodactylus* cf. *arcuatus* from three *Pomatoschistus* spp. and *G. arcuatus* from *Gasterosteus aculeatus*: a multivariate approach. *Parasitology* **119**, 27-40.

GHEORGHIU, C., Cable, J., Marcogliese, D.J. Scott, M.E. 2007. Effects of waterborne zinc on reproduction, survival and morphometrics of *Gyrodactylus turnbulli* (Monogenea) on guppies (*Poecilia reticulata*). *International Journal for Parasitology* **37**, 375–381.

GILAD, O., Yun, S., Adkison, M.A., Way, K., Willits, N.H., Bercovier, H., Hedrick, R.P. 2003. Molecular comparison of isolates of an emerging fish pathogen, koi herpesvirus, and the effect of water temperature on mortality of experimentally infected koi. *Journal of General Virology* **84**, 2661-2668.

HARRIS, P.D. 1993. Les interactions entre la reproduction et la biologie des populations chez les Monogenes Gyrodactylidae: revue. *Bulletin Francais de la Peche et de la Pisciculture* **328**, 47–65.

HARRIS, P.D. 1998(a). Extreme morphological variation between related individuals of *Gyrodactylus pungitii* Malmberg, 1964 (Monogenea). *Systematic Parasitology* **39**, 137-140.

HARRIS, P.D. 1998(b). Ecological and genetic evidence for clonal reproduction in *Gyrodactylus gasterostei* Gläser, 1974. *International Journal of Parasitology* **28**, 1595-1607.

HARRIS, P.D., Cable, J., Tinsley, R.C. 1999. Combined ribosomal DNA and morphological analysis of individual Gyrodactylid monogeneans. *The Journal of Parasitology* **85** (2), 188-191.

HARRIS, P.D., Shinn, A.P., Cable, J., Bakke, T.A. 2004. Nominal species of the genus *Gyrodactylus* von Nordmann 1832 (Monogenea: Gyrodactylidae), with a list of principal host species. *Systematic Parasitology* **59**, 1–27.

HARRIS, P.D., Soleng, A., Bakke, T.A. 2000. Increased susceptibility of salmonids to the monogenean *Gyrodactylus salaris* following administration of hydrocortisone acetate. *Parasitology* **120**, 57-64.

HAYWARD, C.J., Iwashita, M., Ogawa, K., Ernst, I. 2001. Global spread of the eel parasite *Gyrodactylus anguillae* (Monogenea). *Biological Invasions* **3**, 417–424.

HAYWARD, C.J., Bott, N.J., Itoh, N., Iwashita, M., Okihiro, M., Nowak, B.F. 2007. Three species of parasites emerging on the gills of mulloway, *Argyrosomus japonicus* (Temminck and Schegel, 1843), cultured in Australia. *Aquaculture* **265**, 27-40.

HECHT, T., Endemann, F. 1998. The impact of parasites, infections and diseases on the development of aquaculture in sub-Saharan Africa. *Journal of Applied Ichthyology* **14**, 213-221.

HEDRICK, P.W., Kim, T.J., Parker, K.M. 2001. Parasite resistance and genetic variation in the endangered Gila topminnow. *Animal Conservation* **4**, 103-109.

HILL, B.J. 2005. The need for effective disease control in international aquaculture. *Developmental Biology* **123**, 3-12.

HOFFMAN, G.L. 1998. Parasites of North American Freshwater Fishes. Comstock Publishing Associates, Ithaca and London, 539 pp.

HOFFMAN, L.C., Swart, J.J., Brink, D. 2000. The 1998 production and status of aquaculture in South Africa. *Water SA* **26** (1), 133-136.

HUTORAN, M., Ronen, A., Perelberg, A., Ilouze, M., Dishon, A., Bejerano, I., Chen, N., Kotler, M, 2005. Description of an as yet unclassified DNA virus from diseased *Cyprinus carpio* species. *Journal of Virology* **79**, 1983-1991.

HUYSE, T., Volkaert, F.A.M. 2005. Comparing host and parasite phylogenies: *Gyrodactylus* flatworms jumping from goby to goby. *Systematic Biology* **54**, 710-718.

IMPSON, N.D. 2007. State of Biodiversity: Western Cape Province. Chapter 3: Freshwater fishes, Western Cape State of Biodiversity 2007. Western Cape Conservation Board, Cape Town ISBN 978-0-620-39289-1.

IMPSON, N.D., Bills, I.R., Cambray, J.A. 2000. State of Biodiversity: Western Cape Province, South Africa.Freshwater Fishes. Western Cape State of Biodiversity 2002. Western Cape Nature Conservation Board, CapeTown. ISBN: 0-620-29893-6.

JACKSON, J.A., Tinsley, R.C. 1998. Effects of temperature on oviposition rate in *Protopolystoma xenopodis* (Monogenea: Polystomatidae). *International Journal for Parasitology* **28**, 309-315.

JALALI, B., Shamsi, S., Barzegar, M. 2005. Occurrence of *Gyrodactylus* spp. (Monogenea: Gyrodactylidae) from Iranian freshwater fish. *Iranian Journal of Fisheries Sciences* **4**, 19-30.

KING, T.A., Cable, J. 2007. Experimental infections of the monogenean *Gyrodactylus turnbulli* indicate that it is not a strict specialist. *International Journal for Parasitology* **37**, 663-672.

KING, T.A., van Oosterhout, C., Cable, J. 2009. Experimental infections with the tropical monogenean, *Gyrodactylus bullatarudis*: Potential invader or experimental fluke? *Parasitology International* **58,** 249-254.

KIR, I., Tekin Ozan, S. 2007. Helminth Infections in common carp, *Cyprinus carpio* L., 1758 (Cyprinidae) from Kovada Lake (Turkey). *Türkiye Parazitoloji Dergisi,* **31** (3), 232-236.

KOEHN, J.D. 2004. Carp (*Cyprinus carpio*) as a powerful invader in Australian waterways. *Freshwater Biology* **49**, 882–894.

LESTER, R.J.G. 1972. Attachment of *Gyrodactylus* to *Gasterosteus* and Host Response. *The Journal of Parasitology* **58** (4), 717-722.

LEUNG, K.M.Y., Dudgeon, D. 2008. Ecological risk management of exotic organisms associated with aquaculture activities. In: M.G. Bondad-Reantaso, J.R.Arthur and R.P

Subasinghe (eds). Understanding and applying risks analysis in aquaculture. FAO Fisheries and Aquaculture Technical Paper. No. 519. Rome, FAO. 67-100 pp.

LINTERMANS, M. 2004. Human-assisted dispersal of alien freshwater fish in Australia. *New Zealand Journal of Marine and Freshwater Research* **38**, 481–501.

LUX, E. 1987. Neues zum Artenbestand von *Gyrodactylus* bei Karpfen in der DDR. *Angewandte Parasitologie* **28**, 159-164.

LUX, E. 1990. Population dynamics and interrelationships of some *Dactylogyrus* and *Gyrodactylus* species on *Cyprinus carpio. Angewandte Parasitologie* **31** (3), 143-149.

MARGOLIS, L., Esch, G.W., Holmes, J.C., Kuris, A.M., Schad, G.A. 1982. The use of ecological terms in parasitology (Report of an ad hoc committee of the American Society of Parasitologists). *Journal of Parasitology* **68**, 131-133.

MATEJUSOVA, I., Morand, S., Gelnar, M. 2000. Nestedness in assemblages of gyrodactylids (Monogenea: Gyrodactylidae) parasitizing two species of cyprinid, with reference to generalists and specialists. *International Journal of Parasitology* **30**, 1153-1158.

MCHUGH, E.S., Shinn, A.P., Kay, J.W. 2000. Discrimination of the notifiable pathogen *Gyrodactylus salaris* from *G. thymalli* (Monogenea) using statistical classifiers applied to morphometric data. *Parasitology* **121**, 315-323.

MO, T.A. 1991. Seasonal variations of opisthaptoral hard parts of *Gyrodactylus salaris* Malmberg, 1957 (Monogenea: Gyrodactylidae) on parr of Atlantic salmon *Salmo salar* L. in the River Batnfjordselva, Norway. *Systematic Parasitology* **19** (3), 231-240.

MO, T.A. 1994. Status of *Gyrodactylus salaris* problems and research in Norway. In: Parasitic Diseases of Fish (ed. Pike, A. W. & Lewis, J. W.), Samara Publishing Ltd, Dyfed, 43-56 pp.

MOUTON, A. Basson, L., Impson, D. 2001. Health status of ornamental freshwater fishes imported to South Africa: a pilot study. *Aquarium Sciences and Conservation* **3**, 327-333.

MOYLE, P.B., Light, T. 1996. Biological invasions of freshwater: empirical rules and assembly theory. *Biological Conservation* **78**, 149-161.

MUIR, J. 2005. Managing to harvest? Perspectives on the potential of aquaculture. *Philosophical Transactions of the Royal Society* B: *Biological Sciences* **360** (1453), 191-218.

MURRAY, A.G., Peeler, E.J. 2005. A framework for understanding the potential for emerging diseases in aquaculture. *Preventative Veterinary Medicine* **67**, 223-235.

MYERS, N., Mittermeier, R.A., Mittermeier, C.G., da Fonseca, G.A.B., Kent, J. 2000. Biodiversity hotspots for conservation priorities. *Nature* **403**, 853–858.

NAYLOR, R.L., Goldburg, R.J., Primavera, J.H. Krautsky, N. Beveridge, M.C.M., Clay, J., Folke, C., Lubchenco, J., Mooney, H., Treoll, M. 2000. Effect of aquaculture on world fish supplies. *Nature* **405**, 1017-1024.

OGAWA, K. 1994. Monogenean parasites of freshwater fishes of Hokkaido, Japan. *Scientific Report of Hokkaido Fish Hatchery* **48**, 59-67.

OGAWA, K., Egusa, S. 1978. Seven species of *Gyrodactylus* (Monogenea: Gyrodactylidae) from *Plecoglossus altivelis* (Plecoglossidae), *Cyprinus carpio* (Cyprinidae) and *Anguilla* spp. (Anguillidae). *Bulletin of the Japanese Society of Scientific Fisheries* **44** (6), 613-618.

OLSTAD, K., Cable, J., Robertson, G., Bakke, T.A. 2006. Unpredicted transmission strategy of *Gyrodactylus salaris* (Monogenea: Gyrodactylidae): survival and infectivity of parasites on dead hosts. *Parasitology* **133**, 33-41.

OLSTAD, K., Bachmann, L., Bakke, T.A. 2009. Phenotypic plasticity of taxonomic and diagnostic structures in gyrodactylosis-causing flatworms (Monogenea, Platyhelminthes). *Parasitology* **136**, 1305–1315.

OLSTAD, K., Shinn, A., Bachmann, L., Bakke, T.A. 2007. Host-based identification is not supported by morphometrics in natural populations of *Gyrodactylus salaris* and *G. thymalli* (Platyhelminthes, Monogenea). *Parasitology* **134**, 2041-2052.

PAPERNA, I. 1996. Parasites, infections and disease of fishes in Africa: An update. CIFA Technical Paper No. 31 Food and Agriculture Organization of the United Nations, Rome. 220 pp.

PEELER, E.J., Gardiner, R., Thrush, M.A. 2004. Qualitative risk assessment of routes of transmission of the exotic fish parasite *Gyrodactylus salaris* between river catchments in England and Wales. *Preventive Veterinary Medicine* **64**, 175–189.

PLOEG, A. 2007. The volume of the ornamental fish trade. In: S. Fosså, G.M.O. Bassleer, L.L. Chuan 1462 & A. Ploeg (Eds.) *International transport of live fish in the ornamental aquatic industry.* OFI 1463 Educational publication 2. OFI, Maarssen, The Netherlands. 48-64 pp.

POKOROVA, D., Vesely, T., Piackova, V., Reschova, S., Hulova, J. 2005. Current knowledge on koi herpervirus (KHV): a review. *Veterinary Medicine-Czech* **4**, 139-147.

PONPORNPISIT, A., Endo, M., Murata, H. 2000. Experimental infections of a ciliate *Tetrahymena pyriformis* on ornamental fish. *Fisheries Sciences* **66**, 1026-1031.

POULIN, R., Keeney, D.B. 2008. Host specificity under molecular and experimental scrutiny. *Trends in Parasitology* **24** (1), 24-28.

PRESSEY, R.L., Cowling, R.M., Rouget, M. 2003. Formulating conservation targets for biodiversity pattern and process in the Cape Floristic Region, South Africa. *Biological Conservation* **112**, 99-127.

PŘIKRYLOVÁ, I., Matějusova, I., Musilová, N., Gelnar, M. 2009. *Gyrodactylus* species (Monogenea: Gyrodactylidae) on the cichlid fishes of Senegal, with the description of *Gyrodactylus ergensi* n. sp. From the Mango tilapia, *Sarotherodon galilaeus* L. (Teleostei: Cichlidae). *Parasitology Research* **106** (1): 1-6.

RAHEL, F.J. 2002. Homogenization of freshwater faunas. *Annual Review of Ecological Systems* **33**, 291–315.

REBELO, A.G. 1992. Red data book species in the Cape Floristic region: Threats, priorities and target species. *Transactions of the Royal Society of South Africa* **48** (1), 55-86.

RENO, P.W. 1998. Factors involved in the dissemination of disease in fish populations. *Journal of Aquatic Animal Health* **10**, 160-171.

RETIEF, N., Avenant-Oldewage, A., du Preez, H.H. 2007. Ecological aspects of the occurrence of asian tapeworm, *Bothriocephalus acheilognathi* Yamaguti, 1934 infection in the largemouth yellowfish, *Labeobarbus kimberleyensis* (Gilchrist and Thompson, 1913) in the Vaal Dam, South Africa. *Physics and Chemistry of the Earth* **32**, 1384-1390.

SALGARDO-MALDONADO, G., Pineda-Lopez, R.F. 2003. The Asian fish tapeworm *Bothriocephalus acheilognathi*: a potential threat to native freshwater fish species in Mexico. *Biological Invasions* **5**, 261–268.

SCHOLZ, T. 1999. Parasites in cultured and feral fish. *Veterinary Parasitology* **84**, 317–335.

ŠEFROVÁ, H., Laštůvka, Z. 2005. Catalogue of alien animal species in the Czech Republic. *Acta Universitatis Agriculturae et Slviculturae Mndelianae Brunensis Sbornik Mendelovy Zemedelske a Lesnicke Univerzity v Brne* **53**, 151-170.

SHINN, A.P., Christison K.W., Garcia-Vasquez A. 2009. The *Gyrodactylus* von Nordmann, 1832 (Gyrodactylidae: Monogenea) fauna of Africa including a re-description of species of the genus. Personal communication.

SHINN, A.P., Gibson, D.I., Sommerville, C. 2001. Morphometric discrimination of *Gyrodactylus salaris* Malmberg (Monogenea) from species of *Gyrodactylus* parasitising British salmonids using novel parameters. *Journal of Fish Diseases* **24**, 83-97.

SHINN, A.P., Hansen, H., Olstad, K., Bachmann, L., Bakke, T.A. 2004. The use of morphometric characters to discriminate specimens of laboratory-reared and wild populations of *Gyrodactylus salaris* and *G. thymalli* (Monogenea). *Folia Parasitologica* **51**, 239-252.

SIMKOVA, A.S., Verneau, O., Gelnar, M., Morand, S. 2006. Specificity and specialization of congeneric monogeneans parasitizing cyprinid fish. *Evolution* **60** (5), 1023–1037.

SKELTON, P. 2001. A Complete Guide to the Freshwater Fishes of Southern Africa. Struik Publishers, Cape Town, South Africa, 395 pp.

SKELTON, P.H. 1980. Systematics and biogeography of the redfin *Barbus* species (Pisces: Cyprinidae) from southern Africa. Ph.D. thesis, RhodesUniversity, Grahamstown, South Africa.

SKELTON, P.H., Cambray, J.A., Lombard, A., Benn, G.A. 1995. Patterns of distribution and conservation status of freshwater fishes in South Africa. *South African Journal of Zoology* **30**, 71-81.

SOLENG, A, Bakke, T.A. 2001. The susceptibility of grayling (*Thymallus thymallus*) to experimental infections with the monogenean *Gyrodactylus salaris*. *International Journal for Parasitology* **31**, 793-797.

SWARTZ, E.R. 2005. Phylogeography, phylogenetics and evolution of the redfins (Teleostei, Cyprinidae, *Pseudobarbus*) in southern Africa. PhD thesis, University of Pretoria, Pretoria, South Africa.

SWARTZ, E.R., Impson, D. 2007. *Pseudobarbus burchelli*. In: IUCN 2008. 2008 IUCN Red List of Threatened Species. <http://www.iucnredlist.org/>. Downloaded on 09 March 2009.

SWARTZ, E.R., Skelton, P.H., Bloomer, P. 2009. Phylogeny and biogeography of the genus *Pseudobarbus* (Cyprinidae): Shedding light on the drainage history of the rivers associated with the Cape Floristic Region. *Molecular Phylogenetics and Evolution* **51**, 75-84.

TEKIN OZAN, S., Kir, I., Barlas, M. 2008. Helminth parasites of common carp (*Cyprinus carpio* L., 1758) in Beyşehir Lake and population dynamics related to month and host size. *Turkish Journal of Fisheries and Aquatic Sciences* **8**, 201-205.

THONEY, D.A., Hargis, W.J. 1991. Monogenea (Platyhelminthes) as hazards for fish in confinement. *Annual Review of Fish Diseases* **1**, 133-153.

TIDWELL, J.H., Allan, G.L.A. 2001.Fish as food: aquaculture's contribution: Ecological and economic impacts and contributions of fish farming and capture fisheries. *European Molecular Organisation Reports* **2** (11), 958–963.

TUBBS, L.A., Poortenaar, C.W., Sewell, M.A., Diggles, B.K. 2005. Effects of temperature on fecundity in *in vitro*, egg hatching and reproductive development of *Benedenia seriolae* (Monogenea) parasitic on yellowtail kingfish *Seriola lalandi*. *International Journal for Parasitology* **35**, 315-327.

VAN OOSTERHOUT, C., Harris, P.D., Cable, J. 2003. Marked variation in parasite resistance between two wild populations in the Trinidadian guppy, *Poecilia reticulata* (Pisces: Poecilidae). *Biological Journal of the Linnean Society* **79**, 645-651.

WATT, R., De Kock, S. 1996. Living Jewels: Koi keeping in South Africa. Jonathan Ball Publishers, Jeppestown, South Africa, 159 pp.

WHITTINGTON, I.D., Cribb, B.W., Hamwood, T.E., Halliday, J.A. 2000. Host-specificity of monogenean (Platyhelminth) parasites: a role for anterior adhesive areas. *International Journal of Parasitology* **30** (3), 305-320.

WHITTINGTON**,** RJ**,** Chong**,** R. 2007. Global trade in ornamental fish from an Australian perspective: The case for revised import risk analysis and management strategies**'** *Preventive Veterinary Medicine* **81,** 92-116.

WINGER, A.C., Primicerio, R., Kristoffersen, R., Siikavuopio, S.I., Knudsen, R. 2008. *Gyrodactylus salaris* infecting allopatric Arctic charr *Salvelinus alpinus* fry: an experimental study of host survival. *Journal of Fish Biology* **73**, 2198-2209.

WYNBERG**,** R. 2002. A decade of biodiversity conservation and use in South Africa: tracking progress from the Rio earth summit to the Johannesburg world smmit on sstainable dvelopment. *South African Journal of Science* **98**, 233-243.

XU, D., Shoemaker, C.A., Klesius, P.H. 2007. Evaluation of the link between gyrodactylosis and streptococcosis of Nile tilapia, *Oreochromis niloticus* (L.). *Journal of Fish Diseases* **30**, 233–238.

ZIETARA, M.S., Kuusela, J., Veselov, Lumme, J. 2008. Molecular faunistics of accidental infections of *Gyrodactylus* Nordmann, 1832 (Monogenea) parasitic on salmon *Salmo salar* L. and brown trout *Salmo trutta* in NW Russia. *Systematic Parasitology* **69**, 123-135.

ZIETARA, M.S., Lumme, J. 2003. The crossroads of molecular, typological and biological species concepts: two new of *Gyrodactylus* Nordmann, 1832 (Monogenea: Gyrodactylidae). *Systematic Parasitology* **55**, 39–52.

Appendix

Appendix 1

Univariate analyses of the various attachment organs of *G. kherulensis*

Levene's test of homogeneity for the different opisthaptoral characters of the different *G. kherulensis* populations. Significant values (p>0.05) are bold.

	SS	df	MS	SS	df	MS	F	p
Hapert	**40.3248**	**7**	**5.76069**	**144.918**	**110**	**1.31743**	**4.372667**	**0.000260**
HPrSW	**6.7883**	**7**	**0.96975**	**29.744**	**110**	**0.27040**	**3.586326**	**0.001628**
HPL	**184.7157**	**7**	**26.38795**	**429.859**	**110**	**3.90781**	**6.752624**	**0.000001**
HDSW	**2.8372**	**7**	**0.40531**	**11.010**	**110**	**0.10009**	**4.049370**	**0.000552**
HSL	**112.2991**	**7**	**16.04273**	**369.198**	**110**	**3.35634**	**4.779826**	**0.000101**
HPCurv	**25.0397**	**7**	**3.57710**	**54.335**	**110**	**0.49396**	**7.241730**	**0.000000**
HAA	**63.0894**	**7**	**9.01276**	**367.145**	**110**	**3.33768**	**2.700306**	**0.012753**
HPCA	**263.3826**	**7**	**37.62608**	**626.726**	**110**	**5.69751**	**6.603949**	**0.000002**
HICO	**267.9389**	**7**	**38.27698**	**1103.857**	**110**	**10.03507**	**3.814323**	**0.000956**
HRL	**93.7353**	**7**	**13.39076**	**434.618**	**110**	**3.95107**	**3.389146**	**0.002581**
HTL	90.0033	7	12.85762	747.498	110	6.79544	1.892096	0.077506
VBTW	5.4255	7	0.77507	114.069	110	1.03699	0.747417	0.632375
VBTL	33.0952	7	4.72788	332.951	110	3.02682	1.561994	0.154271
VBPML	1.0220	7	0.14600	19.355	110	0.17596	0.829761	0.564781
VBML	4.8617	7	0.69452	46.544	110	0.42313	1.641397	0.131243
VBProL	0.4849	7	0.06927	8.582	110	0.07802	0.887780	0.518769
VBMemL	25.3785	7	3.62550	195.716	110	1.77923	2.037676	0.056557
MHTL	87.2443	7	12.46347	879.528	110	7.99571	1.558770	0.155277
MHSL	18.1056	7	2.58652	549.142	110	4.99220	0.518112	0.819243
MHSickL	**0.7543**	**7**	**0.10775**	**5.453**	**110**	**0.04957**	**2.173500**	**0.041946**
MHPW	**2.6194**	**7**	**0.37420**	**10.729**	**110**	**0.09753**	**3.836653**	**0.000907**
MHToe	0.1141	7	0.01631	2.450	110	0.02227	0.732174	0.645070
MHDW	**0.9574**	**7**	**0.13678**	**5.684**	**110**	**0.05168**	**2.646848**	**0.014416**
MHAp	**1.0389**	**7**	**0.14841**	**6.027**	**110**	**0.05479**	**2.708593**	**0.012513**
MHIns	0.1389	7	0.01985	1.316	110	0.01196	1.658902	0.126602

Parametric statistics

ANOVA post hoc test for the comparison of various morphological characters for the 8 populations of *G. kherulensis.* Significant values (p>0.05) are bold

Ventral bar total length

	1	2	3	4	5	6	7	8
1								
2	**0.000797**							
3	0.728211	**0.000279**						
4	0.999993	**0.000415**	0.810899					
5	0.999993	**0.021805**	0.571068	0.999827				
6	0.763364	**0.000123**	0.991305	0.898972	0.917910			
7	1.000000	**0.000945**	0.803465	1.000000	0.999865	0.984594		
8	0.684989	**0.000121**	0.994752	0.845122	0.890330	1.000000	0.973942	

Ventral bar median length

	1	2	3	4	5	6	7	8
1								
2	0.668689							
3	0.999752	0.686061						
4	0.374748	**0.027070**	0.998323					
5	0.953165	0.183243	0.999813	0.999999				
6	0.780121	0.093289	0.999986	0.998464	1.000000			
7	0.998040	0.307844	1.000000	0.989994	0.999479	0.999908		
8	0.484031	**0.038730**	0.999386	1.000000	1.000000	0.999820	0.996030	

Marginal hook shaft length

	1	2	3	4	5	6	7	8
1								
2	0.999997							
3	0.958855	0.984877						
4	0.978421	0.999839	0.999001					
5	0.877164	0.946290	1.000000	0.994595				
6	0.999708	1.000000	0.990910	0.999772	0.965060			
7	0.998851	0.999962	0.998325	1.000000	0.991686	0.999996		
8	0.976503	0.999813	0.999079	1.000000	0.994965	0.999722	1.000000	

Non-parametric statistics

Kruskal-Wallis post hoc test for the comparison of various morphological characters for the 8 populations of *G. kherulensis.* Significant values (p>0.05) are bold

Hamulus aperture length

	1	2	3	4	5	6	7	8
1								
2	1.000000							
3	0.383511	0.309927						
4	**0.034398**	0.092051	1.000000					
5	**0.000816**	**0.002032**	1.000000	1.000000				
6	**0.000395**	**0.004527**	1.000000	1.000000	1.000000			
7	1.000000	1.000000	1.000000	1.000000	0.120225	0.502537		
8	**0.006055**	**0.028053**	1.000000	1.000000	1.000000	1.000000	1.000000	

Hamulus proximal shaft width

	1	2	3	4	5	6	7	8
1								
2	1.000000							
3	0.704033	0.129766						
4	**0.000015**	**0.000019**	1.000000					
5	1.000000	0.670529	1.000000	1.000000				
6	**0.002072**	**0.000834**	1.000000	1.000000	1.000000			
7	0.843971	0.136724	1.000000	1.000000	1.000000	1.000000		
8	**0.041080**	**0.009319**	1.000000	1.000000	1.000000	1.000000	1.000000	

Hamulus point length

	1	2	3	4	5	6	7	8
1								
2	0.746820							
3	1.000000	0.101593						
4	**0.001456**	**0.000009**	1.000000					
5	0.087528	**0.000894**	1.000000	1.000000				
6	0.090567	**0.000520**	1.000000	1.000000	1.000000			
7	1.000000	0.341139	1.000000	1.000000	1.000000	1.000000		
8	0.096090	**0.000554**	1.000000	1.000000	1.000000	1.000000	1.000000	

Hamulus distal shaft width

	1	2	3	4	5	6	7	8
1								
2	**0.000160**							
3	0.240835	1.000000						
4	**0.026491**	1.000000	1.000000					
5	**0.000113**	1.000000	1.000000	0.813279				
6	0.637092	0.318557	1.000000	1.000000	0.116793			
7	0.068835	1.000000	1.000000	1.000000	1.000000	1.000000		
8	**0.003213**	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	

Hamulus shaft length

	1	2	3	4	5	6	7	8
1								
2	1.000000							
3	0.091844	**0.010282**						
4	**0.001853**	**0.000375**	1.000000					
5	**0.000489**	**0.000061**	1.000000	1.000000				
6	**0.003518**	**0.000633**	1.000000	1.000000	1.000000			
7	1.000000	0.622180	1.000000	1.000000	0.304789	1.000000		
8	**0.000901**	**0.000209**	1.000000	1.000000	1.000000	1.000000	1.000000	

Hamulus outer aperture angle

	1	2	3	4	5	6	7	8
1								
2	1.000000							
3	1.000000	1.000000						
4	1.000000	0.320171	1.000000					
5	1.000000	1.000000	1.000000	0.297358				
6	0.740052	1.000000	1.000000	0.053494	1.000000			
7	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000		
8	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	

Hamulus point curve angle

	1	2	3	4	5	6	7	8
1								
2	1.000000							
3	1.000000	1.000000						
4	**0.000800**	1.000000	1.000000					
5	**0.000045**	**0.042587**	0.171564	1.000000				
6	1.000000	1.000000	1.000000	0.385141	**0.009278**			

7	**0.019820**	1.000000	1.000000	1.000000	1.000000	0.942803		
8	**0.000000**	**0.034007**	0.261645	1.000000	1.000000	**0.001999**	1.000000	

Hamulus inner aperture angle

	1	2	3	4	5	6	7	8
1								
2	1.000000							
3	1.000000	1.000000						
4	1.000000	1.000000	1.000000					
5	1.000000	1.000000	1.000000	1.000000				
6	1.000000	1.000000	1.000000	1.000000	1.000000			
7	1.000000	1.000000	1.000000	1.000000	1.000000	0.734739		
8	0.467804	1.000000	1.000000	1.000000	1.000000	0.319384	1.000000	

Hamulus root length

	1	2	3	4	5	6	7	8
1								
2	**0.000006**							
3	1.000000	**0.000474**						
4	1.000000	**0.006453**	1.000000					
5	1.000000	**0.007371**	1.000000	1.000000				
6	1.000000	**0.002698**	1.000000	1.000000	1.000000			
7	1.000000	0.286752	0.977888	1.000000	1.000000	1.000000		
8	1.000000	**0.002569**	1.000000	1.000000	1.000000	1.000000	1.000000	

Marginal hook sickle length

	1	2	3	4	5	6	7	8
1								
2	1.000000							
3	0.628786	1.000000						
4	1.000000	1.000000	1.000000					
5	1.000000	1.000000	1.000000	1.000000				
6	1.000000	1.000000	1.000000	1.000000	1.000000			
7	1.000000	1.000000	0.110238	1.000000	1.000000	1.000000		
8	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	

Marginal hook sickle proximal width

	1	2	3	4	5	6	7	8
1								
2	1.000000							
3	1.000000	1.000000						
4	1.000000	1.000000	1.000000					
5	**0.020862**	**0.040821**	1.000000	0.193355				
6	1.000000	1.000000	1.000000	1.000000	0.689485			
7	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000		
8	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	

Appendix 2

Multivariate analyses of *G. kherulensis* features

Multivariate analyses of the opisthaptoral features of *G. kherulensis.* Factor loadings greater than 0.7 are highlighted. The factor score plots all morphological features are shown.

All opisthaptoral features

Factor loadings

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6
Hapert	**0.824039**	0.116843	-0.326210	-0.173398	0.250534	0.092955
HPrSW	**0.764221**	-0.109734	-0.085445	0.067841	0.079096	0.054167
HPL	**0.848248**	-0.427676	-0.143852	0.019314	0.065107	0.024236
HDSW	0.345027	0.284154	0.536356	0.269118	-0.367424	-0.050316
HSL	**0.857833**	-0.313998	-0.206599	-0.091944	0.129911	0.018115
HAA	0.102730	**-0.801115**	0.305345	0.267984	-0.281305	-0.080120
HPCA	0.495568	-0.696741	-0.313506	-0.019854	0.124181	0.030929
HICO	0.096408	**-0.847635**	0.176110	0.156132	-0.157070	-0.105881
HRL	**0.869245**	0.121596	0.221165	0.037946	-0.061170	-0.088416
HTL	**0.889121**	0.217993	0.196661	-0.047342	0.016577	-0.066289
VBTW	**0.824298**	0.154849	0.139746	0.117989	0.038823	-0.039531
VBTL	**0.857154**	0.229407	0.281904	-0.088464	-0.132317	-0.060176
VBML	0.625135	0.191584	-0.117955	-0.050233	0.451925	-0.067036
VBMemL	**0.753230**	0.225118	0.319706	-0.100445	-0.264522	-0.044209
MHTL	0.198389	0.256497	-0.504019	0.665518	-0.242681	0.052914
MHSL	0.227595	0.215831	-0.542781	0.671696	-0.159188	0.060921
MHSickL	0.130400	0.134470	-0.475289	-0.309839	-0.371107	-0.585273
MHPW	0.299176	0.014710	-0.071615	-0.302293	-0.413070	**0.720840**
MHAp	0.061357	-0.066883	-0.468663	-0.511636	-0.511207	-0.035135
Expl.Var	7.222841	2.595099	1.989337	1.593791	1.295518	0.927353
Prp.Totl	0.380150	0.136584	0.104702	0.083884	0.068185	0.048808

Hamuli features

Factor loadings

	Factor1	Factor2	Factor3	Factor4	Factor5	Factor6
HAPERT	**-0.844046585**	-0.162028055	0.28962935	0.04589474	-0.26966	-0.01653
HPRSW	**-0.770210301**	0.107356519	0.07045385	-0.0782481	-0.00311	0.086046
HPL	**-0.850441182**	0.423025217	0.13091067	0.00264694	-0.07874	-0.01228
HDSW	-0.308069143	-0.180635762	-0.6474358	-0.0545994	0.377688	0.119425
HSL	**-0.864343911**	0.292613608	0.18760433	0.05499675	-0.16976	-0.06643
HAA	-0.080619111	**0.854398583**	-0.24293582	-0.0767401	0.316063	0.060228
HPCA	-0.503906229	0.66706096	0.33821055	0.03773064	-0.17163	-0.05965
HICO	-0.080311784	**0.87624961**	-0.10249841	-0.0392633	0.165685	-0.04956
HRL	**-0.854249682**	-0.080514775	-0.29146729	-0.0164357	0.071422	-0.07349
HTL	**-0.879909975**	-0.19464601	-0.25151942	-0.0102028	0.002162	-0.07259
VBTW	**-0.819093309**	-0.126212404	-0.18442998	-0.132141	0.063239	-0.00145
VBTL	**-0.837359044**	-0.187915868	-0.37454359	0.10840817	0.052268	-0.0578
VBML	-0.633169347	-0.215521344	0.10048518	-0.1849576	-0.34814	-0.24634
VBMEML	**-0.733317109**	-0.179106961	-0.4058588	0.17349117	0.150442	0.008658
MHTL	-0.458760484	-0.229429605	0.66142268	-0.2849339	0.354604	0.207949
MHSL	-0.42957517	-0.181480358	0.66947992	-0.3289551	0.33438	0.246963
MHSICKL	-0.150442527	-0.181327836	0.38293341	0.50341601	0.433538	-0.43988
MHPW	-0.288819053	-0.004136476	-0.0544941	0.52886664	-0.17796	0.694456
MHAP	-0.06572196	0.029326057	0.31540991	**0.8033638**	0.09937	-0.03193
Expl.Var	7.457846944	2.571231863	2.37637061	1.48491222	1.029925	0.891935
Prp.Totl	0.39251826	0.135327993	0.12507214	0.07815327	0.054207	0.046944

Ventral bar features

Factor loadings

	Factor 1	Factor 2	Factor 3	Factor 4
VBTW	**-0.867775**	-0.131355	0.478471	-0.027883
VBTL	**-0.959221**	0.192730	-0.114511	0.172153
VBML	-0.652380	**-0.720022**	-0.233645	-0.037132
VBMemL	**-0.855680**	0.466114	-0.178734	-0.136398
Expl. Var	2.830927	0.790093	0.328583	0.050398
Prp. Totl	0.707732	0.197523	0.082146	0.012599

Marginal hooklet features

Factor loadings

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
MHTL	**-0.93289**	0.284298	-0.08566	-0.06186	-0.19421
MHSL	**-0.91337**	0.329796	-0.12363	-0.07037	0.19172
MHSICKL	-0.51348	-0.52438	0.51267	0.445465	0.009631
MHPW	-0.12555	-0.52528	**-0.80851**	0.233729	-0.00197
MHAP	-0.2815	**-0.82145**	0.110454	-0.48349	0.004866
Expl.Var	2.063203	1.415254	0.951342	0.495607	0.074595
Prp.Totl	0.412641	0.283051	0.190268	0.099121	0.014919

Appendix 3

Univariate analyses of the various features of the attachment organ of *G. kobayashii*

Levene's test of homogeneity for the different opisthaptoral characters of the different *G. kobayashii* populations. Significant values (p>0.05) are in bold.

	SS	df	MS	SS	df	MS	F	p
HApert	23.7791	7	3.39702	178.5611	98	1.82205	1.864391	0.083614
HPrSW	**2.1394**	**7**	**0.30562**	**10.3762**	**98**	**0.10588**	**2.886516**	**0.008741**
HPL	**80.3164**	**7**	**11.47378**	**167.2839**	**98**	**1.70698**	**6.721687**	**0.000002**
HDSW	**1.2967**	**7**	**0.18524**	**5.6383**	**98**	**0.05753**	**3.219627**	**0.004094**
HSL	16.4061	7	2.34372	175.1414	98	1.78716	1.311425	0.252970
HPCurv	**5.2419**	**7**	**0.74885**	**16.5396**	**98**	**0.16877**	**4.437044**	**0.000255**
HAA	37.3086	7	5.32979	447.9831	98	4.57126	1.165937	0.329375
HPCA	**208.6576**	**7**	**29.80823**	**989.9819**	**98**	**10.10186**	**2.950768**	**0.007554**
HICO	**164.6161**	**7**	**23.51659**	**488.2090**	**98**	**4.98172**	**4.720572**	**0.000134**
HRL	**20.1019**	**7**	**2.87170**	**100.4277**	**98**	**1.02477**	**2.802281**	**0.010580**
HTL	**34.0783**	**7**	**4.86832**	**183.8382**	**98**	**1.87590**	**2.595194**	**0.016879**
VBTW	2.8864	7	0.41235	56.6324	98	0.57788	0.713547	0.660629
VBTL	8.3300	7	1.19001	106.7424	98	1.08921	1.092542	0.374003
VBPML	1.3313	7	0.19018	11.7860	98	0.12027	1.581327	0.149869
VBML	0.9291	7	0.13272	15.8589	98	0.16183	0.820159	0.572817
VBProL	**1.2472**	**7**	**0.17817**	**7.6350**	**98**	**0.07791**	**2.286983**	**0.033532**
VBMemL	**18.2153**	**7**	**2.60219**	**101.8703**	**98**	**1.03949**	**2.503330**	**0.020737**
MHTL	14.9686	7	2.13837	103.4847	98	1.05597	2.025032	0.059366
MHSL	**19.3123**	**7**	**2.75891**	**89.3600**	**98**	**0.91184**	**3.025657**	**0.006371**
MHSickL	1.0665	7	0.15236	9.2709	98	0.09460	1.610594	0.141294
MHPW	0.7976	7	0.11394	9.1350	98	0.09321	1.222363	0.297859
MHToe	0.1068	7	0.01525	2.0928	98	0.02135	0.714278	0.660020
MHDW	11.8832	7	1.69761	125.5179	98	1.28079	1.325432	0.246437
MHAp	1.6908	7	0.24154	18.6586	98	0.19039	1.268641	0.273804
MHIns	0.1478	7	0.02112	1.2033	98	0.01228	1.719923	0.113032

Parametric statistics

ANOVA post hoc analysis for each of the morphological characters of the 8 different populations of *G. kobayashii*

Hamulus shaft length

	1	2	3	4	5	6	7	8
1								
2	1.000000							
3	0.999999	1.000000						
4	0.704866	0.997942	0.580625					
5	**0.000196**	0.188564	**0.000158**	**0.013856**				
6	**0.000120**	0.133986	**0.000121**	**0.000120**	**0.000120**			

7	0.990755	0.999956	0.999826	0.307672	**0.000127**	**0.000120**		
8	0.234236	0.984930	0.681016	**0.016058**	**0.000120**	**0.000125**	0.718537	

Hamulus outer aperture angle

	1	2	3	4	5	6	7	8
1								
2	1.000000							
3	0.992489	1.000000						
4	**0.000557**	0.580001	**0.007100**					
5	0.999888	0.999942	0.961958	**0.001545**				
6	**0.000139**	0.753811	**0.028442**	0.998818	**0.008320**			
7	0.963878	0.999620	0.729623	**0.000135**	0.999998	**0.000120**		
8	0.051386	0.987998	0.744361	0.351999	0.277652	0.347870	**0.001287**	

Ventral bar total width

	1	2	3	4	5	6	7	8
1								
2	0.865489							
3	**0.000137**	0.987900						
4	**0.000130**	0.967739	0.999959					
5	0.982927	0.987058	**0.029795**	**0.011852**				
6	**0.000120**	0.335659	**0.036122**	0.135020	**0.000121**			
7	**0.000120**	0.997311	0.999852	0.993435	0.077742	**0.000251**		
8	**0.000150**	0.999976	0.914552	0.774003	0.311965	**0.000121**	0.963336	

Ventral bar median length

	1	2	3	4	5	6	7	8
1								
2	0.995017							
3	0.050082	0.999216						
4	**0.018971**	0.993689	0.999736					
5	0.999630	0.999741	0.512511	0.269699				
6	**0.000120**	0.906265	0.713446	0.955802	**0.029604**			
7	**0.016732**	0.999918	0.999949	0.990050	0.712497	0.142160		
8	**0.000143**	0.975670	0.976339	0.999833	0.122146	0.986195	0.631522	

Marginal hook sickle length

	1	2	3	4	5	6	7	8
1								
2	0.999607							
3	0.996029	0.991442						
4	0.945448	0.969647	0.999836					
5	0.999958	0.999985	0.980468	0.887667				
6	0.623881	0.952761	0.997458	0.999999	0.812161			
7	0.871609	1.000000	0.626659	0.385608	0.999739	**0.033125**		
8	0.999936	0.998167	0.999895	0.989524	0.998706	0.834528	0.620004	

Marginal hook sickle proximal width

	1	2	3	4	5	6	7	8
1								
2	0.983424							
3	0.937929	0.999895						

4	0.952113	0.999893	1.000000				
5	0.983871	0.999877	1.000000	1.000000			
6	0.152715	1.000000	0.985886	0.989138	0.995813		
7	0.995687	0.997513	0.998900	0.999225	0.999818	0.513398	
8	0.996944	0.997190	0.998467	0.998923	0.999742	0.486292	1.000000

Non-parametric statistics

Kruskal-Wallis post hoc analysis for each of the morphological characters of the 8 different populations of *G. kobayashii*

Hamulus point length

	1	2	3	4	5	6	7	8
1								
2	1.000000							
3	0.760170	1.000000						
4	1.000000	1.000000	0.633525					
5	1.000000	1.000000	**0.013392**	1.000000				
6	**0.026975**	1.000000	1.000000	**0.043506**	**0.000360**			
7	0.353418	1.000000	1.000000	0.362502	**0.004711**	1.000000		
8	0.716631	1.000000	1.000000	0.664760	**0.009943**	1.000000	1.000000	

Hamulus distal shaft width

	1	2	3	4	5	6	7	8
1								
2	0.780666							
3	1.000000	1.000000						
4	1.000000	1.000000	1.000000					
5	0.075873	1.000000	1.000000	0.219739				
6	**0.000025**	1.000000	0.147192	**0.001308**	1.000000			
7	1.000000	1.000000	1.000000	1.000000	0.524477	**0.001326**		
8	1.000000	1.000000	1.000000	1.000000	0.431865	**0.000760**	1.000000	

Hamulus point curve angle

	1	2	3	4	5	6	7	8
1								
2	0.915201							
3	1.000000	1.000000						
4	**0.004233**	1.000000	0.557417					
5	**0.001102**	1.000000	0.142796	1.000000				
6	**0.000024**	1.000000	0.072556	1.000000	1.000000			
7	1.000000	1.000000	1.000000	0.065048	**0.015525**	**0.001435**		
8	1.000000	1.000000	1.000000	0.067117	**0.016009**	**0.001387**	1.000000	

Hamulus inner aperture angle

	1	2	3	4	5	6	7	8
1								
2	1.000000							

3	1.000000	1.000000					
4	**0.000464**	1.000000	0.146824				
5	1.000000	1.000000	1.000000	0.172466			
6	**0.000051**	1.000000	0.111932	1.000000	0.161492		
7	1.000000	1.000000	1.000000	**0.000043**	1.000000	**0.000002**	
8	0.260840	1.000000	1.000000	0.769504	1.000000	0.663923	0.039252

Hamulus root length

	1	2	3	4	5	6	7	8
1								
2	1.000000							
3	0.177653	1.000000						
4	1.000000	1.000000	1.000000					
5	1.000000	1.000000	**0.008922**	0.432337				
6	**0.000000**	1.000000	0.644276	**0.008761**	**0.000000**			
7	0.597662	1.000000	1.000000	1.000000	**0.028435**	**0.011178**		
8	1.000000	1.000000	1.000000	1.000000	0.237275	**0.000180**	1.000000	

Appendix 4

Multivariate analyses of the opisthaptoral features of *G. kobayashii.* Factor loadings greater than 0.7 are highlighted. The factor score plots all morphological features are shown.

All opisthaptoral features

Factor loadings

	Factor1	Factor2	Factor3	Factor4	Factor5	Factor6
HAPERT	**-0.885**	-0.02561	-0.22488	0.25793	0.077683	0.082407
HPRSW	-0.42582	-0.56709	-0.30014	-0.16159	-0.17666	0.039624
HPL	-0.50659	**-0.80381**	-0.00555	0.075393	-0.0019	0.027392
HSDW	-0.2753	0.209578	0.333292	-0.62634	-0.10913	0.248526
HSL	**-0.79418**	-0.44902	-0.12844	0.099711	-0.05113	0.089078
HAA	0.60558	-0.54889	0.291846	-0.37266	-0.10294	-0.07594
HPCA	0.101028	**-0.89262**	-0.1535	0.192138	-0.04531	-0.01319
HICO	0.548506	-0.67493	0.177239	-0.29696	-0.16007	-0.05148
HRL	**-0.80871**	-0.3685	0.079311	0.007442	0.061171	-0.03145
HTL	**-0.90922**	0.063735	0.172962	-0.15014	0.052983	0.087654
VBTW	**-0.79216**	0.18732	0.236759	0.07481	-0.0007	0.114147
VBTL	**-0.7535**	-0.00562	0.415523	-0.27767	0.204003	-0.23932
VBML	-0.6188	0.080014	0.148536	0.232868	-0.07216	0.400787
VBMEML	-0.5888	-0.04591	0.437195	-0.35204	0.261397	-0.36777
MHTL	-0.35089	0.162096	-0.68443	-0.51834	-0.05515	-0.02866
MHSL	-0.25323	0.068212	**-0.72505**	-0.50965	0.208765	0.081516
MHSICKL	-0.32732	0.281434	-0.1416	-0.06602	-0.60305	-0.54318
MHPW	0.255715	-0.12987	-0.21608	0.135414	0.671711	-0.33791
MHAP	-0.59015	0.087141	-0.09862	0.402007	-0.17122	-0.39552
Expl.Var	6.719096	3.085562	1.957156	1.760727	1.102556	1.035398
Prp.Totl	0.353637	0.162398	0.103008	0.09267	0.058029	0.054495

Hamuli features

Factor loadings

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6
HApert	**-0.820858**	0.219488	0.018339	0.332512	-0.166280	0.262779
HPrSW	-0.693823	-0.076470	0.196483	-0.476710	0.374810	0.306554
HPL	**-0.786202**	0.084306	0.387655	-0.249716	0.012491	-0.346697
HDSW	-0.259582	0.126052	**-0.884130**	-0.268644	0.160173	-0.130068
HSL	**-0.928835**	0.136260	0.117420	-0.001819	-0.036239	0.018829
HAA	-0.364552	**-0.826734**	-0.060354	0.299010	0.230153	-0.064843
HPCA	-0.192974	**-0.705879**	-0.085923	-0.422376	-0.521407	0.063155
HICO	-0.358346	**-0.882359**	-0.069501	0.194994	0.092628	-0.025142
HRL	**-0.879777**	0.210213	0.074923	0.116217	-0.068992	-0.205870
HTL	**-0.801258**	0.269797	-0.422011	0.157180	-0.121157	0.077417
Expl.Var	4.418001	2.172877	1.184254	0.816391	0.548110	0.357700
Prp.Totl	0.441800	0.217288	0.118425	0.081639	0.054811	0.035770

Ventral bar features

Factor loadings

	Factor 1	Factor 2	Factor 3	Factor 4
VBTW	**-0.841312**	0.359032	0.403240	0.026205
VBTL	**-0.918849**	-0.328213	-0.073026	-0.206542
VBML	-0.656887	0.684814	-0.313587	0.034545
VBMemL	**-0.804751**	-0.559583	-0.082211	0.180232
Expl.Var	2.631215	1.018731	0.273031	0.077023
Prp.Totl	0.657804	0.254683	0.068258	0.019256

Marginal hooklet features

Factor loadings

	Factor1	Factor2	Factor3	Factor4
MHTL	**-0.93563**	-0.24124	0.115625	0.036699
MHSL	**-0.82741**	-0.50159	-0.01975	-0.13348
MHSICKL	-0.51969	0.662658	-0.09203	0.526701
MHPW	0.143497	-0.45509	**-0.85186**	0.213136
MHAP	-0.35276	0.654988	-0.4713	-0.47349
Expl.Var	1.975106	1.385017	0.970017	0.566194
Prp.Totl	0.395021	0.277003	0.194003	0.113239

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