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Species composition and phylogenetic relationships among monogenean parasites (Platyhelminthes: Dactylogyridae) of catfishes (Siluriformes) from the Amazon River basin

Ph.D. Thesis

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ANOTATION

This thesis evaluates the diversity and phylogenetic relationships of monogenean parasites of the family Dactylogyridae of catfishes (Siluriformes) from the Peruvian Amazonia combining morphological and molecular approaches. A total of 99 dactylogyrids were found on 42 species of catfishes of 33 genera of five families. Six were described as new for science, the status of 3 species was reviewed and new morphological data and geographical records for six known species were provided. The status of all monogenean parasites of freshwater fishes from the Neotropical region was updated and all available records of these parasites, along with new data presented here, were summarized in a checklist. Additionally, the phylogenetic relationships of monogenean parasites on Neotropical catfishes of the subfamily Ancyrocephalinae, along with African and Asian representatives of the Ancylodiscoidinae, were assessed for the first time using partial sequences of the 28S rRNA gene. Based on Bayesian inference (BI) and maximum likelihood (ML) criteria, the monophyly of the monogenean parasites on catfishes from the Neotropical region, along with African and Asian species, was evaluated.

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DECLARATION

I hereby declare that I did all the work presented in this thesis by myself or in collaboration with co-authors of the presented papers and only using the cited literature.

České Budějovice, 17 October 2014 Carlos Alonso Mendoza Palmero

PROHLÁŠENÍ

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List of papers and author's contribution

The thesis is structured based on the following papers (listed chronologically):

I. **Mendoza-Palmero, C. A.** and T. Scholz. 2011. New species of *Demidospermus* (Monogenea: Dactylogyridae) of pimelodid catfish (Siluriformes) from Peruvian Amazonia and the reassignment of *Urocleidoides lebedevi* Kritsky and Thatcher, 1976. *Journal of Parasitology* 97: 586–592 (IF = 1.405).

Carlos Alonso Mendoza Palmero conceived the study, carried out the sampling, collection of parasites, identification, description and drafted the manuscript. Overall author's contribution is c. 90%.

II. **Mendoza-Palmero, C. A.**, T. Scholz, E. F. Mendoza-Franco, and R. Kuchta. 2012. New species and geographical records of dactylogyrids (Monogenea) of catfish (Siluriformes) from the Peruvian Amazonia. *Journal of Parasitology* 98: 484–497 (IF = 1.405).

Carlos Alonso Mendoza Palmero conceived the study, carried out the sampling, collection of parasites, identification, description and drafted the manuscript. Overall author's contribution is c. 90%.

III. **Mendoza-Palmero, C. A.** and T. Scholz. An updated checklist of monogeneans (Platyhelminthes: Monogenea) parasites of freshwater fishes from the Neotropical region. Target journal: *Systematic Parasitology* (Manuscript in preparation) (IF = 1.035).

Carlos Alonso Mendoza Palmero conceived the study, carried out the literature review, verified the records, summarized the data and drafted the manuscript. Overall author's contribution is c. 90%.

IV. **Mendoza-Palmero, C. A.**, I. Blasco-Costa, and T. Scholz. Molecular phylogeny of Neotropical monogeneans (Platyhelminthes) from catfishes (Siluriformes): new insights into the systematics of Ancyrocephalinae (Dactylogyridae). *Parasites & Vectors* (Manuscript in review) (IF = 3.25).

Carlos Alonso Mendoza Palmero conceived the study, carried out DNA extraction, PCR amplification and sequencing, discussed the results and drafted the manuscript. Overall author's contribution is c. 80%.

In all four papers, Carlos Alonso Mendoza Palmero was the corresponding author.

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People involved

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Dedication

For my dear friends Mario Morales Flores and Richard Klee

Para mi abuelo Fernando Palmero Jiménez, este trabajo es para todos ustedes!

CONTENT

CONTENTS

GENERAL INTRODUCTION

General features of the monogeneans

Monogeneans (Platyhelminthes: Monogenea) are hermaphroditic ectoparasites primarily infecting gills and external surfaces of marine and freshwater fishes, with some, less abundant groups parasitic in internal organs of amphibians and reptiles (e.g., species of *Polystoma* Zeder, 1800). Others have been reported from invertebrates (e.g., *Isancistrum loliginis* de Beauchamp, 1912, a parasite of the cephalopod *Loligo media*) and only one species (*Oculotrema hippopotami* Stunkard, 1924) has been reported from mammals (Stunkard, 1924; Kearn, 1998). Due to the extraordinary morphological diversity of monogeneans and the fact that the present PhD study was focused particularly on monogeneans of the family Dactylogyridae Bychowsky, 1933, special emphasis will be given to these monogeneans.

Dactylogyrid monogeneans are small, usually less than 1 mm in length. Their body can be divided into the anterior region, trunk, peduncle and haptor. The cephalic glands and head organs are located in the anterior region, which secrete adhesive substances for attachment to the host and facilitate movement on the host body. In some groups eyespots (one or two pairs) composed of several spherical or semi-ovoid granules are present in the anterior region. The pharynx and oesophagus are also present in the anterior region and the mouth is surrounded by the musculature of the pharynx. Two intestinal branches emerge from the oesophagus and run all along the body up to the anterior part of the peduncle where the two branches may or not connect each other forming a blind digestive system.

The trunk contains all the organs and structures related to reproduction. The genital pore is located near to the intestinal bifurcation. The gonads can be in tandem position or overlapped. The testis, usually one in number (in species of *Linguadactyla* Brinkmann, 1940 [Linguadactylinae] the testis is divided into several follicles) is located in dorsal position in relation to the germarium. The vas deferens originates from the anterior part of the testis; loops the left intestinal caecum and expands at its distal region forming the seminal vesicle (two vesicles may be present in monogeneans of the subfamily Ancylodiscoidinae Gussev, 1961 – see Lim et al., 2001). The position of the prostatic reservoir and the seminal vesicle is variable but usually are associated with the copulatory complex; delicate ducts connect the prostatic reservoir and the seminal vesicle to the base of the male copulatory organ (MCO). In dactylogyrid monogeneans, the copulatory complex exhibits an extraordinary variety of forms; it is located just posterior to the intestinal bifurcation and includes two, usually sclerotized components:

the male copulatory organ (MCO) and the accessory piece. The MCO is a hollow tube that may be straight or coiled, forming ½, 1 or several rings, and at its base may present some ornamentations. The base of the MCO may be either articulated or not to the accessory piece. The morphology of the accessory piece is highly variable among groups, but it is generally a rod-shaped structure that can be composed of two subunits [e.g., *Ameloblastella chavarriai* (Price, 1968) Kritsky, Mendoza-Franco & Scholz, 2000]; the function of this structure is to guide the distal part of the MCO during copulation.

The female reproductive system is composed of the germarium, which is located in the middle part of the body in ventral position respective to the testis. The vaginal opening is located approximately at the mid-length of the trunk either on its dextral, sinistral or ventral side. The vaginal opening may be sclerotized or not and it is connected to the seminal receptacle by a vaginal channel which also can be sclerotized. The seminal receptacle is associated to the germarium, which is usually located at the same position of the vaginal opening. The oviduct and ootype arise from the germarium forming a channel, which leads to the uterus. The vitellarium consists of numerous vitelline follicles spread throughout the trunk except for the midline of the body where the reproductive organs are located.

The peduncle is a small, sometimes inconspicuous region that connects the trunk and the haptor. The haptor or attachment organ is one of the more distinctive morphological features of the monogeneans. It is armed with anchors, hooks, clamps, bars, spines or some other sclerotized structures. The haptor provides secure attachment to the host surfaces and resists all water current occurring in the aquatic environment. This structure shows an extraordinarily variable morphology depending on the groups, and along with the copulatory complex, both are considered to be of a great taxonomic value for distinguishing between species.

In the last decades, the taxonomy of monogeneans was focused mainly on the haptoral armament and copulatory complex morphology, whereas the internal organs were not considered to be of a taxonomic importance. In the recent years, however the internal anatomy of monogeneans has been used to circumscribe genera or even subfamilies (see Kritsky et al., 1986; Lim et al., 2001).

Life cycle

Unlike other platyhelminths such as trematodes and cestodes, the monogeneans exhibit a direct life cycle, i.e. they do not require intermediate hosts to complete their development and reproduction (Kearn, 1998). Dactylogyrids are oviparous, but in monogeneans of the family Gyrodactylidae van Beneden & Hesse, 1863, the viviparity has evolved as a unique and complex reproduction mode (see Cable and Harris, 2002). In oviparous monogeneans, a single egg is produced and then released to the water; in the case of marine monogeneans, dozens of eggs can be produced (see Kearn, 1998). In some cases, the eggs possess filaments and other ornamentations, which serve for the attachment to the surrounding elements such as the gills of the host, aquatic vegetation or substrate. Larval development takes between 2 days and 4 weeks, depending on the group and the water temperature. A free-swimming ciliated larva called oncomiracidium hatches from the egg and its function is to find a suitable host, either the same or a new one. Once the larva finds a suitable host, the development starts very fast and continues until reproductive organs are fully developed (Euzet and Combes, 1998; Kearn, 1998; Williams and Jones, 1994).

The route of invasion by the oncomiracidium is still not fully understood. It is suggested that in the case of fish monogeneans, the larva firstly attaches to the skin of the host and then migrates to the gill chamber, but the passive invasion through the mouth of the fish facilitated by the water current has been also suggested (Euzet and Combes, 1998).

Host specificity

Monogeneans may be highly specific to their hosts, i.e., they parasitize a particular fish species or closely related species either of the same genus or family. For instance, species of *Dactylogyrus* Diesing, 1850 have shown different levels of specificity to their hosts, mainly cyprinid fishes, from specialist to generalist (see Šimková et al., 2001, 2004 and references therein).

Whereas the monogenean fauna in the Neotropical region is still far from being well known, narrow parasite-host associations have been identified. For instance, species of *Trinigyrus* Hanek, Molnar & Fernando, 1974 and *Aphanoblastella* Kritsky, Mendoza-Franco & Scholz, 2000 solely have been recorded on catfishes of the families Loricariidae and Heptapteridae (both from the order Siluriformes), respectively, *Tucunarella cichlae* Mendoza-Franco, Scholz & Rozkošná, 2010 is specific to *Cichla*

Bloch & Schneider, 1801 (Perciformes: Cichlidae), whereas species of *Gussevia* Kohn & Paperna, 1964 and *Sciadicleithrum* Kritsky, Thatcher & Boeger, 1989 parasitise species of other cichlid genera, and species of *Dawestrema* Price & Nowlin, 1967 occur exclusively on *Arapaima gigas* (Schinz, 1822) (Osteoglossiformes: Osteoglossidae), among others associations (Thatcher, 2006; Cohen et al., 2013; see Publication III in Results section).

In contrast, one particular fish species can harbour several species of monogeneans of the same genus or different genera, such as the red piranha *Pygocentrus nattereri* Kner, 1858 (Characiformes: Serrasalmidae), which hosts about 25 species of gill monogeneans in the Amazon River basin of the genera *Amphithecium* Boeger & Kritsky, 1988, *Anacanthorus* Mizelle & Price, 1965, *Calpidothecium* Kritsky, Boeger, & Jégu, 1997, *Enallothecium* Kritsky, Boeger & Jégu, 1998, *Mymarothecium* Kritsky, Boeger & Jégu, 1996, *Notozothecium* Boeger & Kritsky, 1988, *Pithanothecium* Kritsky, Boeger & Jégu, 1997 and *Rhinoxenus* Kritsky, Boeger & Thatcher, 1988 (Thatcher, 2006; Cohen et al., 2013, see Publication III in Results section).

Siluriform catfishes and their monogenean fauna

The monophyletic order Siluriformes, commonly known as catfishes, comprises more than 2,800 species with worldwide distribution. A total of 1,700 species including undescribed species, occur in the American continent, mostly distributed in the Neotropical region (Nelson, 2006; Albert and Reis, 2011). These fishes support an important aquarium trade industry; several species are exported worldwide, e.g., the redtail catfish *Phractocephalus hemiliopterus* (Bloch & Schneider, 1801), small callichthyids (Callichthyidae) belonging to the genus *Corydoras* Lacépède, 1803, the suckermouth catfish of the species complex *Hypostomus* Lacépède, 1803 (Loricariidae), among others. Large catfishes are also greatly appreciated for local consumption, such as the pimelodids (Pimelodidae), *Zungaro zungaro* (Humboldt, 1821), *Pseudoplatystoma fasciatum* (Linnaeus, 1766), *P. tigrinum* (Valenciennes, 1840) and *Platynematichthys notatus* (Jardine, 1841).

On the other hand, the limited ability of siluriform catfishes to cross oceanic barriers (most species are confined to freshwater environments) which predates major intercontinental tectonic fragmentations (<90 Mya), make them of a great interest for biogeographical and evolutionary studies (see Albert & Reis, 2011, Cheng et al., 2013 and references therein). From a parasitological point of view, Neotropical catfishes harbour a highly diverse fauna of helminth parasites including monogeneans, but less than 10% of them have been examined for parasites (see Moravec, 1998, Thatcher, 2006; Luque & Poulin, 2007; Cohen et al., 2013; de Chambrier et al., 2014).

The global monogenean fauna of the family Dactylogyridae infecting siluriform catfishes is very diverse and includes 379 species belonging to 31 genera. Of these, 75 species in 14 genera are distributed only in the Neotropical region (Kohn & Cohen, 1998; Lim et al., 2001; Cohen & Kohn, 2008; Cohen et al., 2013; Braga et al., 2014; Branches & Domingues, 2014). Traditionally, all these monogenean species are included in the dactylogyrid subfamilies Ancylodiscoidinae Gussev, 1961 and Ancyrocephalinae Bychowsky, 1937.

Mendoza-Palmero et al. (2012) listed only from the Peruvian Amazonia a plethora of undescribed dactylogyrids (almost 60 spp.) from catfishes, thus providing evidence that the current number of nominal species represents only a very small proportion of the actual species richness and diversity of these parasitic flatworms. Given this scenario, it is clear that monogenean fauna, particularly that from the Neotropics, is far from being well known.

The family Dactylogyridae and the phylogenetic relationships of its subfamilies

Boeger and Kritsky (1993) provided the first cladistic analysis of the class Monogenea Bychowsky, 1937 based on the analysis of morphological characters of all monogenean families and proposed a new scheme of classification. In their study, a close relationship between the families Dactylogyridae, Pseudomurraytrematidae Kritsky, Mizelle & Bilqees, 1978 and Diplectanidae Monticelli, 1903 within the order Dactylogyridea Bychowsky, 1937 was revealed. In the last decades, the classification and evolutionary relationships among monogeneans of the subfamily Ancyrocephalinae Bychowsky, 1937 within the Dactylogyridae have become one of the most active fields in phylogenetic systematics of monogeneans (Šimková et al., 2003, 2006; Plaisance et al., 2005; Wu et al., 2007; Blasco-Costa et al., 2012 and references therein).

Kritsky and Boeger (1989) reviewed the status of the Ancyrocephalidae *sensu* Bychowsky & Nagibina (1978) based on the analysis of the morphological characters of some of its members. They found no support for the family, suggesting that the Ancyrocephalidae should be considered as a junior synonym of the Dactylogyridae. They included nine subfamilies within this family, namely Anacanthorinae Price, 1967, Ancylodiscoidinae Gussev, 1961, Ancyrocephalinae Bychowsky, 1937, Dactylogyrinae

Bychowsky, 1933, Hareocephalinae Young, 1968, Heterotesiinae Euzet & Dossou, 1979, Linguadactylinae Bychowsky, 1957, Linguadactyloidinae Thatcher & Kritsky, 1983, and Pseudodactylogyrinae Ogawa, 1986.

Recent phylogenetic studies based on different molecular markers (e.g., 16S, 18S, ITS1, 28S), have revealed that the subfamily Ancyrocephalinae represents a paraphyletic assemblage, composed of two ecologically divergent clades: (i) freshwater Ancyrocephalinae clustering together with the Ancylodiscoidinae (with species parasitizing freshwater catfishes), and (ii) marine Ancyrocephalinae as the sister group to both the Dactylogyrinae and Pseudodactylogyrinae (see Šimková et al., 2003; Plaisance et al., 2005; Wu et al., 2007; Blasco-Costa et al., 2012). These studies included representative species from Europe, North America and Asia.

Given the enormous dactylogyrid diversity infecting freshwater catfishes from the Neotropical region, the inclusion of these species in phylogenetic analyses could shed light on the evolutionary relationships among the subfamilies in the Dactylogyridae.

Difficulties faced during the PhD study

In the original research plan of the present PhD thesis, which I started on 2nd June 2008, the key idea was to use ancyrocephaline monogeneans parasitizing catfishes, especially those of the family Pimelodidae, from the Peruvian Amazonia, as a model of study to assess possible co-evolution between these parasites and their siluriform hosts. This parasite-host system appeared at first glance to be promising because of several reasons: (i) phylogeny of siluriform fishes including pimelodids is relatively well known, which would facilitate the planned co-evolutionary study, (ii) extensive material of these monogeneans was collected from pimelodids and other catfishes in the Peruvian Amazonia by Dr. Tomáš Scholz during his field trips to Peru in 2004–2006, and (iii) specimens of monogeneans found were fixed with all methods necessary for morphological and molecular evaluation, i.e. with GAP (glycerin: ammonium picrate) to study sclerotized parts, hot formaldehyde solution (formalin) for staining reproductive organs and ethanol for DNA sequencing.

However, this original idea soon appeared to be unrealizable in some aspects. In 2008, I carried out a preliminary taxonomic evaluation of the material collected in Iquitos, Peru, which revealed that the material comprised an unexpectedly high number of species (several tens), most of them being new to science and thus not characterized morphologically. Moreover, I found mixed infections of monogeneans in the same fish host (in some cases up to 6 species), which required considerable time to separate them in different species. Despite the high number of species found, most of them were not represented by a sufficient number of specimens, which impeded their formal description as new species or genera.

As a result of these obstacles, the focus of the PhD study and strategy of sample processing had to be modified. As the first step, all morphotypes, including undescribed species that could not be assigned to any of the known genera of ancyrocephalines, were characterized by representative illustrations of their taxonomically important structures, with the aim to enable reliable identification for additional fieldworks in Iquitos, Peru. It is obvious that this step took much more time than I originally expected, but made it possible to present taxonomic descriptions of six new species of two genera, add new morphological data and geographical distributional records of six already known species, among other results which are summarized in the Results section of this thesis.

Another step that made it possible to obtain the first molecular data on ancyrocephalines from Neotropical catfishes was to modify the collecting protocols applied during two additional trips to Iquitos in the Peruvian Amazonia I carried out in 2009 and 2011 with the aid of T. Scholz, R. Kuchta and A. de Chambrier. Based on the morphological evaluation of the material collected, I elaborated an 'atlas' which includes about 85 undescribed species (not included in the thesis) and it served as a field guide to facilitate the separation of the monogeneans collected and their proper fixation for further analyses. As a result, new and reliably identified material suitable for molecular analyses as well as for morphological characterization was obtained using the methodology described in the Materials and Methods section below.

All the above-mentioned methodological difficulties as well as the necessity to collect additional material in Peru obviously represented serious obstacles to achieve the goals of this project in the originally planned period of 4 years. However, these delays were compensated by the possibility to present the first molecular phylogenetic analysis of monogenean parasites from Neotropical catfishes based on an unprecedented dataset of new sequences of these parasites (see the Results section).

AIM AND OBJECTIVES

AIM

This study aims to advance the knowledge on the diversity and taxonomic composition of the monogenean fauna of the family Dactylogyridae infecting freshwater catfishes of the order Siluriformes from the Peruvian Amazonia, which represents one of the most diverse groups of freshwater fishes in the Neotropical region as well as worldwide. This study examines previous records of all monogeneans in the Neotropics and applies a molecular phylogenetic approach to examine the evolutionary history of Neotropical dactylogyrid monogeneans infecting siluriforms.

OBJECTIVES

(1) To assess species richness and morphology of dactylogyrid monogeneans from gills of catfishes (Siluriformes) from the Peruvian Amazonia based based on morphological evaluation of newly collected material.

(2) To prepare a checklist of all monogenean species parasitizing freshwater fishes from the Neotropical region based on the compilation of literary and newly obtained data, including information on their host and geographic distribution.

(3) To assess the phylogenetic relationships of dactylogyrid monogeneans infecting catfishes using sequences of the 28S rRNA gene and define their position among other ancyrocephaline monogeneans.

GENERAL MATERIALS AND METHODS

Morphological studies

Fish sampling and parasite collection

Fishes examined in this study were caught in the tributaries of the Amazon River basin, such as Río Nanay, Río Itaya and Río Momón, off Iquitos, in the region of Loreto, Peru, by local fishermen, purchased at local markets, or obtained from ornamental fish companies from 2004 to 2011. A complete list of host species and localities is given in Table 1 (see Appendix II for fish illustrations). Most fish were fresh, i.e. examined immediately after killing by dorsal pithing (spinal cord and blood vessels were cut just behind the head), which is a method to lessen the suffering of animals.

Gills were excised, separated individually and immediately placed in Petri dishes with tap water. All branquial arches were examined individually for monogeneans using a stereomicroscope. Monogeneans found were isolated by fine needles and fixed in a mixture of glycerin-ammonium picrate (GAP) to study their sclerotized structures (Ergens, 1969). After morphological evaluation, these specimens fixed in GAP were remounted in Canada balsam according to the procedure of Ergens (1969). In order to study internal organs some other specimens were fixed in hot 4% formalin, stained with Gomori's trichrome, and mounted in Canada balsam.

Morphological analysis

All measurements in morphological descriptions are expressed in micrometres and represent the distance between extreme points; they are presented as the mean followed by the range and number of specimens measured (n) in parentheses. In the case of species of *Demidospermus* Suriano, 1983 where the haptoral bars may be V- or W-shaped, the total length for the bars was considered as the total of the distances (A–B) + (B–D) + (D–E) and the distance between the ends was considered as the distance between the points A and E (Figure 1).

Drawings were made with the aid of an Olympus BX51 microscope (Olympus Corporation, Tokyo, Japan) equipped with a drawing tube. Numbering of hook pairs followed Mizelle (1936). Direction of the male copulatory organ (MCO) was determined according to Kritsky et al. (1985). Type and voucher specimens of new taxa described were deposited in the helminthological collection of the Institute of Parasitology, České Budějovice, Czech Republic (IPCAS), the United States National Parasitic Collection, Beltsville, Maryland, USA (USNPC), and the Natural History Museum, London, UK (BMNH). Scientific names of hosts follow those provided by FishBase (Froese and Pauly, 2014) and complemented with Reis et al. (2003).

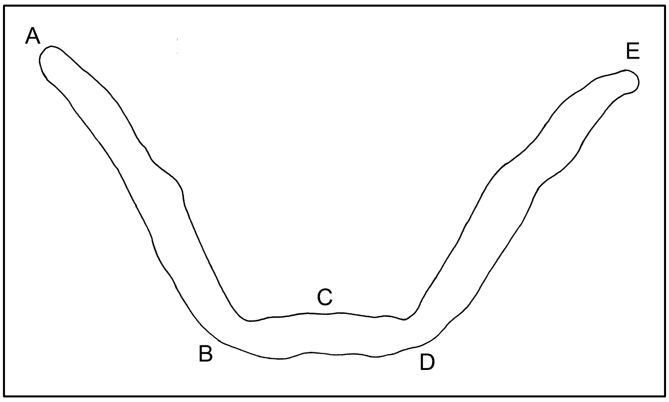

Figure 1. Measurements scheme for the haptoral bars in *Demidospermus* spp. (for explanation see text above).

For comparative purposes, the following specimens of *Demidospermus* and *Urocleidoides* were examined to verify data from the original descriptions: *Demidospermus anus* Suriano, 1983 (USNPC 87159 – 2 vouchers); *Demidospermus armostus* Kritsky & Gutiérrez, 1998 (USNPC 87144 – 3 paratypes), and *D. armostus* (USNPC 102359 – 3 vouchers); *D. bidiverticulatum* (Suriano & Incorvaia, 1995) Kritsky & Gutiérrez, 1998 (USNPC 87147 – 5 vouchers; USNPC 102364 – 2 vouchers); *D. centromochli* Mendoza-Franco & Scholz 2009 (USNPC 101538 – 1 paratype; IPCAS M-483/1 – 1 paratype); *D. cornicinus* Kritsky & Gutiérrez, 1998 (USNPC 87156 – 4 paratypes); *D. idolus* Kritsky & Gutiérrez, 1998 (USNPC 87152 – 2 paratypes); *D. leptosynophallus* Kritsky & Gutiérrez, 1998 (USNPC 87150 – 4 paratypes); *D. luckyi* (Kritsky, Thatcher & Boeger, 1987) Kritsky & Gutiérrez, 1998 (USNPC 78795 – 1 paratype); *D. macropteri* Mendoza-Franco & Scholz 2009 (USNPC 101542 – 1 paratype); *D. majusculus* Kritsky & Gutiérrez, 1998 (USNPC 87154 – 3 paratypes); *D. paravalenciennesi* Gutiérrez & Suriano, 1992 (USNPC 102366 – 2 vouchers); *D. pinirampi* (Kritsky, Thatcher & Boeger, 1987) Kritsky & Gutiérrez, 1998 (USNPC 78798 – 2 paratypes); *D. uncusvalidus* Gutiérrez & Suriano, 1992 (USNPC 87148 – 3 vouchers; USNPC 102367 – 4 vouchers); *D. valenciennesi* Gutiérrez & Suriano, 1992 (USNPC 87157 – 5 vouchers); and *Urocleidoides lebedevi* Kritsky & Thatcher, 1976 (USNPC 73279 – 4 paratypes).

Table 1. Catfishes infected with monogeneans examined in this study from Iquitos, Peru (2004–2011).

Host species	Field code[i]	Locality
Auchenipteridae		
Ageneiosus vittatus Steindachner, 1908	PI 546	off Santa Clara
	PI 547	off Santa Clara
	PI 767	Río Nanay
	PI 768	Río Nanay
Ageneiosus sp.	PI 567	off Santa Clara
Auchenipteridae gen. sp.	PI 574	Belén-Market
Trachelyopterus galeatus (Linnaeus, 1766)	PI 779	Belén-Market
	PI 786	Belén-Market
Callichthyidae		
Brochis multiradiatus (Orcés V., 1960)	PI 328	Aquarium Río Momón
	PI 834	Río Nanay
	PI 904	Aquarium Río Momón
	PI 908	Aquarium Río Momón
Doradidae		
Acanthodoras cataphractus (Linnaeus, 1758)	PI 411	off Santa Clara
	PI 431	off Santa Clara
Anadoras weddellii (Castelnau, 1855)	PI 813	Belén-Market
Hassar orestis (Steindachner, 1875)	PI 909	Aquarium Río Momón
	PI 910	Aquarium Río Momón
Hassar sp.	PI 46a	off Iquitos
	PI 169	Aquarium Río Momón
	PI 170	Aquarium Río Momón
	PI 749	Río Momón
	PI 752	Río Momón
Nemadoras hemipeltis (Eigenmann, 1925)	PI 788	Belén-Market
Oxydoras niger (Valenciennes, 1821)	PI 313	Aquarium Río Momón
	PI 797	Belén-Market
Pterodoras granulosus (Valenciennes, 1821)	PI 634	Río Itaya
	PI 637	Río Itaya
Heptapteridae		
Goeldiella eques (Müller & Troschel, 1849)	PI 255	off Santa Clara
	PI 256	off Santa Clara
	PI 405	off Santa Clara
	PI 422	off Santa Clara
	PI 639	off Santa Clara
	PI 643	off Santa Clara
	PI 650	off Santa Clara
	PI 651	off Santa Clara
	PI 652	off Santa Clara
	PI 844	Río Nanay
Pimelodella cristata (Müller & Troschel, 1849)	PI 734	Río Momón
Pimelodella gracilis (Valenciennes, 1835)	PI 55	off Iquitos

Table 1. Continued.

Host species	Field code	Locality
Pimelodella sp. (4 lines)	PI 856	Río Nanay
	PI 858	Río Nanay
Pimelodella sp.	PI 856	Río Nanay
Loricariidae		
Pterygoplichthys anisitsi Eigenmann & Kennedy, 1903	PI 624	Belén-Market
	PI 832	Belén Marquet
	PI 833	Belén Marquet
Rineloricaria malabarbai Rodriguez & Reis, 2008	PI 722	Río Momón
Sturisoma barbatum (Kner, 1853)	PI 784	Belén-Market
Pimelodidae		
Aguarunichthys torosus Stewart, 1986	PI 548	off Santa Clara
Brachyplatystoma juruense (Boulenger, 1898)	PI 430	off Santa Clara
	PI 572	off Santa Clara
	PI 808	Belén-Market
	PI 870	Río Nanay
Brachyplatystoma vaillantii (Valenciennes, 1840)	PI 789	Río Nanay
	PI 854	Río Nanay
	PI 855	Río Nanay
	PI 894	Río Nanay
	PI 897	Río Nanay
Brachyplatystoma sp.	PI 735	Rio Momón
Calophysus macropterus (Lichtenstein, 1819)	PI 528	Belén-Market
	PI 549	off Santa Clara
Duopalatinus peruvianus Eigenmann & Allen, 1942	PI 403	off Santa Clara
	PI 404	off Santa Clara
Exallodontus aguanai Lundberg, Mago-Leccia & Nass, 1991	PI 766	Río Nanay
Hemisorubim platyrhynchos (Valenciennes, 1840)	PI 432	Aquarium Río Momón
	PI 433	Aquarium Río Momón
Hypophthalmus edentatus Spix & Agassiz, 1829	PI 871	Río Nanay
Perrunichthys perruno Schultz, 1944	PI 162	Aquarium Río Momón
	PI 166	Aquarium Río Momón
	PI 189	Aquarium Río Momón
	PI 224	Belén-Market
Pimelodina flavipinnis Steindachner, 1876	PI 839	Río Nanay
Pimelodus blochii Valenciennes, 1840	PI 67	off Iquitos
Pimelodus ornatus Kner, 1858	PI 104	off Iquitos
	PI 105	off Iquitos
	PI 106	off Iquitos
	PI 113	off Iquitos
	PI 407	off Santa Clara
	PI 410	off Santa Clara
	PI 519	off Santa Clara

Table 1. Continued.

Host species	Field code	Locality
Pimelodus sp.	PI 102	off Iquitos
	PI 112	off Iquitos
	PI 551	off Santa Clara
	PI 552	off Santa Clara
	PI 553	off Santa Clara
	PI 656	off Santa Clara
	PI 679	off Santa Clara
	PI 682	off Santa Clara
	PI 683	off Santa Clara
	PI 737	Río Momón
Pimelodidae gen. sp.	PI 314	off Iquitos
	PI 525	off Santa Clara
	PI 540	off Santa Clara
	PI 541	off Santa Clara
	PI 542	off Santa Clara
	PI 543	off Santa Clara
	PI 544	off Santa Clara
	PI 545	off Santa Clara
	PI 568	off Santa Clara
	PI 659	off Santa Clara
Pinirampus pirinampu (Spix & Agassiz, 1829)	PI 659	off Santa Clara
	PI 676	off Santa Clara
	PI 815	Río Nanay
Platynematichthys notatus (Jardine, 1841)	PI 532	off Santa Clara
	PI 691	off Santa Clara
	PI 798	Río Nanay
	PI 802	Río Nanay
	PI 804	Río Nanay
Platysilurus mucosus (Vaillant, 1880)	PI 859	off Santa Clara
Pseudoplatystoma fasciatum (Linnaeus, 1766)	PI 78	off Iquitos
	PI 509	off Santa Clara
	PI 818	Río Nanay
Sorubim lima (Bloch & Schneider, 1801)	PI 51	off Iquitos
	PI 52	off Iquitos
	PI 59	off Iquitos
	PI 598	Belén-Market
	PI 647	Belén-Market
	PI 862	Belén Market
Sorubimichthys planiceps (Spix & Agassiz, 1829)	PI 314	Belén-Market
Zungaro zungaro (Humboldt, 1821)	PI 61	off Iquitos
	PI 467	Belén-Market
	PI 845	Río Nanay

[i]Each field code represents an individual fish.

Molecular studies

DNA extraction, amplification and sequencing

For molecular analyses, individual worms were placed in a drop of water between a slide and coverslip, identified to the level of species or morphotype with the aid of an ocular microscope, unmounted and fixed separately in 95% ethanol. In the case of heavily infected fishes, entire gill arcs were fixed in hot water (~60ºC) and kept in plastic containers with 100% ethanol. In order to ensure the identity of each isolate, either the haptor or the medial part of trunk of each isolate used for molecular analysis was separated from the body, mounted in a slide with a mixture of glycerin and picric acid (GAP) and kept as a voucher for morphological identification (hologenophore – see Astrin et al., 2013 for terminology). The rest of the body was used for molecular characterization.

Genomic DNA was extracted in 200 µl of a 5% suspension of Chelex™ in deionized water containing 0.1 mg/ml proteinase K, followed by incubation at 56°C for 3 h, boiling at 90°C for 8 min and centrifugation at 14,000 rpm for 10 min. Polymerase chain reaction (PCR) was performed in 20 µl reactions containing 4 µl of extraction supernatant (~10–20 ng of template DNA), 2x MyFi™ Mix (Bioline, USA) and 8 µM of each PCR primer. Partial 28S rDNA (D1–D3) sequences were amplified using primers U178 (5′-GCA CCC GCT GAA YTT AAG-3′) and L1642 (5′-CCA GCG CCA TCC ATT TTC A-3′) (Lockyer et al., 2003), and the following profile was utilized: denaturation of DNA (95°C for 3 min); 34 cycles of amplification (94°C for 30 s, 56°C for 30 s and 72°C for 1:30 min); and 4 min extension hold at 72°C. PCR products were purified prior to sequencing using either exonuclease I and shrimp alkaline phosphatase enzymes (Werle et al., 1994) or gel-excised using High Pure PCR product purification kit™ (Roche, Germany). Amplicons were cycle-sequenced from both strands with the PCR primers and L1200R (5′-GCA TAG TTC ACC ATC TTT CGG-3′) (Littlewood et al., 2000), using ABI BigDye™ chemistry (ABI Perkin-Elmer, UK), alcohol precipitated and run on an ABI Prism 3130x1 (Applied Biosystems, Foster City, California) automated sequencer. Contiguous sequences were assembled and edited using Sequencer™ (GeneCodes Corp. v. 5) and submitted to GenBank (see Table 1).

Alignment and phylogenetic analyses

Forty newly generated sequences (900–1500bp long) of the partial (D1–D3) 28S rDNA fragment of 25 monogenean species all parasites of catfishes, 23 from the Neotropical region, one from Africa (*Schilbetrema* sp.), and one from Asia (*Thaparocleidus* sp.) (see Table 2) were aligned together with 42 published sequences of species belonging to the subfamilies Ancylodiscoidinae, Ancyrocephalinae, Dactylogyrinae and Pseudodactylogyrinae retrieved from GenBank (see Table 3). Three sequences of species belonging to the order Monocotylidea Lebedev, 1988, sister group of the Dactylogyridea Bychowsky, 1937, were used as outgroups, together with six sequences of species belonging to families sister to the Dactylogyridae Bychowsky, 1933, i.e. Diplectanidae Monticelli, 1903, Pseudomurraytrematidae Kritsky, Mizelle & Bilqees, 1978, and Tetraonchidae Monticelli, 1903. Sequences were aligned using MUSCLE implemented in MEGA v. 5 (Tamura et al., 2011). Both ends of the alignment were trimmed to match the sorted sequences; the dataset included a total of 695 nucleotide positions.

Phylogenetic analyses were run under Bayesian inference (BI) and maximum likelihood (ML) criteria, employing the nucleotide substitution model GTR+Γ+I estimated using jModelTest 2.1.1 (Guindon and Gascuel, 2003; Darriba et al., 2012). BI trees were constructed using MrBayes v.3.2 (Ronquist et al., 2012), running two independent MCMC runs of four chains for 10^7 generations and sampling tree topologies every 10^3 generations. Burn-in periods were set to 10^6 generations according to the standard deviation of split frequency values (<0.01). A consensus topology and nodal support estimated as posterior probability values (Huelsenbeck et al., 2001) were calculated from the remaining trees. MrBayes analyses were performed on the computational resource CIPRES (Miller et al., 2010). ML analyses were performed with PhyML 3.0 (Guindon et al., 2010) using the best result of subtree pruning and regrafting and nearest-neighbour interchange tree search strategies starting with 10 random trees, with a non-parametric bootstrap validation based on 1,000 replicates. Topological differences among the BI and the ML reconstructions, and an alternative hypothesis enforcing the monophyly of the Ancyrocephalinae were tested against each other using the approximately unbiased (AU) test implemented in Consel (Shimodaira, 2002; Shimodaira and Hasegawa, 2001). ML branch lengths of the constrained topology were computed in PhyML and the per-site log-likelihood values of both unconstrained and constrained trees were computed in PAUP using the ML settings described above. Subsequently, *p* values of different likelihood-based tests were calculated with Consel.

Table 2. Monogenean parasites infecting siluriform catfishes newly sequenced in this study.

Parasite	Host	Host family	Locality	Isolates
Ameloblastella chavarriai (Price, 1938)	*Rhamdia quelen*	Heptapteridae	Lago de Catemaco, Veracruz, Mexico	2
Ameloblastella sp.	*Hassar* sp.	Doradidae	Aquarium Río Momón, Iquitos, Peru	1
Ameloblastella sp. 8	*Sorubim lima*	Pimelodidae	Iquitos, Peru	1
Ameloblastella sp. 16	*Hypophthalmus edentatus*	Pimelodidae	Río Nanay, Iquitos, Peru	1
Ameloblastella sp. 23	*Hypophthalmus edentatus*	Pimelodidae	Río Nanay, Iquitos, Peru	1
Ancyrocephalinae gen. sp. 4	*Ageneiosus vittatus*	Auchenipteridae	Río Nanay, Iquitos, Peru	1
Ancyrocephalinae gen. sp. 9	*Platynematichthys notatus*	Pimelodidae	Santa Clara, Iquitos, Peru	5
Ancyrocephalinae gen. sp. 10	*Platynematichthys notatus*	Pimelodidae	Santa Clara, Iquitos, Peru	3
Ancyrocephalinae gen. sp. 12	*Sorubim lima*	Pimelodidae	Iquitos-Belén, Peru	1
Ancyrocephalinae gen. sp. 13	*Hypophthalmus edentatus*	Pimelodidae	Río Nanay, Iquitos, Peru	2
Ancyrocephalinae gen. sp. 18	*Pseudoplatystoma fasciatum*	Pimelodidae	Santa Clara, Iquitos, Peru	1
Ancyrocephalinae gen. sp. 23	*Platysilurus mucosus*	Pimelodidae	Santa Clara, Iquitos, Peru	1
Ancyrocephalinae gen. sp. 26	*Platynematichthys notatus*	Pimelodidae	Santa Clara, Iquitos, Peru	1
Aphanoblastella aurorae n. sp.	*Goeldiella eques*	Heptapteridae	Santa Clara, Iquitos, Peru	1
Aphanoblastella sp. 3	*Goeldiella eques*	Heptapteridae	Río Nanay, Iquitos, Peru	2
Chauhanellus boegeri Domingues & Fehlauer, 2006	*Genidens genidens*	Ariidae	Baia de Antonina, municipality of Antonina, Paraná, Brazil	1
Chauhanellus sp.	*Genidens genidens*	Ariidae	Baia de Antonina, municipality of Antonina, Paraná, Brazil	1
Cosmetocleithrum sp. 8	*Hassar* sp.	Doradidae	Aquarium Río Momón, Iquitos, Peru	2
Demidospermus mortenthaleri n. sp.	*Brachyplatystoma juruense*	Pimelodidae	Santa Clara, Iquitos, Peru	2
Demidospermus sp. 11	*Brachyplatystoma vaillantii*	Pimelodidae	Río Nanay, Iquitos, Peru	1
Demidospermus sp. 23	*Brachyplatystoma vaillantii*	Pimelodidae	Río Nanay, Iquitos, Peru	1
Schilbetrema sp.	*Pareutropis debauwi*	Schilbeidae	Aquarium from Czech Republic, origin West Africa	2
Thaparocleidus sp.	*Pangasius* sp.	Pangasidae	Aquarium from Czech Republic, origin Asia	2
Unibarra paranoplatensis Suriano & Incorvaia, 1995	*Aguarunichthys torosus*	Pimelodidae	Santa Clara, Iquitos, Peru	1
Vancleaveus janauacaensis Kritsky, Thatcher & Boeger, 1986	*Pterodoras granulosus*	Doradidae	Río Itaya, Iquitos, Peru	3

Table 3. List of monogenean species used in this study whose genetic sequences were retrieved from GenBank.

Parasite	Codes*	Host species	Host family	Locality	GenBank ID	Reference
Bychowskyella pseudobagri	ANCL	*Tachysurus fulvidraco*	Bagridae	Shaoguan, Guangdong, China	EF100541	Wu et al. (2008)
Quadriacanthus kobiensis	ANCL	*Clarias batracus*	Clariidae	Guangzhou, China	AY841874	Ding and Liao (2005)
Thaparocleidus campylopterocirrus	ANCL	*Pangasianodon hypophthalmus*	Pangasidae	Guangzhou, China	AY841872	Ding and Liao (2005)
Thaparocleidus asoti	ANCL	*Silurus asotus*	Pangasidae	Chongqing City, China	DQ157669	Wu et al. (2006)
Thaparocleidus siluri	ANCL	*Silurus glanis*	Siluridae	River Morava, Czech Republic	AJ969940	Šimková et al. (2006)
Thaparocleidus vistulensis	ANCL	*Silurus glanis*	Siluridae	River Morava, Czech Republic	AJ969941	Šimková et al. (2006)
Actinocleidus recurvatus	ANCR	*Lepomis gibbosus*	Centrarchidae	River Dunaj, Slovak Republic	AJ969951	Šimková et al. (2006)
Aliatrema cribbi	ANCR	*Chaetodon citrinellus*	Chaetodontidae	French Polynesia	AY820612	Plaisance et al. (2005)
Ancyrocephalus morgundae	ANCR	*Siniperca chuatsi*	Percichthyidae	Wuhan, China	AY841871	Ding and Liao (2005)
Ancyrocephalus mogurndae	ANCR	*Siniperca chuatsi*	Percichthyidae	Fuzhou, Fujian Province, China	DQ157667	Wu et al. (2006)
Ancyrocephalus paradoxus	ANCR	*Sander lucioperca*	Percidae	River Morava, Czech Republic	AJ969952	Šimková et al. (2006)
Ancyrocephalus percae	ANCR	*Perca fluviatilis*	Percidae	Lake Constance, Germany	KF499080	Behrmann-Godel et al. (2013)
Bravohollisia rosetta	ANCR	*Pomadasys maculatus*	Haemulidae	Guangdong, China	DQ537364	Wu et al. (2007)
Cichlidogyrus sclerosus	ANCR	*Oreochromis niloticus*	Cichlidae	Panyu, Guangdong Province, China	DQ157660	Wu et al. (2006)
Cichlidogyrus tilapiae	ANCR	*Hemichromis fasciatus*	Cichlidae	Senegal, Africa	HQ010029	Mendlová et al. (2010)
Dactylogyrus nanus	ANCR	*Rutilus rutilus*	Cyprinidae	River Morava, Czech Republic	AJ969942	Šimková et al. (2006)
Dactylogyrus petruschewskyi	ANCR	*Megalobrama amblycephala*	Cyprinidae	China	AY548927	Ding and Liao (unpublished)
Ergenstrema mugilis	ANCR	*Liza ramada*	Mugilidae	Ebro Delta, Spain	JN996800	Blasco-Costa et al. (2012)
Euryhaliotrema perezponcei	ANCR	*Lutjanus guttatus*	Lutjanidae	Bay Cerritos, Mazatlan, Mexico	HQ615996	Soler-Jiménez et al. (unpublished)
Euryhaliotrematoides pirulum	ANCR	*Chaetodon lunula*	Chaetodontidae	French Polynesia	AY820618	Plaisance et al. (2005)
Haliotrema cromileptis	ANCR	*Epinephelus coioides*, *E. bleekeri*	Serranidae	Nha Trang Bay, Vietnam	EU523146	Dang et al. (2010)
Haliotrema platycephali	ANCR	*Platycephalus indicus*	Platycephalidae	Weihai, Shangdong Province, China	DQ157662	Wu et al. (2006)
Haliotrematoides guttati	ANCR	*Lutjanus guttatus*	Lutjanidae	Bay Cerritos, Mazatlan, Mexico	HQ615993	Soler-Jiménez et al. (unpublished)
Haliotrematoides spinatus	ANCR	*Lutjanus guttatus*	Lutjanidae	Pacific Coast, Mexico	KC663679	García-Vázquéz et al. (unpublished)
Ligictaluridus pricei	ANCR	*Ameiurus nebulosus*	Ictaluridae	River Vltava, Czech Republic	AJ969939	Šimková et al. (2006)
Ligophorus augustus	ANCR	*Chelon labrosus*	Mugilidae	off Cullera, Spain	JN996803	Blasco-Costa et al. (2012)
Ligophorus cephali	ANCR	*Mugil cephalus*	Mugilidae	off Cullera, Spain	JN996830	Blasco-Costa et al. (2012)
Ligophorus chabaudi	ANCR	*Mugil cephalus*	Mugilidae	Ebro Delta, Spain	JN996834	Blasco-Costa et al. (2012)
Ligophorus confusus	ANCR	*Liza ramada*	Mugilidae	off Cullera, Spain	JN996810	Blasco-Costa et al. (2012)
Ligophorus heteronchus	ANCR	*Liza saliens*	Mugilidae	Ebro Delta, Spain	JN996812	Blasco-Costa et al. (2012)
Ligophorus imitans	ANCR	*Liza ramada*	Mugilidae	Ebro Delta, Spain	JN996815	Blasco-Costa et al. (2012)
Ligophorus mediterraneus	ANCR	*Mugil cephalus*	Mugilidae	off Cullera, Spain	JN996828	Blasco-Costa et al. (2012)
Ligophorus szidati	ANCR	*Liza aurata*	Mugilidae	Ebro Delta, Spain	JN996806	Blasco-Costa et al. (2012)
Ligophorus vanbenedenii	ANCR	*Liza aurata*	Mugilidae	Ebro Delta, Spain	JN996802	Blasco-Costa et al. (2012)
Metahaliotrema mizellei	ANCR	*Scatophagus argus*	Scatophagidae	Panyu, Guangdong Province, China	DQ157647	Wu et al. (2006)
Onchocleidus similis	ANCR	*Lepomis gibbosus*	Centrarchidae	River Dunaj, Slovak Republic	AJ969938	Šimková et al. (2006)

Table 3. Continued.

Parasite	Codes*	Host species	Host family	Locality	GenBank ID	Reference
Onchocleidus sp.	ANCR	*Lepomis macrochirus*	Centrarchidae	Guangzhou, China	AY841873	Ding and Liao (2005)
Protogyrodactylus alienus	ANCR	*Gerres filamentosus*	Gerreidae	Dayawan, Guangdong Privince, China	DQ157650	Wu et al. (2006)
Protogyrodactylus hainanensis	ANCR	*Therapon jarbua*	Terapontidae	Yangjiang, Guangdong Province, China	DQ157653	Wu et al. (2006)
Pseudodactylogyrus anguillae	ANCR	*Anguilla anguilla*	Anguillidae	River Dunaj, Slovak Republic	AJ969950	Šimková et al. (2006)
Pseudodactylogyrus bini	ANCR	*Anguilla anguilla*	Anguillidae	Neusiedler Lake, Austria	AJ969949	Šimková et al. (2006)
Pseudohaliotrema sphincteroporus	ANCR	*Siganus doliatus*	Siganidae	Green Island, Australia	AF382058	Olson and Littlewood (2002)
Scutogyrus longicornis	ANCR	*Oreochromis niloticus*	Cichlidae	Panyu, Guangdong Province, China	DQ157659	Wu et al. (2006)
Tetrancistrum sp.	ANCR	*Siganus fuscescens*	Siganidae	Heron Island, Queensland, Australia	AF026114	Mollaret et al. (1997)
Murraytrema pricei	DIPL	*Nibea albiflora*	Sciaenidae	Panyu, Guangdong Province, China	DQ157672	Wu et al. (2006)
Pseudorhabdosynochus epinepheli	DIPL	*Epinephelus bruneus*	Serranidae	Huidong, Guangdong Province, China	AY553622	Wu et al. (2006)
Pseudorhabdosynochus lantauensis	DIPL	*Epinephelus bruneus*	Serranidae	Huidong, Guangdong Province, China	AY553624	Wu et al. (2006)
Sinodiplectanotrema argyromus	DIPL	*Pennahia anea*, *Nibea albiflora*	Sciaenidae	Panyu, Guangdong Province, China	DQ157673	Wu et al. (2006)
Clemacotyle australis	MONO	*Aetobatus narinari*	Myliobatidae	Heron Island, Australia	AF348350	Chislom et al. (2001)
Decacotyle lymmae	MONO	*Aetobatus narinari*	Myliobatidae	Heron Island, Australia	AF348359	Chislom et al. (2001)
Dendromonocotyle octodiscus	MONO	*Dasyatis americana*	Dasyatidae	Gulf of Mexico, Mexico	AF348352	Chislom et al. (2001)
Pseudomurraytrema sp.	PSEU	*Catostomus ardens*	Catostomidae	Snake River, Idaho, USA	AF382059	Olson and Littlewood (2002)
Tetraonchus monenteron	TETR	*Exos lucius*	Escocidae	River Morava, Czech Republic	AJ969953	Šimková et al. (2006)

*ANCY = Ancylodiscoidinae; ANCR = Ancyrocephalinae; DIPL = Diplectyanidae; MONO = Monocotylidea; PSEU = Pseudomurraytrematidae; TETR = Tetraonchidae.

RESULTS

Diversity of dactylogyrid monogeneans infecting Peruvian catfishes

As a result of extensive collecting trips to Iquitos, Peru (2004–2011) focused on monogenean parasites of freshwater catfishes (Siluriformes), a total of 99 species of dactylogyrids were found on 42 species of teleost fishes (121 individuals) of 33 genera of the families Auchenipteridae, Callichthyidae, Doradidae, Heptapteridae, Loricariidae and Pimelodidae (see Table 1, 4 and Appendix II).

The genus *Demidospermus* Suriano, 1983 was the most diverse group with 23 species followed by the genera *Ameloblastella* Kritsky, Mendoza-Franco & Scholz, 2000 (22 spp.), *Costemocleithrum* Kritsky, Thatcher & Boeger, 1986 (13 spp.), *Aphanoblastella* Kritsky, Mendoza-Franco & Scholz, 2000 (4 spp.), *Vancleaveus* Kritsky, Thatcher & Boeger, 1986 (3 spp.), *Unilatus* Mizelle & Kritsky, 1967 (2 spp.), and *Amphocleithrum* Price & Gonzalez-Romero, 1969, *Philocorydoras* Suriano, 1986 and *Unibarra* Suriano & Incorvaia, 1995 with only one species each (see Table 4). A total of 29 species were recorded as Ancyrocephalinae gen. sp. because their morphology (internal anatomy and sclerotized structures) did not fit with that of any known genus recorded from Neotropical catfishes and they may represent new genera.

Taxonomic study of monogeneans infecting catfishes from the Peruvian Amazonia

Six new for science were described: *Aphanoblastella aurorae* n. sp., *Demidospermus brevicirrus* n. sp., *D. curvovaginatus* n. sp., *D. mortenthaleri* n. sp., *D. peruvianus* n. sp. and *D. striatus* n. sp. (see **Publications I** and **II**).

The species '*Urocleidoides*' *lebedevi* Kritsky & Thatcher, 1976, a parasite of *Pimelodus grosskopfii* Steindachner, 1879 from Colombia, was relocated as *Demidospermus lebedevi* (Kritsky & Thatcher, 1976) Mendoza-Palmero & Scholz, 2011 n. comb. on the basis of the re-evaluation of the type material (see **Publication II**).

New morphological and geographical data on the previously described dactylogyrids *Cosmetocleithrum bulbocirrus* Kritsky, Thatcher & Boeger, 1986, *Vancleaveus fungulus* Kritsky, Thatcher & Boeger, 1986, *V. janauacaensis* Kritsky, Thatcher & Boeger, 1986, *V. platyrhynchi* Kritsky, Thatcher & Boeger, 1986, *Unilatus brittani* Mizelle, Kritsky & Crane, 1968, and *U. unilatus* Mizelle & Kritsky, 1967 were provided. All these 6 species were registered in the Peruvian Amazonia for the first time and findings of *U. brittani* and *U. unilatus* on the gills of *Pterygoplichthys anisitsi* (new represent host record for both species (see **Publication II**).

Morphological evaluation of newly collected specimens of *U. brittani* and *U. unilatus* revealed morphological and meristic similarities with *U. longispinus* Suriano, 1985 and *U.*

brevispinus Suriano, 1985. Based on the close similarity of new specimens from Peru with those described by Suriano (1985), *U. longispinus* and *U. brevispinus* were synonymized with *U. brittani* and *U. unilatus*, respectively (see **Publication II**).

Dactylogyrid monogeneans of Neotropical freshwater fishes: an updated checklist

The status of all species of monogenean parasites of freshwater fishes from the Neotropical region was updated based on an exhaustive literature review carried out until September 2014. All available records of these parasites, along with new data presented in this thesis, were summarized in a checklist. A total of 396 monogenean species of 73 genera of 3 families, infecting 246 species of freshwater fishes of 131 genera of 31 families were recorded. The checklist summarizes all parasite-host associations and geographical records of almost 400 valid species parasitizing Neotropical freshwater fishes (see Manuscript in preparation – **Publication III**).

Phylogenetic relationships of dactylogyrid parasites of catfishes

The phylogenetic relationships of monogenean parasites on Neotropical catfishes of the subfamily Ancyrocephalinae Bychowsky, 1937, along with African and Asian representatives of the Ancylodiscoidinae Gussev, 1961, were assessed for the first time using partial sequences of the 28S rRNA gene. Based on Bayesian inference (BI) and maximum likelihood (ML) criteria, the monophyly of the monogenean parasites on catfishes from the Neotropical region, along with African and Asian species, was tested. This analysis revealed that the subfamily Ancyrocephalinae is a paraphyletic group of species with 3 main clades. None of the three major clades of Ancyrocephalinae taxa seemed to share obvious morphological synapomorphies, nor clear patterns in host-associations, zoogeographical distribution or ecology. In contrast with the evolutionary history of the Siluriformes, ancyrocephalines parasitizing catfishes do not represent a monophyletic group. The position of the members of the Ancylodiscoidinae among species of the Ancrycephalinae indicates that the subfamily does not represent a natural group (see Manuscript in review – **Publication IV**).

Table 4. Dactylogyrid monogenean parasites found in this study infecting siluriform catfishes from off Iquitos, Peru.

Species	Field code	Host	Family	Locality
Ameloblastella sp. 1	PI 467	*Zungaro zungaro*	Pimelodidae	Belén-Market
Ameloblastella sp. 2	PI 432b	*Hemisorubim platyrhynchos*	Pimelodidae	Aquarium Río Momón
	PI 51	*Sorubim lima*	Pimelodidae	off Iquitos
	PI 52a			
	PI 598			Belén-Market
	PI 647			
	PI 862b			
Ameloblastella sp. 3	PI 328a	*Brochis multiradiatus*	Callichthyidae	Aquarium Río Momón
	PI 904			
	PI 905			
	PI 908			
	PI 844a	*Goeldiella eques*	Heptapteridae	Río Nanay
Ameloblastella sp. 4	PI 169b,d	*Hassar* sp.	Doradidae	Aquarium Río Momón
	PI 909b	*Hassar orestis*	Doradidae	
	PI 910b			
Ameloblastella sp. 5	PI 313a	*Oxydoras niger*	Doradidae	Aquarium Río Momón
	PI 313b			
	PI 856d	*Pimelodella* sp.	Heptapteridae	Río Nanay
	PI 734	*Pimelodella cristata*	Pimelodidae	Río Momón
Ameloblastella sp. 6	PI 411b	*Acanthodoras cataphractus*	Doradidae	off Santa Clara
Ameloblastella sp. 7	PI 162	*Perrunichthys perruno*	Pimelodidae	Aquarium Río Momón
	PI 166a			
	PI 189a			
Ameloblastella sp. 8	PI 734	*Pimelodella cristata*	Heptapteridae	Río Momón
	PI 528a	*Calophysus macropterus*	Pimelodidae	Belén-Market
	PI 112a	*Pimelodus* sp.	Pimelodidae	Iquitos
	PI 544	Pimelodidae gen. sp.	Pimelodidae	off Santa Clara
	PI 51a	*Sorubim lima*	Pimelodidae	off Iquitos
	PI 52a			
Ameloblastella sp. 9	PI 574	Auchenipteridae gen. sp.	Auchenipteridae	Belén-Market
	PI 525a	Pimelodidae gen. sp.	Pimelodidae	off Santa Clara
Ameloblastella sp. 10	PI 55a	*Pimelodella gracilis*	Pimelodidae	off Iquitos
	PI 314a	Pimelodidae gen. sp.	Pimelodidae	
	PI 544	Pimelodidae gen. sp.	Pimelodidae	off Santa Clara
	PI 52a	*Sorubim lima*	Pimelodidae	off Iquitos
Ameloblastella sp. 11	PI 634	*Pterodoras granulosus*	Doradidae	Río Itaya
	PI 637			Río Itaya
Ameloblastella sp. 12	PI 647	*Sorubim lima*	Pimelodidae	Belén-Market
Ameloblastella sp. 13	PI 735	*Brachyplatystoma* sp.	Pimelodidae	Rio Momón
Ameloblastella sp. 14	PI 78c	*Pseudoplatystoma fasciatum*	Pimelodidae	off Iquitos
Ameloblastella sp. 15	PI 102a	*Pimelodus* sp.	Pimelodidae	off Iquitos

Table 4. Continued.

Species	Field code	Host	Family	Locality
Ameloblastella sp. 16	PI 871b	*Hypophthalmus edentatus*	Pimelodidae	Río Nanay
Ameloblastella sp. 17	PI 871c	*Hypophthalmus edentatus*	Pimelodidae	Río Nanay
Ameloblastella sp. 18	PI 818f	*Pseudoplatystoma fasciatum*	Pimelodidae	Río Nanay
Ameloblastella sp. 19	PI 779b	*Trachelyopterus galeatus*	Auchenipteridae	Belén-Market
Ameloblastella sp. 20	PI 767h	*Ageneiosus vittatus*	Auchenipteridae	Río Nanay
Ameloblastella sp. 21	PI 815e	*Pinirampus pirinampu*	Pimelodidae	Río Nanay
Ameloblastella sp. 22	PI 786a	*Trachelyopterus galeatus*	Auchenipteridae	Belén-Market
Amphocleithrum paraguayensis	PI 78c	*Pseudoplatystoma fasciatum*	Pimelodidae	off Iquitos
Price & Gonzalez-Romero, 1969	PI 818c			Río Nanay
Aphanoblastella aurorae n. sp.	PI 405b	*Goeldiella eques*	Heptapteridae	off Santa Clara
	PI 639			
	PI 643			
	PI 650			
	PI 651			
	PI 652			
Aphanoblastella sp. 1	PI 734	*Pimelodella cristata*	Heptapteridae	Río Momón
	PI 544	Pimelodidae gen. sp.	Pimelodidae	off Santa Clara
Aphanoblastella sp. 3	PI 422a	*Goeldiella eques*	Heptapteridae	off Santa Clara
	PI 844a			Río Nanay
Aphanoblastella sp. 4	PI 255b,c	*Goeldiella eques*	Heptapteridae	off Santa Clara
	PI 256c,d			
	PI 405b			
	PI 844b			Río Nanay
Cosmetocleithrum bulbocirrus	PI 634	*Pterodoras granulosus*	Doradidae	Río Itaya
Kritsky, Thatcher & Boeger, 1986	PI 637			Río Itaya
Cosmetocleithrum confusus	PI 797	*Oxydoras niger*	Doradidae	Belén-Market
Kritsky, Thatcher & Boeger, 1986				
Cosmetocleithrum gussevi	PI 797	*Oxydoras niger*	Doradidae	Belén-Market
Kritsky, Thatcher & Boeger, 1986				
Cosmetocleithrum parvum	PI 797	*Oxydoras niger*	Doradidae	Belén-Market
Kritsky, Thatcher & Boeger, 1986				
Cosmetocleithrum rarum	PI 797	*Oxydoras niger*	Doradidae	Belén-Market
Kritsky, Thatcher & Boeger, 1986				
Cosmetocleithrum sp. 1	PI 169b,d	*Hassar* sp.	Doradidae	Aquarium Río Momón
	PI 170			
Cosmetocleithrum sp. 2	PI 46a	*Hassar* sp.	Doradidae	off Iquitos
	PI 169b,d		Doradidae	Aquarium Río Momón

Table 4. Continued.

Species	Field code	Host	Family	Locality
	PI 170			
Cosmetocleithrum sp. 3	PI 797	*Oxydoras niger*	Doradidae	Belén-Market
Cosmetocleithrum sp. 4	PI 788	*Nemadoras hemipeltis*	Doradidae	Belén-Market
Cosmetocleithrum sp. 5	PI 786c	*Trachelyopterus galeatus*	Auchenipteridae	Belén-Market
Cosmetocleithrum sp. 6	PI 786b,d	*Trachelyopterus galeatus*	Auchenipteridae	Belén-Market
Cosmetocleithrum sp. 7	PI 779C	*Trachelyopterus galeatus*	Auchenipteridae	Belén-Market
	PI 786b,d			Belén-Market
Cosmetocleithrum sp. 8	PI 909a	*Hassar orestis*	Doradidae	Aquarium Rio Momón
	PI 910a			
Demidospermus brevicirrus n. sp.	PI 656	*Pimelodus* sp.	Pimelodidae	off Santa Clara
	PI 679			
	PI 682			
	PI 683			
Demidospermus curvovaginatus n. sp.	PI 104a,b	*Pimelodus ornatus*	Pimelodidae	off Iquitos
	PI 113a,b			
	PI 540-5	Pimelodidae gen. sp.		off Santa Clara
Demidospermus macropteri Mendoza-Franco & Scholz, 2009	PI 839c	*Pimelodina flavipinnis*	Pimelodidae	Río Nanay
Demidospermus mortenthaleri n. sp.	PI 430a	*Brachyplatystoma juruense*	Pimelodidae	off Santa Clara
	PI 572			
Demidospermus peruvianus n. sp.	PI 67a	*Pimelodus blochii*	Pimelodidae	off Iquitos
	PI 104a,b	*Pimelodus ornatus*		
	PI 106a	*Pimelodus* sp.		
	PI 540-5	Pimelodidae gen. sp.		off Santa Clara
Demidospermus striatus n. sp.	PI 67a	*Pimelodus blochii*	Pimelodidae	off Iquitos
	PI 112a	*Pimelodus* sp.	Pimelodidae	
Demidospermus sp. 1	PI 224a	*Perrunichthys perruno*	Pimelodidae	Belén-Market
Demidospermus sp. 2	PI 224a	*Perrunichthys perruno*	Pimelodidae	Belén-Market
Demidospermus sp. 3	PI 734	*Pimelodella cristata*	Heptapteridae	Río Momón
Demidospermus sp. 4	PI 528a	*Calophysus macropterus*	Pimelodidae	Belén-Market
Demidospermus sp. 5	PI 104a	*Pimelodus ornatus*	Pimelodidae	off Iquitos
	PI 105b		Pimelodidae	
	PI 106a+b		Pimelodidae	
	PI 407		Pimelodidae	off Santa Clara
	PI 410b		Pimelodidae	
	PI 519b			
	PI 737	*Pimelodus* sp.	Pimelodidae	Río Momón

Table 4. Continued.

Species	Field code	*Host*	Family	Locality
	PI 540	*Pimelodidae* gen. sp.	Pimelodidae	off Santa Clara
	PI 568			
	PI 856b	*Pimelodella* sp. (4 lines)	Heptapteridae	Río Nanay
	PI 858a	*Pimelodella* sp. (4 lines)	Heptapteridae	
Demidospermus sp. 6	PI 722	*Rineloricaria malabarbai*	Loricariidae	Río Momón
Demidospermus sp. 7	PI 659	*Pinirampus pirinampu*	Pimelodidae	off Santa Clara
Demidospermus sp. 8	PI 676	*Pinirampus pirinampu*	Pimelodidae	off Santa Clara
	PI 815f			Río Nanay
Demidospermus sp. 9	PI 407	*Pimelodus ornatus*	Pimelodidae	off Santa Clara
	PI 410b			
Demidospermus sp. 10	PI 549b	*Calophysus macropterus*	Pimelodidae	off Santa Clara
	PI 854c	*Brachyplatystoma vaillantii*	Pimelodidae	Río Nanay
	PI 855c			
	PI 894b			
	PI 897b			
Demidospermus sp. 11	PI 403d	*Duopalatinus peruvianus*	Pimelodidae	off Santa Clara
	PI 404d			
	PI 789	*Brachyplatystoma vaillantii*	Pimelodidae	Río Nanay
	PI 854c			
	PI 855c			
	PI 894c			
	PI 897b			
Demidospermus sp. 12	PI 551	*Pimelodus* sp.	Pimelodidae	off Santa Clara
	PI 552			
	PI 553			
Demidospermus sp. 13	PI 737	*Pimelodus* sp.	Pimelodidae	Río Momón
	PI 856a	*Pimelodella* sp. (4 lines)	Heptapteridae	Río Nanay
Demidospermus sp. 14	PI 737	*Pimelodus* sp.	Pimelodidae	Río Momón
Demidospermus sp. 15	PI 676	*Pinirampus pirinampu*	Pimelodidae	off Santa Clara
	PI 659	Pimelodidae gen. sp.	Pimelodidae	
Demidospermus sp. 17	PI 818g	*Pseudoplatystoma fasciatum*	Pimelodidae	Río Nanay
Demidospermus sp. 18	PI 815g	*Pinirampus pirinampu*	Pimelodidae	Río Nanay
Demidospermus sp. 19	PI 766a	*Exallodontus aguanai*	Pimelodidae	Río Nanay
	PI 815c	*Pinirampus pirinampu*	Pimelodidae	
Demidospermus sp. 20	PI 839b	*Pimelodina flavipinnis*	Pimelodidae	Río Nanay
Demidospermus sp. 21	PI 839b	*Pimelodina flavipinnis*	Pimelodidae	Río Nanay
Demidospermus sp. 22	PI 766a	*Exallodontus aguanai*	Pimelodidae	Río Nanay
Demidospermus sp. 23	PI 789	*Brachyplatystoma vaillantii*	Pimelodidae	Río Nanay

Table 4. Continued.

Species	Field code	Host	Family	Locality
	PI 854c			
Philocorydoras sp.	PI 834b	*Brochis multiradiatus*	Callichthyidae	Río Nanay
Unibarra paranoplatensis	PI 548b	*Aguarunichthys torosus*	Pimelodidae	off Santa Clara
Suriano & Incorvaia, 1995	PI 532b	*Platynematichthys notatus*	Pimelodidae	
	PI 691			
	PI 798d			Río Nanay
	PI 802			
	PI 804			
Unilatus brittani	PI 624	*Pterygoplichthys anisitsi*	Loricariidae	Belén-Market
Mizelle, Kritsky & Crane, 1968				
Unilatus unilatus	PI 624	*Pterygoplichthys anisitsi*	Loricariidae	Belén-Market
Mizelle & Kritsky, 1967	PI 832a			
	PI 833			
Urocleidoides sp.	PI 102	*Pimelodus* sp.	Pimelodidae	off Iquitos
Vancleaveus fungulus	PI 509a	*Pseudoplatystoma fasciatum*	Pimelodidae	off Santa Clara
Kritsky, Thatcher & Boeger, 1986				
Vancleaveus janauacaensis	PI 634	*Pterodoras granulosus*	Doradidae	Río Itaya
Kritsky, Thatcher & Boeger, 1986	PI 637			Río Itaya
Vancleaveus platyrhynchi	PI 432b	*Hemisorubim platyrhynchos*	Pimelodidae	Aquarium Río Momón
Kritsky, Thatcher & Boeger, 1986	PI 433b			
Ancyrocephalinae gen. sp. 1	PI 509a	*Pseudoplatystoma fasciatum*	Pimelodidae	off Santa Clara
Ancyrocephalinae gen. sp. 2	PI 767g	*Ageneiosus vittatus*	Auchenipteridae	Río Nanay
	PI 768c			
	PI 467	*Zungaro zungaro*	Pimelodidae	Belén-Market
Ancyrocephalinae gen. sp. 3	PI 567	*Ageneiosus* sp.	Auchenipteridae	off Santa Clara
Ancyrocephalinae gen. sp. 4	PI 767f	*Ageneiosus vittatus*	Auchenipteridae	Río Nanay
	PI 768d			
	PI 567	*Ageneiosus* sp.	Auchenipteridae	off Santa Clara
Ancyrocephalinae gen. sp. 5	PI 767e	*Ageneiosus vittatus*	Auchenipteridae	Río Nanay
	PI 567	*Ageneiosus* sp.	Auchenipteridae	off Santa Clara
Ancyrocephalinae gen. sp. 6	Pi 767j	*Ageneiosus vittatus*	Auchenipteridae	Río Nanay
	PI 567	*Ageneiosus* sp.	Auchenipteridae	off Santa Clara
Ancyrocephalinae gen. sp. 7	PI 546a	*Ageneiosus vittatus*	Auchenipteridae	off Santa Clara
	PI 547a			off Santa Clara
Ancyrocephalinae gen. sp. 9	PI 61a	*Zungaro zungaro*	Pimelodidae	off Iquitos
	PI 870b	*Brachyplatystoma juruense*	Pimelodidae	Río Nanay
	PI 532b	*Platynematichthys notatus*	Pimelodidae	off Santa Clara
	PI 798c			Río Nanay
	PI 314a	*Sorubimichthys planiceps*	Pimelodidae	Belén-Market

Table 4. Continued.

Species	Field code	Host	Family	Locality
Ancyrocephalinae gen. sp. 10	PI 808b	*Brachyplatystoma juruense*	Pimelodidae	Belén-Market
	PI 845g	*Zungaro zungaro*	Pimelodidae	Río Nanay
	PI 532b	*Platynematichthys notatus*	Pimelodidae	off Santa Clara
	PI 691			
	PI 798b			Río Nanay
	PI 314a	*Sorubimichthys planiceps*	Pimelodidae	Belén-Market
Ancyrocephalinae gen. sp. 11	PI 59a	*Sorubim lima*	Pimelodidae	off Iquitos
	PI 647			Belén-Market
	PI 862c			
Ancyrocephalinae gen. sp. 12	PI 526a	*Sorubim lima*	Pimelodidae	Belén-Market
	PI 598			
	PI 647			
	PI 862a			
Ancyrocephalinae gen. sp. 13	PI 547	Auchenipteridae gen sp.	Auchenipteridae	Belén-Market
	PI 871a	*Hypophthalmus edentatus*	Pimelodidae	Río Nanay
	PI 525a	Pimelodidae gen. sp.	Pimelodidae	off Santa Clara
Ancyrocephalinae gen. sp. 15	PI 431a	*Acanthodoras cataphractus*	Doradidae	off Santa Clara
Ancyrocephalinae gen. sp. 16	PI 411b	*Acanthodoras cataphractus*	Doradidae	off Santa Clara
Ancyrocephalinae gen. sp. 17	PI 102a	*Pimelodus* sp.	Pimelodidae	off Iquitos
	PI 102c			
Ancyrocephalinae gen. sp. 18	PI 78b	*Pseudoplatystoma fasciatum*	Pimelodidae	off Iquitos
	PI 818d			Río Nanay
Ancyrocephalinae gen. sp. 20	PI 61c	*Zungaro zungaro*	Pimelodidae	off Iquitos
Ancyrocephalinae gen. sp. 22	PI 749	*Hassar* sp.	Doradidae	Río Momón
	PI 752			
Ancyrocephalinae gen. sp. 23	PI 859	*Platysilurus mucosus*	Pimelodidae	off Santa Clara
Ancyrocephalinae gen. sp. 24	PI 784b	*Sturisoma barbatum*	Loricariidae	Belén-Market
Ancyrocephalinae gen. sp. 25	PI 871d	*Hypophthalmus edentatus*	Pimelodidae	Río Nanay
Ancyrocephalinae gen. sp. 26	PI 61a	*Zungaro zungaro*	Pimelodidae	off Iquitos
	PI 845h			Río Nanay
	PI 691	*Platynematichthys notatus*	Pimelodidae	off Santa Clara
Ancyrocephalinae gen. sp. 27	PI 813	*Anadoras weddellii*	Doradidae	Belén-Market
Ancyrocephalinae gen. sp. 28	PI 813	*Anadoras weddellii*	Doradidae	Belén-Market
Ancyrocephalinae gen. sp. 29	PI 870	*Brachyplatystoma juruense*	Pimelodidae	Río Nanay
Ancyrocephalinae gen. sp. 30	PI 813	*Anadoras weddellii*	Doradidae	Belén-Market
Ancyrocephalinae gen. sp. 31	PI 767k	*Ageneiosus vittatus*	Auchenipteridae	Río Nanay

Table 4. Continued.

Species	Field code	Host	Family	Locality
Ancyrocephalinae gen. sp. 32	PI 797	*Oxydoras niger*	Doradidae	Belén-Market
Ancyrocephalinae gen. sp. 33	PI 768e	*Ageneiosus vittatus*	Auchenipteridae	Río Nanay

Note: numbers given to species do not represent consecutive numbers and were used as a mere reference.

PUBLICATION I

New species of *Demidospermus* (Monogenea: Dactylogyridae) of pimelodid catfish (Siluriformes) from Peruvian Amazonia and the reassignment of *Urocleidoides lebedevi* Kritsky and Thatcher, 1976

Mendoza-Palmero, C. A. and T. Scholz

Journal of Parasitology 2011, 97: 586–592

PUBLICATION II

New species and geographical records of dactylogyrids (Monogenea) of catfish (Siluriformes) from the Peruvian Amazonia

Mendoza-Palmero, C. A., E. F. Mendoza-Franco, T. Scholz and R. Kuchta

Journal of Parasitology 2012, 97: 586–592

PUBLICATION III

An updated checklist of monogeneans (Platyhelminthes: Monogenea) parasites of freshwater fishes from the Neotropical region

Mendoza-Palmero C. A. and T. Scholz

Manuscript in preparation (target journal *Systematic Parasitology*)

PUBLICATION IV

Molecular phylogeny of Neotropical monogeneans (Platyhelminthes) from catfishes (Siluriformes): new insights into the systematics of the Dactylogyridae

Mendoza-Palmero, C. A., I. Blasco-Costa and T. Scholz

Manuscript in review (*Parasites* & *Vectors*)

CONCLUSIONS AND PERSPECTIVES

This thesis aimed to evaluate the diversity and taxonomic composition of dactylogyrid monogeneans infecting siluriforms from the Peruvian Amazonia and, for the first time, to assess their evolutionary history in the Neotropics applying a molecular phylogenetic approach. As a result of an intensive fieldwork carried out in Iquitos, Peru from 2004 to 2011, a remarkable number of monogenean species (99) of the family Dactylogyridae infecting 42 species of catfishes of the families Auchenipteridae, Callichthyidae, Doradidae, Heptapteridae, Loricariidae and Pimelodidae was found. This resulted in a more accurate estimation of the species richness, morphology and systematic position of the most diverse group of parasites infecting Neotropical freshwater catfishes. Finally, the application of molecular approach provided the first test of the taxonomic framework and phylogenetic relationships of Neotropical dactylogyrids of catfishes, and permitted an evaluation of their monophyly and a re-evaluation of the monophyly of Ancyrocephalinae. As a summary of the study, the following main conclusions can be drawn:

The more abundant species found in these fishes belong to the genus *Demidospermus* Suriano, 1983 (with 23 species) followed by the genera *Ameloblastella* Kritsky, Mendoza-Franco & Scholz, 2000 (22 spp.), *Costemocleithrum* Kritsky, Thatcher & Boeger, 1986 (13 spp.), *Aphanoblastella* Kritsky, Mendoza-Franco & Scholz, 2000 (4 spp.), *Vancleaveus* Kritsky, Thatcher & Boeger, 1986 (3 spp.), *Unilatus* Mizelle & Kritsky, 1967 (2 spp.), and *Amphocleithrum* Price & Gonzalez-Romero, 1969, *Philocorydoras* Suriano, 1986 and *Unibarra* Suriano & Incorvaia, 1995 with only one species each. Furthermore, 29 species are recorded here as Ancyrocephalinae gen. sp. since their morphology (internal anatomy and sclerotized structures) did not fit to any known genus reported in Neotropical catfishes, therefore these may represent new genera.

Of these 99 species recorded here, 6 are described as new for science: *Aphanoblastella aurorae* Mendoza-Palmero, Scholz, Mendoza-Franco & Kuchta, 2012 from *Goeldiella eques* (type and only host) (Heptapteridae), *Demidospermus brevicirrus* Mendoza-Palmero, Scholz, Mendoza-Franco & Kuchta, 2012 from *Pimelodus* sp. (type host), *D. curvovaginatus* Mendoza-Palmero & Scholz, 2011 from *Pimelodus* sp. (type host) and Pimelodidae gen. sp., *D. mortenthaleri* Mendoza-Palmero, Scholz, Mendoza-Franco & Kuchta, 2012 from *Brachyplatystoma juruense* (type host), *D. peruvianus* Mendoza-Palmero & Scholz, 2011 from *Pimelodus ornatus* (type host), *Pimelodus* sp., *P. blochii* and Pimelodidae gen. sp. and *D. striatus* Mendoza-Palmero & Scholz, 2011 from *Pimelodus* sp. (type host) and *Pimelodus blochii*.

On the basis of the re-evaluation of the type material of '*Urocleidoides*' *lebedevi* Kritsky & Thatcher, 1976 from *Pimelodus grosskopfii* in Colombia, this species is relocated as *Demidospermus lebedevi* (Kritsky and Thatcher, 1976) Mendoza-Palmero & Scholz, 2011 n. comb. New morphological and geographical data on the previously described dactylogyrids *Cosmetocleithrum bulbocirrus* Kritsky, Thatcher & Boeger, 1986, *Vancleaveus fungulus* Kritsky, Thatcher & Boeger, 1986, *V. janauacaensis* Kritsky, Thatcher & Boeger, 1986, *V. platyrhynchi* Kritsky, Thatcher & Boeger, 1986, *Unilatus brittani* Mizelle, Kritsky & Crane, 1968, and *U. unilatus* Mizelle & Kritsky, 1967 are provided. For the first time all these 6 species are registered in the Peruvian Amazonia and the presence of *U. brittani* and *U. unilatus* parasitizing the gills of *Pterygoplichthys anisitsi* (new host record for both species) is also recorded.

Morphological evaluation of newly collected specimens of *U. brittani* and *U. unilatus* revealed morphological and meristic similarities with *U. longispinus* Suriano, 1985 and *U. brevispinus* Suriano, 1985. Therefore based on the characteristics observed between the specimens studied in the present thesis and the original descriptions provided by Suriano (1985), the latter 2 species are synonymized with *U. brittani* and *U. unilatus*, respectively. The type specimens of *U. longispinus* and *U. brevispinus* were not found in any parasitological collection (see Lamothe-Argumedo et al., 2010), this highlights the importance of the deposition of the type material in stable parasitological collections for future studies where the confirmation of the species identity is needed.

The finding of 10 previously described species from the genera *Cosmetocleithrum*, *Vancleaveus* and *Unilatus* is related to the distributional range of their hosts in the Amazon River basin, including the Peruvian Amazonia: *Cosmetocleithrum bulbocirrus* Kritsky, Thatcher & Boeger, 1986 and *V. janauacaensis* Kritsky, Thatcher & Boeger, 1986 both parasites of the doradid *Pterodoras granulosus*, *C. confusus* Kritsky, Thatcher & Boeger, 1986, *C. gussevi* Kritsky, Thatcher & Boeger, 1986, *C. parvum* Kritsky, Thatcher & Boeger, 1986 and *C. rarum* Kritsky, Thatcher & Boeger, 1986, the latter 4 species infecting the doradid *Oxydoras niger*, *V. fungulus* Kritsky, Thatcher & Boeger, 1986 found on the gills of *Pseudoplatystoma fasciatum* (Pimelodidae), *V. platyrhynchi* Kritsky, Thatcher & Boeger, 1986 parasite of *Hemisorubim platyrhynchos* (Pimelodidae), *U. brittani* and *U. unilatus* infecting *Pterygoplichthys anisisti* (Loricariidae), respectively.

In catfishes from the Peruvian Amazonia, monogenean species of the genera *Demidospermus*, *Ameloblastella*, *Cosmetocleithrum* and the distinct morphotypes recorded herein as Ancyrocephalinae gen. spp. are represented, in most cases, by few individuals of each species/morphotypes; which impedes a detailed morphological analysis that would allow their formal description. However, in some other cases, combining traditional taxonomic methods along with novel molecular approaches will facilitate the future characterization of new taxa, especially of those morphotypes herein recorded as Ancyrocephalinae gen. spp.

In order to update the status of all species of monogenean parasites of freshwater fishes from the Neotropical region, an exhaustive literature review is carried out (up to September, 2014) to compile all available records of these parasites, along with data generated in this thesis, which all in conjunction are summarized in a checklist herein. Thus, this checklist also intends to assist and point at future parasitological research and surveys needed. The checklist includes a total of 396 monogenean species of 73 genera of three families, infecting 246 species of freshwater fishes of 131 genera of 31 families (Cohen et al., 2013 listed 337 species of monogeneans recorded only from South America). The checklist summarizes all parasite-host associations and geographical records of almost 400 known monogenean species parasitizing Neotropical freshwater fishes. Since the checklist only includes taxa identified to the species level, the total number of monogenean species recorded (396) should be taken with caution in future interpretations and conclusions regarding the diversity of this group of helminths in the Neotropics. A considerable number of species found in this literature will further require detailed morphological study for accurate identification.

Considering recent estimations of the freshwater fish fauna inhabiting in the Neotropics (about 7,000 species), only 3.5% of the potential host species have been ever studied for monogeneans. Consequently, the latter percentage indicates that an extraordinary rich fauna of these helminth parasites still remain undiscovered.

Before this work, the monogenean fauna of the family Dactylogyridae infecting Neotropical catfishes was composed of 75 species belonging to 14 genera. Adding the undescribed species recorded in this study (80 spp.) only from the Peruvian Amazonia, which doubles the current number of species of monogeneans of siluriforms recorded from the Neotropical region, it is possible to conclude that the monogenean fauna previously recorded in siluriforms represents a small fraction of the actual richness and diversity of these parasites in the Neotropical region.

Despite the large dactylogyrid diversity found infecting freshwater catfishes in the Neotropical region, none of these species had been included in molecular phylogenetic analyses before. For the first time, the phylogenetic relationships of monogenean parasites on Neotropical catfishes of the subfamily Ancyrocephalinae Bychowsky, 1937, along with

African and Asian representatives of the Ancylodiscoidinae Gussev, 1961, are assessed using partial sequences of the 28S rRNA gene. Bayesian inference (BI) and maximum likelihood (ML) analyses reveal that dactylogyrids of Neotropical catfishes do not represent a monophyletic group.

The Ancyrocephalinae results as paraphyletic group composed of three main clades. The first (clade A, see Publication IV) includes species of *Ameloblastella*, *Ligictaluridus*, *Unibarra* and *Vancleaveus* and the second (clade C) comprise species of *Aphanoblastella*, *Bychowskyella*, *Chauhanellus*, *Cosmetocleithrum*, *Demidospermus*, *Quadriacanthus*, *Schilbetrema*, *Thaparocleidus* plus several new undescribed species. The former lineage contains other ancyrocephaline genera (*Actinocleidus*, *Ancyrocephalus* and *Onchocleidus*) parasitizing Holarctic perciforms, whereas the latter lineage comprises only species infecting freshwater catfishes from the Neotropical, Palaearctic and Ethiopian regions.

The third well-supported lineage (clade C) comprises worldwide distributed marine species of the genera *Aliatrema*, *Bravohollisia*, *Ergenstrema*, *Euryhaliotrema*, *Euryhaliotrematoides*, *Haliotrema*, *Haliotrematoides*, *Ligophorus*, *Metahaliotrema*, *Pseudohaliotrema*, *Protogyrodactylus* and *Tetrancistrium* parasites of scorpaeniform and perciform fishes, along with freshwater species of *Cichlidogyrus* and *Scutogyrus*, which are specific parasites of African tilapias (Cichlidae).

The Ancylodiscoidinae is found to represent an artificial group since it is placed among members of the freshwater Ancyrocephalinae. Therefore, a new classification based on both phylogenetic information and morphological diagnostic characters is much needed.

None of the three major clades of the Ancyrocephalinae taxa are known to share obvious morphological synapomorphies, nor clear patterns in host-associations, zoogeographical or ecological distributions that would allow us to establish a new systematic classification. The phylogenetically diverse group of Neotropical ancyrocephalines should be considered in future attempts to propose a new classification of the subfamilies whitin the Dactylogyridae based on the phylogenetic relationships among its members. This new classification will require search for morphological and other synapomorphies to circumscribe adequately these genetically well-delimited clades (clades A–C).

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APPENDIX I

Parallel publications carried out during the PhD

Record of *Urocleidoides vaginoclaustrum* Jogunoori, Kritsky and Venkatanarasaiah, 2004 (Monogenea: Dactylogyridae) from a freshwater fish in Mexico

Mendoza-Palmero, C. A. and R. Aguilar-Aguilar

Parasitology Research 2008, 103: 1235–1236

Two new species of *Gyrodactylus* von Nordmann, 1832 (Monogenea: Gyrodactylidae) parasitizing *Girardinichthys multiradiatus* (Cyprinodontiformes: Goodeidae), an endemic freshwater fish from central Mexico

Mendoza-Palmero, C. A., A. L. Sereno-Uribe and G. Salgado-Maldonado

Journal of Parasitology 2009, 95: 315–318

Helminth parasites of freshwater fishes, Nazas River basin, northern Mexico

Pérez-Ponce de León, G., R. Rosas-Valdez, R. Aguilar-Aguilar, B. Mendoza-Garfias, **C. A. Mendoza-Palmero,** L. García-Prieto, A. Rojas- Sánchez, R. Briosio-Aguilar, R. Pérez-Rodríguez and O. Domínguez-Domínguez

Check List 2010, 6: 26–35

Checklist of helminth parasites of Goodeinae (Osteichthyes: Cyprinodontiformes: Goodeidae), as endemic subfamily of freshwater fishes from Mexico

Martínez-Aquino, A., **C. A. Mendoza-Palmero,** R. Aguilar-Aguilar and G. Pérez-Ponce de León

Zootaxa 2014, 3856: 151–191

APPENDIX II

Representative species of the siluriform fish families examined in this study

Auchenipteridae

Trachelyopterus galeatus
(Linnaeus, 1766)

Callichthydae

Brochis multiradiatus
(Orcés V., 1960)

Doradidae

Hassar orestis
(Steindachner, 1875)

Heptapteridae

Goeldiella eques
(Müeller & Troschel, 1849)

Loricariidae

Pterygoplichthys anisitsi
Eigenmann & Kennedy, 1903

Pimelodidae

Brachyplatystoma juruense
(Boulenger, 1898)

CURRICULUM VITAE

Carlos Alonso Mendoza Palmero, MSc.

Research area. Systematics of helminth parasites of fish, with special focus on monogeneans (Platyhelminthes: Monogenea) of freshwater fish.

EDUCATION

2004. Bachelor thesis. Taxonomy of monogeneans of freshwater fish from Los Tuxtlas region, including Catemaco Lake, Veracruz, Mexico (in Spanish). Instituto de Biología, Universidad Nacional Autónoma de México (UNAM).

2007. Master thesis. Monogenean parasites of freshwater fish of Goodeinae (Pisces: Cyprinodontiformes) with a geographical distribution analysis (in Spanish). Instituto de Biología, Universidad Nacional Autónoma de México (UNAM).

2008-present. PhD in Parasitology, Institute of Parasitology, Biology Centre of the Academy of Sciences and Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic.

PUBLICATIONS

Journals with impact factor

Salgado-Maldonado, G., G. Cabañas-Carranza, R. Aguilar-Aguilar, E. Soto-Galera & **C. Mendoza-Palmero.** 2005. Helminth parasites in freshwater fish from the Papaloapan river basin, Mexico. *Parasitology Research* 96: 69–89.

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Andrés Martínez-Aquino, **Carlos A. Mendoza-Palmero**, Rogelio Aguilar-Aguilar & Gerardo Pérez-Ponce de León. 2014. Checklist of helminth parasites of Goodeinae (Osteichthyes: Cyprinodontiformes: Goodeidae), an endemic subfamily of freshwater fishes from Mexico. *Zootaxa* 3856: 151–191.

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CURSES ATTENDANCE

“Integrando la Diversidad del Reino Animal: un enfoque filogenético”
Imparted by Dr. Estela Lopreto, Faculty of Science, UNAM.
10–14 July 2000.

“Nomenclatura Zoológica”
Imparted by Dr. Nelson Papavero, Faculty of Science, UNAM.
14–18 June 2004.

“**Enfermedades en Peces de Producción, Silvestres y de Ornato**”
Coordinators: M. V. Z. Carlos Cedillo Peláez and MSc. José Ramírez Lezama.
Faculty of Medicine and Veterinary, UNAM.
21 and 22 September 2006.

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