Parasitology International 63 (2014) 492–499

Contents lists available at ScienceDirect

Parasitology International

journal homepage: www.elsevier.com/locate/parint

The phylogenetic position of the Loimoidae Price, 1936 (Monogenoidea: Monocotylidea) based on analyses of partial rDNA sequences and morphological data

W.A. Boeger [a], D.C. Kritsky [b,*], M.V. Domingues [c], M. Bueno-Silva [a]

[a] Laboratory of Molecular Ecology and Evolutionary Parasitology, Departamento de Zoologia, Universidade Federal do Paraná, PR 81531–980, Brazil
[b] Health Education Program, School of Health Professions, Campus Box 8090, Idaho State University, Pocatello, ID 83209, USA
[c] Universidade Federal do Pará, Campus Universitário de Bragança, Instituto de Estudos Costeiros; Alameda Leandro Ribeiro s/n., 68600–000 Bragança, PA, Brazil

ARTICLE INFO

Article history:
Received 14 October 2013
Received in revised form 21 January 2014
Accepted 23 January 2014
Available online 31 January 2014

Keywords:
Monogenoidea
Monocotylidae
Loimoidae
Loimosina sp.
Hammerhead shark
Sphyrna sp.

ABSTRACT

Phylogenetic analyses of partial sequences of 18S and 28S rDNA of some monogenoids, including monocotylids and a specimen of *Loimosina* sp. collected from a hammerhead shark off Brazil, indicated that the Loimoidae (as represented by the specimen of *Loimosina* sp.) represents an in-group taxon of the Monocotylidae. In all analyses, the Loimoidae fell within a major monocotylid clade including species of the Heterocotylinae, Decacotylinae, and Monocotylinae. The Loimoidae formed a terminal clade with two heterocotyline species, *Troglocephalus rhinobatidis* and *Neoheterocotyle rhinobatis*, for which it represented the sister taxon. The following morphological characters supported the clade comprising the Loimoidae, Heterocotylinae, Decacotylinae and Monocotylinae: single vagina present, presence of a narrow deep anchor root, and presence of a marginal haptoral membrane. The presence of cephalic pits was identified as a putative synapomorphy for the clade (Loimoidae (*T. rhinobatidis*, *N. rhinobatis*)). Although rDNA sequence data support the rejection of the Loimoidae and incorporating its species into the Monocotylidae, this action was not recommended pending a full phylogenetic analysis of morphological data.

1. Introduction

Phylogenetic analyses using molecular data have been responsible for some unforeseen results concerning the taxonomy, classification, and evolutionary relationships of taxa comprising the Class Monogenoidea. One of the more trenchant examples was the determination of the putative phylogenetic position of *Udonella* Johnston, 1835 within the Platyhelminthes. Johnston [1] initially described the type species, *Udonella caligorum* Johnston, 1835 as an annelid leech. The genus was subsequently included as a member of the Udonellidae (labeled a subfamily of the Family Tristomeae) within the trematode Order Monogenea by Taschenberg [2]. The subfamily was elevated to superfamily status by Yamaguti [3] and to the level of class by Ivanov [4,5], with both authors using the epithet Udonelloidea. Ehlers [6] considered the taxon (as Udonellida) a provisional member of an apparently unnatural group of non-parasitic flatworms, the Dalyellioida, while Brooks [7] considered it to represent the Class Udonellidea serving as sister group to the Class Cercomeridea which included the remaining parasitic platyhelminths (the trematodes, monogenoids and cestodes). Later, molecular evidence suggested that the taxon represented a family of the Subclass Polyonchoinea (Monogenoidea) in proximity to the Gyrodactylidae [8]. This hypothesis was subsequently supported by Boeger and Kritsky [9], who included the taxon as the Udonellidae within the polyonchoinean Order Gyrodactylidea based on their phylogenetic analyses of morphological data.

Phylogenetic analyses using molecular data followed by evaluation of morphological data are almost necessary for determining relationships of some monogenoidean groups (see Zietara and Lumme [10] and Kuusela et al. [11] who evaluated the phylogenetic relationships of the viviparous Gyrodactylidae). However, serious conflict within some taxa of the Monogenoidea often occurs between respective phylogenetic hypotheses derived from molecular and morphological data sets (i.e., Perkins et al. [12]), suggesting that reconciliation between competing hypotheses will often require additional study and perhaps new methodology.

The Loimoidae comprises a small group (three genera and about nine species) of monogenoids parasitic on the gills of carcharhinid and sphyrnid sharks and dasyatid rays. The family is currently considered to be the sister group of the Monocotylidae within the Order Monocotylidea [9,13]. Members of the family are rarely encountered by investigators and comparatively little is known regarding their total diversity, geographic and host distributions, and biology. The most recent studies are those of Justine and Mattei [14] and Rohde et al. [15], who investigated the ultrastructure of the sperm cell and flame bulb of the protonephridial system of *Loimosina wilsoni* Manter,

* Corresponding author at: Campus Box 8090, Idaho State University, Pocatello, Idaho 83209, USA. Tel.: +1 208 282 3726; fax: +1 208 282 4645.
E-mail addresses: wboeger@gmail.com (W.A. Boeger), kritdela@isu.edu (D.C. Kritsky), mvdomingues71@gmail.com (M.V. Domingues).

1383-5769/$ – see front matter © 2014 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.parint.2014.01.005

Table 1
Species used in the phylogenetic analyses with respective GENBANK accession numbers.

Family	Species	18S	28S long	28S short
Loimoidae	*Loimosina* sp.	KF 908849	KF 908848	KF 908848
Anoplodiscidae	*Anoplodiscus cirrusspiralis*	AJ287475	AF382060	–
Capsalidae	*Benedenia lutjani*	–	AY033939	–
	Benedenia rohdei	–	AY033940	–
	Benedenia sp.	AJ228774	–	AF382052
	Capsala martinieri	AJ276423	AF382053	AF382053
	Encotyllabe chironemi	AJ228780	–	–
	Entobdella australis	–	AF026108	–
	Neobenedenia sp.	–	AF382056	AF382056
Concinnocotylidae	*Concinnocotyla australensis*	AM157183	AM157197	–
Dactylogyridae	*Actinocleidus recurvatus*	–	AJ969951	–
	Cichlidogyrus sp. *1 XW-2006*	–	DQ537367	–
	Cleidodiscus pricei	–	AJ969939	–
	Dactylogyrus extensus	–	AJ969944	–
	Euryhaliotrema cribbi	AY820601	AY820612	–
	Euryhaliotrema chrysotaeniae	–	AF026115	–
	Euryhaliotrema johni	–	DQ157657	–
	Euryhaliotrema grandis	AY820605	AY820616	–
	Haliotrema angelopterum	AY820609		–
	Ligophorus leporinus	–	DQ537380	–
	Metahaliotrema geminatohamula	–	DQ157646	–
	Pseudodactylogyroides apogonis	AB065115		–
	Pseudodactylogyrus anguillae	–	AJ969950	–
	Pseudodactylogyrus bini	AB065113	–	–
	Pseudohaliotrema sphincteroporus	AJ287568	–	–
	Quadriacanthus kobiensis	–	AY841874	–
	Scutogyrus longicornis	–	DQ157659	–
	Tetrancistrum sp.	–	AF026114	–
	Thaparocleidus campylopterocirrus	–	EF100546	–
	Urocleidus similis	–	AJ969938	–
Diplectanidae	*Acleotrema* sp.	–	AF026118	–
	Diplectanum blairense	–	AY553627	–
	Lamellodiscus acanthopagri	–	DQ054822	–
	Lamellodiscus japonicus	EU836236	–	–
	Lamellodiscus pagrosomi	EU836235	–	–
	Laticola paralatesi	–	DQ054826	–
	Pseudorhabdosynochus coioidesis	–	DQ054828	–
Gyrodactylidae	*Aglaiogyrodactylus ctenistus*	JX840354	–	–
	Gyrodactyloides bychowskii	AJ566379	–	–
	Gyrodactylus gobiensis	AJ566375	–	–
	Gyrodactylus rutilensis	AJ566376	–	–
	Gyrodactylus salaris	EF612463	–	–
	Gyrodactylus sedelnikovi	AJ566378	–	–
	Gyrodactylus superbus	JX840357	–	–
	Macrogyrodactylus polypteri	AJ567671	–	–
	Phanerothecium spinulatum	JX840360	–	–
Iagotrematidae	*Euzetrema knoepffleri*	AJ564212	–	–
Microbothriidae	*Leptocotyle minor*	AJ228784	AF382063	–
Monocotylidae	*Calicotyle affinis*	AJ228777	–	–
	Calicotyle kroyeri	–	AF279744	AF279744
	Calicotyle palombi	–	–	AF131709
	Calicotyle sp.	–	–	FJ971978
	Calicotyle stossichi	–	–	AF279751
	Calicotyle urolophi	–	–	AF279753
	Clemacotyle australis	–	AF348350	AF348350
	Decacotyle floridana	–	AF348357	AF348357
	Decacotyle lymmae	–	AF348359	AF348359
	Decacotyle tetrakordyle	–	AF348358	AF348358
	Dendromonocotyle ardea	–	AF348351	AF348351
	Dendromonocotyle octodiscus	–	–	AF348352
	Dictyocotyle coeliaca	AJ228778	AF382062	AF382062
	Empruthotrema dasyatidis	–	AF348345	AF348345
	Empruthotrema quindecima	–	AF348346	AF348346
	Heterocotyle capricornensis	–	–	AF348360
	Merizocotyle australensis	–	AF348348	AF348348
	Merizocotyle icopae	–	AF348349	–
	Merizocotyle sinensis	–	FJ514075	FJ514075
	Monocotyle corali	–	AF348353	AF348353
	Monocotyle helicophallus	–	–	AF348355
	Monocotyle multiparous	–	–	AF348356
	Monocotyle spiremae	–	AF348354	AF348354
	Neoheterocotyle rhinobatis	–	AF348361	AF348361
	Troglocephalus rhinobatidis	AJ228795	AF348364	AF348364
Polystomatidae	*Diplorchis ranae*	AM157184	AM157198	–

(continued on next page)

Table 1 (*continued*)

Family	Species	18S	28S long	28S short
Pseudomurraytrematidae	*Pseudomurraytrema* sp.	–	AF382059	–
	Pseudomurraytrema ardens[a]	AJ228793	–	–
Sundanonchidae	*Sundanonchus micropeltis*	AJ287579	–	–
Udonellidae	*Udonella caligorum*	FJ946831	–	–

[a] This species is identified in GenBank as *Pseudomurraytrema ardens*, a nomen nudum. In Fig. 3, it is referred to as *Pseudomurraytrema* sp.*.

1944, respectively. Molecular sequences from loimoid species are lacking from DNA databanks.

In October, 2009, a hammerhead shark, *Sphyrna* sp., was purchased from fishermen at the fish market in the city of Barra Velha, State of Santa Catarina, Brazil; the shark was apparently caught in the Atlantic Ocean near Barra Velha the evening before its purchase. A single specimen, a member of the Loimoidae, was collected from the gills of the shark, which permitted the sequencing of nuclear DNA fragments. In the present study, the resulting data, including incomplete sequences of 18S and 28S rDNA of the loimoid specimen, were used to evaluate the phylogenetic position of the Loimoidae within the Monogenoidea and to test the current hypothesis of the family serving as the sister group of the Monocotylidae.

2. Materials and methods

The gills of the hammerhead shark were surgically removed and placed in hot water (~70 °C) for approximately 1 min, after which 70% ethanol was added to the washings and gills for preservation. The loimoid specimen was subsequently removed from the washings with a fine probe under low power microscopy and placed in a small drop of 70% ethanol on a slide where it was cut into three parts using a razor blade. The haptor and anterior end containing the copulatory complex were mounted on a microscope slide in Hoyer's mounting medium [16] for later identification of the helminth; the mid-section of the worm was immediately replaced in 70% ethanol and retained for subsequent DNA extraction. The slide containing the worm fragments was deposited in the helminthological collection of the Instituto Oswaldo Cruz, Rio de Janero, Brazil (CHIOC 37742).

DNA was extracted and purified from the middle section of the worm with the DNAEasy tissue kit (Qiagen). Primers used to amplify and sequence fragments of 18S rDNA were18S7F 5′-GCCCTATCAACT TACGATGGTA-3′, 18SF 5′-CCAGCTTGATCCTTCTGCAGGTTCACCTAC-3′ and of 28S rDNA, LSU5-F 5′-TAGGTCGACCCGCTGAAYTTAAGCA-3′, ECD2R 5′-CCTTGGTCCGTGTTTCAAGACGGG-3′ [12,17]. The polymerase chain reaction (PCR) mix for amplification of 18S rDNA contained 3 μl DNA template, 0.4 mM dNTP, 3 mM $MgCl_2$, 1 U Taq Platinum (Invitrogen), PCR-buffer (1X), 1 μm of each primer and autoclaved ultrapure water to complete a 25 μl final volume. The PCR program for 18S rDNA was: 5 min at 95 °C, after which 40 cycles of 45 s at 95 °C, 1 min at 60 °C, 1 min at 72 °C and 5 min at 72 °C. The PCR mix for 28S rDNA contained 3 μl DNA template, 0.4 mM dNTP, 3 mM $MgCl_2$, 1 U Taq Platinum (Invitrogen), PCR-buffer (1X), 0.6 μm of each primer and autoclaved ultrapure water to complete a 25 μl final volume; the PCR-program was: 5 min at 94 °C, followed by 40 cycles of 45 s at 94 °C, 45 s at 62 °C, 45 s at 72 °C and 5 min at 72 °C.

Confirmation of amplification of the fragments by PCR was achieved through electrophoresis on a 1.5% agarose gel, subsequent staining in ethidium bromide, and visualization under UV light. Amplicons were purified using the MinElute Purification kit (Qiagen). Sequences were obtained with the Big Dye 3.1 chemistry in a 3130 DNA Analyser (Applied Biosystems) with the same programs and primers used in the amplifications. Sequences were edited and aligned using Genious 5.1 [18].

Bayesian phylogenetic analyses were performed with the software MrBayes in the Cipres platform [19] using 4 chains, 10,000,000 generations, burnin of 100,000, and with initial model (18S and 28S large — GTR + I + G; 28S small — TVM + G) chosen by the software JModeltest [20] with Phyml [21]. Three analyses were performed. Two, using 18S and 28S rDNA, respectively, were designed to evaluate the phylogenetic position of the Loimoidae, as represented by the sequenced loimoid specimen, within the Polyonchoinea. In addition to those obtained from the loimoid specimen, these analyses

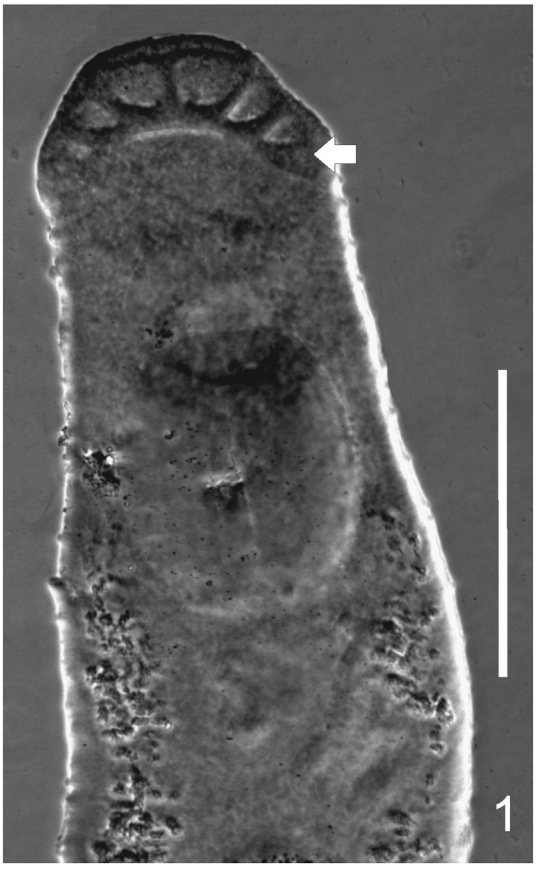

Fig. 1. Phase contrast photomicrograph of *Loimosina* sp. from *Sphyrna* sp.: Cephalic region. Arrow indicates cephalic pits. Scale = 500 μm.

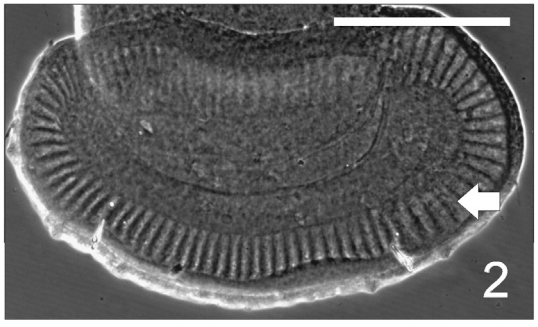

Fig. 2. Phase contrast photomicrograph of *Loimosina* sp. from *Sphyrna* sp.: Haptor. Arrow indicates radial ridges. Scale = 250 μm.

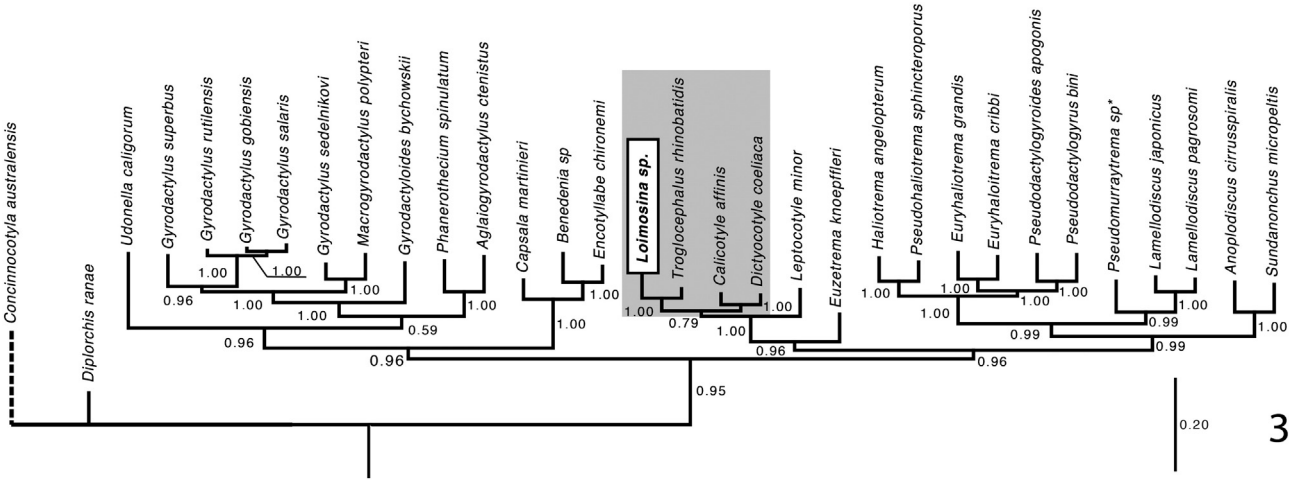

Fig. 3. Relative position of *Loimosina* sp. (white rectangle) within selected representatives of Monogenoidea based on a Bayesian phylogeny of the fragment 18S rDNA; gray rectangle indicates species of Monocotylidae.Posterior probability values are presented by each respective branch. Scales refer to genetic distance based on the selected model of each analysis. Branch lengths reflect substitutions per site; the dashed branch is not to scale.

included available sequences from representative species of several families of the Polyonchoinea (Table 1). The third analysis was performed with a short segment of 28S rDNA sequences of species from the taxa shown to be close to the loimoid specimen by the two prior analyses and was used to interpret historical changes associated with morphology of the Loimoidae and respective sister groups. This analysis was considered an additional test of the phylogenetic position of the Loimoidae.

Morphological information (homologous series presented in Appendix 1) was traced on the phylogeny obtained from the short 28S rDNA analysis using the Parsimony Ancestral Character Reconstruction module of the software Mesquite [22] in order to evaluate the consistency of the putative phylogenetic position of the Loimoidae with morphological characters.

3. Results

The specimen from the hammerhead shark was provisionally identified as an undescribed species of *Loimosina* Manter, 1944 (Loimoidae) based on the presence of more than two pairs of cephalic pits, a rudimentary male copulatory organ, and a haptor lacking dorsal cuticular ridges (Figs. 1–2). However, morphological features in the two fragments of the worm were insufficient for species identification and description. Two attributes apparently precluded the assignment of the specimen to either of the currently described species of *Loimosina*. The specimen possessed four pairs of cephalic pits (Fig. 1, arrow) (three pairs in both *L. wilsoni* and *L. parawilsoni* Bravo-Hollis, 1970) and a transversely oval haptor with numerous submarginal radial ridges and loculi (Fig. 2, arrow) (haptor subcircular in *L. parawilsoni*) (see Manter [23]; Bravo-Hollis [24]).

Bayesian reconstructions with 18S, 28S (long) and 28S (short) rDNA suggested the Loimoidae, as represented by the *Loimosina* specimen, to be embedded within clades composed of species of the Monocotylidae (Figs. 3–5, respectively). That the Loimoidae represents a subordinate taxon of the Monocotylidae was consistently supported by posterior probability (PR) values for each of these clades (PR = 0.79, 1.00 and 0.98, respectively). The three analyses placed the Loimoidae as sister

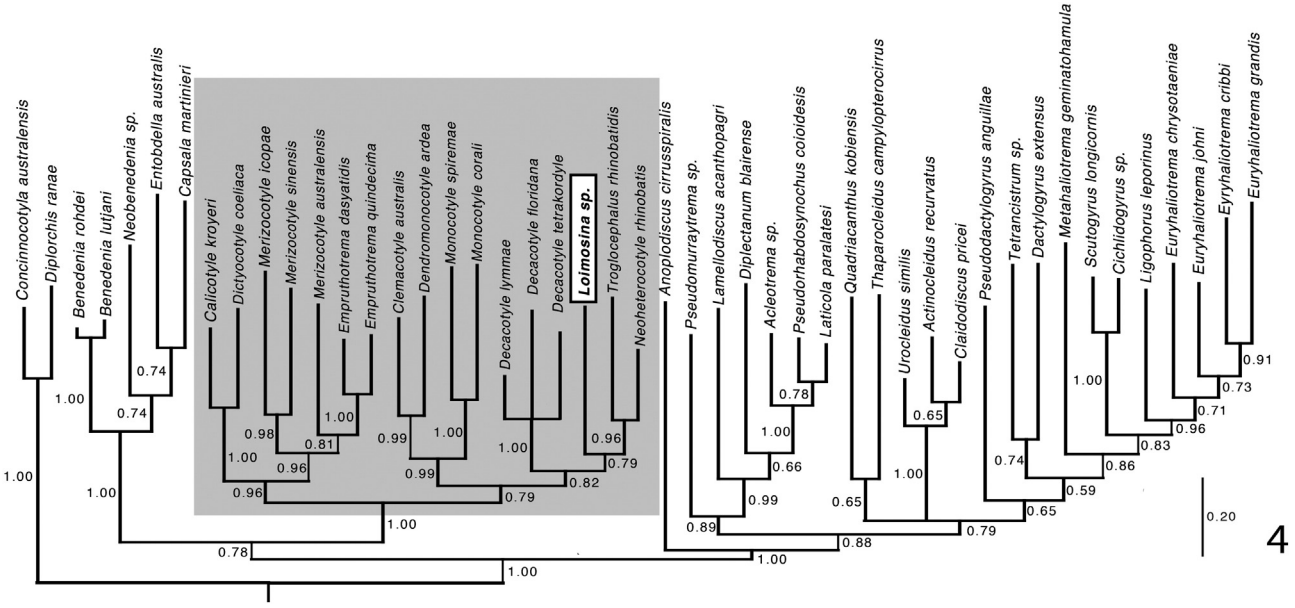

Fig. 4. Relative position of *Loimosina* sp. (white rectangle) within selected representatives of Monogenoidea based on a Bayesian phylogeny of the long fragment of 28S rDNA; gray rectangle indicates species of Monocotylidae.Posterior probability values are presented by each respective branch. Scales refer to genetic distance based on the selected model of each analysis. Branch lengths reflect substitutions per site.

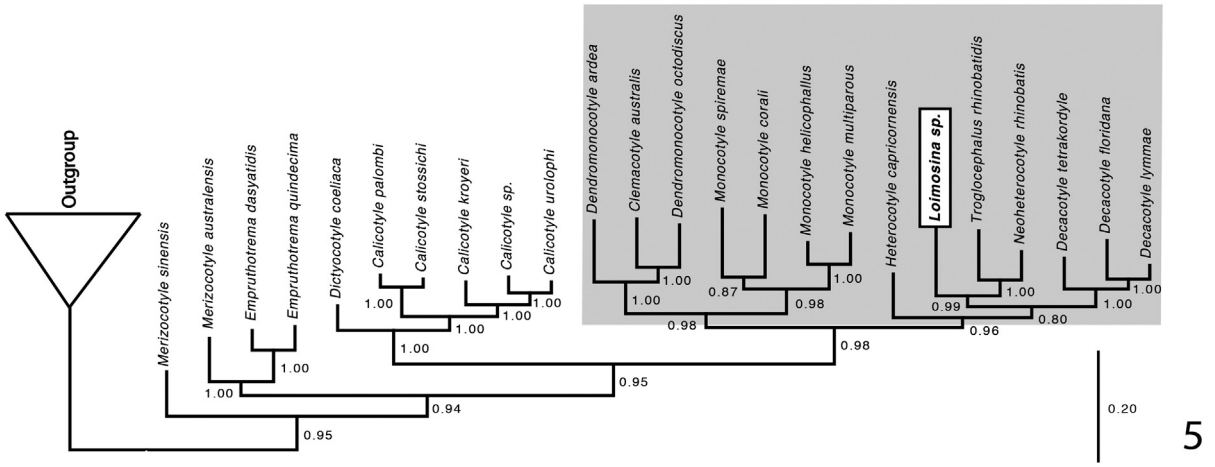

Fig. 5. Relative position of *Loimosina* sp. (white rectangle) within selected representatives of Monocotylidae based on a Bayesian phylogeny of a short fragment 28S rDNA; gray rectangle indicates species of Monocotylidae most closely related to *Loimosina*. Posterior probability values are presented by each respective branch. Scales refer to genetic distance based on the selected model of each analysis. Branch lengths reflect substitutions per site.

taxon to a clade containing *Troglocephalus rhinobatidis* Young, 1967 and *Neoheterocotyle rhinobatis* (Pillai & Pillai, 1976) Chisholm & Whittington, 1997 (PR = 1.00, 0.70 and 0.99, respectively), with a clade comprised of three species of *Decacotyle* Young, 1967 serving as its sister group in analyses using long and short 28S rDNA sequences (PR = 0.82 and 0.80, respectively) (Figs. 4, 5); sequences of 18S rDNA from *Decacotyle* species were not available for the present study.

Reconstruction of the evolutionary history of morphological character states on the 28S (short) cladogram (Fig. 5) provided additional support for the proposed phylogenetic position of the Loimoidae within the Monocotylidae. Three characters present in *Loimosina* spp. represented synapomorphies for a major branch of Monocotylidae that contained the *Loimosina* specimen: the presence of 1) a single vagina (Fig. 6); 2) a narrow deep root of the anchor (Fig. 7) (see Figs. 20–24, 29, 30 in Chisholm et al. [25] for various monocotylid anchors having a "narrow deep root of the anchor"), and 3) a marginal haptoral membrane (Fig. 8). The presence of cephalic pits provided a synapomorphy for the clade containing *Loimosina*, *T. rhinobatidis*, and *N. rhinobatis* (Fig. 9), while no morphological characters were found to support the sister group relationship of *Decacotyle* species with those of the clade comprising *Loimosina*, *Troglocephalus*, and *Neoheterocotyle* spp.

While providing no phylogenetic information, four morphological characters represented autapomorphies of the Loimoidae, as represented by the *Loimosina* sp. These included an ovate egg (as opposed to a tetrahedric egg), an intercecal germarium (as opposed to a germarium looping the gut), a lobulate germarium (as opposed to a compact

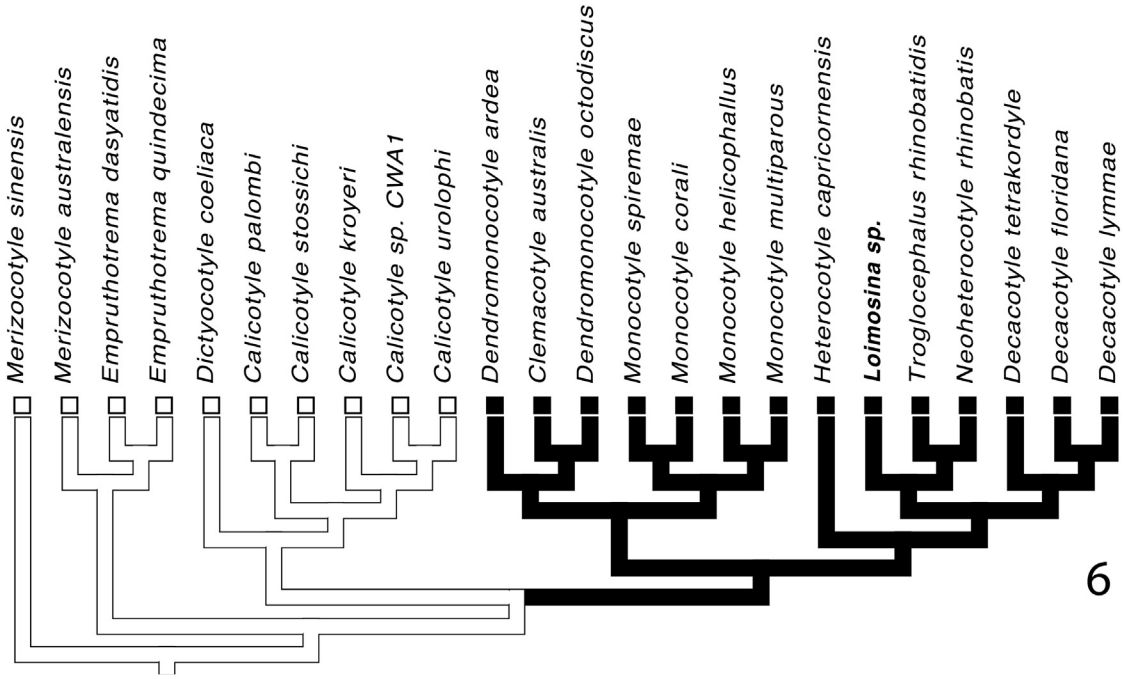

Fig. 6. Reconstruction of the history of relevant morphological characters (probable synapomorphies) onto the short-fragment 28S rDNA phylogenetic hypothesis. Black squares at the terminus of each branch indicate presence of the morphological character; white squares indicate absence of the character; absence of squares represents an unknown or irrelevant character; arrows indicate the relative position of *Loimosina* sp. in the phylogeny. Single vagina present.

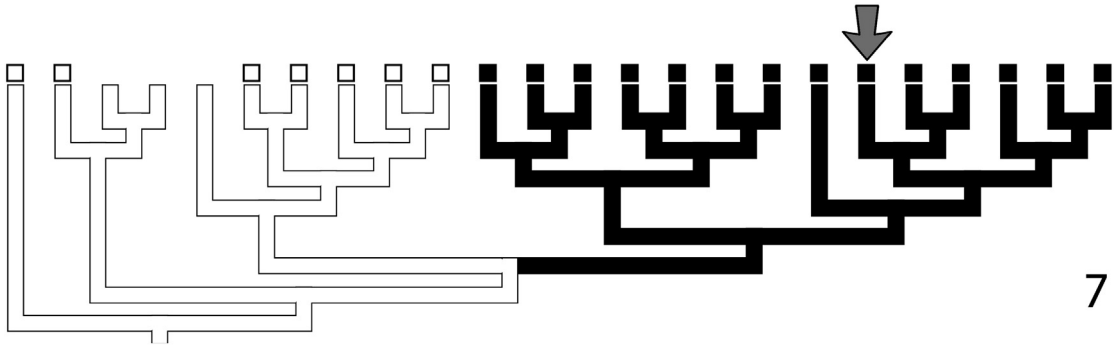

Fig. 7. Reconstruction of the history of relevant morphological characters (probable synapomorphies) onto the short-fragment 28S rDNA phylogenetic hypothesis. Black squares at the terminus of each branch indicate presence of the morphological character; white squares indicate absence of the character; absence of squares represents an unknown or irrelevant character; arrows indicate the relative position of *Loimosina* sp. in the phylogeny. Presence of narrow deep anchor root.

germarium), and spermatids with one normal and one altered axoneme during spermiogenesis (see Boeger and Kritsky [9] and Justine and Mattei [14]).

4. Discussion

That organizational taxonomic units (OTUs) are monophyletic is a necessary assumption in phylogenetic analyses conducted at supraspecific levels. As a result, Boeger and Kritsky [9,13] considered a priori that the Loimoidae and Monocotylidae represented monophyletic taxa in their phylogenetic analyses of the families comprising the Class Monogenoidea. Boeger and Kritsky's [9,13] analyses, based on morphological data, clustered the Loimoidae and Monocotylidae as sister taxa, which in their overall hypothesis, formed the basal clade that they identified as the Order Monocotylidea within the Subclass Polyonchoinea. The Monocotylidea (sensu Boeger and Kritsky [9,13]) was supported by three putative synapomorphic characters: 14 marginal hooks in the larva, 14 marginal hooks in the adult, and distal regions of the mature spermatozoon with a portion of the nucleus. Putative synapomorphies of the Loimoidae included pigmented eyes absent in the adult and 1 + 1-altered axonemes in the spermatid during spermiogenesis, while those of the Monocotylidae included the germarium/oviduct looping the gut and presence of tetrahedric eggs. Chisholm et al. [25] suggested that the division of the haptor into one central and eight peripheral loculi represented an additional synapomorphy for the Monocotylidae.

Whereas the Loimoidae was not represented in their set of OTUs, Chisholm et al. [26] proposed a hypothesis for phylogenetic relationships within the Monocotylidae inferred from 28S rDNA sequences obtained from 26 species representing the six monocotylid subfamilies listed by Chisholm et al. [25], who also considered the Loimoidae to be the sister taxon of the Monocotylidae. Although the three synapomorphies of the Monocotylidae presented above were absent in species of Loimoidae, each character had apparently undergone subsequent transformation during the evolutionary development of the Order Monocotylidea. Tetrahedric eggs were apparently secondarily modified into ovate eggs in species of *Dictyocotyle* Nybelin, 1941 and *Potamotrygonocotyle* Mayes, Brooks & Thorson, 1981 [27]. In species of *Mehracotyle* Neifar, Euzet & Ben Hassine, 2002, a taxon not included in the analyses of Chisholm et al. [25], the intercecal germarium [28] was apparently secondarily developed. While division of the haptor into one central and eight peripheral loculi may be a basal character for Monocotylidae, Chisholm et al. [25] indicated that subdivision or fusion of haptoral loculi had secondarily occurred in some monocotylid lineages.

Hypotheses resulting from the present investigation are highly congruent with that presented by Chisholm et al. [26], who did not have a representative sequence from the Loimoidae. Molecular analyses of the two nuclear fragments (18S and 28S rDNA) strongly supported the position of the Loimoidae (as represented by the *Loimosina* specimen) as an in-group taxon of the Monocotylidae and as sister group to species parasitizing rhinobatid rays, i. e., *T. rhinobatidis* and *N. rhinobatis*. Three morphological characters serving as synapomorphies supported the position of the Loimoidae within a major monocotylid clade containing the Heterocotylinae, (including *Troglocephalus* and *Neoheterocotyle*), Decacotylinae, and Monocotylinae: a single vagina (Fig. 6), a narrow deep root of anchor (Fig. 7), and the presence of a marginal haptoral membrane (Fig. 8). The presence of cephalic pits apparently represented a putative synapomorphy for a smaller subordinate clade

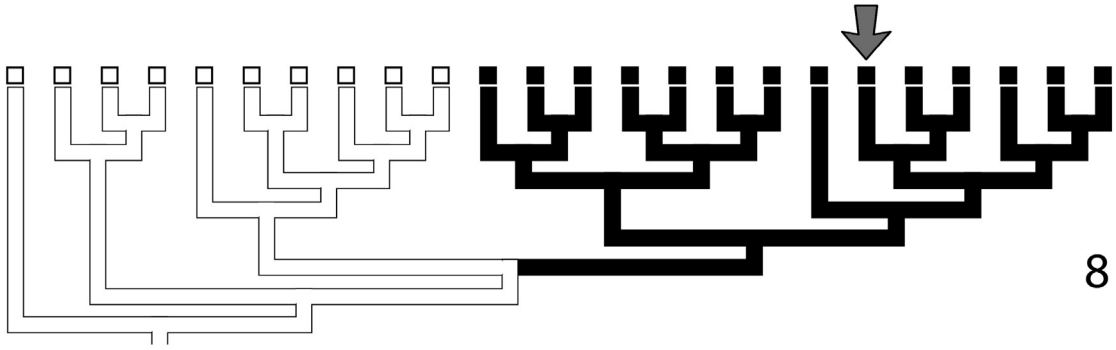

Fig. 8. Reconstruction of the history of relevant morphological characters (probable synapomorphies) onto the short-fragment 28S rDNA phylogenetic hypothesis. Black squares at the terminus of each branch indicate presence of the morphological character; white squares indicate absence of the character; absence of squares represents an unknown or irrelevant character; arrows indicate the relative position of *Loimosina* sp. in the phylogeny. Presence of marginal haptoral membrane.

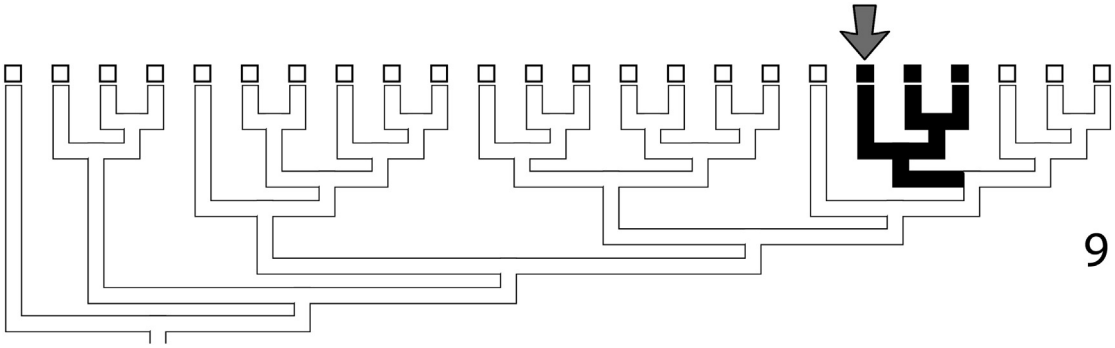

Fig. 9. Reconstruction of the history of relevant morphological characters (probable synapomorphies) onto the short-fragment 28S rDNA phylogenetic hypothesis. Black squares at the terminus of each branch indicate presence of the morphological character; white squares indicate absence of the character; absence of squares represents an unknown or irrelevant character; arrows indicate the relative position of *Loimosina* sp. in the phylogeny. Presence of cephalic pits.

of this larger group composed of loimoids, *T. rhinobatidis* and *N. rhinobatis* (Fig. 9). In addition, the putative synapomorphies of the Loimoidae and Monocotylidea listed above were easily accommodated within the short 28S rDNA hypothesis (Fig. 5). While not intended as a revision of the Monocotylidae, the results strongly suggested that the Loimoidae be rejected. However, a detailed phylogenetic anaysis of morphological data should be conducted prior to any taxonomic decision regarding the validity of the family.

Mehracotyle was recently proposed by Neifar et al. [28] for *Mehracotyle insolita* Neifar, Euzet & Ben Hassine, 2002 from the rhinobatid ray *Rhinobatos cemiculus* Geoffroy Saint-Hilaire, 1817 (Rhinobatidae) from the Mediterreanean Sea off Tunisia. Although not included in the present analyses due to the lack of molecular data, the monotypic *Mehracotyle* included a species lacking a germarium/oviduct looping the gut, which suggested a phylogenetic relationship with the Loimoidae. Added support for this putative relationship was the presence of 4 pairs of cephalic pits ("Huit sacs céphaliques ventraux" of Neifar et al. [28]) in *M. insolita* and the *Loimosina* specimen. Presence of cephalic pits also suggested a proximity of *Mehracotyle* to *Neoheterocotyle* and *Troglocephalus*, species of both of which possessed the character.

Finally, loimoids have been reported from carcharhinid and sphyrnid sharks and dasyatid rays. Since the Carcharinidae and Sphyrnidae are apparently closely related, if not synonymous taxa [29–31], the origin of the putative clade containing the loimoid specimen apparently was associated with dispersal of their common ancestor into carcharhinids and sphyrnids from ray-like fishes. For instance, taxa basal to the Loimoidae include *N. rhinobatis* and *T. rhinobatidis*, both of which parasitize rhinobatid rays and species of *Decacotyle* and *Heterocotyle* Scott, 1904 that parasitize myliobatid or dasyatid rays.

Acknowledgments

The authors gratefully acknowledge Luciana Patella and Renata C. Santos Ferreira, Laboratory of Molecular Ecology and Evolutionary Parasitology, Departamento de Zoologia, Universidade Federal do Paraná, for laboratory support. WAB was supported as a research fellow with the CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico, Brasil).

Appendix 1. Homologous series used in the Parsimonious Ancestral Character State Reconstruction. Each homologous series is presented with respective states following a colon

1. Cephalic organs: more than three pairs; three pairs; one pair. 2. Number of cephalic-gland openings: more than one pair; one pair. 3. Cephalic pit: absent; present. 4. Shape of the intestinal ceca: tubular; dendritic. 5. Haptoral septa and locules: absent; present. 6. Sclerotized ridges on the septa: absent; sinuous single ridges. 7. Haptoral sclerites: absent; present. 8. Marginal loculi: absent; present. 9. Marginal haptoral membrane: absent; present. 10. Marginal haptoral papillae: absent; present. 11. Papillary sclerites (when marginal haptoral papillae are present): absent; present. 12. Dorsal haptoral accessory structure associated with posterior loculi: absent; one; two; six. 13. Dorsal haptoral accessory structure associated with peripheral-posterior loculi: absent; one pair; two pairs. 14. Sclerites associated with the dorsal haptoral accessory structure: absent; present. 15. Anchors: present; absent. 16. Anchor deep root: narrow; expanded. 17. Anchor superficial root: well developed; poorly developed; inconspicuous. 18. Anchor: evenly curved point/shaft of similar length; long shaft/short point. 19. Accessory piece: absent; present. 20. Germarium: intercecal; loops gut. 21. Distal portion of the germarium: uniform; lobate. 22. Vagina: single; double; absent. 23. Vagina: non-sclerotized; sclerotized. 24. Number of hooks in adult: 16, 14, absent. 25. Number of axonemes during spermiogenesis: 2; 1 + 1 altered;1 + 1 disappearing; 1. 26. Nucleus of distal region of mature spermatozoon: distal region with nucleus and other cytoplasmic elements; distal region with nucleus only. 27. Cytoplasmic middle process and flagella: separated, then fused during spermiogenesis; fused from start of spermiogenesis. 28. External ornamentation of cell membrane in zone of differentiation of spermatid: present; absent. 29. Egg: ovate; tetrahedric.

References

[1] Johnston G. Art. IV. Illustrations in British zoology. Mag Nat Hist Lond 1835;8:494–8.
[2] Taschenberg EO. Zur Systematik der monogenetischen Trematoden. Z Gesamte Naturwiss 1879;52:232–65.
[3] Yamaguti S. Systema Helminthum, vol. 4. New York: Interscience Publishers; 1963.
[4] Ivanov AV. *Udonella caligorum* Johnston, 1835, a representative of a new class of flatworms. Zool Zhurnal 1952;31:175–8 [In Russian].
[5] Ivanov AV. Morphology of *Udonella caligorum* Johnston, 1835, and the position of Udonellidae in the systematics of platyhelminths. Parazitol Sb 1952;14:112–63 [Translated from Russian by M. Kassatkin & S. Kassatkin, Virginia Institute of Marine Sciences, Translation series 25, 1981, 57 pp.].
[6] Ehlers U. Das Phylogenetische System der Plathelminthes. Stuttgart: Gustav Fischer Verlag; 1985.
[7] Brooks DR. The phylogeny of the Cercomeria (Platyhelminthes: Rhabdocoela) and general evolutionary principles. J Parasitol 1989;75:606–16.
[8] Littlewood DTJ, Rohde K, Clough KA. The phylogenetic position of *Udonella* (Platyhelminthes). Int J Parasitol 1998;28:1241–50.
[9] Boeger WA, Kritsky DC. Phylogenetic relationships of the Monogenoidea. In: Littlewood DTJ, Bray RA, editors. Interrelationships of the Platyhelminthes. London: Taylor & Francis; 2001. p. 92–102.
[10] Zietara MS, Lumme J. Comparison of molecular phylogeny and morphological systematics in fish parasite genus *Gyrodactylus* Nordmann, 1832 (Monogenea, Gyrodactylidae). Zool Pol 2004;49:5–28.
[11] Kuusela J, Zietara MS, Lumme J. Description of three new European cryptic species of *Gyrodactylus* Nordmann, 1832 supported by nuclear and mitochondrial phylogenetic characterization. Acta Parasitol 2008;53:120–6.
[12] Perkins EM, Donnellan SC, Bertozzi T, Chisholm LA, Whittington I. Looks can deceive: molecular phylogeny of a family of flatworm ectoparasites (Monogenea: Capsalidae) does not reflect current morphological classification. Mol Phylogen Evol 2009;52:705–14.

[13] Boeger WA, Kritsky DC. Phylogeny and a revised classification of the Monogenoidea Bychowsky, 1937 (Platyhelminthes). Syst Parasitol 1993;26:1–32.
[14] Justine JL, Mattei X. Ultrastructure de la spermiogenèse et du spermatozoïde de *Loimosina wilsoni* et affinités phylétiques des Loimoidae (Plathelminthes, Monogenea, Monopisthocotylea). Zool Scr 1985;14:169–75.
[15] Rohde K, Justine J-L, Watson N. Ultrastructure of the flame bulbs of the monopisthocotylean Monogenea *Loimosina wilsoni* (Loimoidae) and *Calceostoma herculanea* (Calceostomatidae). Ann Parasitol Hum Comp 1989;64:433–42.
[16] Humason GL. Animal tissue techniques. 4th ed. San Francisco: W.H. Freeman and Company; 1979.
[17] Littlewood DTJ, Olson PD. Small subunit rDNA and the Platyhelminthes: signal, noise, conflict and compromise. In: Littlewood DTJ, Bray RA, editors. Interrelationships of the Platyhelminthes. London: Taylor & Francis; 2001. p. 262–78.
[18] Drummond AJ, Ashton B, Buxton S, Cheung M, Cooper A, Heled J, et al. Geneious v5.1. http://www.geneious.com; 2010.
[19] Miller MA, Holder MT, Vos R, Midford PE, Liebowitz T, Chan L, et al. The CIPRES portals. http://www.phylo.org/sub_sections/portal; 2009.
[20] Posada D. jModelTest: phylogenetic model averaging. Mol Biol Evol 2008;25:1253–6.
[21] Guindon S, Gascuel O. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst Biol 2003;52:696–704.
[22] Maddison WP, Maddison DR. Mesquite: a modular system for evolutionary analysis. Version 2.73. http://mesquiteproject.org; 2010.
[23] Manter HW. Notes on the trematode subfamily Loimoinae (Monogenea), with a description of a new species. J Wash Acad Sci 1944;34:85–9.
[24] Bravo-Hollis M. Helmintos de peces del Parífico Mexicano. XXXI. Descripcion de *Loimosina parawilsoni* sp. nov. (Familia Loimoidae Bychowsky, 1957), de *Sphyrna lewini* (Griffith) de Mazatlán, Sinaloa. An Inst Biol, Univ Nal Autó México, 41; 1970 147–52.
[25] Chisholm LA, Wheeler TA, Beverley-Burton M. A phylogenetic analysis and revised classification of the Monocotylidae Taschenberg, 1879 (Monogenea). Syst Parasitol 1995;32:159–91.
[26] Chisholm LA, Morgan JAT, Adlard RD, Whittington I. Phylogenetic analysis of the Monocotylidae (Monogenea) inferred from 28S rDNA sequences. Int J Parasitol 2001;31:1537–47.
[27] Domingues MV, Marques FPL. Revision of *Potamotrygonocotyle* Mayes, Brooks & Thorson, 1981 (Platyhelminthes: Monogenoidea: Monocotylidae), with descriptions of four new species from the gills of the freshwater stingrays *Potamotrygon* spp. (Rajiformes: Potamotrygonidae) from the La Plata river basin. Syst Parasitol 2007;67:157–74.
[28] Neifar L, Euzet L, Ben Hassine OK. Une nouvelle espèce de Monocotylidae (Monogenea) parasite branchial de *Rinobatos cemiculus* (Euselachii, Rhinobatidae), avec proposition d'un nouveau genre et d'un amendement à la diagnose des Monocotylidae. Zoosystema 2002;24:699–706.
[29] Deets GB. Copepod–chondrichthyan coevolution: a cladistic consideration. Ph.D. Thesis University of British Columbia; 1994.
[30] Shirai S. Phylogenetic interrelationships of neoselachians (Chondrichthyes: Euselachii). In: Stiassny MLJ, Parenti LR, Johnson GD, editors. Interrelationships of fishes. New York: Academic Press; 1996. p. 9–32.
[31] Vélez-Zuazo X, Agnarsson I. Shark tales: a molecular species-level phylogeny of sharks (Selachimorpha, Chondrichthyes). Mol Phylogenet Evol 2011;58:207–17.