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Systematic assessment of nymphs of Diesingiinae (Pentastomida: Sebekidae) parasitizing *Palloceros harpagos* (Cyprinodontiformes: Poeciliidae) from Parque Nacional Iguazú, Argentina: filling gaps in an incomplete phylogenetic framework

MARTÍN MIGUEL MONTES, JORGE BARNECHE, NICOLAS LEGUNDA, WALTER FERRARI, ROGELIO AGUILAR-AGUILAR & SERGIO ROBERTO MARTORELLI

Abstract: During a search for parasites in fishes from Iguazu River, Argentina, a nymph of pentastomid was found encysted on mesenteries of *Phalloceros harpagos*. The 28S rDNA and COI mt-DNA were used to compare with the sequences deposited in the GenBank. Pentastomid nymphs belong to the subfamily Diesingiinae (Sebekidae) for having chloride cell pores distributed in a single row per annulus; also, the hooks and rows of spines of our material differ to other genera. Present specimens are most likely *Diesingia* sp., having 63-74 annuli, a U shaped oral cadre with fibers closing anteriorly and a peg like extension of the oral cadre. The 28S rDNA analysis places our samples into a sister group of *Alofia* sp., but the COI mt-DNA situate them on the base of the clade. In conclusion, our pentastomid positively belongs to *Diesingia* sp., however, indisputable assignation requires a reliable description of the nymph, or the availability of sequences linking nymphs and adults, which even could provide evidence of a new hitherto undescribed genus. Aditionally, the systematic position of *Sebekia oxycephala* previously described by *P. harpagos* is discussed on the basis of our results, allowing us to suggest a re-assignation of such specimens to the genus *Diesingia*.

Key words: Iguazu, COI mtDNA analysis, Misiones, Pentastomid, 28S rDNA analysis.

INTRODUCTION

As part of a series of ongoing studies on parasites of freshwater fishes from Iguazu River, a pentastomid in the juvenile stage (nymph) was found encysted in the mesentery of *Phalloceros harpagos* (Cyprinodontiformes: Poeciliidae). This host is a small fish that prefers shallow waters with a muddy and sandy bottom, near the margin of the stream, where the water flow is low (Mazzoni et al. 2011). This fish species has a great variation in diet, consisting of detritus and aquatic insects (Ephemeroptera, Chironomidae, and Simuliidae), being considered as detritivore/insectivore (Uieda & Pinto 2011, Almeida Monaco et al. 2014, Pereira Neves et al. 2015, Carvalho Leitão et al. 2018).

Freshwater fishes have been reported as intermediate hosts for pentastomids of the families Sebekidae and Subtriquetridae. The adult species of Sebekidae are mainly parasites of crocodilians, chelonians, and monitor lizards (Christoffersen & De Assis 2013). In a monograph of Pentastomida, Christoffersen & De Assis (2013) evaluated the classification

of this subclass, retaining into Sebekidae the subfamilies Samboninae (*Sambonia* sp.), Diesingiinae (*Diesingia* sp., *Alofia* sp., *Selfia* sp.), and Sebekinae (*Agema* sp., *Pelonia* sp., *Sebekia* sp.), and erecting the new subfamily Leiperiinae to include three species of the genus *Leiperia*. The current arrangement in the classification of this group includes an additional genus, *Levisunguis*, which was described by Curran et al. (2014) and assigned to this latter subfamily.

There are few records of pentastomids in South America. According to Luque et al. (2013), there are reports for *Leiperia* sp. parasitizing freshwater fishes of the families Characidae, Erythrinidae, Pimelodidae, and *Sebekia* sp. found in fish hosts of the families Apteronotidae, Arapaimidae, Ariidae, Characidae, Cichlidae, Cynodontidae Electrophoridae, Osteoglossidae, Pimelodidae, Pistigasteridae, Poeciliidae, and Synbranchidae. Among the poeciliid fishes, the species *P. harpagos*, examined from the Cambé River, Brazil, was recorded as a host of *Sebekia oxycephala* (Almeida et al. 2009). Additional records for pentastomids have been done from some Argentinian vertebrates such as snakes (Cavalieri 1967, 1970, Troiano & Repetto 1994, Martinez et al. 1999, 2000), cayman (Fernandez et al. 2016), and armadillo (Martinez 1982, Martinez & Resoagli 1982). However, there are no records of other aquatic vertebrates such as Chelonians or fishes. This work aims to provide the record of Sebekidae nymphs found in the poeciliid fish *P. harpagos*, collected from the National Park Iguazu, Argentina, to describe their morphological features, and to apply molecular phylogenetic tools in order to explore the location of these specimens in the phylogenetic context of Pentastomida.

MATERIALS AND METHODS

Sampling and morphological study

A total of 30 *Phalloceros harpagos* were caught with hand nets in the Yacaratia stream, which belongs to the flood plain of Iguazu River up to the waterfalls (25°40´34¨ S 54° 27´ 08¨W) inside the Iguazu National Park, Argentina. The fishes were carried to the field camp in the Centro de Investigaciones Ecológicas Subtropicales (CIES), euthanized and examined for parasites under a magnifier glass. Some of the pentastomids were fixed in 10% formalin for morphological procedures, or in 96% ethanol for DNA extraction and sequencing. Samples were transported to the Laboratory of Fishes, Mollusks and Crustaceans Parasites (in the CEPAVE), where some individuals were cleared using lactophenol, and then observed under the microscope. The specimens were photographed with an AmScope MU 1000 10 MP digital camera (Irvine, Wisconsin) attached to an Olympus Bx51 microscope (Tokyo, Japan). The structures captured on microphotographs were measured using the ImageJ software. All measurements are given in micrometers unless otherwise stated, and expressed as the minimum and maximum values, followed by the mean in parentheses. The hooks measurements were taken according to Barton & Morgan (2016). The drawings were made with the aid of a camera lucida attached to an optical interference microscope Olympus BX53 (Tokyo, Japan). Additional specimens preserved in formalin were used for the observation under scanning electron microscopy (SEM).

DNA extraction, PCR amplification and sequencing

Some of the individuals conserved in 96% ethanol were used for the DNA analysis. The extraction was performed using a Wizard® Genomic DNA Purification Kit (Promega) according to the

manufacturer's protocol. The 28S rDNA and COI gene fragments were amplified by Polymerase-Chain Reaction (PCR) on an Eppendorf Mastercycler thermal cycler, using the forward primers LSU-5 (5'-TAG GTC GAC CCG CTG AAY TTA AGC A-3') and 1500R (5'-GCT ATC CTG AGG GAA ACT TCG-3') for the 28S rDNA (Tkatch et al. 2003), and the LCO1490 forward primer (5'- GGT CAA CAA ATC ATA AAG ATA TTG G-3') and the HCO2198 reverse primer (5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3') for the COI gen (Folmer et al. 1994). The reactions were prepared using Green GoTaq 5X Buffer (Promega), 2.5 mM $MgCl_2$ (Promega), 0.2 mM of NEB Nucleotide Mix, and Flexi GoTaq polymerase enzyme (Promega).

The PCR reactions were performed according to the protocols described by Tkach et al. (2003) for the 28s rDNA and by Folmer et al. (1994) for the COI mt-DNA gene. The PCR products were analyzed by electrophoresis in 1% agarose gel using TAE 1X buffer and observed under ultraviolet light. PCR products were purified and sequenced using an ABI 3730XLs sequencer, Macrogen Inc. (Korea).

Molecular data and phylogenetic reconstruction

The sequences obtained were edited using the platform Geneious Pro v.10 (Drummond et al. 2016) and used in the Genbank blast-n tool, searching for homologue sequences for comparison (Table I). For each gene, the sequences were separately aligned using the online version of MAFFT v.7 (Katoh & Standley 2016). The 28S rDNA dataset was analyzed on the Gblocks Website (Castresana 2000, Talavera & Castresana 2007) to detect ambiguously aligned hypervariable regions, and were excluded from the analysis using a less stringent selection (allowing smaller final blocks, gap position within the final blocks, and less strict flanking positions).

The best partitioning scheme and substitution model for the 28S rDNA and COI mt-DNA were chosen under the Bayesian Information Criterion (BIC; Schwarz 1978) using the 'greedy' search strategy in Partition Finder v.1.1.1 (Lanfear et al. 2012). The appropriate nucleotide substitution model implemented for the 28S rDNA matrix resulting after the Gblock program was K80, and for COI mt-DNA was TrN+G, for the first, F81+G for the second, and HKY+G for the third position.

Additionally, the proportion (*p*) of absolute nucleotide sites (*p-distance*) (Nei & Kumar 2000) was obtained to compare the genetic distance between selected copepod species. The *p*-value matrix was obtained using MEGA X (Kumar et al. 2018), with variance estimated with the bootstrap method (1000 replicates) and with a nucleotide substitution (transition + transversions) uniform rate.

Phylogenetic reconstruction was carried out using Bayesian Inference (BI) through MrBayes v.3.2.3 (Ronquist et al. 2012). Phylogenetic trees were constructed using two parallel analyses of Metropolis-Coupled Markov Chain Monte Carlo (MCMC) for 20 million generations each, to estimate the posterior probability (PP) distribution. Topologies were sampled every 1,000 generations and the average standard deviation of split frequencies was observed to be less than 0.01, as suggested by Ronquist et al. (2012).

The robustness of the clades was assessed using Bayesian Posterior Probability (PP), where PP > 95 (in percentage) was considered strongly supported. A majority consensus tree with branch lengths was reconstructed for the two runs after discarding the first 25% of the sampled trees.

Table I. Sequences utilized in the molecular analysis based on the 28S rDNA and COI mtDNA genes.

Family	Specie	host	Country	Sequence		Author
				COI	28s RDNA	
Linguatulidae	*Linguatula artica*	*Rangifer tarandus*	Norway	KF029443		a
Linguatulidae	*Linguatula serrata*	*Bos indicus*	Bangladesh	LC150783 LC150784		b
Porocephalidae	*Armillifer agkistrodontis*		China	FJ607340		c
Porocephalidae	*Kiricephalus coarctatus*	*Python bivittatus*	USA	MG559656 MG559657 MG559658		d
Porocephalidae	*Porocephalus cotali*	*Python bivittatus*	USA Germany	MG559647 MG559652 MG559655	EF417058	d,e
Porocephalidae	*Waddycephalus* sp.	*Stegonotus cucullatus*	Australia	KF885748 KF885768 KF885769 KF885780		f
Raillietiidae	*Raillietiella* sp.				AY744894	g
Sebekidae	*Alofia Merki*		Australia	KU975383 KU975384	KU975382	h
Sebekidae	*Alofia* sp.	Pseudoplatystoma fasciatum	Peru	MH484065		i
Sebekidae	**"*Diesingia* sp."**	***Phalloceros harpagos***	**Iguazú, Argentina**	**MN518003**	**MN521701 MN521702**	**This manuscript**
Sebekidae	*Sebekia mississippiensis*	*Lepisosteus oculatus*		MK248486 MK248488 MK248489		j
Sebekidae	*Sebekia purdieae*		Australia	KU975386	KU975381	h
Sebekidae	*Sebekia* sp. 2		Australia	KU975387	KU975379 KU975380	h

Authors: a= Gjerde (2013), b= Itagaki & Mohanta (unpublished) c= Chen et al. (unpublished) d= Miller et al. (2017), e= Sonnenberg et al. (2007), f= Kelehear et al. (unpublished), g= Giribet et al. (2005), h= Barton & Morgan (2016), i= Gomez-Puerta et al. (unpublished), j= Woodyard et al. (2019).

RESULTS

Based on the morphological features and the molecular analysis, the examined specimens were tentatively assigned to the genus *Diesingia* Sambon, 1922.

Description of the specimens

Infective nymphs (measurements based on 12 specimens) were found attached to the body wall cavity of the hosts, encapsulated in a thin layer. Fresh specimens were white in color and had intestines with a red content. The body was 3,576-4,387 (3,982 mm) long and 660-956 (808) wide. Annuli margins were easily discernible in whole mounts (Fig 1a), with a total count of 63-74 (67). The annuli lacked interrupted rows of spines, which were similar in length and distributed all over the body. Between each annulus, the chloride cell pore was distributed in a single row (Fig 1b).

Hooks were arranged around mouth opening (Fig. 1c). The U-shaped buccal open anteriorly

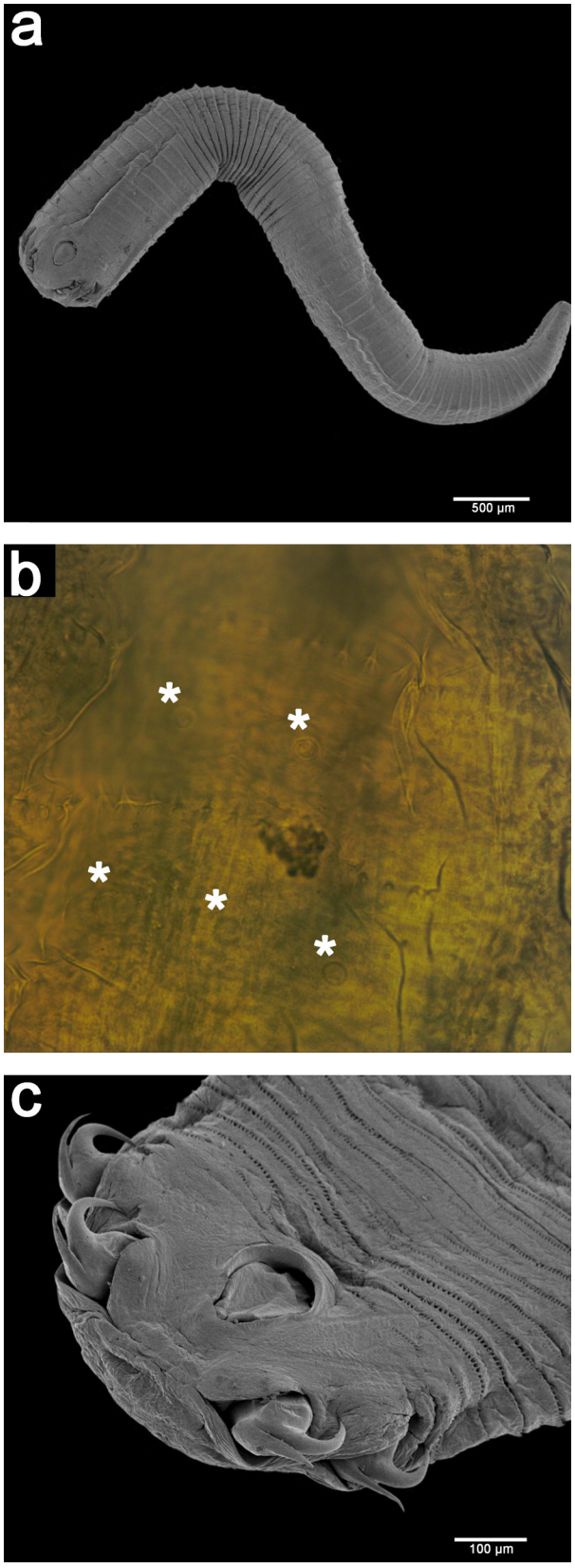

Figure 1. ***Diesingia*** **sp from** ***Phalloceros harpagos*****. a.- entire nymph. b.- Chloride cell pore distributed in a single row. c.- Detail of the anterior end with the four hooks and the oral cadre.**

cadre presented almost parallel sides of thick cadre (Fig.2a-c). The posterior edge of the oral cadre was rounded and thick, situated between the fulcra of the anterior hook pair. The anterior edge or oral cadre was approximately at the level of the posterior edge of the anterior hooks. The buccal cadre was 282-319 (300) long and 151-163 (157) wide. The posterior lateral extensions of the cadre were 102-122 (112).

Anterior and posterior hooks were sharp tipped, all with dorsal accessory pieces (Fig. 1c, 2a, 3a-e). Hooks and dorsal accessory pieces were long, stout, slightly curved fulcra, visible under the dissection microscope. Anterior hooks (Fig 3b, 3d): dorsal accessory piece (DAP) 137-195 (166), blade length (AC) 136-149 (143), hook length (AD) 144-172 (158), base length (BC) 79-93 (86), plateau length (CD) 36-67 (51), hook gape (AB) 55-61 (58), fulcrum length (FL) 216-314 (265). Posterior hooks (Fig 3c, 3e): DAP 143-166 (155), AC 136-149 (143), AD 136-149 (143), BC 90-98 (94), CD 41-52 (46), AB 53-61 (57), FL 271-296 (284). Total hooks: DAP 145-161 (153), AC 138-146 (142), AD 138-146 (142), BC 89-95 (92; 1.5), CD 41-49 (45), AB 55-61 (58), FL 278-297 (287).

Prevalence: 50 %

Intensity: 2,07.

Mean abundance: 0,99.

Genetic results

One sequence of COI mt-DNA with 582bp was obtained and compared with those downloaded from the GenBank (Table I). Almost all the sequences downloaded from GenBank are well supported by the literature, but some of them as *Linguatula serrata*, *Armilifer agkistrodontis*, *Waddycephalus* sp. and *Alofia* sp. are unpublished. Most of these sequences

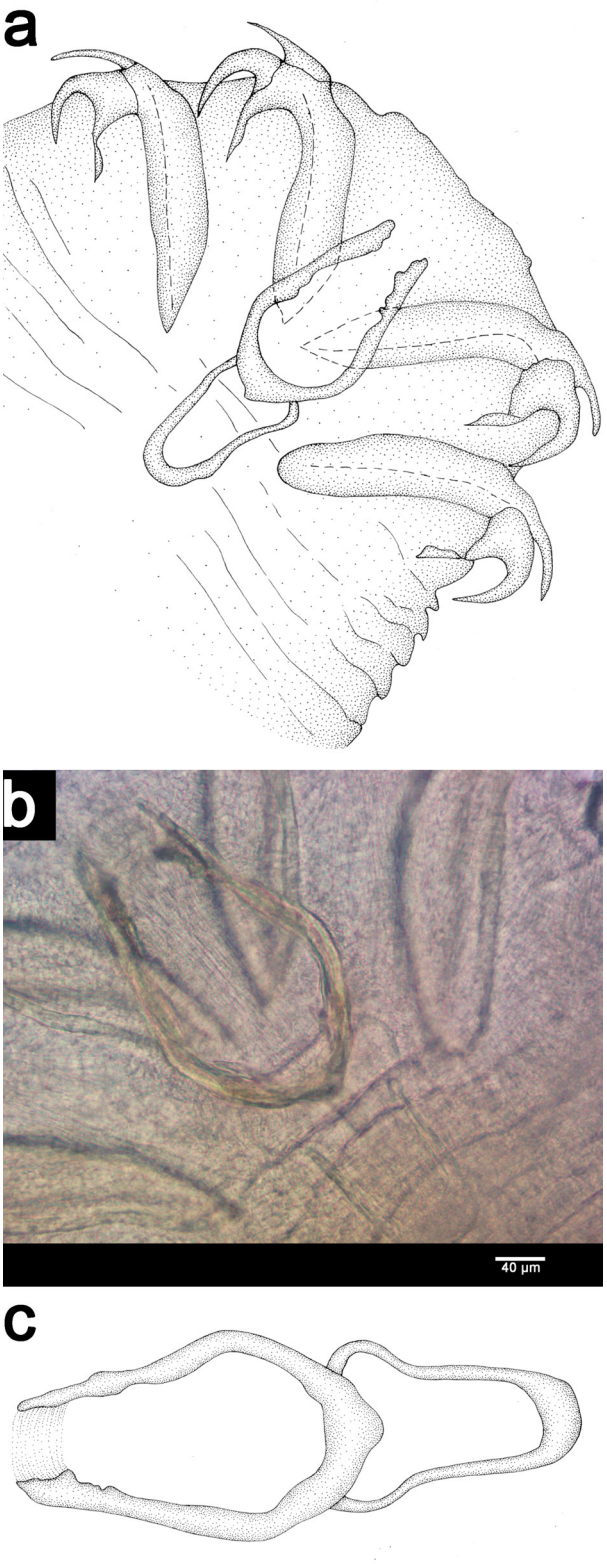

Figure 2. a.- Drawing of the anterior end of *Diesingia* sp. showing the position of the oral cadre between the hooks. b.- Photograph of the oral cadre. c.- Drawing of the oral cadre.

were reported from Europe, North America and Oceania, while only one was reported from Peru. The Sebekidae family is represented by two genera (*Alofia* and *Sebekia*) in the sequences deposited in GenBank. A matrix with 723 bp was performed and used to construct a phyllogram, using the species *Raillietiella hebitihamata* as outgroup, (Fig 4). The resultant tree showed well resolution. The analyzed sequence constitutes a well-supported clade, having the clade containing species attributed to the families Sebekidae and Porocephalidae as sister group. The clade formed by species belonging to the genus *Kiricepahlus*, *Porocephalus* and *Amilifer* (all of them assigned to Porocephalidae) was well supported (97% PP), but was related (86% PP) to the genus *Alofia* sp. (assigned to Sebekidae). These relationships, along with those showed in the sister clade, where taxa of the genus *Waddycephalus* (Porocephalidae) arising as sister group of the specimens of *Sebekia* (Sebekidae) (PP value = 100%; being the PP between them also higher than 99%), strongly suggest that these families have a paraphyletic arrangement. The *p*-distance showed values over 20% to separate different genus, and less than 20% to separate between species. The distance between our specimen and the other genera varied from 21 to 25 % (Table II).

Two sequences of 28s rDNA with 1167 and 1280 bp, respectively, were obtained and used to download the homologue sequences deposited in the GenBank (Table I). Contrary to the sequences of COI downloaded from GenBank, all of the sequences are well supported by the literature. These sequences are reported from Europe, North America and Oceania..The matrix constructed after excluding the hypervariable

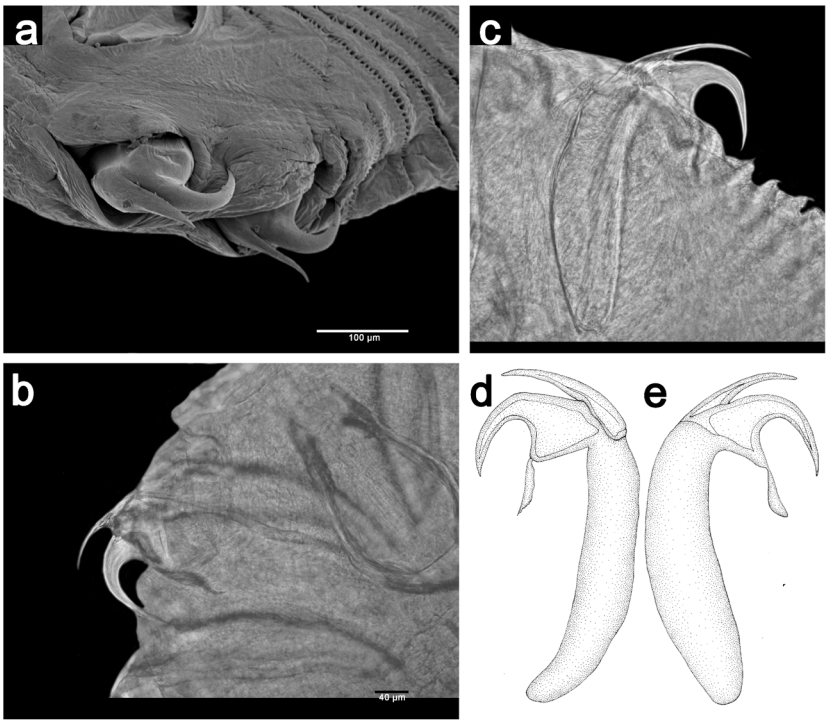

Figure 3. Hooks of *Diesingia* sp. a.- Microphotograph of the anterior end showing the nymphal doble hooks. b.- Photograph of the anterior hook. c.-Photograph of the posterior hook. d.- Drawing of the anterior hook. e.- Drawing of the posterior hook.

regions had 1090 bp, with *Raillietiella* sp. used as outgroup. The tree obtained (Fig 5) showed that the sequences obtained were related to those assigned to *Alofia merki*. However, this relationship showed a low support (55% PP). This tree was also related to those sequences assigned to *Sebekia* with the available sequence of *Porocephalus* sp. (65% PP). The *p*-distance between our specimens and the closest genus, *Alofia*, was 12%, while between the genus *Sebekia* and *Porocephalus* varied between 18% and 19% (Table III).

DISCUSSION

Pentastomida has been recently confirmed by genetic analysis as a subclass inside the Crustacea with a much modified parasitic life (Regier et al. 2010, Poore 2012). However, the phylogenetic relationships inside this group are still uncertain. Therefore, the current taxonomical arrangement among families, and genera inside them, arises as a promissory research topic for future years. Since the molecular approach is still far to provide an accurate taxonomical resolution, more species and genera must be sequenced in order to clarify the phylogeny of this fascinating group.

A factor increasing the issues to understand the relationships among Pentastomida are the poor knowledge of the real diversity of this group. In this sense, the nymphs of some genera, as well as their life cycles, and potential hosts remain unknown. Information about pentastomids in Argentina is limited to some eventual records in Ophidia and Dasypodidae (Cavalieri 1967, 1970, Martinez 1982, Martinez & Resoagli 1982, Troiano & Repetto 1994, Martinez et al. 1999, 2000, Fernandez et al. 2016), and nothing is known concerning nymphs or adults of other groups of aquatic vertebrate hosts, like turtles or fishes. In other South American countries such as Brazil and Peru, some works about the diversity of pentastomids have been published (see Garate et al. 2007, Luque et al. 2013), providing some light about this diverse

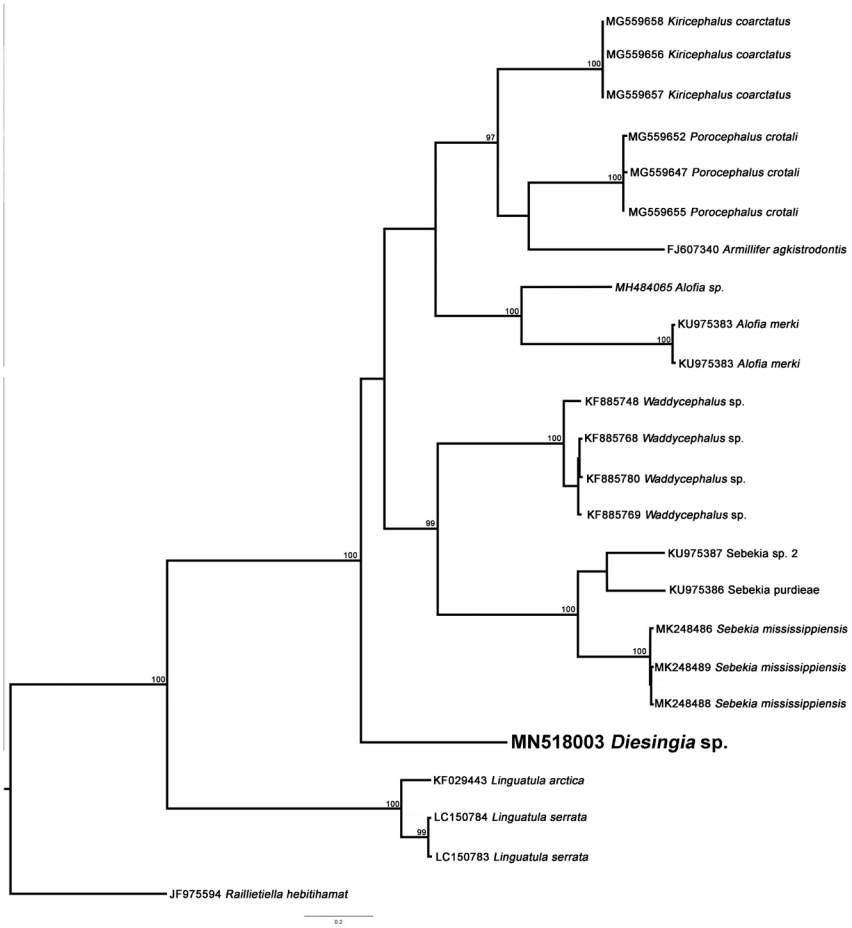

Figure 4. Phylogram resulting from Bayesian Inference (20,000,000 generations) of partial COI mtDNA gene sequences of "*Diesingia* sp." Branch support values indicate posterior probabilities.

parasitic group. However, the use of molecular tools in these works is very limited.

Most of the published studies in South America have been made for adult pentastomids, being relatively few those records of nymphs infecting different species and organs of fishes (Almeida et al. 2009, Giesen et al. 2013, Luque et al. 2013). In Australia, Barton & Morgan (2016) recently started the study of this group using DNA tools, bringing some new sequences and morphological information about nymphs, which was used here to elucidate the affiliation of the nymphs found inside the poeciliid host *Phalloceros harpagos*.

The morphology of the nymphs found here agrees with the description of Sebekidae family (Pentastomidae), having a vermiform body, anterior and posterior hooks with two blades (Luque et al. 2013), sinuous alimentary canal longer than the body, and distinctly convex hooks (Christoffersen & De Assis 2013). Inside this family, the subfamily Diesingiinae is composed of *Diesignia* (2 species), *Alofia* (3 species), and *Selfia* (1 species), which are characterized by having a chloride cell pore distributed in a single row per annulus, (Christoffersen & De Assis 2013). This feature agrees with the material examined here, which allows us to distinguish between Sebekinae and Diesingiinae. The hooks of the nymphs of *Alofia* and *Selfia* have rows of reduced spines (Christoffersen & De Assis 2013), which were not present in our specimens. However, nymphs of the remaining genus *Diesingia* had not been described or reported until now. *Diesingia* was described from material collected from turtles

Table II. Genetic distances matrix (uncorrected p-distance) for pentastomids. The percentage values are derived from the COI mt-DNA.

	1	2	3	4	5	6	7	8	9	10	11	12
1.- *Raillietiella habitihamat* JF975594 (Outgroup)												
2.- *Kiricephalus coarctatus* MG559656-58	29											
3.- *Porocephalus crotali* MG559647,52,55	31	20										
4.- Amilifer agkistrodontis FJ607340	30	21	20									
5.- Alofia sp. MH484065	29	23	23	24								
6.- Alofia merki KU975383-84	32	25	23	24	18							
7.- Waddycephalus sp. KF885748, 68-70	31	21	23	25	25	26						
8.- Sebekia sp. 2 KU975387	32	24	23	24	26	25	23					
9.- Sebekia purdiae KU975386	31	23	24	23	24	26	22	15				
10.- Sebekia mississippiensis MK248486, 88-89	32	23	23	24	24	25	23	18	16			
11.- *Diesingia* sp. MN518003	32	21	22	25	24	26	23	22	23	21		
12.- Linguatula ártica KF029443	28	29	28	28	31	31	29	32	32	31	**30**	
13.- Linguatula serrata LC150783-84	28	28	30	27	30	30	29	33	32	30	**30**	10

in Brazil (Heymons 1941, Da Fonseca & Ruiz 1956, Self & Rego 1985, Junker et al. 2003), and because the concordant morphological features between this genus and our material, this was tentatively assigned to *Diesignia*. Further collections of specimens linking the nymphal stage with adults will allow to corroborate this assignation or to suggest the presence of a new genus. In order to evaluate some morphological traits between previously described adults and our material, the species *Diesingia megastoma* have between 65-70 annuli (Heymons 1941), and the oral cadre is almost twice larger than wide (Heymons 1941, Da Fonseca & Ruiz 1956, Self & Rego 1985, Junker et al. 2003). In our specimens, the oral cadre was U-shaped, with fibers anteriorly closing, as was mentioned by Self & Rego (1985). Junker et al. (2003) confirmed the U-shape. However, they suggest that the presence of the fibers could be caused by pressure during the sample mounting process. About that, we observed that the specimens mounted under different pressure conditions always showed those fibers. Another feature supporting the nearness of our material to *Diesingia* is the clear peg-like extension of the oral cadre, present in the analyzed nymphs, and also mentioned by Junker et al. (2003) in the re-description of *D. megastoma*. According to Junker et al. (2003), the oral cadre of *D. megastoma* exceeds the size of the hooks. Even though this corresponds to the description of adults, Giesen et al. (2013) considered that this feature could be reflected in the larvae. This is not the case of our specimens, where the relation is quite similar between oral cadre and hooks. However, it is possible that in the last

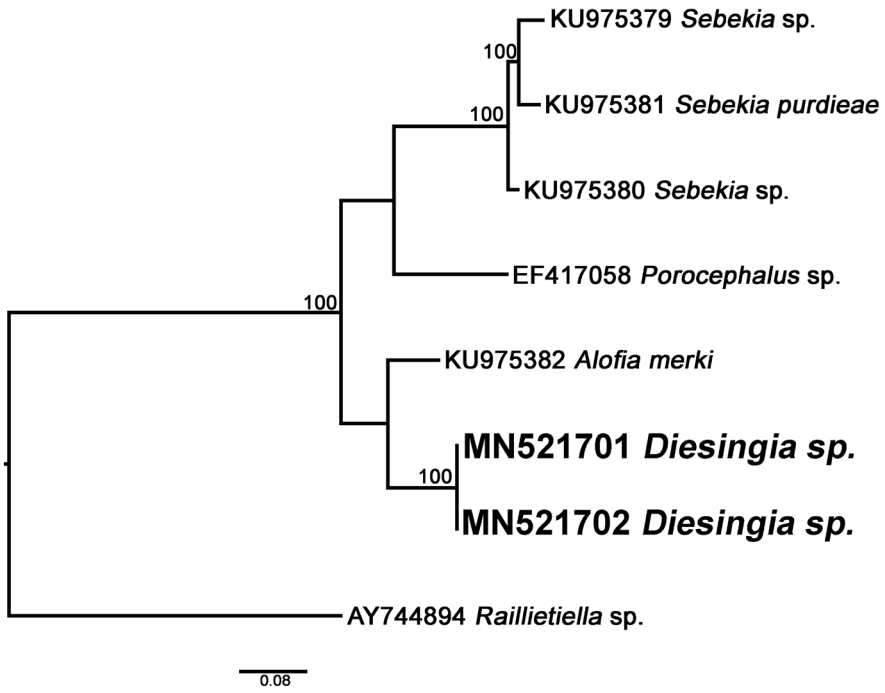

Figure 5. Phylogram resulting from Bayesian Inference (20,000,000 generations) of partial 28 rDNA gene sequences of "*Diesingia* sp." Branch support values indicate posterior probabilities.

moult previous to the adult stage, the oral cadre grew bigger than the hooks. Even if it was not the case, the morphology of the nymphs matches better with the genus *Diesingia* than the other members of Sebekidae. Further morphological and molecular analyzes, including samples of nymphs and adults from Brazil and Argentina, are necessary to solve the taxonomical identity of the specimens here described.

The only published report of pentastomid nymphs in the fish *P. harpagos* corresponds to specimens collected from Brazil, which show morphological features very similar to those presented here but registered as *Sebekia oxycephala* (Almeida et al. 2009). Clearly, this taxonomical assignation must be re-evaluated, since the morphological information linked to the genetic analysis suggests that specimens of the genus *Sebekia* are phylogenetically far from our samples, with a *p*-distance of 18-19% in the 28S-rDNA gen and 21-23% in the COI mt-DNA gen. These values of genetic divergence support the inclusion of the samples in another genus (in this case, conservatively assigned to *Diesingia*).

The phylogenetic analysis based on the COI mt-DNA presented here situates our material as the sister group of a big clade conformed by specimens assigned to different genera belonging to the families Sebekidae and Porocephalidae. These relationships, where taxa such as *Porocephalus*, *Kiricephalus*, *Armillifer*, and *Waddycephalus* (Porocephalidae) are inserted among specimens of *Diesingia*, *Sebekia*, and *Alofia* (Sebekidae), strongly suggest that these families have a paraphyletic arrangement. This assumption is reinforced by the phylogenetic analysis of the 28S rDNA, which shows a well-supported clade including specimens of *Alofia*, *Sebekia*, and the analyzed *Diesingia* (Sebekidae), with a sequence of *Porocephalus* inserted as sister of *Sebekia*. Both phylogenetic trees corroborating the molecular distinction of our samples from the genera *Sebekia* sp. and *Alofia* sp. leading us to choose the more parsimonious possibilities, where the

Table III. Genetic distances matrix (uncorrected p-distance) for pentastomids. The percentage values are derived from the 28S rDNA.

	1	2	3	4	5	6	7
1.- *Raillietiella* sp. (outgroup)							
2.- *Alofia merki* KU975382	30						
3.- *Diesingia* sp. MN521701-02	**30**	**12**					
4.- *Sebekia* sp. KU975379	32	18	**19**				
5.- *Sebekia purdieae* KU975381	32	17	**18**	5			
6.- *Sebekia* sp. KU975380	32	18	**19**	5	4		
7.- *Porocephalus* sp. EF417058	31	17	**18**	19	19	18	

nymphs found parasitizing *P. harpagos* belongs to *Diesingia* sp. Christoffersen & De Assis (2013) differenced morphologically the families inside the Pentastomida characterizing Porocephalidae by the hooks aligned in adults and Sebekidae by having alimentary canal sinous (longer than body), hooks distinctly convex (with spines large and proeminent), and nymphal double hooks. These features could be insignificant and both families could be synonymized in the future. These observations are concordant with the suggestions of Barton & Morgan (2016) who, based on the analysis of the 18S rDNA and the COI mt-DNA markers, noticed that *Sebekia* and *Alofia* showed affiliations with members of the family Porocephalidae.

As a conclusion, the DNA analysis situates our specimens far to the genus *Sebekia*, and the morphological analysis shows that they are similar to *Diesignia*. The present finding represents the first mention for this genus in Argentina. Further analysis of adult and nymph samples will allow to corroborate the specific identity of the specimens. *Diesingia* is the only genus that exclusively uses chelonian as definitive host, and there are reports of this pentastomid in the species *Hydromedusa tectifera* and *Phrynops geoffroanus* (= *Hydraspis geoffroyana*) from Brazil (Diesing 1836, Sambon 1922, Heymons 1941, Da Fonseca & Ruiz 1956, Overstreet et al. 1985, Self & Rego 1985, Riley 1994). Based on these records, future collections must be done having aquatic fish-eating turtles as a principal target, being also advisable to examine other aquatic reptiles such as fish-eating snakes.

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MARTÍN MIGUEL MONTES[1]
https://orcid.org/0000-0002-7177-333X

JORGE BARNECHE[1]
https://orcid.org/0000-0002-8451-6906

NICOLAS LEGUNDA[1]
https://orcid.org/0000-0001-9703-7368

WALTER FERRARI[1]
https://orcid.org/0000-0003-3995-5122

ROGELIO AGUILAR-AGUILAR[2]
https://orcid.org/0000-0002-2414-6384

SERGIO ROBERTO MARTORELLI[1]
https://orcid.org/0000-0002-5375-9155

[1]Centro de Estudios Parasitológicos y Vectores (CEPAVE-UNLP-CONICET-), Boulevard 120 s/n e/60 y 64, C.P. 1900, La Plata, Buenos Aires, Argentina

[2]Departamento de Biología Comparada, Facultad de Ciencias, Universidad Nacional Autónoma de México, Circuito Exterior s/n, C.P. 04510, Ciudad Universitaria, Mexico

Correspondence to: **Martín Miguel Montes**
E-mail: martinmiguelmontes@gmail.com.

Author contributions

Martin Montes: fish collect, conceptualization of the manuscript, parasite study, measurement of structures, phyllogenetical analysis, principal writer of the manuscript. *Jorge Barneche*: Fish collect, parasite search, photos and figures edition, and help with the conclusion part. *Nicolas Legunda*: Parasite search, measurement of structures and help with the Material and Methods part (measurements, and preparation of parasites). *Walter Ferrari*: DNA extraction, and phyllogenetical studies and help with the Material and Methods part (molecular part). *Rogelio Aguilar-Aguilar*: analysis of the bibliography, editing and revision and help with the introduction and conclusion part. *Sergio R. Martorelli*: Source founding and manuscript supervision and coherence between each part.