FISH PARASITOLOGY - ORIGINAL PAPER

New insights on *Pomphorhynchus sphaericus* Gil de Pertierra, Spatz et Doma, 1996 (Acanthocephala: Pomphorhynchidae)

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Abstract

The finding of *Pomphorhynchus sphaericus* in new localities from La Plata River allowed the reevaluation of the species using a taxonomic integrative approach. The newly found specimens in *Pimelodus maculatus* from Samborombon Bay differ from *P. sphaericus* by the roots of hooks 1–6 which not form a wide sheet split into 2 apophysis, the slender, separated and equatorial testicles, the position of the cement glands, the shape of the proboscis, the shape and length of lemnisci, and the eggs size. Despite the notorious observed morphological differences, the COI mtDNA analysis confirmed that *Pomphorhynchus* individuals are the same conspecific, and showed that there is a high phenotypical plasticity in this species. *Pomphorhynchus sphaericus* is the first South American species analyzed to a DNA level (COI mtDNA, ITS, and 18S rDNA genes). The molecular analysis relates *P. sphaericus* to *P. bulbocolli* and *P. purhepechus*.

Keywords Pomphorhynchus · Acanthocephalan · Argentina · Brackish waters · Pimelodus

Introduction

The genus *Pomphorhynchus* Monticelli, 1905 currently includes 31 valid species (Amin 2013; Garcia-Varela et al. 2017; Li et al. 2017). Species of *Pomphorhynchus* shows a worldwide distribution and with most of them known from freshwater fishes. To date, only five species were reported in freshwater fishes of South America. These are *Pomphorhynchus moyanoi* Olmos & Habit, 2007 and *Pomphorhynchus yamagutii* Schmidt & Hugghins, 1973 from Chile parasitizing *Percilia gillissi* Girard and *Percichthys melanops* Girard (Percichthyidae), respectively; and three species from Argentina, *Pomphorhynchus omarsegundoi* Arredondo & Gil de Pertierra, 2010 parasitizing *Gymnotus carapo* Linnaeus (Gymnotidae); *Pomphorhynchus patagonicus* Ortubay et al., 1991 parasitizing several freshwater fish species of Patagonia; and *Pomphorhynchus sphaericus* Gil de Pertierra et al., 1996 parasitizing freshwater pimelodids from the Parano-Platense River basin (Schmidt and Hugghins 1973; Ortubay et al. 1991; Gil de Pertierra et al. 1996; Olmos and Habit 2007; Arredondo and Gil de Pertierra 2010). Recently,

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Hernández-Orts et al. (2019) provided a complete list of Argentinean *Pomphorhynchus* fish hosts.

Another pomphorhynchid species, *Pomphorhynchus patii*, was described by Lunaschi, 1997 parasitizing *Luciopimelodus pati* (Valenciennes) and *Parapimelodus valenciennis* (Lütken) (both Pimelodidae), but it was considered a junior synonym of *P. sphaericus* based on similarities in morphological features, fish host, and geographical distribution (Amin et al. 2003). Nevertheless, some relevant differences can be observed between these, mainly with respect to the proboscis armature and the morphology of hook roots (Gil de Pertierra et al. 1996; Lunaschi 1997).

During surveys of fish parasites from Samborombón Bay (located in the brackish waters area La Plata River estuary) and Parana River basin, specimens of an acanthocephalan species identified as *P. sphaericus* were found in *Pimelodus maculatus* Lacepède (yellow-mandi catfish). The finding of these individuals leads us to study their morphology, and to make a molecular approach using the COI mtDNA, ITS, and 18S rDNA genes to elucidate the real filiation of this species.

Materials and methods

Collection of samples and morphological study

Ten *P. maculatus* were collected from Salado Relief Channel (35° 50′ S, 57° 25′ W) using cast nets and hand nets. Alive fishes were carried in bags to the laboratory with water from the sample site and added oxygen, and then kept in aquariums in the laboratory. Finally, the fishes were euthanized, dissected under a stereomicroscope, and the intestines examined for acanthocephalans.

Acanthocephalans found in the intestine were carefully detached from the intestinal wall, washed in saline solution, placed in distilled water at 4 °C for a few hours to relax and evaginate proboscides, fixed in 10% formalin, and stored in 70% ethanol. Some of the recovered *Pomphorhynchus* specimens were conserved in 96% alcohol for molecular studies. For morphological studies, the specimens were stained with chlorhydric carmine, dehydrated in a graded ethanol series according to the laboratory protocols (Pritchard and Kruse 1982), cleared in clove oil, and mounted in Canada balsam. Other specimens were unstained and cleared in lactophenol. The drawings were made with the aid of a drawing tube attached to an optical interference Olympus BX53 microscope. Measurements (expressed as the range, followed by the mean in parentheses) are given in millimeters (mm), unless otherwise stated. The hook ranges are given in micrometers. The trunk length does not include the neck, bulb, or proboscis. Parasitological descriptors were calculated according to Bush et al. (1997).

The vouchers were deposited in the Helminthological Collection of the Museo de La Plata, Buenos Aires, Argentina (MLP).

Molecular analysis

Parasite DNA was extracted from two individual specimens using Wizard® Genomic DNA Purification Kit (Promega) and according to the manufacturer's protocol. To secure the extraction and presence of DNA, no hologenophore specimens were saved. Instead, entire acantocephalan specimens were used.

The COI mtDNA gene was amplified by PCR on an Eppendorf Mastercycler thermal cycler using the Folmer et al. (1994) primers: 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′).

The partial segment 18S rDNA gene was amplified by PCR using the Near et al. (1998) primers: 1073F forward primer (5′-CGG GGG GAG TAT GGT TGC-3′) and the 18SR reverse primer (5′-TGA TCC TTC TGC AGG TTC ACC TAC-3′).

The partial ITS region was amplified by PCR using the Králóvá-Hromadová et al. (2003) primers: BD1 forward primer (5′-GTC GTA ACA AGG TTT CCG TA-3′) and the BD2 reverse primer (5′-TAT GCT TAA ATT CAG CGG GT-3′).

The reactions were carried out with GoTAQ Master Mix (Promega) according to the manufacturer's protocol, using the thermocycling conditions proposed by Gomez et al. (2002) for a portion of COI mtDNA gene, Perrot-Minnot (2004) for the partial 18S rDNA gene, and Králóvá-Hromadová et al. (2003) for the ITS rDNA gene.

The PCR products were analyzed by electrophoresis in 1% agarose gel using TAE 1 × buffer supplemented with 2 µl of ethidium bromide in the presence of UV light. Sequencing for each sample was carried out for both stands in a specialized laboratory (Macrogen, Korea).

Additionally, one specimen of *P. sphaericus* ex *Pimelodus maculatus* from Colastiné River (tributary of Parana River, 31° 39′S 60° 46′W) was used to extract the DNA and sequence the COI mtDNA.

The accuracy of the sequencing data was confirmed by sequencing in both directions. All sequences were edited using the platform Geneiuos R11 under free trial (http://www.geneious.com, Kearse et al. 2012) and the consensus sequence was built with the MUSCLE (Edgar 2004) alignment tool within Geneious with final edition "by eye" in the same platform. For the barcode sequences, we checked the nucleotide alignment, and for the presence of pseudogenes in Geneious, we used the translated amino acid sequences based on the invertebrate mitochondrial genetic code.

The consensus of each pair of COI mtDNA, ITS, and 18S rDNA sequence obtained after MUSCLE alignment was used to search homologues in the GenBank with the BLASTn tool (Table 1) and then the sequences were aligned using the online version of MAFFT v.7 (Katoh et al. 2017). The alignment was trimmed to the length of the shortest sequence, eliminating any poorly aligned regions of the rDNA using the online program Gblocks v0.91 (Castresana 2000; Talavera and Castresana, 2007) with relaxed parameters.

The best partitioning scheme and substitution model for each DNA partition were chosen under the Akaike information criterion (AIC; Posada and Buckley 2004) in Jmodeltest2.1 (Darriba et al. 2012). The barcode fragment dataset was partitioned into first, second, and third codon positions with the appropriate nucleotide substitution model implemented for each codon position (TIM2 + I + G for the first, TRN + G for the second, and TPM1uf + G for the third codon position). The appropriate nucleotide substitution models for the ITS and 18S rDNA were TVM + G and TIM2 + I + G, respectively.

According to the analysis made by Li et al. (2017), sequences of *Acanthocephalus nanus* were used as outgroup taxa (Table 1).

The phylogenetic reconstruction was conducted using Bayesian Inference (BI) through MrBayes v. 3.2.1 (Ronquist et al. 2012). The COI mtDNA, 18 s rDNA, and ITS rDNA trees were constructed using 628, 1770, and 612 bp with 19, 11, and 21 taxa included in the analysis. In addition, a concatenated tree was constructed including all the species.

The phylogenetic trees were reconstructed using two parallel analyses of Metropolis-Coupled Markov Chain Monte Carlo (MCMC) for 20×10^6 generations each, to estimate the posterior probability (PP) distribution using Bayesian Inference through MrBayes v. 3.2.1 (Ronquist et al. 2012). Topologies were sampled every 1000 generations. The first 25% of the sampled trees were discarded as "burn in." The consensus tree was visualized in FigTree 1.4.2 (Rambaut 2009).

The proportion (*p*) of absolute nucleotide sites (*p*-distance) was obtained to compare the genetic distance among and between lineages as was described by Castro-Romero et al. (2016) using Mega X (Kumar et al. 2018).

Results

Pomphorhynchidae Yamaguti, 1939.
    Pomphorhynchus Monticelli, 1905.
    Pomphorhynchus sphaericus Gil de Pertierra et al., 1996 (Fig. 1 and Table 2).
    Palaeacanthocephala, Pomphorhynchidae, with the characters of the genus *Pomphorhynchus*. Fixed white to

light orange individuals. Cylindrical proboscis, enlarged at its anterior third. Hooks arranged in 12 slightly spiralling longitudinal rows, each one armed with 15 hooks with simple roots. Basal crown with large hooks separated from the proper proboscis. Bulb like a posterior expansion of the proboscis, spherical to subspherical. Neck without bulb, shorter than the trunk. Cylindrical trunk with swollen anterior region and slightly thinner at the posterior end. Proboscideal receptacle with a double wall, bag shape, usually extending barely into the trunk. Unequal, short, and cylindrical lemnisci.

Male (based on 7 specimens): trunk 5.24–6.99 (6.14) long, 0.35–0.59 (0.47) wide (Fig. 1A). Proboscis 0.41–0.68 (0.51) long, 0.14–0.26 (0.19) wide, with 12 hook rows, each row with 15 hooks (Fig. 1B). Proboscis hooks length and root length in Table 1 (Fig. 1C). Bulb 0.86–1.11 (0.95) long, 0.78–1.16 (0.93) wide. Neck without bulb 1.84–2.13 (1.96) long, 0.32–0.49 (0.41) wide. Proboscideal receptacle 2.90–3.50 (3.20) long, 0.08–0.12 (0.1) wide. The longest lemnisci with 0.44–0.78 (0.57) long, 0.10–0.14 (0.12) wide. The shortest lemnisci with 0.41–0.62 (0.5) long, 0.08–0.14 (0.11) wide. Oval, equatorial testes, in tandem and slightly separated from each other, anterior testis 0.43–0.57 (0.51) long, 0.24–0.32 (0.28) wide, posterior testis 0.49–0.57 (0.53) long, 0.24–0.35 (0.3) wide. Six pyriform cement glands, similar in shape and arranged 1–1–2–2, without the conducts, 0.3–0.38 (0.35) long, 0.05–0.14 (0.09) wide. Ovoid Saefftigen's pouch, 0.65–0.78 (0.69) long, 0.16–0.19 (0.18) wide.

Females (based on 10 gravid specimens from *P. maculatus*): trunk 4.37–8.4 (6.69) long, 0.46–0.65 (0.55) wide. Proboscis 0.38–0.49 (0.43) long, 0.16–0.22 (0.18) wide. Proboscis hook length and root length in Table 2. Bulb 0.97–1.57 (1.21) long, 0.95–1.38 (1.10) wide. Neck without bulb 1.46–2.65 (1.82) long, 0.27–0.41 (0.34) wide. Proboscideal receptacle 2.0–4.2 (2.8) long, 0.09–0.13 (0.10) wide. Longest lemnisci 0.34–0.44 (0.38) long, 0.07–0.17 (0.10) wide. Shortest lemnisci 0.23–0.38 (0.29) long, 0.07–0.08 (0.08) wide. Ovary along the anterior 2/3 of the trunk with 2.35–4.86 (4.07) × 0.11–2.97 (0.17). Uterine bell located in the beginning of the posterior 1/3 of body. From there to the posterior end of the trunk, we find the uterus measuring 1.19–2.03 (1.64) × 0.08–0.14 (0.11). Fusiform eggs (in μm) 52–76 (62) × 8–12 (11), with polar prolongations (Fig. 1E).

Taxonomic summary

Host: *Pimelodus maculatus* Lacepède (Characiformes: Pimelodidae).

Site of infection: Attached to the intestine; proboscis and bulb penetrating into or through intestinal wall and body in intestinal lumen. Some specimens induced a host

Table 1 Species, host, locality, and accession numbers of sequences of COI, 18S, and ITS of the acanthocephalan species included in the phylogenetic analyses

Species	Host	Locality	COI	18S	ITS	References
Acanthocephalus nanus	*Cynops pyrrhogaster* (Boie, 1826)	Japan	LC100070	LC129889	LC100043	Nakao 2016
Longicolllum pagrosomi	*Oplegnathus fasciatus* (Temminck & Schlegel, 1844)	China	KY490048	KY490052	KX245131	Li et al. 2017
Pomphorhynchus bosniacus	*Barbus barbus* Linnaeus, 1758	Bosnia and Herzegovina	MH319900 MH319901 MH282839			Nedic and Vardic Smrzlic 2018 (direct submission to GenBank)
	Alburnus alburnus Linnaeus, 1758				MK133340	
Pomphorhynchus bulbocolli	*Moxostoma erythrurum* (Rafinesque, 1818)	Canada	KY911323			Garcia-Varela et al. 2017
	Catostomus nebuliferus Garman, 1881		KY911293			
	Onchorhynchus mykiss Walbaum, 1792			AF001841		Near et al. 1998
Pomphorhynchus lucyi	*Micropterus salmonoides* Lacépède, 1802	USA			AY135418	Král'ová-Hromadová et al. 2003
Pomphorhynchus laevis		France			MF563527	David et al. 2018
					EF051062 EF051063	Moret et al. 2007
	Squalius cephalus Linnaeus, 1758	Croatia			KF559284 KF559285	Valic et al. 2013 (direct submission to GenBank)
					KJ819957 KJ819958	Valic et al. 2014 (direct submission to GenBank)
	Barbus barbus Linnaeus, 1758	France	LN994842			Perrot and Tougard 2015 (direct submission to GenBank)
	Barbatula barbatula Linnaeus, 1758		LN994843			
			MF563495			David et al. 2018
	Silurus glanis Linnaeus, 1758	Bosnia and Herzegovina	MH282838 MK133342			Nedic 2018 (direct submission to GenBank)
	Gammarus roeseli Gervais, 1835	Hungary	AY423349 AY423350			Perrot-Minnot 2004
	Gammarus pulex (Linnaeus, 1758)	France		AY423346		
	Squalius cephalus (Linnaeus, 1758)	Italy		AY135416		Kral'ova-Hromadova et al. 2003
	Barbus tyberinus Bonaparte, 1839	Italy		AY135417		
	Squalius cephalus Linnaeus, 1758	Croatia		KF559305	KF559306	Valic et al. 2013 (direct submission to GenBank)
	Dikerogammarus villosus (Sowinsky, 1894)	Germany			KJ756498	Emde et al. 2014
	Neogobius melanostomus (Pallas, 1814)				KJ756499	
	Silurus glanis Linnaeus, 1758	Bosnia and Herzegovina			MH319898 MH319899	Paras and Nikolic 2018 (direct submission to GenBank)
Pomphorhynchus perhepechus	*Moxostoma austrinum* Bean, 1880	Mexico	KY911289 KY911290			Garcia-Varela et al. 2017
Pomphorhynchus sphaericus (from brackish waters)	**Pimelodus maculatus Lacépéde, 1803**	**Argentina**	**MK429836 MK429837**	**MK411251 MK411252**	**MK411253 MK411254**	**Present study**
Pomphorhynchus sphaericus (from freshwaters)			**MN305321**			

Table 1 (continued)

Species	Host	Locality	COI	18S	ITS	References
Pomphorhynchus tereti-collis	*Gammarus roeseli* Gervais, 1835	France	AY423352 AY423353			Perrot-Minnot 2004
	Gammarus pulex (Linnaeus, 1758)			AY423347	AY424670	
	Platichthys flesus (Linnaeus, 1758)	Germany			JF706705	Spakulova et al. 2011
Pomphorhynchus zhoush-anensis	*Oplegnathus fasciatus* (Temminck & Schlegel, 1844)	China	KY490045 KY490046 KY490047	KY490049 KY490050 KY490051	KY472821 KY472822 KY472823	Li et al. 2017
Tenuiproboscis	*Lutjanus argentimaculatus* (Forsskål, 1775)		JF694276	JF694275	JF694277	Sanil et al. 2011 (direct submission to Gen-Bank)
Tenuiproboscis keralensis	*Siganus javus* (Linnaeus, 1766)	India			KU726605	Kaur et al. 2017

encapsulation reaction that causes deformities or atrophy of the proboscis and/or bulb.

Locality: Salado Relief Channel (35°50′10″ S, 57°50′20″ W), Samborombón Bay (Buenos Aires province, Argentina).

Prevalence: 10% (1/10) in *P. maculatus* from Salado River Channel (S.R.C.).

Mean intensity: 17 in *P. maculatus*.

Mean abundance: 1.7 in *P. maculatus*.

Deposited specimens: Helminthological Collection of *Museo de La Plata*, Argentina. Under the voucher number MLP-He 7727.

Remarks

As it was mentioned before, Gil de Pertierra et al. (1996) described *P. sphaericus* from several pimelodids hosts from La Plata River near the port of Buenos Aires City collected during a 2-year period. Almost at the same time, *P. patii* was described by Lunaschi (1997) from another locality in the same estuary. Based on similarities in morphology, fish host, and geographical distribution, *P. patii* was considered a junior synonym of *P. sphaericus* by Amin et al. (2003). However, several morphological differences can be noted among the specimens described by those authors (see Table 2).

One of the most noticeable features observed in *P. sphaericus* and described by Gil de Pertierra et al. (1996) is the morphology of the hook roots, with the roots 1 to 6 formed by a wide sheet that splits into two apophyses, and root 7 and subsequents with slender roots directed posteriorly, and quadrangular sheets directed anteriorly (see Fig. 1B Gil de Pertierra et al. 1996). Gil de Pertierra et al. (1996) also remarked the morphology of the hooks, mainly of the fourth hook which is described as "stout." Another particular feature is the presence of a penial stylet present in the males of these specimens. The mentioned morphological features are almost unique among pomphorhynchids, mainly the presence of two types of hooks, which it is not usual in *Pomphorhynchus*.

The newly collected specimens from Samborombon Bay water share host with *P. sphaericus*. Also, both acanthocephalans share 12 slightly spiralling longitudinal rows, unequal lemniscus, and neck forming a spherical or subspherical bulb. Despite these similarities observed, the specimens described by Gil de Pertierra et al. (1996) differ from the new material from Samborombon Bay, mainly by the following features: the number of hooks per row (14–16 vs 15, respectively); the shape of hooks roots (1–6 formed by a wide sheet split into 2 apophysis vs simple roots, respectively); the size of the hooks (smaller in the Samborombon material with a similar morphology); the size of the female proboscis (0.55–0.81 (0.66) vs 0.38–0.49 (0.43), respectively); the length of the lemniscus (half-length in the new specimens), and the size and arrangement of the testes (pre-equatorial, larger, and close together vs equatorial to post-equatorial, slender, and separated, respectively).

The specimens described by Lunaschi (1997) belong undoubtedly to *P. sphaericus*, but it is worthwhile to note that several dimensions of the structures given by the author are not reliable, as there were apparently erroneous measurements (see Table 2). However, some relevant features can be recognized, for example, the proboscis hook roots morphology, which shows simple roots like in the Samborombon specimens. Additionally, in these specimens, the proboscideal hooks morphology is similar to that showed by the specimens described by Gil de Pertierra et al. (1996), including the "stout" fourth hook. Another similarity could be seen in the proboscideal receptacle, which extends deeply into the trunk, according to both authors.
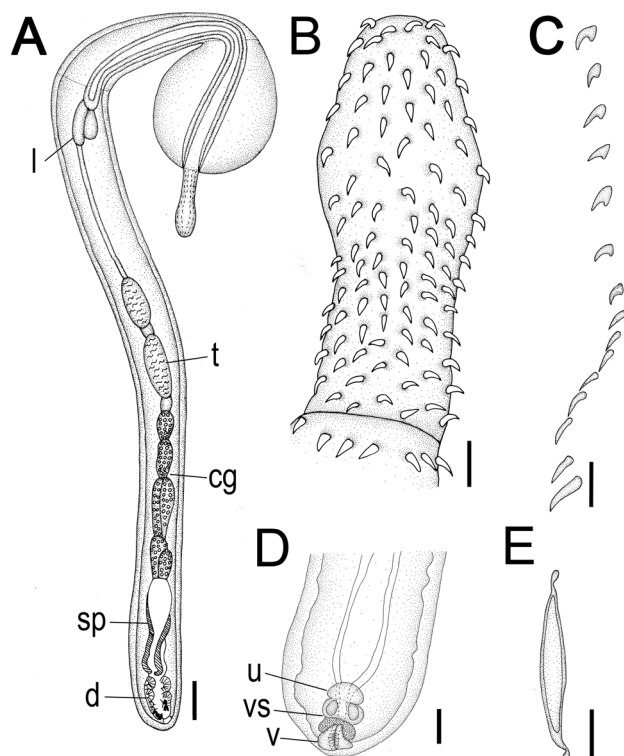
Fig. 1 A *Pomphorhynchus sphaericus* lateral view complete male specimen. **B** Armature of male *Pomphorhynchus sphaericus* proboscis. **C** Detail of hook showing roots. **D** Female reproductive system. **E** Eggs with polar prolongations. Abbreviations: cg, cement glands; d, copulatory bursa; sp, saefftigen's pouch; u, uterus; vs, vaginal sphincter; v, vagina. Scale bar: A = 400 μm, B = 60 μm, C = 33 μm, D = 85 μm, E = 16 μm

The results obtained here disclose that there is a high intraspecific variability in several of the morphological characteristics showed by the specimens of *P. sphaericus* from different localities. This fact could suggest the existence of two morphotypes in *P. sphaericus*, but the presence of mixed characters in the specimens of Lunaschi (1997) makes difficult this assumption.

One possible explanation to the apparent phenotypic plasticity in *P. sphaericus* is the geographical distribution, which involves slightly different ecosystems, despite all the localities registered belong to the Parano-Platense River basin or, more accurately, to the La Plata River estuary. La Plata River comprises three well-defined areas or ecosystems: the continental or riverine (freshwater), the estuarial (brackish water), and the marine ecosystem. Each one is characterized not only by the environment but also by the fish species assemblages which inhabit it (Baigún et al. 2016; García et al. 2010).

Pomphorhynchus sphaericus could be found in the riverine (Buenos Aires City and Punta Lara) and also in the

estuarial areas (Salado relief channel). Four of the five registered hosts (*P. albicans*, *P. maculatus*, *L. pati*, and *P. valenciennis*) are present in both areas (García et al. 2010).

Molecular analyses

The COI mtDNA analysis related the acanthocephalans found on *P. maculatus* from Samborombon River with *P. sphaericus*. The genetic distance between both parasites is 1%, showing them as the same entity (Fig. 2).

The phyllogram constructed on COI mtDNA (Fig. 2) established that *Tenuiproboscis* Yamaguti, 1935 is the first separated clade, but with a low probability (only 76% PP). After that arise *Pomphorhynchus tereticollis* (Rudolphi, 1809) at the base of the branch with 34% PP, later the clade of *P. bosniacus* Kiskaroly & Cankovic, 1969 and *P. laevis* (Zoega in Muller, 1776) with a high posterior probability (100%). The next node emerges with a low posterior probability (44%) with *P. zhoushanensis* Li et al., 2017 and *L. pagrosomi* Yamaguti, 1935 (100% PP), and then a node with a 93% PP, emerging the *P. sphaericus* specimens, followed by a node with 93% PP and two branches, one belonging to *Pomphorhynchus purhepechus* García-Varela et al., 2017, and the other with *Pomphorhynchus bulbocolli* Linkins in Van Cleave, 1919. The *p*-value calculated for COI mtDNA shows 23–30% of distance among the *P. sphaericus* and the other species (Table 3). The relationship between *P. zhoushanensis* and *L. pagrosomi* stated by Li et al. (2017) is also confirmed.

The relation of *P. sphaericus*, according to the 18S rDNA (Fig. 3), is close to the node composed by *P. tereticollis* and *P. laevis*, but with a low posterior probability (only 46%), and as the sister clade appears *Tenuiproboscis* with 92% PP. The *p*-value shows a distance of 1% among *P. sphaericus* and both *P. laevis* and *P. tereticollis* (Table 4).

The phyllogram based on ITS gen (Fig. 4) shows that *P. sphaericus* is closer to *Pomphorhynchus lucyi* Williams & Rogers, 1984 with 100% PP. The other branches of the phylogenetic tree are the same as stated by Li et al., 2017, *P. tereticollis* is the sister group of *P. laevis* + *P. bosniacus*, and with *Tenuiproboscis* at the base of that branch. On the other hand, *Pomphorhynchus zhoushanensis* and *Longicollum pagrosomi* Yamaguti, 1935 appear to be the same species. The *p*-value between *P. sphaericus* and *P. lucyi* is 5%, and compared with the other species used in the analysis, the distance of these to *P. sphaericus* is between 23 and 25% (Table 4).

The concatenated tree (Fig. 5) was obtained from all the species including in this study but there is no 18S rDNA sequences for *P. bosniacus*, *P. lucyi*, and *P. purhepechus*; ITS sequences for *P. bulbocoli* and *P. purhepechus*; COI mtDNA sequences for *P. lucyi* and *L. pagrosomi*. The configuration of this concatenated tree is in accordance with the results

Table 2 Morphometric ranges for *Pomphorhynchus sphaericus* Gil de Pertierra et al., 1996 according to different authors

	P. sphaericus after Gil de Pertierra et al. 1996	*P. sphaericus* (syn. *P. patii*) after Lunaschi, 1997	*P. sphaericus* present study
Body length	–	M: 3.44–4.66 F: 3.5–6.39	–
Male trunk (L×W)	2.6–8.2 (5.4)×0.4–1.1 (0.7)	2.03–3.39×*51–79	5.24–6.99 (6.14)×0.35–0.59 (0.47)
Female trunk (L×W)	3.2–9.5 (6.0)×0.5–1.0 (0.8)	1.63–3.90×53–80	4.37–8.40 (6.69)×0.46–0.65 (0.55)
Male proboscis (L×W)	0.51–0.72 (0.61)×0.11–0.24 (0.20)	*48–60×*16–20	0.41–0.68 (0.51)×0.14–0.26 (0.19)
Female proboscis (L×W)	0.55–0.81 (0.66)×0.14–0.29 (0.21)	*54–78×*15–18	0.38–0.49 (0.43)×0.16–0.22 (0.18)
Rows of hooks	12	12	12
Hooks per row	14–16	14–15	15
Hooks length (hooks roots length)	1–3°: M: 24–31 F: 24–36 (25–46)	1–3°: M: 28–33 (11–21)	1–3°: M: 19–22 (11–21) F: 21–24 (11–21)
	4°: M: 25–30 F: 23–39 (25–46) Stout	4°: M: 30–33 (22–25) Stout	4°: M: 16–23 (11–16) F: 19–24 (11–16)
	5°: M: 21–25 F: 21–38 (25–46) Shorter and slenderer	5–12°: M: 22–40 (hook 5 = 15–21, hook 6 = 11)	5°: M: 19–22 (7–13) F: 21–38 (7–13)
	6° and 7°: M: 22–28 F: 22–36 (hook 6 = 25–46 and hook 7 = 11–19) Larger		6° and 7°: M: 16–22 (6–8) F: 17–21 (6–10)
	8° and subs.: M: 23–35 F: 28–38 (11–19) Longer		8–14°: M: 16–27 (5–9) F: 16–28 (6–9)
	Basal circle: M: 35–37 F: 37–44 (11–19)	Basal circle: M: 28–35	Basal circle: M: 30–32 (8–10) F: 30–35 (8–12)
Hook roots	1–6° wide sheet splits into 2 apophyses	Simple	Simple
	7–16° slender and directed posteriorly with quadrangular sheet directed anteriorly		
Male bulb (L×W)	0.64–1.40 (1.02)×0.66–1.58 (1.16)	*27–69×0.54–1.07	0.86–1.11 (0.95)×0.78–1.16 (0.93)
Female bulb (L×W)	0.78–1.64 (1.13)×0.96–1.17 (1.28)	*29–75×*77–80	0.97–1.57 (1.21)×0.95–1.38 (1.10)
Male neck (without bulb) (L×W)	0.96–2.39 (1.66)×0.25–0.46 (0.35)	*44–56×*16–35	1.84–2.13 (1.96)×0.32–0.49 (0.41)
Female neck (without bulb) (L×W)	1.07–2.39 (1.65)×233–490 (381)	0.80–1.20×*16–20	1.46–2.65 (1.82)×0.27–0.41 (0.34)
Male proboscideal receptacle (L×W)	1.9–4.2 (3.10)×0.10–0.15 (0.13)	1.57–2.22×0.93–1.15	2.9–3.5 (3.2)×0.08–0.12 (0.10)
Female proboscideal receptacle	2.4–3.8 (3.15)×0.12–0.19 (0.16)	1.89–2.43×*65–93	2.0–4.2 (2.8)×0.09–0.13 (0.10)
Male larger lemnisci (L×W)	0.75–1.61 (1.19)×0.10–0.21 (0.16)	0.54–1.09×0.10–0.14	0.44–0.78 (0.57)×0.10–0.14 (0.12)
Female larger lemnisci (L×W)	0.98–2.04 (1.37)×0.14–0.22 (0.17)	0.44–1.09×*9–5	0.34–0.44 (0.38)×0.07–0.17 (0.10)
Male shorter lemnisci (L×W)	0.61–1.48 (1.07)×0.10–0.24 (0.16)		0.41–0.62 (0.50)×0.08–0.14 (0.11)
Female shorter lemnisci (L×W)	0.81–1.66 (1.20)×0.12–0.24 (0.19)		0.23–0.38 (0.29)×0.07–0.08 (0.08)
Anterior testis (L×W)	0.43–1.16 (0.69)×0.31–0.64 (0.48)	*25–35×*18–34	0.43–0.57 (0.51)×0.24–0.32 (0.28)
Posterior testis length (L×W)	0.42–1.23 (0.68)×0.30–0.62 (0.39)	*27–39×*17–33	0.49–0.57 (0.53)×0.24–0.35 (0.30)
Cement glands (L×W)	0.24–0.75 (0.44)	–	0.30–0.28 (0.35)×0.05–0.14 (0.09)
Saefftigen's pouch (L×W)	–	*45–82×*11–16	0.65–0.78 (0.69)×0.16–0.19 (0.18)

Table 2 (continued)

	P. sphaericus after Gil de Pertierra et al. 1996	*P. sphaericus* (syn. *P. patii*) after Lunaschi, 1997	*P. sphaericus* present study
Penis (L×W)	0.11×0.81		
Bursal suckers (L×W)	0.12–0.14 (0.13)×0.85–0.98 (0.92)		
Penial stylet (L×W)	0.96×0.19		
Uterine bell length/uterus	–	*15–27	11.19–2.03 (1.64)×0.08–0.14 (0.11)
Vagina		*0.76–1.15	
Eggs (in μm)	34.5–57.5 (46.8)×6.9–9.2 (7.7)	51–67×11	57–76 (62)×8–12 (11)
Locality	La Plata River near port of Buenos Aires city (34°70′S; 58°22′W)	La Plata River, Punta Lara, Ensenada, Buenos Aires province (34°49′S; 57°57′W)	Salado relief chanel, Samborombon Bay, Buenos Aires province (35°50′S; 57°50′W)
Hosts	*Pimelodus maculatus* (type), *P. albicans*, *Luciopimelodus pati*, and *Bergiaria platana* (syn: *Iheringhichthys platanus*)	*Luciopimelodus pati* and *Parapimelodus valenciennis*	*Pimelodus maculatus*

obtained for the trees of 18S rDNA, ITS, and COI mtDNA. The *Pomphorhynchus* sp. is divided in two groups, in the low branch shows *P. bulbocoli* and *P. purhepechus* with high PP value. The other big node shows a close relation between *P. zhoushanensis* and *L. pagrosomi* (100% PP); *P. sphaericus* and *P. lucyi* (94% PP); and among *Tenuiproboscis*, *P. tereticollis*, *P. bosniacus*, and *P. laevis*, respectively.

Discussion

The Pomphorhynchidae Yamaguti, 1939 is composed currently by around 55 species distributed in 5 genera *Longicollum* Yamaguti, 1955, *Parallongicollum* Amin et al., 1991, *Pomphorhynchus* Monticelli, 1905, *Pyriproboscis* Amin et al., 2003, and *Tenuiproboscis* Yamaguti, 1935 (Amin 2013). Like in the rest of the Acanthocephala, the members of the family were characterized by a few morphological features, namely the morphology of the neck and bulb (uniformly cylindrical or not, with a more or less developed bulb), the morphology of the proboscis (cylindrical and filiform or not cylindrical and anteriorly enlarged), and the type of hooks (one type of hook or two types of hooks) (Amin et al. 2003; Amin 2013). However, the recent studies in pomphorhynchids, involving taxonomic integrative approaches, provide new insights into this interesting acanthocephalan genus (Spakulova et al. 2011; Li et al. 2017; Garcia-Varela et al. 2017). The most outstanding of these results show that there is a high phenotypic plasticity in *Pomphorhynchus*, and that the genus is not a monophyletic group, resulting in the opened question about the systematic status of the other genus in the family.

Taking into account only the information provided by the morphology, we could assume that the specimens from Samborombon Bay represent a new species, but the COI mtDNA analysis related those with the species *P. sphaericus*. This fact highlights the advantages of using integrative morphological and molecular approaches to confirm the taxonomic status of the species. In this way, the sequences here reported of *P. sphaericus* from Argentina represent an advance in the knowledge of the phylogenetic analysis inside the Pomphorhynchidae.

Recently, Li et al. (2017) stablished important evidence about the morphology of this genus and the genetic similarity between *P. zhoushanensis* and *Longicollum pagrosomi* Yamaguti, 1935, and among the clade of *P. tereticollis* + *P. laevis* with *Tenuiproboscis* sp. These authors found that the presence of symmetrical or asymmetrical bulb in the same species is possible, as they reported for *P. zhoushanensis* but, as the authors claim, this could not be true for all the species inside the genus. According to this, the bulb is not so important to discriminate species. The authors also suggest that in order to eliminate the polyphyly of *Pomphorhynchus*, it was necessary to determine the relations among *Pomphorhynchus*, *Longicollum*, and *Tenuiprobosis* (Li et al. 2017).

On the other hand, Spakulova et al. (2011) resurrect *P. tereticollis*, which was previously considered synonym of *P. laevis* (Amin et al. 2003), based on the presence of two types of hook, and the morphology of hook roots. Additionally, the molecular evidence obtained supports the existence of two different species in several fish hosts (including fresh and brackish water) in the same geographical area and emphasizes the need for taxonomical and molecular studies to clarify the status of cryptic species (Spakulova et al. 2011).

The findings about *P. sphaericus* are noteworthy despite the morphological differences observed among specimens from different localities, mainly in the shape and size of the hooks and hooks roots, genetically—when the COI mtDNA
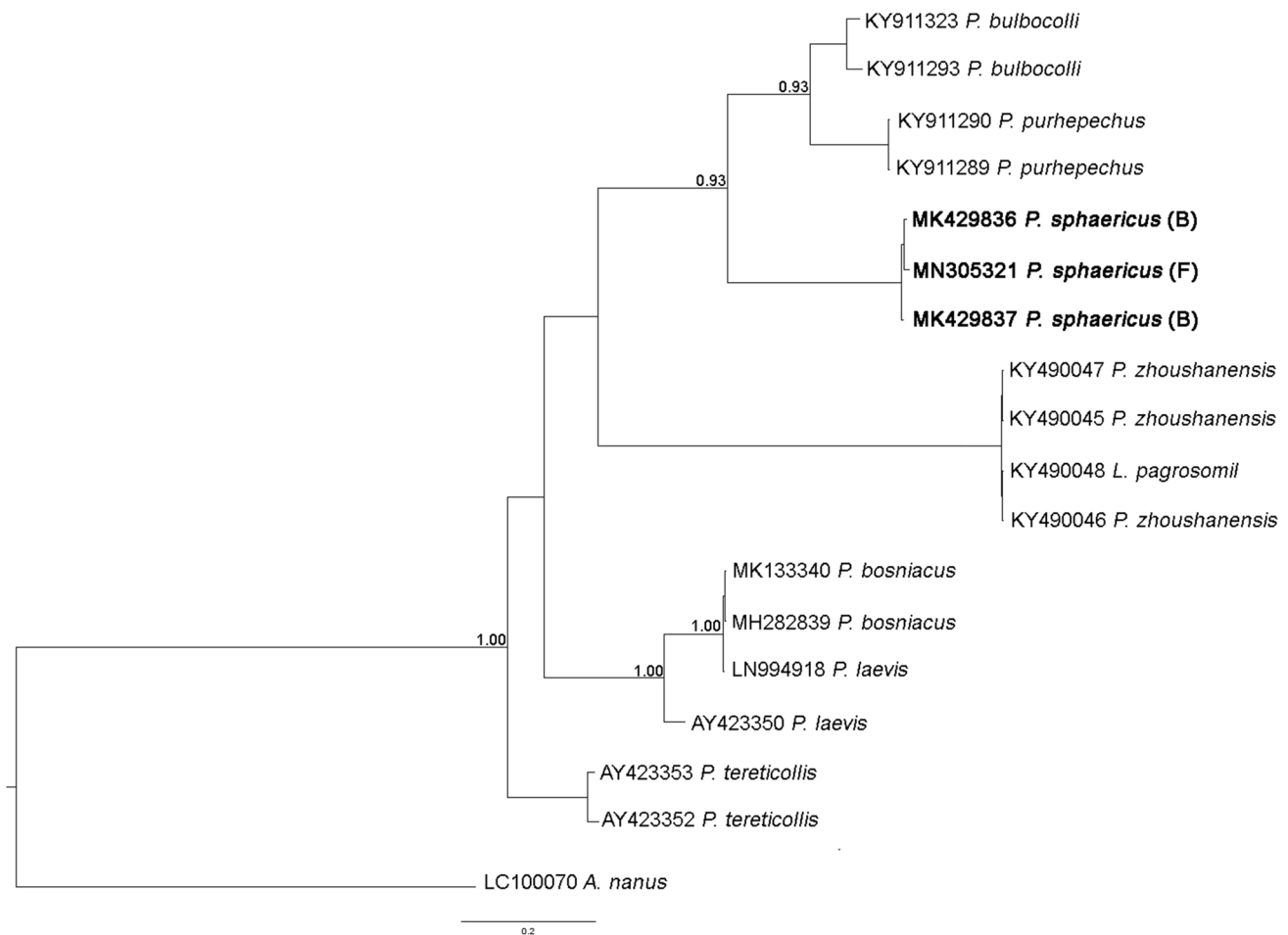
Fig. 2 Phylogenetic tree based on COI mtDNA sequences by Bayesian Inference (evolutionary parameters used were TIM2+I+G for the first, TRN+G for the second, and TPM1uf+G for the third codon position). The new sequenced forms are in bold. Numbers given at nodes represent posterior probability value (<0.90 are not shown). B, brackish waters; F, freshwaters

is analyzed—they are the same species. In particular, given the significance of the hooks and hook roots for the characterization of the species, it is remarkable that, while in the case of *P. tereticollis* and *P. laevis*, this feature is crucial for discriminating species (Spakulova et al. 2011). In *P. sphaericus*, it could be considered as phenotypical plasticity.

This is not strange that the Pomphorhynchidae family could show high morphological variability and plasticity with different morphotypes (see, for example, Spakulova et al. 2011; Li et al. 2017). According to several authors, the microenvironment could lead to phenotypic plasticity (Stunkard 1957; Mouhaid et al. 1997; Nolan and Cribb 2005; Poulin 2007). Also, according to Amin and Redlin (1980) and Shostack et al. (1986), the age, sex, and geographical location can alter characters in acanthocephalans. García Varela et al. (2017) described *P. purhepechus* in *Moxostoma austrinum* Bean from central Mexico and analyzed the genetic divergence of *P. bulbocolli*, another North

American species with a widely distribution and numerous fish hosts. Additionally, the authors analyzed the genetic divergence in *P. bulbocolli*, distribution and host associations, hypothesizing that North and South America would form a distinct monophyletic assemblage with the North American species (*P. bulbocolli*, *P. lucyi*, and *P. purhepechus*) nesting with the other Palearctic species (*P. laevis* and *P. tereticollis*) (Laurasian origin), whereas the South American species would show a separate but common origin (Gondwanan), revealing that its distribution is not the result of the faunal interchange through the Great American Biotic Interchange (García Varela et al. 2017).

As expected, the addition of new sequenced species to the molecular analysis helps to clarify the systematic status of the genus. The COI mtDNA sequences show that *P. sphaericus* is closely related to *P. bulbocolli* and *P. purhepechus*, both species from North and Central America (93% PP).

Table 3 p-distance values of the COI mtDNA calculated in MEGA X with variance estimation, with bootstrap method (500 replicates), and with nucleotide substitution (transition + transversion) uniform rate. Intraspecific divergence in bold font (*n/c*, not calculated)

|	0	1	2	3	4	5	6	7	8	9	10
0. *A. nanus*	nc										
1. *P. tereticolis*	0.34	**0.02**									
2. *P. laevis*	0.39	0.22	**0.01**								
3. *P. bosniacus*	0.39	0.25	0.06	**0**							
4. *Tenuiproboscis* sp.	0.36	0.22	0.24	0.24	nc						
5. *P. sphaericus* (brackish waters)	0.38	0.25	0.28	0.30	0.28	**0.01**					
6. *P. bulbocolli*	0.35	0.25	0.26	0.27	0.26	0.23	**0.04**				
7. *P. purhepechus*	0.36	0.25	0.27	0.27	0.27	0.23	0.15	**0**			
8. *P. zhoushanensis*	0.36	0.27	0.28	0.28	0.27	0.27	0.29	0.28	**0**		
9. *L. pagrosomi*	0.36	0.27	0.28	0.28	0.27	0.27	0.29	0.28	0	nc	
10. *P. sphaericus* (freshwater)	0.39	0.25	0.28	0.30	0.29	0.01	0.23	0.24	0.28	0.28	nc
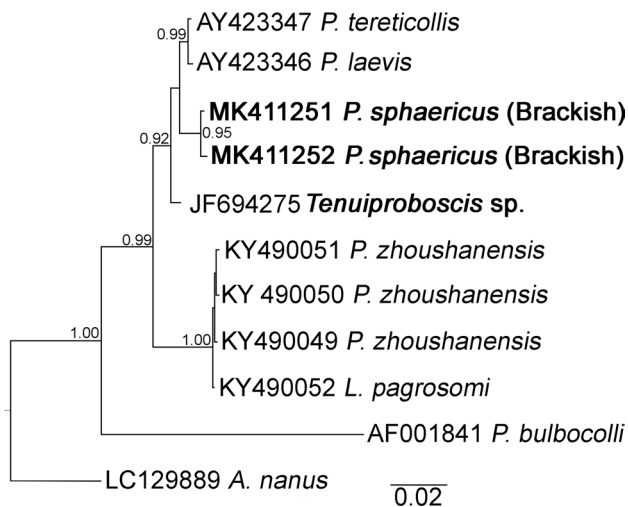
Fig. 3 Phylogenetic tree based on 18S rDNA sequences by Bayesian Inference (evolutionary parameter used was TIM2 + I + G). The new sequenced forms are in bold. Numbers given at nodes branches are the posterior probability value (< 0.90 are not shown)

The real position of *P. lucyi* in the final arrangement could be stated correctly when the COI sequence of that species (or the *P. bulbocolli* and *P. purhepechus* ITS sequences) is reported. In the light of the closeness of *P. sphaericus* with *P. lucyi*, as seen in the ITS analysis, and with *P. bulbocolli* and *P. purhepechus* with the COI gene, it is probable that they could share a node in the phylogenetic tree.

Despite the low number of sequenced species, the COI phyllogram shows an apparently division among continents. *Pomphorhynchus bulbocolli*, *P. purhepechus*, and *P. sphaericus* (plus *P. lucyi* with the ITS gen) belong to America, while *P. tereticollis* and *P. laevis* belong to Europa, and *P. zhoushanensis* and *L. pagrosomi* to Asia. This distribution contradicts, by the moment, the prediction made by Garcia-Varela et al. (2017). The riddle for the future research in the family Pomphorhynchidae will be to obtain specimens reliable and representative of most of the species, mainly of the Indian members of the family, for example, the seven species of *Tenuiproboscis* sp., a poor known genus from Indian marine fishes (Gupta and Naqvi 1992; Amin 2013). Until now, only one species was analyzed using DNA information, *Tenuiproboscis keralensis* Kaur et al., 2017, while future studies of other *Tenuiproboscis* species are needed. It is not clear whether it presents a distribution with a clade from each different continent, a visible pattern in other parasites, for example, in the digenean of the genus *Clinostomum* (Locke et al. 2015; Pérez-Ponce de Leon et al. 2016).

Up to day, of the seven South American species, only *P. sphaericus* was studied using an integrative taxonomic study.

Table 4 p-distance of the 18S rDNA (below diagonal) and ITS rDNA (above diagonal) calculated in MEGA X with variance estimation, with bootstrap method (500 replicates), and with nucleotide substitution (transition + transversion) uniform rate (*n/c*, not calculated)

	0	1	2	3	4	5	6	7	8	9
0.- *A. nanus*	■	0.41	0.41	0.40	0.40	0.41	0.39	n/c	0.40	0.40
1.- *P. tereticolis*	0.08	■	0.04	0.24	0.24	0.15	0.23	n/c	0.04	0.23
2.- *P. laevis*	0.08	0	■	0.25	0.25	0.16	0.24	n/c	0.01	0.24
3.- *P. zhoushanensis*	0.08	0.03	0.03	■	n/c	0.27	0.23	n/c	0.25	0.23
4.- *L. pagrosomi*	0.08	0.02	0.02	0	■	0.27	0.23	n/c	0.25	0.23
5.- *Tenuiproboscis* sp.	0.11	0.01	0.01	0.03	0.02	■	0.25	n/c	0.16	0.25
6.- *P. sphaericus* (brackish waters)	0.07	0.01	0.01	0.04	0.04	n/c	■	n/c	0.24	0.05
7.- *P. bulbocolli*	0.18	0.16	0.16	0.16	0.16	0.18	0.14	■	n/c	n/c
8.- *P. bosniacus*	n/c	n/c	n/c	n/c	n/c	n/c	n/c	n/c	■	0.24
9.- *P. Lucy*	n/c	n/c	n/c	n/c	n/c	n/c	n/c	n/c	n/c	■

Fig. 4 Phylogenetic tree based on ITS sequences by Bayesian Inference (evolutionary parameter used was TVM + G). The new sequenced forms are in bold. Numbers given at nodes branches are the posterior probability value (< 0.90 are not shown). B, brackish waters
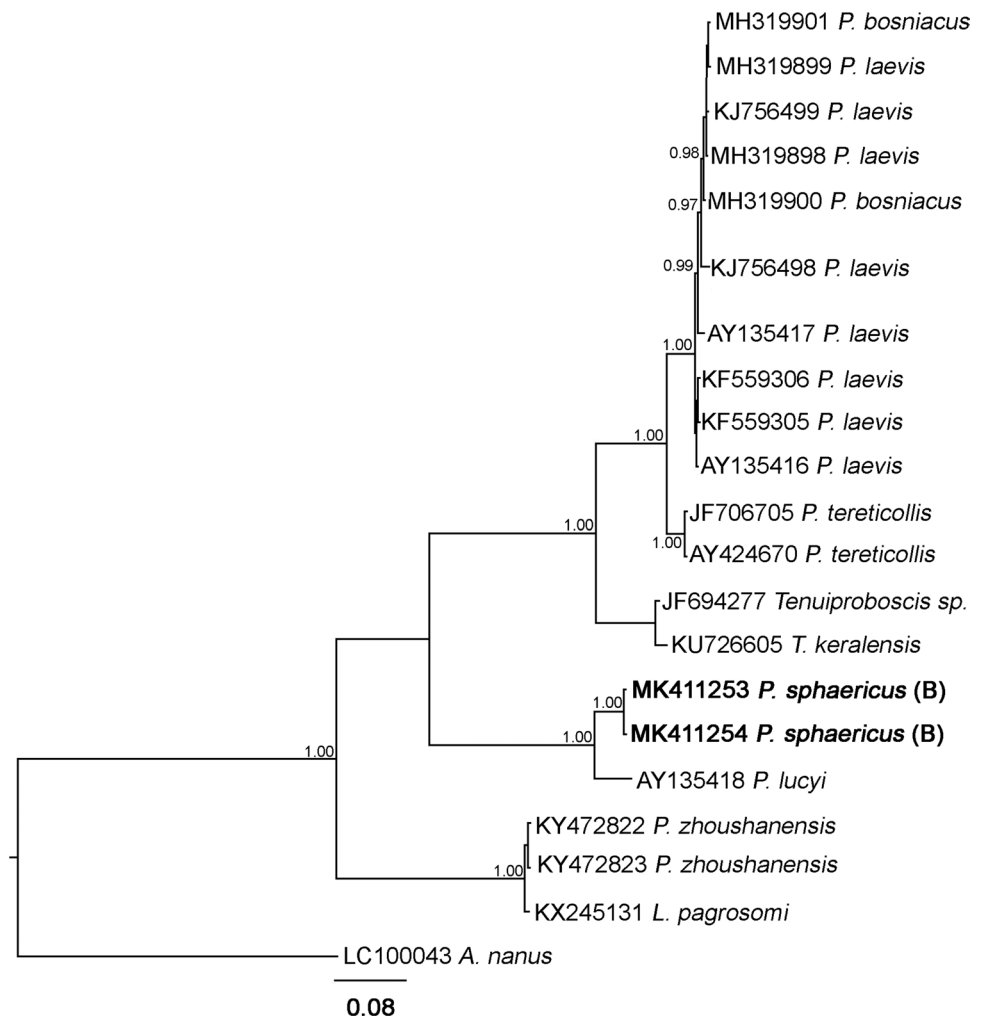
Based on the contradictory results obtained about the interspecific variability inside the genus, it will be interesting to know the relationship among the three Patagonian species (*P. patagonicus*, *P. moyanoi*, and *P. yamagutii*), which show very similar morphological characteristics (see Table 1 in
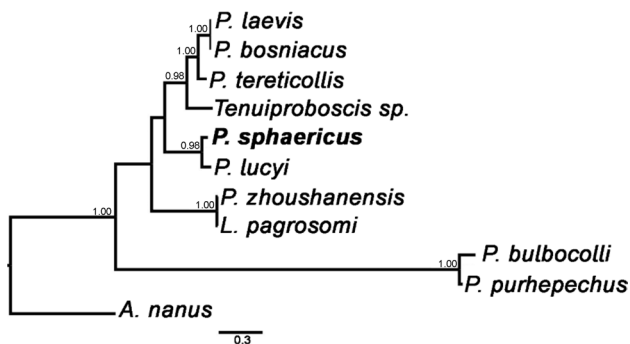
Fig. 5 Phylogenetic tree based on concatenated COI mtDNA, 18S rDNA, and ITS sequences by Bayesian Inference. Numbers given at nodes branches are the posterior probability value (< 0.90 not shown)

Olmos and Habit 2007). On the other hand, *P. omarsegundoi* could be clearly distinguished from *P. sphaericus*, but the species is characterized by a non-spirally twisted long neck with an inconspicuous and asymmetrical bulb, and this feature does not fit well with the traditional definition of the genus (Arredondo and Gil de Pertierra 2010). Unfortunately, we still do not have neither specimens of *P. sphaericus* from the other fish hosts nor the other species from South America. Therefore, its phylogenetic relationships will be more accurate in the future.

Data availability All the material will be deposited in Museums and the sequences deposited on GenBank.

Code availability Not applicable.

Declarations

Ethics approval The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional guides on the care and use of laboratory animals.

Consent to participate All the authors give their consent to participate in this work.

Consent for publication All the authors give their consent to the publication of this work.

Conflict of interest The authors declare no competing interests.

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