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 omarsogundoi* 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,
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 Perttierra 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 acanthocephalan 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 x 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 Geneious 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 Jmodeltest 2.1 (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 \(2 \times 10^6\) 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 (\(\rho\)) of absolute nucleotide sites (\(\rho\)-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 Perttierra 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 spiraling 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 Accession Numbers | 18S Accession Numbers | ITS Accession Numbers | References                      |
|-------------------------------|------------------------|---------------------------------|-----------------------|------------------------|-----------------------|---------------------------------|
| Acanthocephalus nanus         | Cynops pyrhogaster     | Japan                           | LC100070              | LC129889               | LC100043              | Nakao 2016                     |
| Longicolllum pagrosomi        | Oplegnathus fasciatus  | (Temminck & Schlegel, 1844)     |                       |                        |                       |                                 |
| Pomphorhynchus bosniacus      | Barbus barbus Linnaeus | Bosnia and Herzegovina          | MH319900             | MH319901              | MH282839              | Nedic and Vardic Smrzlic 2018 (direct submission to GenBank) |
|                              | Alburnus alburnus      |                                 |                       |                        |                       |                                 |
| Pomphorhynchus bulbocollii    | Mosoxoma erythrum      | Canada                          | KY911323              |                        |                       | Garcia-Varela et al. 2017     |
|                              | Catostomus nebuliferus |                                 | KF559284              | KF559285              |                       |                                 |
|                              | Osteichthys mykiss     | Walbaum, 1792                   | AF001841              |                        |                       |                                 |
| Pomphorhynchus lucyi          | Microterus salmonoides | USA                             | AY133518              |                        |                       |                                 |
| Pomphorhynchus laevis         |                       | France                          | MF563527              | EF051062              | EF051063              | David et al. 2018              |
|                              | Squallus cephalus      | Croatia                         | KF559305              | KF559306              |                       | Valic et al. 2013 (direct submission to GenBank) |
|                              | Barbus barbus Linnaeus | France                          | LN994842              |                        |                       |                                 |
|                              | Barbatula barbatula Linnaeus | 1758       | LN994843              |                        |                       | Perrot and Tougard 2015 (direct submission to GenBank) |
| Silurus glanis Linnaeus, 1758 |                       | Bosnia and Herzegovina          | MH282838              | MK133342              |                       | David et al. 2018              |
| Gammarus rosei Gervais, 1835  |                       | Hungary                         | AY423349              | AY423350              |                       | Nedic 2018 (direct submission to GenBank) Perrot-Minnot 2004 |
| Gammarus pulex (Linnaeus, 1758) |                       | France                          | AY423346              |                        |                       |                                 |
| Squallus cephalus (Linnaeus, 1758) |                       | Italy                           | AY135416              |                        |                       | Kraľ’ová-Hromadová et al. 2003 |
| Barbus tyberinus Bonaparte, 1839 |                       | Italy                           | AY135417              |                        |                       |                                 |
| Squallus cephalus Linnaeus, 1758 |                       | Croatia                         | KF559305              | KF559306              |                       | Valic et al. 2013 (direct submission to GenBank) |
| Dikerogammarus villosus       |                       | 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    | Mosoxoma australinum   | Mexico                          | KY911289              | KY911290              |                       | Garcia-Varela et al. 2017     |
| Pomphorhynchus spharicicus    | Pimelodus maculatus    | Lacedpédé, 1803                 | MK429836              | MK429837              | MK411251              | Present study                  |
|                              |                       | (from brackish waters)          |                       |                       | MK411252              |                                 |
| Pomphorhynchus spharicus      |                       | (from freshwaters)              |                       |                       | MK411253              |                                 |
|                              |                       |                                 |                       |                       | MK411254              |                                 |
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 subsequent roots directed posteriorly, and quadangular 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.
estuarial areas (Salado relief channel). Four of the five registered hosts (P. albicans, P. maculatus, L. pati, and P. valenciennesi) 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 phylogram 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 bulboccoli 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 phylogram 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. bulboccoli and P. purhepechus; COI mtDNA sequences for P. lucyi and L. pagrosomi. The configuration of this concatenated tree is in accordance with the results

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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
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) F: 21–24 (11–21) | 1–3°: M: 19–22 (11–21) F: 19–24 (11–16) |
|                         | 4°: M: 25–30 F: 23–39 (25–46)                        | 4°: M: 30–33 (22–25)                                  | 4°: M: 16–23 (11–16) F: 19–24 (11–16) |
|                         | 5°: M: 21–25 F: 21–38 (25–46)                        | 5°: M: 22–40 (hook 5 = 15–21, hook 6 = 11)           | 5°: M: 19–22 (7–13) F: 21–38 (7–13) |
|                         | Stout                                               | Stout                                                 | Stout                         |
|                         | Shorter and slimmer                                  | Shorter and slimmer                                   | Shorter and slimmer           |
|                         | 6° and 7°: M: 22–28 F: 22–36 (hook 6 = 25–46 and hook 7 = 11–19) | 6° and 7°: M: 16–22 (6–8) F: 17–21 (6–10)            | 6° and 7°: M: 16–22 (5–9) F: 16–28 (6–9) |
| Hook roots              | 1–6° wide sheet splits into 2 apophyses              | Simple                                                | Simple                        |
|                         | 7–16° slender and directed posteriorly with quadrangular sheet directed anteriorly | Simple                                                | Simple                        |
| 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 lemmisci (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 lemmisci (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 lemmisci (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 lemmisci (L×W) | 0.81–1.66 (2.0)×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) |
| Saefitgen’s pouch (L×W)  | –                                                   | *45–82×*11–16                                      | 0.65–0.78 (0.69)×0.16–0.19 (0.18) |
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. bulbocelli* 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) established 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 *Tenuiproboscis* (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
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 australinum* Bean from central Mexico and analyzed the genetic divergence of *P. bulbocalli*, another North American species with a widely distribution and numerous fish hosts. Additionally, the authors analyzed the genetic divergence in *P. bulbocalli*, distribution and host associations, hypothesizing that North and South America would form a distinct monophyletic assemblage with the North American species (*P. bulbocalli, P. lucyi*, and *P. purhepechus*) nesting with the other Paleartic 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. bulbocalli* and *P. purhepechus*, both species from North and Central America (93% PP).
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.

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**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    |
|----------------|------|------|------|------|------|------|------|------|------|------|
| 0. *A. nanus*  |      |      |      |      |      |      |      |      |      |      |
| 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 |      |      |      |      |      |      |
| 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 | 0.27 | 0.27 | 0.29 | 0.28 | 0    |      |      |
| 9. *L. pagrosomi* | 0.36 | 0.27 | 0.28 | 0.27 | 0.27 | 0.29 | 0.28 | 0    |      |      |
| 10. *P. sphaericus* (freshwater) | 0.39 | 0.25 | 0.28 | 0.30 | 0.29 | 0.01 | 0.23 | 0.24 | 0.28 | 0.28 |

**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 | 0.39 | *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 | 0.25 | 0.23 |    |
| 4. *L. pagrosomi* | 0.08 | 0.02 | 0.02 | 0   | *n/c* | 0.27 | 0.23 | 0.25 | 0.23 |    |
| 5. *Tenuiproboscis* sp. | 0.11 | 0.01 | 0.01 | 0.03 | 0.02 | *n/c* | 0.25 | 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 | 0.18 | 0.14 | *n/c* | *n/c* | *n/c* |    |
| 8. *P. bosniacus* | *n/c* | *n/c* | *n/c* | *n/c* | *n/c* | *n/c* | *n/c* | *n/c* | *n/c* |    |
| 9. *P. lucyi* |    | *n/c* | *n/c* | *n/c* | *n/c* | *n/c* | *n/c* | *n/c* | *n/c* |    |

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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).

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Parasitology Research (2021) 120:3725–3737
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 Olmos and Habit 2007). On the other hand, *P. omarsegundoï* 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.

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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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