The systematic position of Opistocystidae (Annelida, Clitellata) revealed by DNA data

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Abstract

Opistocystidae Černosvitov, 1936 is a largely Neotropical oligochaete taxon containing seven species. Its familial status has never been formally challenged, although possible close relationships with Naididae and Phreodrilidae have been noted. Mitochondrial 12S and 16S rDNA, and nuclear 18S rDNA, of a range of aquatic oligochaete taxa, including Trieminentia corderoi (Opistocystidae), were analysed by Bayesian inference. This led to the suggestion to consider Naididae as a subfamily, Pristininae Lastoc ˇkin, 1921, well phylogenetically nested within the latter (Christensen and Theisen, 1998; Erséus et al., 2005). This led to the suggestion to consider Naididae as a subfamily (Naidinae) within Tubificidae (Erséus and Gustavsson, 2002; Erséus et al., 2005), but The International Commission on Zoological Nomenclature (2007) ruled that Naididae maintains its precedence over Tubificidae, with the consequence that all former tubificids instead are proposed to be part of an enlarged Naididae (Erséus et al., 2008). Moreover, as Envall et al. (2006) showed that Naididae in its former, restricted, sense is likely to be polyphyletic, Erséus et al. (2008) advocated that Pristina Ehrenberg, 1828, should be referred to a separate subfamily, Pristininae Lastoc ˇkin, 1921, well separated from Naidinae Ehrenberg, 1828; the latter containing all other former naidid genera. Both Naidinae and Pristininae occur globally in freshwater, but a few naidines are estuarine. A taxonomically and geographically more restricted aquatic oligochaete family, Opistocystidae Černosvitov, 1936 (authorship recognized by intent; Harman, 1969), is a largely Neotropical taxon with seven nominal species (four inadequately described), known from Argentina, Brazil, Paraguay and Uruguay in the South to the southern United States in the North, and classified into three genera (Harman and Loden, 1978): Opistocysta Černosvitov, 1936 (type species, O. funiculus Cordero, 1948), Trieminentia Harman and Loden, 1978 (type species, O. corderoi Harman, 1969), and Crustipellis Harman and Loden, 1978 (type species, O. tribranchiata Harman, 1969). Brinkhurst (1966) also reported a worm, “most likely to belong to O. funiculus”, from Africa, which may reflect a recent introduction of an opistocystid onto this continent. The genera are separated by the location of their genital organs; testes and ovaries are in segments XXI–XXII in Opistocysta, XIV(or XV)–XV(or XVI) in Trieminentia, and XI–XII in Crustipellis. Otherwise, they are morphologically similar, all with a long prostomial protuberance (“proboscis”), three (one median and two lateral) ciliated caudal appendages (gills), hair chaetae in all dorsal bundles (normally together with needle chaetae), atria covered by diffuse prostate glands, and terminating in eversible penial-to-pseudopenial copu-
latory structures, and spermathecae located in the segment imme-
diately posterior to the one bearing the male pores (Harman, 1969; 
Brinkhurst and Jamieson, 1971; Harman and Loden, 1978; Brink-
hurst and Marchese, 1989). The predominant mode of reproduc-
tion seems to be asexual by budding (Harman and Loden, 1978), 
i.e., paratomatic fission (see, e.g., Dehorne, 1916; Bely and Wray, 
2001). All these features except the segmental positions of the gen-
etalia make opistocystids similar to members of Naidinae and Pris-
tininae, a resemblance recognized already by Leidy (1880), who 
regarded it as closely related to “Naididae” (i.e., today’s 
Naidinae and Pristininae together), a view also shared by Čeka-
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Dorydrilidae, and Tubificidae/Naididae, but its exact phylogenetic 
(1978) regarded 
in 
Pristina. However, Harman (1969) and Harman and Loden 
(1978) regarded O. flagellum as a species inquirenda.

Brinkhurst and Jamieson (1971) placed Opistocystidae in an 
unresolved superfamily (Tubificoidea) together with Phreodrilidae, 
Dorydrilidae, and Tubificidae/Naididae, but its exact phylogenetic 
position has only been briefly discussed in the literature. Černosvi-
tov (1936) regarded it as closely related to “Naididae” (i.e., today’s 
Naidinae and Pristininae together), a view also shared by Čeka-
tov (1936) noted that both Opisto-
cystidae and the southern hemisphere family Phreodrilidae have 
their spermathecae placed in a segment posterior to (instead of anterior to) 
that of the atria, an unusual condition among aquatic 
Opistocystidae closest to Phreodrilidae in his intuitive scheme of 
aquatic oligochaete evolution.

New material of Trieminentia corderoi from the Paraná River 
foldplain in Argentina enabled the first examination of DNA se-
quences from an opistocystid. In this study, parts of the mitochon-
drial (12S and 16S rDNA) and nuclear genome (18S rDNA) were 
sequenced and compared to the corresponding sequences of other 
aquatic oligochaete taxa, with the aim to clarify the systematic po-
sition of Opistocystidae.

2. Material and methods

Specimens of the Trieminentia corderoi were collected (August 
18, 2006) in a floodplain lake connected to the Middle Paraná River 
(31°39.9′, 060°35.4′W), NW of Paraná City, Entre Ríos Province, 
Argentina. The site is a silty-sandy bottom, 0.3 m deep, with a rich 
cover of macrophytes (pH 7.4; O₂ 8.23 mg/l). Worms were pre-
collected in 96% ethanol, but in most specimens, the body wall then 
served by Marchese).

All other taxa analyzed here are those studied by Enwall et al. 
(2006, Table 2), with a total of 51 different naidids (4 Tubificinae, 
4 Phallodrilinae, 4 Lnimodriloidinae, 12 Rhyacodrilinae, 23 Naidi-
nae and 4 Pristininae), which together with Trieminentia corderoi 
were regarded as the ingroup, and 5 outgroup species (2 Enchyr-
atica, 1 each of Phreodrilidae, Lumbricidae and Lumbriculidae).

DNA of specimen CE2037 was extracted using the DNeasy® 
Blood and Tissue Kit (Qiagen). PCR of 12S (381 bp), 16S (484 bp) 
and 18S (1726 bp) was carried out with PuReTaq Ready-To-Go 
PCR Beads (GE Healthcare). Primers used and thermocycling proto-
cols followed are listed by Enwall et al. (2006). PCR products were 
checked by electrophoresis on an agarose gel containing ethidium-
 bromide (3%), and then purified using the E.Z.N.A® Cycle-Pure kit 
(GE Healthcare). The PCR products were sequenced by Macrogen Inc., 
using primers listed by Enwall et al. (2006).

The sequences of Trieminentia corderoi (GenBank Nos.: 
GU002446, 12S; GU002447, 16S; GU002448, 18S) were added to 
those used by Enwall et al. (2006), and aligned using ClustalW in 
MegAlign (DNAStar Inc.), with default settings (e.g., pairwise gap 
opening penalty 15/multiple gap opening penalty 15; see Sec-
tion 4). The combined dataset was analyzed by Bayesian inference, 
using MrBayes, version 3.1.2 (Huelsenbeck and Ronquist, 2001). 
The models used in the analysis were selected according to the Akaike 
information criterion in MrModest, version 2.3 (Nylander, 
2004), in conjunction with PAUP, version 4.0b10 (Swofford, 
2002). The GTR+I+G model was selected for all three genes. Substi-
tution rates, character state frequencies, gamma shape parameters, 
and proportions of invariable sites were unlinked between the genes 
(Ronquist and Huelsenbeck, 2003). Four runs times four 
Markov chains (one cold and three heated) were run simulta-
neously for 20 million generations; trees were sampled every 
100th generation. Each of the chains was started from a random 
starting tree. The first 20,000 trees sampled during the burn-in 
phase were discarded. Gaps were treated as missing data.

The dataset was also analysed by parsimony resampling with 
TNT 1.1 (Goloboff et al., 2008), using jacknife (35% removal pro-
bability), and 1000 replicates.

3. Results

The data matrix contains 2727 characters, of which 788 are 
informative. The Bayesian inference tree (Fig. 1) gives maximum 
support (posterior probability, pp 1.00) for the naidid ingroup, 
and although poorly resolved in the basal part, several of its main 
clades also are strongly supported: one consisting of all Phallodril-
inae except Bathhydrilus (pp 1.00), and most closely related to the 
“rhyacodriline” Heterodrilus (pp 1.00); one with all Tubificinae 
(pp 1.00), but with Branchiura (earlier regarded as a rhyacodriline) 
nested within it (pp 0.96); one with all Lnimodriloidinae (pp 1.00); 
and finally, a large clade consisting of all Naidinae, Pristina (Pristin-
inae), Trieminentia and the “rhyacodriline” genera Aimudrilus, Rhy-
acodrilus, Epirodrilus and Monopylephorus (pp 1.00). Within this
Fig. 1. Phylogenetic tree obtained from the Bayesian inference analysis of the combined 12S, 16S and 18S rDNA dataset, alignment 15/15. Posterior probabilities (pp) > 0.90 indicated at nodes. Abbreviations after taxon names give classification within Naididae: Lim, Limnodriloidinae; Nai, Naidinae; Opi, ‘former Opistocystidae’; Pha, Phallodrilinae; Pri, Pristininae; Rhy, Rhyacodrilinae; Tub, Tubificinae.
large clade, Pristina (pp 1.00) and Trieminentia are sister groups (pp 1.00), and together form the sister of all remaining taxa (pp 1.00). Among the remaining taxa the two species of Ainudrilus (pp. 1.00) are the sister of a less supported clade (pp 0.94), within which Naidinae (pp 1.00) is the sister of a group comprising Rhyacodrilus, Epipodrilus and Monopylephorus (pp 1.00). Thus, Trieminentia is with maximum support placed as the sister group of Pristina.

For all jackknife frequencies >50% (not shown in Fig. 1) except one, the parsimony resampling supports nodes also present in the Bayesian tree. The exception is that the parsimony analysis places Chaetogaster (instead of Dero) as the sister to all remaining Naidinae (latter with 86% jackknife support). The sister group relationship between Trieminentia and Pristina is supported by 100% jackknife support, as is the placement of these two taxa as the sister to the clade comprising Naidinae, Ainudrilus, Rhyacodrilus, Epipodrilus and Monopylephorus.

4. Discussion

The analyses of the combined ribosomal mitochondrial and nuclear data give maximum support for Trieminentia being a member of the family Naididae sensu Erßéus et al. (2008), and more specifically, with a position within the large clade also comprising Naidinae, Pristina (Pristininae), and a paraphyletic assemblage of various “Rhyacodrilinae”; and with Pristina as its sister group in this taxon sample (Fig. 1). Although Trieminentia is not the type genus of Opistocystidae, it seems reasonable to assume that Opistocystidae as defined by Harman and Loden (1978) is monophyletic (morphological evidence for this is discussed below). If this assumption proves correct, the type (Opistocysta) of Opistocystidae would be nested within, and Opistocystidae would become a junior synonym of, Naididae Ehrenberg, 1828.

The phylogenetic position of Opistocystidae proposed herein is corroborated by morphological evidence. All “opistocystids” fully described to date have male ducts with diffuse prostate glands, and eversible pseudopenes, two features typical also of the rest of the large naidine–rhyacodriline–pristinid group. At the same time, the opistocystid genera have hairs and needles – or hair-like needles – in all dorsal bundles, as is common for freshwater naidids, including Pristina but excluding the naidines; all Naidinae show reductions in the dorsal distribution of chaetae. A possible synapomorphy of Pristininae, Opistocysta, Trieminentia and Crustipellis is the proboscis, but a similar prostomial appendage is present also in the naidine genera Arcteomnais, Ripistes and Stylaria. Opistocysta and Trieminentia have their sexual organs in an unusually posterior position, whereas Crustipellis have them in the position (testes in XI; ovaries and male pores in XII) that has been regarded as the plesiomorphic state for aquatic microdrilid groups (Brinkhurst, 1994). However, as the present study suggests that the former opistocystids are terminally nested members of Naididae, which normally have the genitalia in X–XI or even anterior to that, it is likely that they share a derived state of this character, i.e., a general backward shift of genitalia. Regardless of this, however, two other characters support the monophyly of the former opistocystids: the posterior position of the spermathecae vis-à-vis the atria, and the particular arrangement of caudal gills, including two longer ventro-lateral and one shorter dorso-median appendages. Members of the naidine genus Dero also possess posterior gills, which are lamelliform or digitiform processes around the anus, but they are always in multiples of two (all paired) and may have evolved convergently to those of Opistocysta, Trieminentia and Crustipellis.

Expanding Pristininae Lastockin, 1921 to include the former Opistocystidae would be in accordance with the tree obtained (Fig. 1). Other alternatives, e.g., lowering the rank of Opistocystidae Černosvitov (1936) to yet another subfamily within Naididae, are also possible, as the morphological coherence of Opistocysta, Trieminentia, and Crustipellis support their identity as a separate lineage. On the basis of the present molecular study, we are only able to conclude that Trieminentia is closely related to Pristina; Opistocysta (the family type), e.g., was not included in our molecular study. However, as a working hypothesis, and for the time being, we suggest to regard all former opistocystids as members of the Pristininae.

Whether or not the paratonic reproduction is homologous in Naidinae, Pristininae and Opistocystinae, is still an enigma. Functionally, the process appears similar in all three groups, in that the secondary zooid regenerates a new head end in the fission zone of cloning individuals (for Opistocysta Junicus (sensu Harman and Loden, 1978), see Černosvitov (1936, Fig. 9)), but the number of regenerated segments differs between taxa (Bely and Wray, 2004). The tree obtained in the present study, as well as those from the previous studies by Erßéus et al. (2002) and Enval et al. (2006), suggest that, in the Pristininae + Trieminentia clade, this mode of reproduction has evolved convergently from that in Naidinae. Bely and Wray (2004), who also found Pristina to be a group separate from Naidinae, noted that these groups “display a remarkable diversity of modes of fission” and also found it possible that these modes may have multiple origins.

Enval et al. (2006) used the data of all taxa of the present study, except Trieminentia corderoi, in Bayesian analyses of datasets of combined 12S + 16S + 18S datasets under two different combinations of alignment parameters (different settings in Clustal X). Trees obtained using alignments “15/45 penalties” and “15/15 penalties (default setting)”, both placed Pristina as sister of Ainuddriuls, but this had support only in the 15/45 alignment. By and large, the basal resolution of the naidinae–rhyacodrine–pristine–opistocystine clade was less supported in the 15/15 alignment of the Enval et al. study than in the single alignment (also 15/15) of the present study. The difference may be attributed to the fact that we added an opistocystid to the sampled taxa, and/ or that our Bayesian analysis was run for ten times as many generations as in the previous study. Still, however, the somewhat different topologies in the two studies suggest that the estimated phylogeny of this large lineage within Naididae may not have yet arrived to its final, fully corroborated, state. Additions of taxa and molecular as well as morphological data, and the use of other analytical methods may still change the conclusions regarding the detailed relationships of the lower-level taxa. Nevertheless, our study has not refuted the previous conclusions by Enval et al. (2006) that Pristininae is a taxon well separated from Naididae, and that Rhyacodrilinae, as previously defined indeed is an artificial taxon.

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