

Syndesmis aethopharynx (Umagillidae, Rhabdocoela, Platyhelminthes)
from the sea urchin Paracentrotus lividus: first record from the Eastern
Mediterranean, phylogenetic position and intraspecific morphological variation
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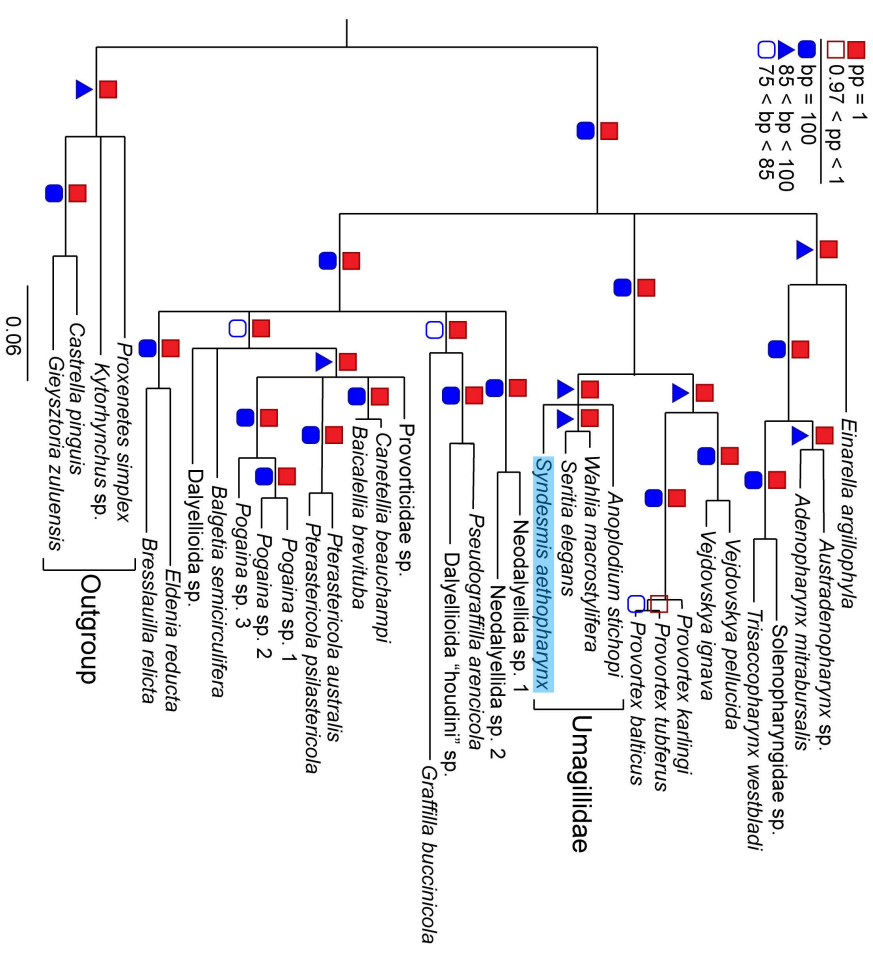
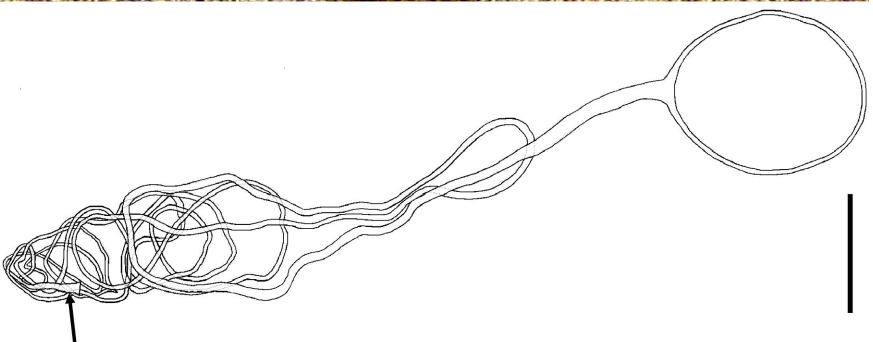
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Highlights

- *Syndesmis aethopharynx* is reported from *Paracentrotus lividus* in Greece, constituting the first record of this species from the Mediterranean.
- Previously-unreported morphological details and intraspecific variation of the species are described.
- The position of *S. aethopharynx* within Umagillidae is confirmed through Bayesian and maximum likelihood analyses (18S rDNA).



Syndesmis aethopharynx (Umagillidae, Rhabdocoela, Platyhelminthes) from the sea urchin *Paracentrotus lividus*: first record from the Eastern Mediterranean, phylogenetic position and intraspecific morphological variation

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Abstract

Specimens of *Syndesmis aethopharynx* Westervelt & Kozloff, 1990 (Umagillidae, Rhabdocoela, Platyhelminthes) were collected from the intestine of several specimens of the sea urchin *Paracentrotus lividus* (Lamarck, 1816) Hansson, 2001 at the Greek coast. This represents the first report of a species of *Syndesmis* from Greece. Our study has revealed several previously-unreported morphological details and intraspecific variation, which are added to the species description. The position of *S. aethopharynx* within Umagillidae is confirmed for the first time through molecular data (based on nuclear 18S rDNA), using both Bayesian and maximum likelihood analyses.

Keywords: taxonomy; phylogeny; marine microturbellarians; Echinoidea

1 Introduction

With about 1700 species described, Rhabdocoela constitutes one of the most species-rich taxa among turbellarian (non-neodermatan) flatworms (Platyhelminthes), and is also one of the most diverse. While the vast majority of species within this group are free-living, several taxa have independently acquired an endosymbiotic lifestyle [3]. Umagillidae Wahl, 1910

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(Neodalyellida, Dalytyphloplanida) is by far the largest family (75 species) of symbiotic turbellarians [5, 6]. Representatives of this group have been reported from echinoderm or sipunculid hosts on all continents. The majority of umagillids live within the intestine or coelomic cavity of either sea cucumbers (51%) or sea urchins (33%).

Despite the well-known ecological importance [7-9] and commercial value [10-12] of echinoids in the Mediterranean, and of the parechinid *Paracentrotus lividus* (Lamarck, 1816) Hansson, 2001 in particular, their endosymbiont fauna remains largely understudied. Occasional studies on this topic have predominantly focused on intestinal bacteria (e.g. Meziti, Kormas, Pancucci-Papadopoulou and Thessalou-Legaki [12]) and ciliates (e.g. Lynn and Strüder-Kypke [15]).

Until now, only three species of endosymbiotic rhabdocoels have been reported from sea urchins from the Mediterranean Sea. All of these are representatives of *Syndesmis* Silliman, 1881, the most species-rich genus of Umagillidae (see Tyler [17]). These three species are *S. echiniacuti* Kozloff, 1997, *S. echinorum* François, 1886 [both described from the echinid *Gracilechinus acutus* (Lamarck, 1816) Fell & Pawson, 1966] and *S. aethopharynx* Westervelt & Kozloff, 1990 (known only from *P. lividus*). Westervelt and Kozloff [1] retrieved what is presumably a fourth Mediterranean species from *P. lividus*, though it was not formally described at the time.

Furthermore, hardly any molecular systematics work has been conducted on Umagillidae, or endosymbiotic rhabdocoels in general. Indeed, in the most-recently-published phylogeny of Dalytyphloplanida (based on 18S and 28S rDNA), only three of the 75 valid umagillid species were included and only one of three subfamilies (Umagillinae Stunkard & Corliss, 1951) was represented [3].

As a first step towards a better understanding of the biodiversity and occurrence of Umagillidae in the Mediterranean, we examined the intestinal rhabdocoels of several specimens of *P. lividus* from the Greek coast. Considering that both *S. aethopharynx* and *S. echinorum* have been reported from *P. lividus* in Banyuls-sur-Mer, France [1] and given the wide distribution range of several species of *Syndesmis* [20], we expected to find specimens of at least some species of *Syndesmis* in local sea urchins. If this was the case, we planned to sequence 18S rDNA of all specimens in order to perform the first molecular phylogenetic analysis including these species of *Syndesmis*.

2 Material & Methods

2.1 Taxon sampling and microscopy

Specimens of *Paracentrotus lividus* were collected by hand in the Saronic Gulf at Anavyssos, off Mavro Lithari (37.73278°N, 23.90361°E) on February 21st, 2013. Their coelom and digestive system were inspected for flatworms under a stereomicroscope. Umagillids were fixed in analytical-grade ethanol; pictures were taken of a subset of specimens prior to fixation, slightly flattened between slide and coverslip in sea water. After fixation, all specimens were cut in half: one part was used for DNA extraction and the other to prepare whole mounts in lactophenol. The internal morphology of 25 whole-mounted specimens was

studied under a Nikon Eclipse 80i compound microscope and a Leica DM 2500 microscope, using interference contrast. Internal organs were photographed and subsequently drawn with the aid of a camera lucida. Measurements were made along the central axis of these structures. The pharynx turned out to be positioned in such a way that it was impossible to capture its entirety on a single photograph. Therefore, we compiled a focus-stacked image of three photographs at different focal depths using Adobe Photoshop CS5 v12.1.

Voucher specimens of the flatworms and their hosts were deposited in the collection of the research group Zoology: Biodiversity and Toxicology of Hasselt University (Diepenbeek, Belgium) (HU nos XXXX-XXXX and HU hostvoucher XXXX-XXXX).

2.2 DNA extraction, PCR and sequencing

DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen) following the manufacturer's instructions. We amplified fragments of the nuclear 18S rDNA gene using a nested PCR approach with primer combinations from Norén and Jondelius [21]. The initial PCR used TimA (5'-AMCTGGTTGATCCTGCCAG-3') and TimB (5'-TGATCCATCTGCAGGTTACCT-3'). Nested PCR was carried out using the internal primer combinations S30 (5'-GCTTGTCTCAAAGATTAAGCC-3') with 5FK (5'-TTCTTGGCAAATGCTTTCGC-3') and 4FB (5'-CCAGCAGCCGCGGTAATTCCAG-3') with 1806R (5'-CCTTGTTACGACTTTTACTTCCTC-3'), respectively. Polymerase Chain Reaction was performed in a GeneAmp PCR system 2700 thermocycler (Applied Biosystems) using Illustra PuReTaq Ready-To-Go PCR Beads (GE Healthcare), adding 2.5 µL of each primer (20 µM) (Sigma Aldrich) (1 µL in the nested PCR), 3 µL of template DNA and 17 µL of double distilled, autoclaved and filter sterilized water (21 µL in the nested PCR). In the initial PCR, after an initial denaturation of 310 s at 95 °C, samples were subjected to 35 cycles of 30 s at 94 °C, 30 s at 55 °C and 90 s at 72 °C. After a final elongation of 300 s at 72 °C, samples were cooled to 4 °C. The nested PCR followed a similar protocol, but with 240 s of initial denaturation and an annealing step at 50 °C for 30 s, an equal duration to the other PCR cycle steps. Final elongation at 72° C took 600 s. PCR products were purified with the QIAquick PCR Purification Kit (Qiagen) following the manufacturer's instructions. Sequencing of both strands was performed using the internal primers with an Applied Biosystems 3730 DNA analyser and BigDye version 1.1. Sequences were deposited in NCBI GenBank under accession numbers xxxxxxxx-xx.

2.3 Phylogenetic analysis

Consensus sequences were assembled from the obtained contigs in GENEIOUS v5.7.5 [22]. The resulting sequences were subsequently subjected to a BLAST search [23] on the NCBI website to screen for contamination. Other neodalyellid and outgroup sequences were selected from Van Steenkiste, Tessens, Willems, Backeljau, Jondelius and Artois [3]. Corresponding GenBank accession numbers of all used sequences are listed in **Table A.1**. Sequences were aligned with the structural Q-INS-I algorithm in MAFFT [24], which accounts for RNA secondary structure. Ambiguously-aligned sites were identified with Aliscore v2.0 [25] with default sliding window settings (w = 6) and removed from the alignment using Alicut v2.3 [26]. The best fitting substitution model for the dataset was determined in jModeltest v2.1.6

[27] on the CIPRES Science Gateway server [28]. The GTR+G+I model was selected based on both the Akaike Information Criterion and the Bayesian Information Criterion.

Model-corrected pairwise genetic distances were calculated in PAUP* v4.0a152 [29]. Phylogenetic trees were constructed using both maximum likelihood (ML) and Bayesian (BI) approaches. ML analysis was carried out in RAxML v8.0.0 [30] on the CIPRES server: 100 independent runs were performed under the GTR+G+I model. Support values were assessed with 1000 non-parametric bootstrap replicates. Bayesian analyses were carried out using the Metropolis coupled Markov chain Monte Carlo (MC³) method under the GTR+G+I model in MrBayes v3.2.6 [31]. Two independent, simultaneous runs, each including one cold and three heated chains, were conducted for 10 million generations. Trees were sampled every 100th generation after a burn-in of 25%. Convergence of the chains was confirmed by the average standard deviation of split frequencies falling below 0.01, the potential scale reduction factor approaching 1.0, and the log-likelihoods reaching a stationary distribution. A majority-rule consensus tree was constructed from all retained topologies. Resulting ML and BI trees were visualized in FigTree v1.4.3 [32].

Intrafamilial relationships of Umagillidae were not dichotomously resolved: while *Wahlia* and *Seritia* together constituted a well-supported clade (bootstrap value = 95, posterior probability = 1), the exact position of *Syndesmis* within the family was left ambiguous (see results in section 3 below). Therefore, we formally tested three hypotheses for the position of *S. aethopharynx* within Umagillidae. From now on, these three topologies will be referred to as topology A (which is part of the ML tree), B and C respectively (as defined in **Fig. 1**). Both the weighted (WSH) and unweighted (SH) Shimodaira-Hasegawa test [33] and the Approximately Unbiased (AU) test [34] were performed in Consel v0.1j [35]. These nonparametric tests use the difference in log-likelihoods of competing topologies as a test statistic and apply bootstrap resampling (multiscale bootstrapping in case of the AU test) to obtain its null distribution. All three tests are valid when comparing *a posteriori* selected topologies, provided the ML tree is included [33, 34]. TREE-PUZZLE v5.2 [36] was used to assess site-wise log likelihoods for the trees, which were subsequently used to construct a suitable input dataset for Consel.

3 Results

3.1 Infection parameters

Out of nine specimens of *P. lividus* collected, six harboured umagillids, all being *S. aethopharynx*, with an infection intensity varying between one and seven flatworms per host. Investigation of ten sympatric specimens of the arbaciid sea urchin *Arbacia lixula* (Linnaeus, 1758) Hansson, 2001 did not yield any turbellarians.

3.2 Taxonomical comments

Syndesmis aethopharynx Westervelt & Kozloff, 1990

New locality. Anavyssos, Mavro Lithari, Greece (37.73278°N, 23.90361°E) (21/02/2013).

Known distribution. Banyuls-sur-Mer, France [1].

Material. Photographs of live animals. Twenty-five specimens, half of each specimen whole-mounted with lactophenol and the other half used for DNA extraction.

Description. The pharynx is situated in the first body half. It has a distinct bipartite structure, consisting of a proximal, bulbous part, followed by a more elongate, cylindrical portion (focus-stacked image in **Fig. 2**; original images provided in **Fig. 6** in appendix). On the only specimen on which the pharynx could be measured adequately, the bulbous part has a diameter of 138 μm , while the cylindrical part is 95 μm long.

The paired testes form a network-like structure and are positioned laterally just behind the pharynx. The sclerotized part of the male copulatory organ consists of a simple, straight stylet, measuring 45 to 67 μm ($\bar{x}$ = 57 μm ; n = 16). It has a funnel-shaped basis and tapers distally towards an open pointed end (**Fig. 3**).

Vitelline glands are paired and confined to the middle third of the body. Each vitellarium is composed of six to nine primary trunks, each dividing dichotomously to form numerous distal branches. Ovaries are also paired and occupy the area just behind the vitellaria. Both consist of one main trunk branching into three to four distal lobes. Filamentous glands are numerous and occupy the major part of the last body third.

Six specimens contain an amber-coloured egg capsule characterized by a very long, tightly-coiled filament (**Fig. 4**). The distal part of the filament shows a small expansion, as indicated in **Fig. 4**. The egg capsules measure 99 to 235 μm in length ($\bar{x}$ = 176 μm , n = 6) and 72 to 164 μm ($\bar{x}$ = 124 μm , n = 6) in width.

3.3 *Phylogenetic analysis*

After processing (alignment and masking), all obtained sequences were identical to each other. Pairwise genetic distances between Umagillidae are shown in **Table 1**. Distances between all species in the analysis are summarized in **Table A.2**. The inferred Bayesian tree topology (**Fig. 5**) is identical to the ML tree. Symbols at each branch indicate posterior probabilities (pp) and bootstrap values (bp). The position of *S. aethopharynx* within Umagillidae is firmly supported (pp = 1, bp = 96), but intrafamilial relationships are not dichotomously resolved.

P-values of the SH, WSH and AU tests are listed in **Table 2**. The AU test rejected topology C ($p < 0.05$) and resulted in a larger p-value for topology B compared to topology A, but neither of these two hypotheses could be rejected statistically ($p > 0.05$). Similarly, the SH and WSH tests resulted in larger p-values for topology B, but neither topology A or C could be rejected ($p > 0.05$).

4 Discussion

4.1 *Species identification and intraspecific variation*

Traditionally, umagillid flatworms from echinoid hosts have been assigned to either *Syndesmis* or '*Syndisyrix*' Lehman, 1946. The validity of the latter genus has been a topic of debate. Here, we will adopt the notion of Marcus [38] that '*Syndisyrix*' is a synonym of *Syndesmis*, which has been agreed upon by Stunkard and Corliss [39], Hyman [40], Jondelius [41] and Doignon and Artois [42]. Our specimens display all typical features of *Syndesmis*: a very long, often tightly-coiled egg filament, a simple, funnel-like stylet, numerous filament

glands in the rear body end, paired ovaries, testes and vitelline glands in discrete pairs and an echinoid host.

The bipartite pharynx is a unique feature for *S. aethopharynx*: it has never been reported in any other syndesmid, or even another umagillid species. In the original description of this species [1], no measurements for the pharynx were provided, but we were able to measure both distinct components of this organ on requested photographs of the paratype specimen (Smithsonian National Museum of Natural History, US National Parasite Collection, USNM 1376373) using Fiji [43]. In the paratype, the bulbous part measures 141 μm in diameter while the cylindrical part measured 96 μm in length, hence closely resembling the pharynx in our specimen, in which these components measure 148 μm and 95 μm respectively.

In their description of *S. aethopharynx*, Westervelt and Kozloff [1] stated that all their specimens possess stylets measuring “about 50 μm ”. The stylets of our specimens largely correspond to this ($\bar{x} = 57 \mu\text{m}$; $n = 16$). We did, however, observe a wide size range (length varying from 45 to 67 μm).

Egg capsule size varies greatly between specimens, ranging from 99 to 235 μm in length and from 72 to 164 μm in width. Two specimens have capsules that are considerably smaller than the rest, measuring only 72 x 107 μm and 80 x 99 μm respectively. The remainder measure between 124-158 μm in length and 187-235 μm in width. Within *Syndesmis*, a comparable degree of variation has only been reported for *S. collongistyla* (Hertel, Duszynski & Ubelaker, 1990) Marcus, 1949 and for *S. dendrastrorum* Stunkard & Corliss, 1951 [45], where the capsule lengths vary over a range of $\pm 90 \mu\text{m}$ and $\pm 100 \mu\text{m}$ respectively. Capsule width does not seem to vary as much in these species ($\pm 60 \mu\text{m}$ in *S. collongistyla* and $\pm 54 \mu\text{m}$ in *S. dendrastrorum*).

To the best of the authors’ knowledge, the only-proposed explanation for large intraspecific variations in syndesmid capsule sizes is a difference in the number of gonads. This has been reported for the three so-called morphs of *S. dendrastrorum* [45]. However, this is not applicable to our specimens. Capsule size might also be correlated with the size of the parent worm, as is the case for the umagillid *Anoplodium hymanae* Shinn, 1983 [47]. Unfortunately, we were unable to adequately measure full body lengths in our specimens and, therefore, could not check whether this correlation also exists in *S. aethopharynx*.

No measurements for the egg capsule were specified by Westervelt and Kozloff [1]. On their photograph, the paratype’s egg measures 124 μm x 65 μm , a bit narrower than the smallest egg in our specimens. Moreover, both the holotype’s [as shown in Fig. 3 in Westervelt and Kozloff [1]] and the paratype’s (as seen on requested photographs) egg capsules are more or less oval, as opposed to the rather spheroid eggs in our specimens. However, the type specimens’ capsule walls appear shrivelled, which may imply these structures have collapsed. It has already been suggested that rhabdocoel egg capsules can sometimes deform during fixation [48] and this might be the case for these specimens. This could also explain the fact that all our specimens’ eggs were a bit wider than the paratype’s egg.

The small expansion at the tip of the egg filament was not mentioned by the original authors. This structure may conform to the ‘solidified shell secretion droplet’ at the end of the filament

which von Graff [48, 49] described in his work on *S. echinorum*. A similar structure was reported in some specimens of *S. aonikenki* Brusa, Montes, Marcotegui and Martorelli, 2017 and *S. selknam* Brusa, Montes, Marcotegui and Martorelli, 2017. It also seems to occur in *S. franciscana* (Lehman, 1946) Marcus, 1949 as shown in Fig. 1 in Shinn and Cloney [51], but this was not discussed by these authors.

Furthermore, photographs as well as the authors' drawings of the holotype [Fig. 1 and Fig. 3 in Westervelt and Kozloff [1]] display the holotype's egg capsule in the first body half, almost at the anterior edge of the testes, but only one of our specimens conforms to this. All other specimens' egg capsules are located about midway along the body and the paratype's egg also seems to be located in the middle of the body. Since no serial sections were available, the position of the uterus in our specimens remains uncertain.

The position of both egg capsule and uterus have been used as diagnostic traits for several species of *Syndesmis*. Examples include the original descriptions of *S. glandulosa* Hyman, 1960 and *S. dendrastrorum*, the redescription of *S. antillarum* Powers, 1936 by Stunkard and Corliss [39], as well as the identification keys by Hickman [53] and Cannon [54]. However, both Marcus [55] and Jennings and Mettrick [56] mentioned a variable position of the egg in their respective descriptions of *S. evelinae* Marcus, 1968 and *S. franciscana*, while Hertel, Duszynski and Ubelaker [44] pointed out that the position of the uterus in *S. collongistyla* varies between specimens. The position of the uterus may be dependent on whether or not an egg capsule is present, as is suggested in the original descriptions of *S. aethopharynx*, *S. rubida* Kozloff & Westervelt, 1990, *S. inconspicua* Westervelt & Kozloff, 1992 and *S. neglecta* Westervelt & Kozloff, 1992. According to these authors, the uterus may extend into the anterior body end in cases where the egg is fully developed. Therefore, and because of the variable position of the eggs in our specimens, the positions of the uterus and/or egg capsules seem at least to some degree to be determined by the stage of development. Consequently, we believe these criteria are not suitable for distinguishing between species of *Syndesmis* and should be avoided as diagnostic characters in future taxonomic work on this taxon, and perhaps umagillids in general.

Only two other umagillids are known from *P. lividus*: *S. echinorum* and another, unnamed *Syndesmis* sp., which may in fact represent some very aberrant specimens of *S. echinorum* [1]. Over the years, many different syndesmid species have unjustifiably been attributed to *S. echinorum*, often with little or no notes on morphology. As a result, taxonomic literature on this species has become entangled [42, 59]. Here, we will compare our specimens to the redescription of *S. echinorum* by Kozloff and Westervelt [59]. Both species differ considerably in stylet length: a length of 200 μm is reported in *S. echinorum* [59], compared to an average of 57 μm in our specimens. Furthermore, ovaries in *S. echinorum* have up to eight lobes, as opposed to the three to four terminal lobes in our specimens. Finally, *S. echinorum* possesses a regular pharynx doliiformis, while our specimens' pharynx has an atypical bipartite structure.

Likewise, our specimens differ notably from *Syndesmis* sp.: *Syndesmis* sp. possesses vitelline glands which extend very far anteriorly, reaching even past the testes, whereas vitellaria in our specimens are confined to the middle body third. Secondly, ovaries in *Syndesmis* sp.

appear sinuous and not (or very slightly) branched, as opposed to the distinctly-lobed ovaries in our specimens. Finally, *Syndesmis* sp. has only one well-developed testis, whereas our specimens all possess two distinct testes [1].

From the above, it is clear that our specimens agree in morphology with the description of *S. aethopharynx* based on a combination of traits, the most important ones being the distinct shape (and size) of the pharynx, the short, straight stylet and the relative sizes and positions of testes, ovaries and vitelline glands. Furthermore, they are easily distinguished from other *P. lividus*-infesting umagillids, and hence it seems justified to attribute our specimens to *S. aethopharynx*. So far, this species had only been reported from its type locality in France, and this is the first record of a syndesmid flatworm from Greece.

4.2 Phylogenetic position

Previous phylogenetic analyses have demonstrated that the 18S rDNA gene generally has a sufficiently high substitution rate to differentiate between closely-related rhabdocoels (e.g. Van Steenkiste, Tessens, Willems, Backeljau, Jondelius and Artois [3]; Tessens, Janssen and Artois [60]). Because all of our obtained sequences were identical to each other, the existence of cryptic species within the morphospecies *S. aethopharynx* seems, therefore, unlikely, despite the above-mentioned intraspecific variation.

Phylogenetic inference resulted in strong support for the position of *S. aethopharynx* within Umagillidae, but intrafamilial relationships are not fully resolved. *Syndesmis* did appear as the sister group of *Wahlia* and *Seritia* in the best-scoring ML tree (**Fig. 1a**), but support values are very low (bp = 48). Likewise, topology A was more often retrieved by the Bayesian analysis (pp = 0.664) compared to the two alternate hypotheses (pp = 0.235 and pp = 0.102 respectively), but the posterior probabilities in all cases are low.

In addition, model-corrected pairwise genetic distances also lend support to topology A. Indeed, the genetic distance between *Syndesmis* and *Anoplodium* (6.6%) is considerably greater than the distance between *Syndesmis* and the *Seritia-Wahlia* group (4.0% and 3.6% respectively). Moreover, distances between *Syndesmis* and *Seritia* and between *Syndesmis* and *Wahlia* are considerably lower than the distances between *Anoplodium* and these two species (5.8% and 5.6% vs. 6.8%).

Conversely, non-parametric testing (SH, WSH and AU) generally resulted in larger p-values for topology B. However, only the AU test resulted in formal rejection of a hypothesis (topology C; $p < 0.05$), leaving the exact position of *S. aethopharynx* still ambiguous. Sequencing of more and more-rapidly-evolving molecular markers (e.g. COI) and inclusion of more taxa in the analysis might yield the necessary phylogenetic resolution to gain a better understanding of the phylogenetic relationships within this family.

4.3 Relation to morphology

No morphological evidence for either of the three hypotheses could be obtained from taxonomical literature. Our analysis did confirm the finding of Van Steenkiste, Tessens, Willems, Backeljau, Jondelius and Artois [3] that *Wahlia* and *Seritia* constitute a monophyletic clade. This result was to be expected, since the same molecular markers were

used in both studies. However, the taxonomical implications of this grouping have never been discussed in detail.

The vast majority of proposed hypotheses on the interrelationships among Umagillidae are solely based on morphology. An extensive review of Umagillidae was provided by Cannon [54]. In that contribution, four different subgroups within the subfamily Umagillinae were defined. This classification is based on a combination of traits related to the uterus, the copulatory organ and the vitelline glands. *Wahlia* and *Seritia* were assigned to the so-called *Cleistogamia*-group, together with *Ozametra* Marcus, 1949 and *Cleistogamia* Faust, 1924. This grouping is based on the presence of a secondary uterus, which Cannon [54] considered to be the derived character state.

Unfortunately, no members of *Ozametra* or *Cleistogamia* could be included in our analysis and the overall number of umagillid species in our analysis is evidently very low. However, it is worth mentioning that our results do provide some support for the hypothesis of Cannon [54] and hence a secondary uterus may indeed be an apomorphy for the *Cleistogamia*-group. Evidently, a more extensive analysis including more taxa is necessary to draw definite conclusions.

4.4 Conclusions and future perspectives

Syndesmis aethopharynx is reported for the first time from the Eastern Mediterranean. Until now, this species had only been reported from its type locality. The original description is supplemented with more details concerning the species' internal morphology, and previously unreported intraspecific variation is described. Moreover, the position of *S. aethopharynx* within Umagillidae is genetically confirmed, yet intrafamilial relationships remain obscure. Future research integrating more taxa and including sequence data from additional molecular markers will be mandatory to gain more phylogenetic resolution for this family.

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7 Appendix

Table A.1 Sequences used in phylogenetic analyses

Species name	GenBank accession number
<i>Adenopharynx mitrabortalis</i>	KC529520
<i>Anoplodium stichopi</i>	AF167424
<i>Austradenopharynx</i> sp.	KC529521
<i>Baicalellia brevityba</i>	KC529505
<i>Balgetia semicirculifera</i>	KC529503
<i>Bresslauilla relicta</i>	KC529515
<i>Canetellia beauchampi</i>	KC529504
<i>Castrella pinguis</i>	KC529438
Dalyellioida “houdini” sp.	KC529522
Dalyellioida sp.	KC529523
<i>Einarella agrillophyla</i>	AY775757
<i>Eldenia reducta</i>	KC529502
<i>Gieysztorina zuluensis</i>	KC529465
<i>Graffilla buccincola</i>	AJ012521
<i>Kytorhynchus</i> sp.	KC529400
Neodalyellida sp. 1	KC529524
Neodalyellida sp. 2	KC529525
<i>Pogaina</i> sp. 1	KC529507
<i>Pogaina</i> sp. 2	KC529508
<i>Pogaina</i> sp. 3	KC529506
<i>Provortex balticus</i>	KC529511
<i>Provortex karlingi</i>	KC529510
<i>Provortex tubiferus</i>	AJ312269
Provorticidae sp.	KC529509
<i>Proxenetes simplex</i>	KC529410
<i>Pseudograffilla arenicola</i>	KC529514
<i>Pterastericola australis</i>	AJ012518
<i>Pterastericola psilastericola</i>	KC529516
<i>Seritia elegans</i>	KC529517
<i>Syndesmis aethopharynx</i>	XXXXXXXX
Solenopharyngidae sp.	KC529519
<i>Trisaccopharynx</i> sp.	AY775774
<i>Vejdovskya ignava</i>	KC529513
<i>Vejdovskya pellucida</i>	KC529512
<i>Wahlia macrostylifera</i>	KC529518

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	
1	<i>Proxenetes simplex</i>	-																																		
2	<i>Eimarella argillophylla</i>	29.6	-																																	
3	<i>Vejdovskýa pellicida</i>	40.9	22.0	-																																
4	<i>Placorhynchus octaculeatus</i>	25.5	27.9	36.0	-																															
5	<i>Wahlia macrostylifera</i>	36.2	19.8	9.9	31.9	-																														
6	<i>Neodalyellida</i> sp. 1	39.6	26.1	26.9	36.5	23.9	-																													
7	<i>Pterastericola australis</i>	37.4	24.5	28.5	34.5	27.5	20.3	-																												
8	<i>Vejdovskýa ignava</i>	39.4	22.3	2.8	34.3	10.0	27.0	27.8	-																											
9	<i>Badgetia semicirculifera</i>	32.6	24.1	26.0	30.4	24.0	18.7	16.5	27.3	-																										
10	<i>Dalyellioida</i> sp.	35.4	22.2	22.4	31.8	21.5	16.6	14.1	23.1	11.0	-																									
11	<i>Seritia elegans</i>	35.6	20.4	9.8	31.2	1.8	23.3	26.7	10.0	22.9	20.7	-																								
12	<i>Austradenopharynx</i> sp.	37.2	19.1	24.4	32.8	25.4	27.4	28.4	25.8	26.1	25.2	26.7	-																							
13	<i>Pogonia</i> sp. 1	36.0	23.7	27.2	30.5	24.0	18.1	14.2	26.0	13.7	14.0	23.5	25.4	-																						
14	<i>Kyrtophynchus</i> sp.	21.8	27.9	38.2	26.5	32.9	35.6	33.4	37.1	33.6	31.8	34.7	32.3	32.0	-																					
15	<i>Solenopharyngidae</i> sp.	37.7	24.0	28.7	35.4	28.2	29.6	33.1	28.7	30.2	27.8	28.1	16.1	29.3	33.5	-																				
16	<i>Pterastericola psilastericola</i>	38.4	23.7	25.4	32.8	25.2	18.6	2.9	25.3	15.0	12.6	24.0	27.7	13.1	35.0	32.1	-																			
17	<i>Pseudogriffilla arenicola</i>	38.7	25.1	29.9	30.7	27.1	20.9	19.5	27.6	16.1	16.8	27.1	26.8	16.1	33.6	29.6	17.5	-																		
18	<i>Giesztoria zuluensis</i>	21.9	25.8	34.0	27.1	30.1	39.1	33.5	33.7	38.8	37.5	31.7	36.6	37.5	21.6	41.0	33.7	41.4	-																	
19	<i>Provorticidae</i> sp.	32.6	20.5	23.1	30.3	19.8	15.1	10.8	23.4	10.0	9.3	18.8	23.4	9.2	29.9	26.1	9.4	12.4	37.7	-																
20	<i>Griffilla buccinicola</i>	53.1	40.0	42.5	48.9	43.8	33.7	35.5	41.3	30.1	30.8	42.7	43.0	31.0	50.8	43.1	35.5	28.4	55.9	28.1	-															
21	<i>Eilemia reducta</i>	37.5	27.2	31.4	36.2	26.4	20.9	22.6	30.4	18.0	17.8	26.0	30.8	22.0	37.8	32.6	20.8	21.1	40.7	16.8	36.9	-														
22	<i>Pogonia</i> sp. 2	34.8	23.3	26.7	31.7	23.5	18.3	13.9	25.6	13.3	12.7	23.5	22.8	3.0	32.7	27.8	12.5	16.6	39.8	8.7	29.7	21.8	-													
23	<i>Provortex karingi</i>	38.6	28.9	16.2	38.6	14.2	30.0	35.1	14.8	30.0	27.6	14.5	32.7	30.9	42.6	34.7	33.2	31.1	40.7	24.7	49.9	33.0	31.5	-												
24	<i>Syndesmis aethopharynx</i>	34.7	18.0	10.3	29.8	3.6	23.1	25.9	9.8	22.7	21.4	4.0	27.1	22.7	31.7	26.6	24.1	26.8	29.1	19.4	43.7	25.5	24.0	14.9	-											
25	<i>Provortex tubiferus</i>	38.2	28.9	16.1	38.9	15.0	30.8	34.5	15.1	29.5	28.3	14.9	32.0	32.1	43.3	33.9	33.2	31.4	41.5	25.4	50.4	32.9	32.8	1.3	15.2	-										
26	<i>Neodalyellida</i> sp. 2	45.2	28.2	30.0	40.2	26.2	7.2	23.2	30.7	21.4	20.4	25.5	28.5	23.5	38.4	34.5	22.0	25.6	41.3	18.5	42.0	25.2	22.6	34.9	25.6	35.2	-									
27	<i>Provortex bullicus</i>	38.9	28.7	15.8	38.9	14.8	30.4	35.4	14.9	29.5	28.2	14.7	32.0	31.1	44.1	34.7	33.3	31.4	41.8	25.4	49.7	33.6	32.2	1.1	15.3	0.6	35.5	-								
28	<i>Pogonia</i> sp. 3	35.3	21.2	25.2	30.8	23.6	18.0	12.0	24.4	11.3	11.5	22.9	22.6	5.4	29.8	26.4	11.0	15.2	37.3	7.7	28.5	19.8	5.5	29.3	22.8	29.2	21.6	29.5	-							
29	<i>Adenopharynx nitribursalis</i>	36.1	16.6	23.6	32.7	24.1	25.2	26.6	25.3	26.0	23.3	25.2	6.4	23.4	34.3	13.5	26.3	25.7	34.2	20.8	39.2	29.4	20.8	31.1	23.8	31.4	27.3	31.3	21.5	-						
30	<i>Canetella beauchampi</i>	32.2	19.7	22.7	29.9	21.5	15.5	9.4	22.3	9.8	8.9	20.4	21.8	9.0	27.7	26.7	8.6	12.1	33.8	4.3	28.7	17.2	8.9	27.1	20.1	27.0	19.3	27.0	6.9	18.6	-					
31	<i>Triscecopharynx westbladi</i>	39.4	22.4	27.4	35.6	27.3	28.1	30.2	29.1	28.9	27.2	27.9	14.2	27.7	35.8	7.4	29.2	28.3	42.5	25.4	43.3	31.1	26.0	32.9	28.0	33.1	30.6	33.3	25.4	12.6	25.2	-				
32	<i>Dalyellioida "noudini"</i> sp.	37.3	27.9	31.5	32.7	27.3	22.7	21.7	29.6	20.3	17.9	28.4	25.8	18.3	35.8	28.4	20.1	8.0	43.9	15.6	32.6	24.8	17.7	33.9	28.2	36.2	26.9	35.1	17.6	25.3	15.0	27.9	-			
33	<i>Anoplocladum stichopi</i>	37.0	21.4	12.5	33.7	5.6	27.2	30.9	11.5	28.1	23.5	5.8	27.6	27.2	35.2	30.5	27.9	29.4	32.8	22.8	49.1	28.7	26.2	16.4	6.6	16.5	29.8	16.4	25.4	27.3	23.7	30.5	29.4	-		
34	<i>Castrella pinguis</i>	20.8	24.4	37.1	26.4	29.9	37.0	36.3	37.1	36.1	33.8	31.5	31.9	36.7	20.5	39.1	36.1	41.2	8.4	34.8	56.1	37.2	37.7	38.3	30.6	39.5	40.8	40.2	34.5	32.9	31.8	37.0	40.3	30.7	-	
35	<i>Baicalietta brevintha</i>	31.1	19.6	22.4	30.2	21.0	14.9	8.8	21.9	9.7	8.6	20.1	20.5	8.7	27.7	25.6	7.9	12.5	34.3	3.9	27.7	17.1	8.5	26.4	19.9	26.1	18.9	26.3	6.5	18.3	0.3	24.2	14.7	23.5	33.0	-
36	<i>Bresslanilla relicta</i>	37.7	22.9	28.8	32.7	26.5	20.0	16.9	27.0	16.4	17.2	26.3	25.6	19.2	34.0	27.2	16.8	21.4	37.9	14.4	34.2	16.0	18.4	31.2	24.5	30.7	22.2	31.6	17.1	24.6	14.7	28.9	25.7	28.5	32.9	14.4

Tables

Table 1 GTR+G+I pairwise genetic distances (in %) between the umagillid species included in the analysis

	1	2	3
1 <i>Anoplodium stichopi</i>	-		
2 <i>Seritia elegans</i>	5.8	-	
3 <i>Syndesmis aethopharynx</i>	6.6	4.0	-
4 <i>Wahlia macrostylifera</i>	5.6	1.8	3.6

Table 2 P-values of the SH, WSH and AU tests obtained in Consel v0.1j. Significant p-values ($p < 0.05$) are indicated by an asterisk (*)

	Shimodaira-Hasegawa (SH)	Weighted Shimodaira-Hasegawa (WSH)	Approximately Unbiased (AU)
Topology A (ML tree)	0.302	0.400	0.297
Topology B	0.836	0.800	0.749
Topology C	0.102	0.071	0.011*

Figure legends

Fig. 1 Three hypotheses for the interrelationships of Umagillidae. **a.** Topology A, part of the ML tree as obtained by RAxML (bootstrap values are indicated above branches). **b.** Topology B. **c.** Topology C.

Fig. 2 Pharynx of *Syndesmis aethopharynx*. Focus-stacked image of three photographs at different focal depths. Photographs made on a Leica DM 2500 microscope using interference contrast. Scale bar: 50 μm .

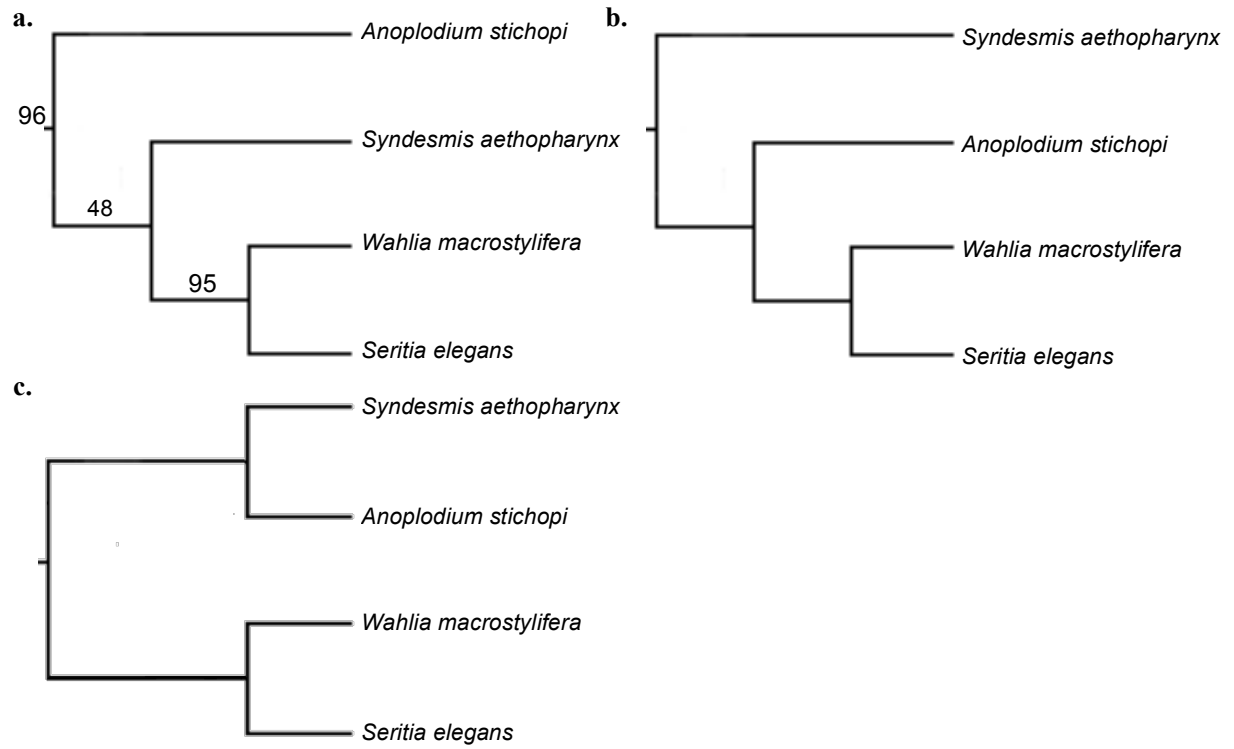
Fig. 3 Stylet of *Syndesmis aethopharynx*. **a.** Camera lucida drawing and **b.** photograph made on a Nikon Eclipse 80i compound microscope, using interference contrast. Scale bars: 20 μm .

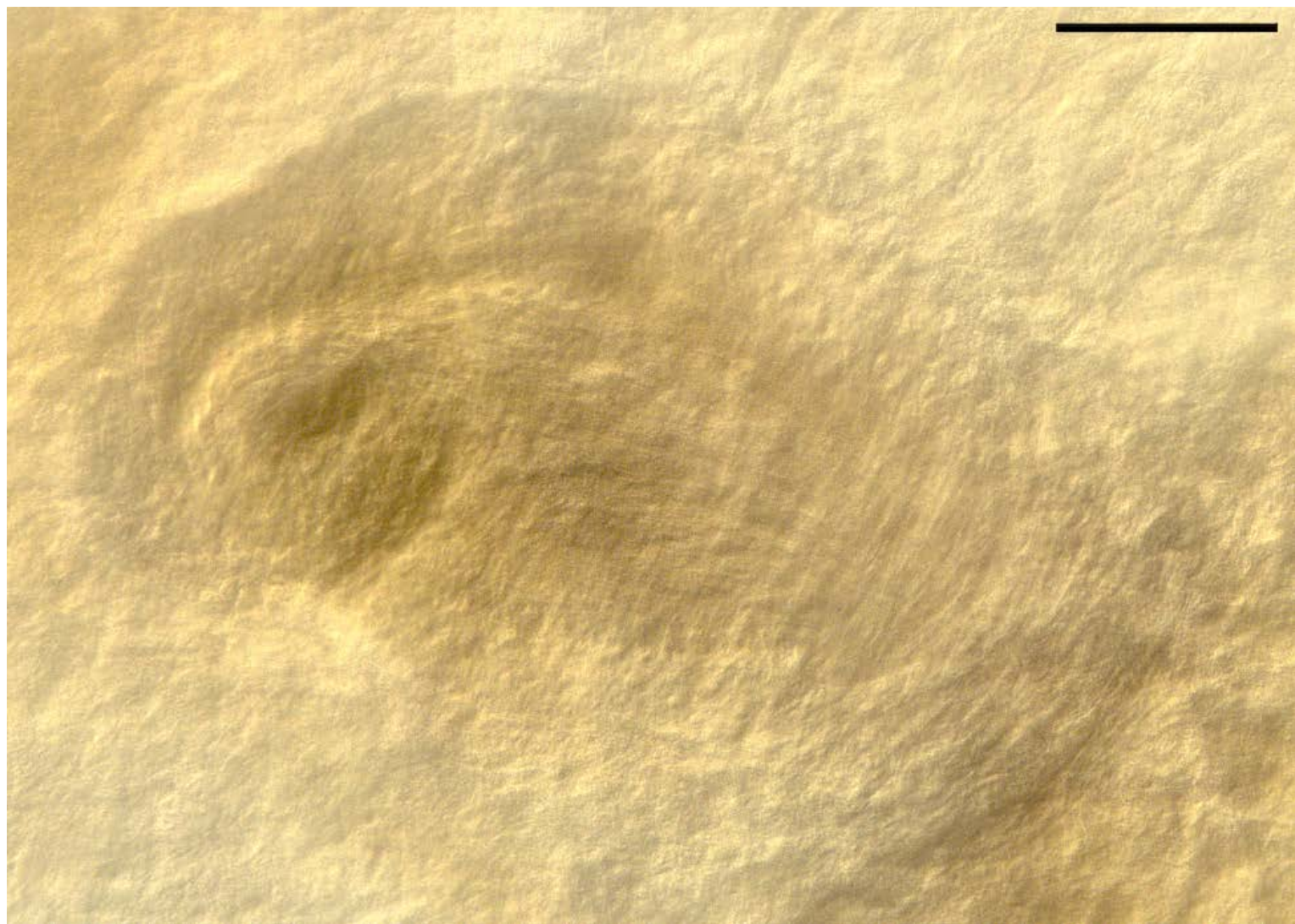
Fig. 4 Egg capsule of *Syndesmis aethopharynx*. **a.** Camera lucida drawing. **b.** and **c.** Photographs made on a Nikon Eclipse 80i compound microscope, using interference contrast. Filament tip expansion is indicated by an arrow. Scale bars 3A and 3B: 100 μm ; 3C: 20 μm .

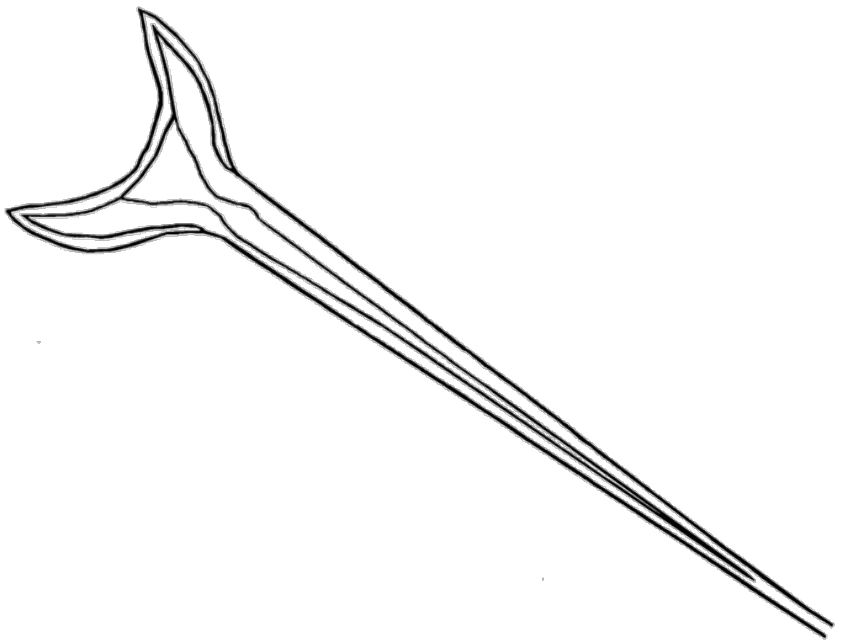
Fig. 5 Majority rule consensus tree of interrelationships within Neodalyellida, obtained from the Bayesian analysis under the GTR+G+I model. Branches with posterior probabilities below 0.97 have been collapsed. Symbols above branches indicate posterior probabilities (pp) and symbols below branches represent bootstrap support values (bp); legend is displayed in the top left. Branch lengths denote the number of expected nucleotide substitutions per site.

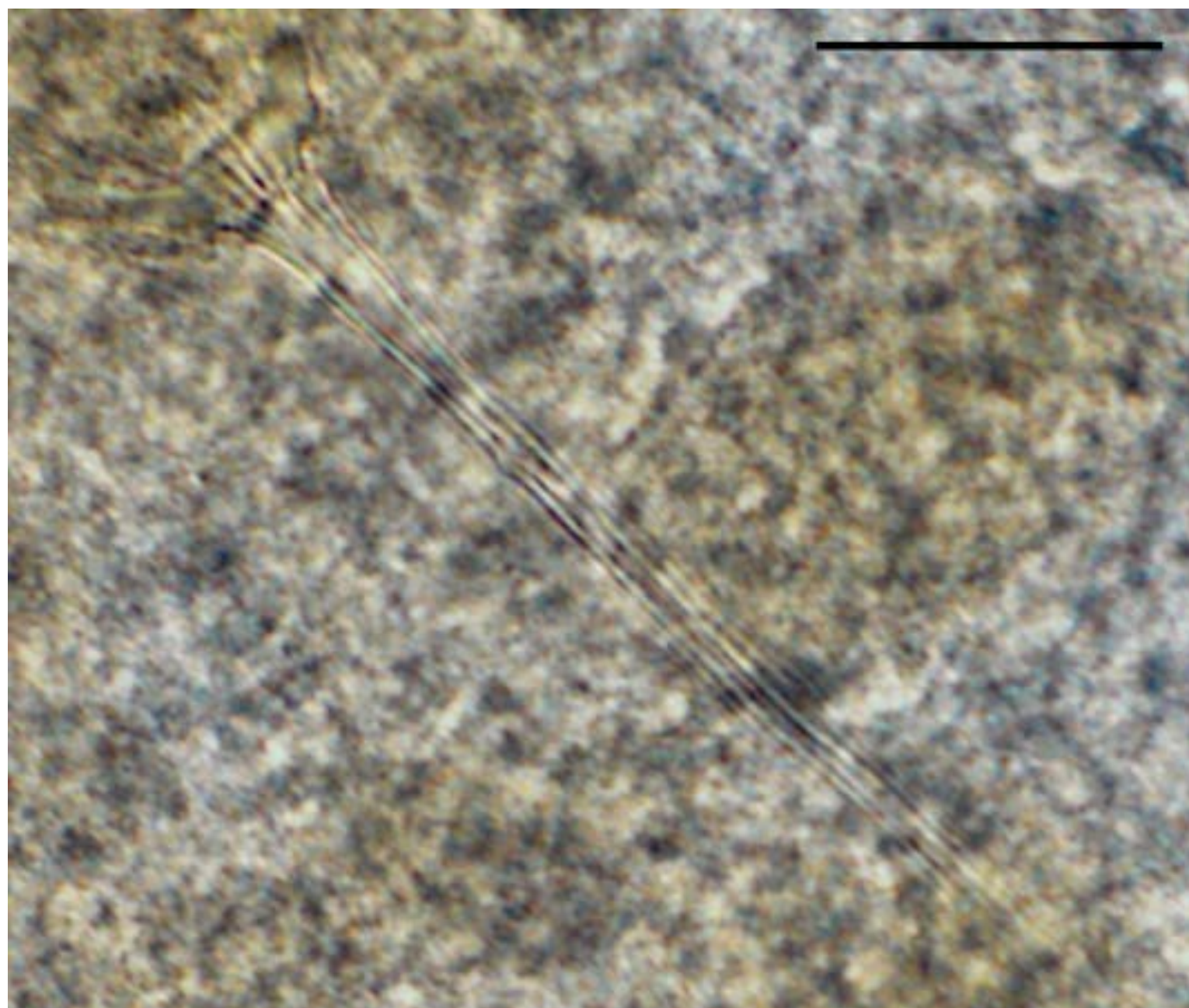
Appendix figure legends

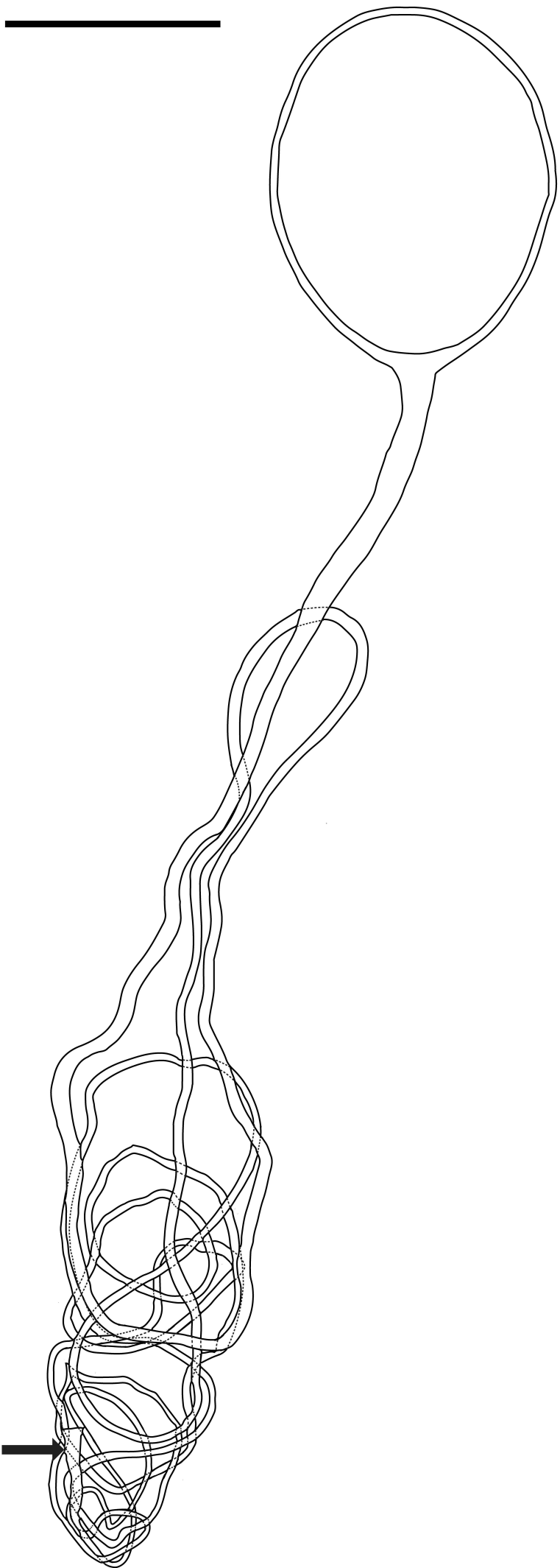
Fig. A.1 Pharynx of *Syndesmis aethopharynx*. Photographs taken at three different focal depths. Scale bars: 50 μm .

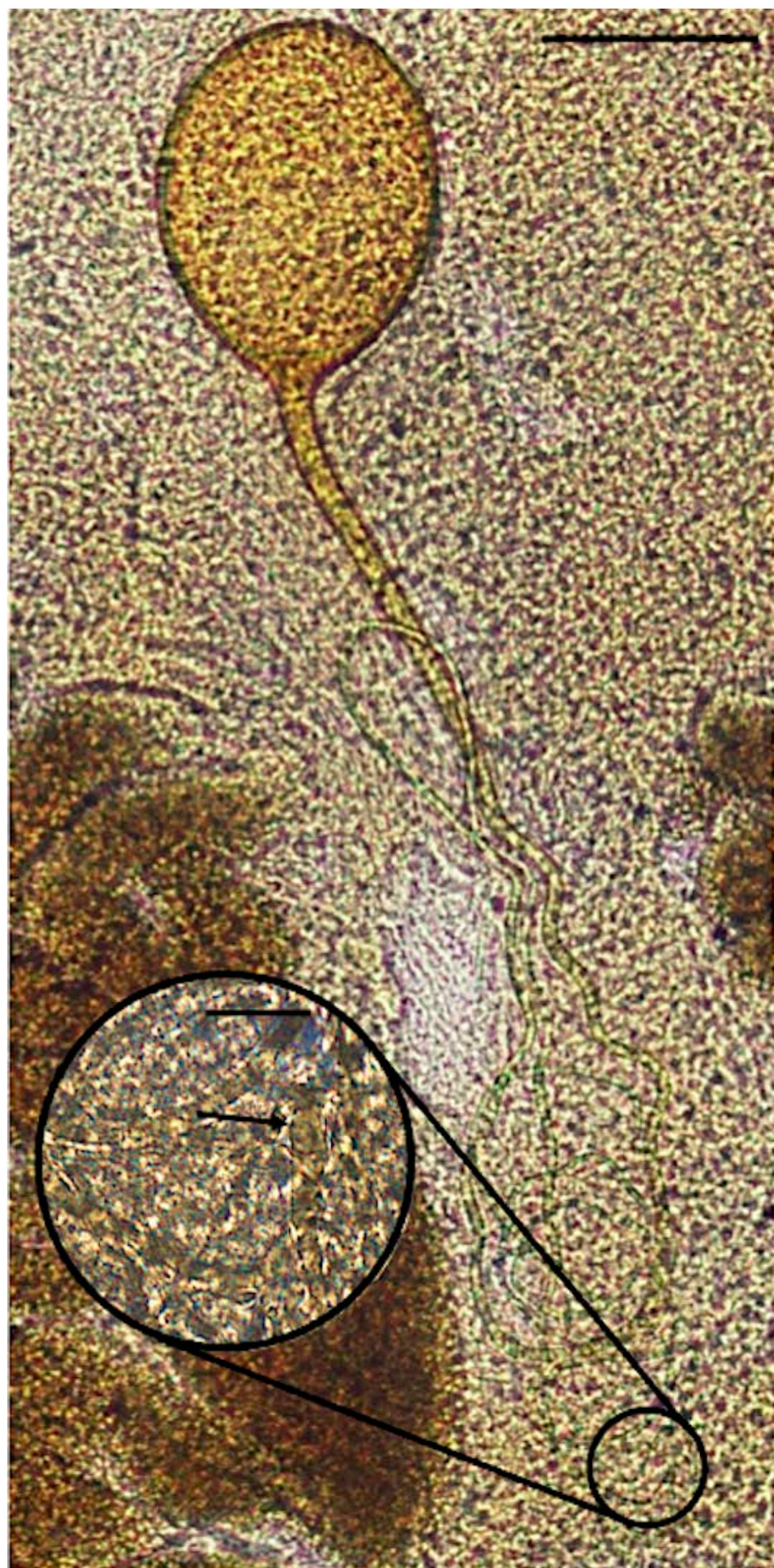




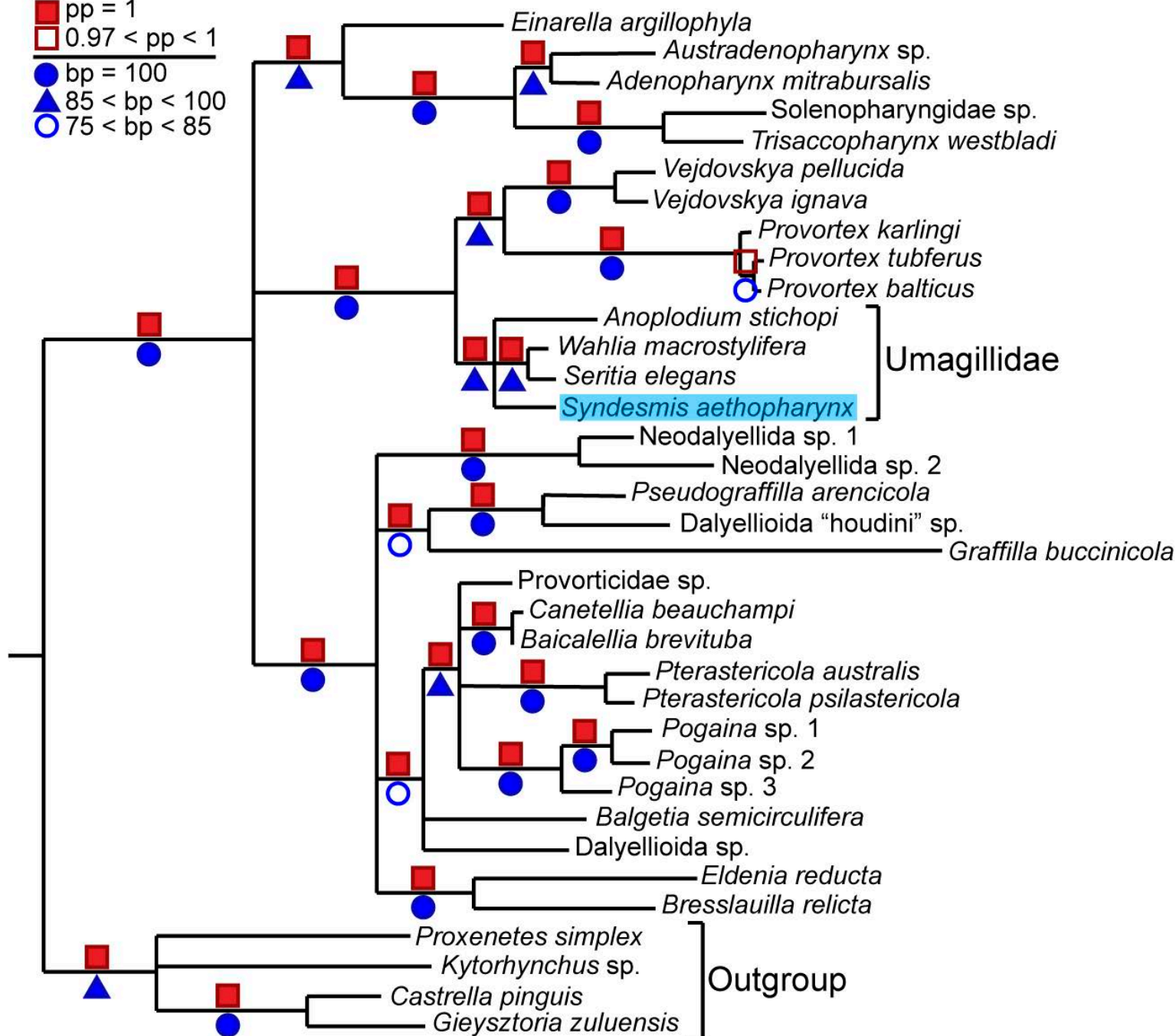








- pp = 1
□ 0.97 < pp < 1
● bp = 100
▲ 85 < bp < 100
○ 75 < bp < 85



0.06

