The anatomy and relationships of *Troschelia* (Neogastropoda: Buccinidae): New evidence for a closer fasciolariid-buccinid relationship?

Alisa R. Kosyan
A.N. Severtsov Institute of Ecology and Evolution
Russian Academy of Sciences
33 Leninski Prospekt
Moscow 119071, RUSSIA
kosalisa@rambler.ru

Maria Vittoria Modica
Marco Oliverio
Dipartimento di Biologia Animale e dell'Uomo
"La Sapienza" Rome University
Viale dell'Università 32
I-00185 Roma, ITALY
mariavittoria.modica@uniroma1.it
marco.oliverio@uniroma1.it

ABSTRACT

Analyses of new anatomical and molecular data confirm the taxonomic position of *Troschelia berniciensis* (King, 1846) within the Buccinidae and provide the framework for a review of the relationships of the families Fasciolariidae and Buccinidae. The morphology of *Troschelia* Mörch, 1876, is similar to that of other Northern Atlantic and Pacific buccinid genera. Anatomical examination of a number of fasciolariid species revealed only a single character, the structure of the proboscis retractor muscles, to be diagnostic of the Fasciolariidae, while other characters are shared with the Buccinidae. A molecular phylogeny also confirms a close relationship between the two groups.

Additional keywords: Taxonomy, phylogeny, anatomy, Buccinidae, Fasciolariidae

INTRODUCTION

Phylogenetic relationships among the more than 200 genera and subgenera included in the gastropod family Buccinidae remain quite ambiguous. The northeastern Atlantic monotypic genus *Troschelia* Mörch, 1876, has been classified by a number of authors (e.g., G.O. Sars (1878), J. Thiele (1929)) in the family Fasciolariidae, due to the peculiar radula of *Troschelia berniciensis* (King, 1846). However, Bouchet and Warén (1985) placed *Troschelia* within Buccinidae, based on the morphology of its lateral teeth, which have multiple, uniform cusps similar to those of some other Buccinidae (*Thalassoplanes* Dall, 1908). To elucidate the phylogenetic relationships of *Troschelia* with the families Buccinidae and Fasciolariidae, morphological and anatomical features were studied, and partial sequences from the mitochondrial 16S rRNA gene were analyzed for *Troschelia berniciensis* as well as for a number of buccinid and fasciolariid taxa.

MATERIALS AND METHODS

Samples for the present study were collected during field work and expeditions to the West Pacific (PANGLAO 2004, Philippines, and SANTO 2006, Vanuatu, organized by the Muséum national d'Histoire naturelle, Paris), to Panama (Neogastropod Workshop 2006, at the Smithsonian Tropical Research Institute, Panama), the Mediterranean Sea, and at other localities, and supplemented by specimens provided by Museums (BMNH – Natural History Museum, London, UK, MNHN – Muséum National d'Histoire naturelle, Paris, France, BAU – Museum of Biologia Animale e dell'Uomo Department, University "La Sapienza", Rome, Italy) and colleagues. The taxa listed in Table 1 were used for anatomical studies. Animals were dissected and radulae examined using an SEM. Sequence data was newly generated for several species, and supplemented with additional taxa that were obtained from GeneBank (see Table 2).

DNA EXTRACTION, PCR, AND SEQUENCING: Total DNA was extracted following a standard Phenol/Chloroform/Ethanol protocol (Hillis et al., 1990) with slight modification as previously described by Oliverio and Mariottini (2001) for mollusks. QIAGEN QiAmp Extraction Kit was used according to manufacturer's instructions for extraction of DNA from difficult samples.

A region of the gene encoding 16SrDNA encompassing the domains IV and V (Gutell and Fox, 1988) was amplified using primers 16SA (5'-CGCCTGTTTATCAAAAACAT-3') (Palumbi et al., 1991) and 16SH (5'-CC GGTCTGAACTCAGATCAC-3') (Espiritu et al., 2001). Amplification conditions were as follows (30–35 cycles): 94° for 30 sec, 45–50°C for 30 sec, 72°C for 60 sec. When a single band was obtained the PCR product was purified using the Exo-Sap enzymatic method. Purified products were then double strand sequenced with BigDye v. 2.0 (Applied Biosystems, Foster City, CA, USA)

Table 1. Species used in the anatomical study.

Family	Subfamily	Species	Locality	Voucher number
Buccinidae		*Troschelia berniciensis* (King, 1846)	Station number: 44/2, Sea Area: S38, 51°19.2′ N, 08°32.2′ W, Depth: 95m, Date: 28/05/1975 RRS Challenger Cruise 8/75	BMNH 20031298 BAU00687
Fasciolariidae	Peristerninae	*Turrilatirus turritus* (Gmelin, 1791)	Sudan, Red Sea, FEL-93 Expedition, Sha'ab Rumi, Stn. 4, N 6, 0.5–1 m, reef-flat brushing, pocilloporids and coral rubble, M/S Felicidad	BAU00691
Fasciolariidae	Peristerninae	*Pustulatirus mediamericanus* (Hertlein and Strong, 1951a),	Panama, Pacific Ocean, Venado beach, 8.89° N, 79.59° W, intertidal, Feb. 2006	BAU00688
Fasciolariidae	Peristerninae	*Latirus polygonus* (Gmelin, 1791)	Sudan, Red Sea, REDSED–5 Cruise, Sanganeb Reef, Stn. RS5–5, N 1, Southern Lagoon, sand and patchy reefs, 2 m, M/S Felicidad – BIORES group, Leg. M. Oliverio, M. Taviani, 3 Sep.1993	BAU00690
Fasciolariidae	Peristerninae	*Peristernia nassatula* (Lamarck, 1822)	Sudan, Red Sea, REDSED-5 Cruise, Sanganeb Reef, Stn. RS5-3, N 1, lagoon plus reef flat, brushing coral rabbish and direct search in situ, 2 Sep. 1993 day – 9 Sep. 1993 night	BAU00692
Fasciolariidae	Peristerninae	*Peristernia ustulata* (Reeve, 1847)	Philippines, Pamilacan, 9.48° N, 123.9°3 E, 10–41 m	BAU00693
Fasciolariidae	Peristerninae	*Opeatostoma pseudodon* (Burrow, 1815)	Panama, Pedro Gonzales Island, Archipelagus Las Perlas, 8.4° N 79.1° W, intertidal, 02.2006	BAU00689
Fasciolariidae	Fusininae	*Fusinus tenerifensis* Hadorn and Rolán, 1999	Canary Islands, Puerta de la Cruz	BAU00694
Fasciolariidae	Fasciolariinae	*Fasciolaria lignaria* (Linnaeus, 1758)	S. Marinella (Italy) 42°02′ N 11°54′ E, intertidal	BAU00227

using the PCR primers and sequences visualized on automatic sequencer. Sequencing was performed by Macrogen Inc. (Seoul, South Korea). Chromatograms were analysed by Staden Package (Version 1.6.0, Staden et al., 1998, 2005). All sequences have been deposited at EMBL (see Table 2 for accession numbers).

Sequences obtained were aligned using ClustalX (Thompson et al., 1994; 1997) with the default settings. The alignments obtained were manually edited. The χ^2 test implemented in PAUP* v. 4b10 (Swofford, 2002) was used to test for base composition homogeneity of the sequence data aligned.

A Bayesian analysis of the aligned sequences was performed using MrBayes v. 3.1.2 (Ronquist and Huelsenbeck, 2003), which sampled trees from posterior densities using the Markov Chain Monte Carlo method (Larget and Simon, 1999; Yang and Rannala, 1997). The substitution model to be used in the Bayesian analysis was chosen after evaluation by the software MrModeltest 2.2 (Nylander, 2004), while base frequencies, relative rates of the six substitution types and model parameters were estimated by MrBayes during phylogenetic reconstruction. A four-chain metropolis-coupled Monte Carlo analysis was run twice in parallel for 10^6 generations, and trees were sampled every 1000 generations, starting after a burn-in of 250000 generations. Stationarity was considered to be reached when the average standard deviation of split frequencies shown in MrBayes was less than 0.01 (Ronquist and Huelsenbeck, 2003). Bayesian posterior probabilities (BPP) of a branch were estimated as the percentage of trees (after burn-in) which showed that specific node.

RESULTS

Anatomy of *Troschelia berniciensis*: External Morphology: Animal (Figures 1–3) is uniform cream in color. Foot (Figure 1, **ft**) partly contracted, with deep propodial groove (**prpg**) separating narrow propodium. Operculum oval, with terminal nucleus (Figure 1, **op**). Head moderately large, broad (Figure 1, **hd**) with pair of long, thick tentacles, each with large black eye on

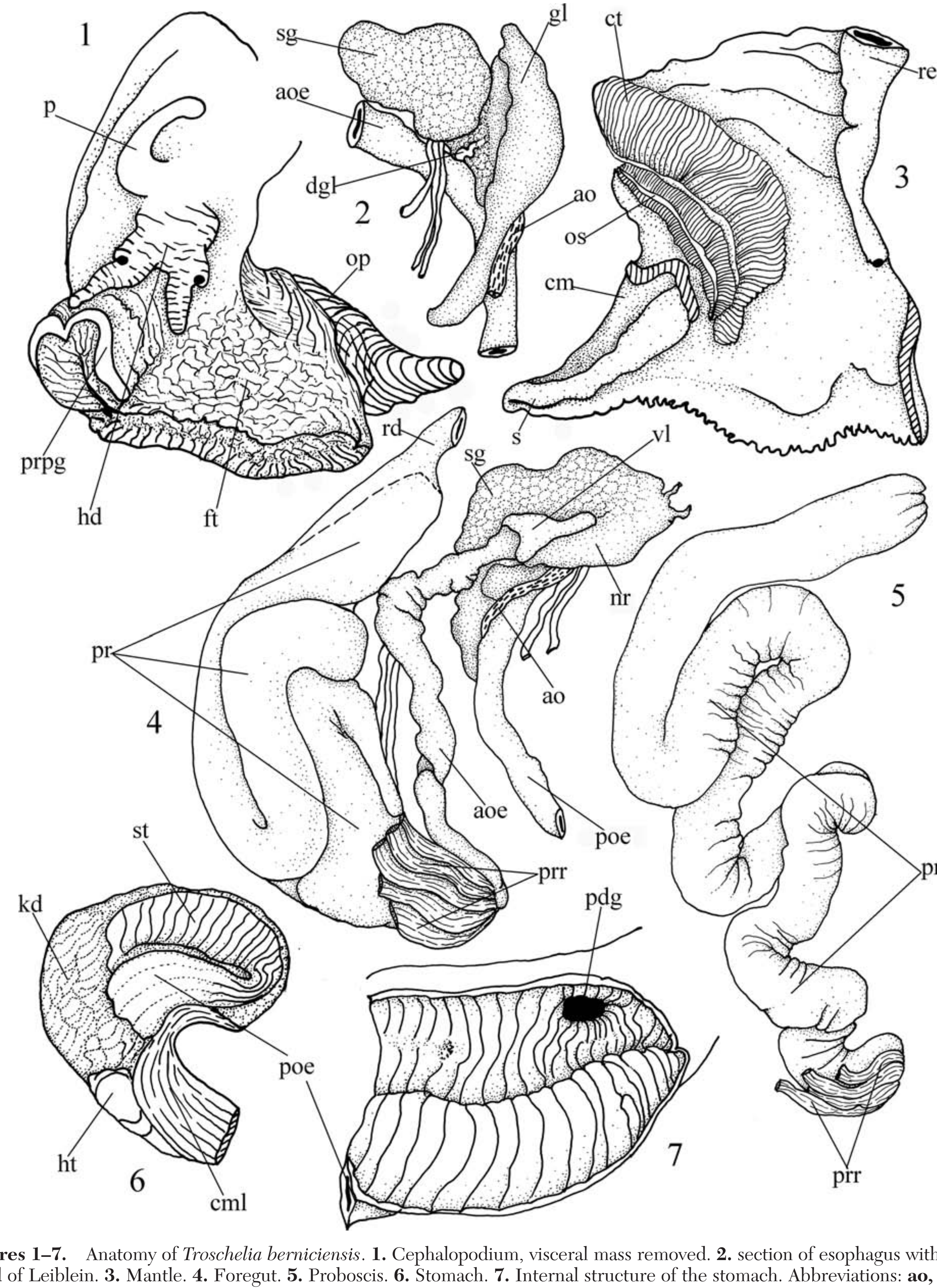
Table 2. Species included in the phylogeny based on partial 16S DNA sequences.

Family	Species	Locality	Voucher number	EMBL	Reference
Cancellariidae	*Cancellaria cancellata* Linné, 1767	Off Malaga (Spain), 40–50 m	BAU00224	FM999105	Oliverio and Modica, in press
Fasciolariidae	*Turrilatirus turritus* (Gmelin, 1791)	Balicasag (Philippines) 9.51° N, 123.68° E, 80–150m	BAU00695	FN394061	this study
Fasciolariidae	*Fasciolaria lignaria* (Linnaeus, 1758)	S. Marinella (Italy) 42.03° N, 11.9° E, intertidal	BAU00227	FN394059	this study
Fasciolariidae	*Fusinus akitai* Kuroda and Habe in Habe, 1961	Off Atsumi, Aichi, central Japan	—	AB044253	Hayashi, 2005
Fasciolariidae	*Granulifusus niponicus* (E.A. Smith, 1879)	Off Kushimoto, Wakayama, central Japan	—	AB044254	Hayashi, 2005
Buccinidae	*Troschelia berniciensis* (King, 1846)	South of Ireland, 51°19.2′ N, 08°32.2′ W	BMNH 20031298 BAU00687	FN394057	this study
Buccinidae	*Pollia tincta* Conrad, 1846	St. Petersburg Beach, Pinellas, Florida, USA	—	AB044270	Hayashi, 2005
Buccinidae	*Pisania striata* Gmelin, 1791	Salina Is. (Italy), 38.58° N, 14.80° E, intertidal	BAU00698	FM999128	Oliverio and Modica, in press
Buccinidae	*Engina pulchra*	Venado (Panama), 8.89° N, 79.59° W intertidal	BAU00276	FN394058	this study
Buccinidae	*Cantharus multangulus* (Philippi, 1848)	Tierre Verde, Pinellas, Florida, USA	—	AB044259	Hayashi, 2005
Buccinidae	*Paraeuthria plumbea* (Philippi, 1841)	Ushuaia (Argentina), 54.78° S, 68.23° W, intertidal	BAU00697 MNHN IM-2009-4613	FM999126	Oliverio and Modica, in press
Buccinidae	*Neobuccinum eatoni* (Smith, 1875)	Terra Nova Bay (Antarctic), 74.69° S, 164.12° E,	BAU00785 MNHN IM-2009-4614	FM999127	Oliverio and Modica, in press
Buccinidae	*Chlanidota densesculpta* Martens, 1885	South Sandwich Islands 56.24° S, 27.44° W	BAU00230	FN394060	this study
Buccinidae	*Buccinulum lineum* (Martyn, 1784)	Leigh Harbour, New Zealand	—	AB044256	Hayashi, 2005
Buccinidae	*Japeuthria ferrea* (Reeve, 1847)	Suga Island, Ise Bay, Mie, central Japan	—	AB044262	Hayashi, 2005
Buccinidae	*Siphonalia cassidariaeformis* (Reeve, 1843)	off Shizuoka, central Japan	—	AB044271	Hayashi, 2005
Buccinidae	*Kelletia kelletii* (Forbes, 1850)	Santa Barbara Island, Los Angeles, USA	—	AB121037	Hayashi, 2005
Buccinidae	*Penion sulcatus* (Lamarck, 1816)	Unknown, New Zealand	—	AB044267	Hayashi, 2005
Buccinidae	*Phos laeve* Kuroda and Habe in Habe, 1961	Off Kushimoto, Wakayama, central Japan	—	AB044268	Hayashi, 2005
Buccinidae	*Neptunea intersculpta* (Sowerby, 1899)	Off Hokkaido, north Japan	—	AB044265	Hayashi, 2005
Buccinidae	*Buccinum opisoplectum* Dall, 1907	unknown	—	AB044257	Hayashi, 2005

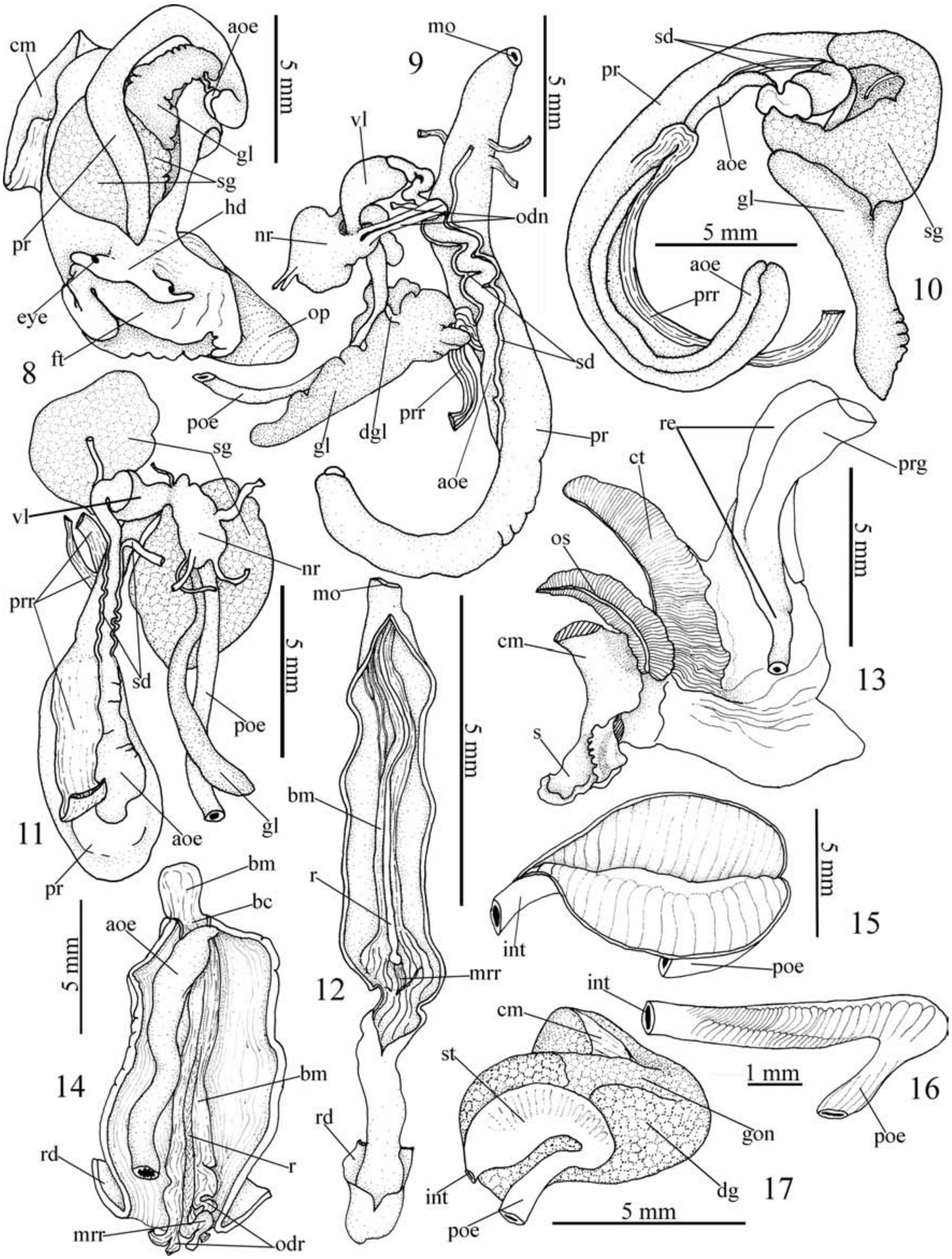
outer side of swelling at its base. Penis (Figure 1, **p**) small, apparently underdeveloped.

MANTLE: Mantle margin indented (Figure 3). Siphon (Figure 3, **s**) moderately long, muscular. Osphradium (**os**) occupies ~ ½ mantle length, ~1/6 mantle width. Ctenidium (**ct**) long, crescent-shaped, occupying 4/5 mantle length. Hypobranchial gland (**hg**) not well developed.

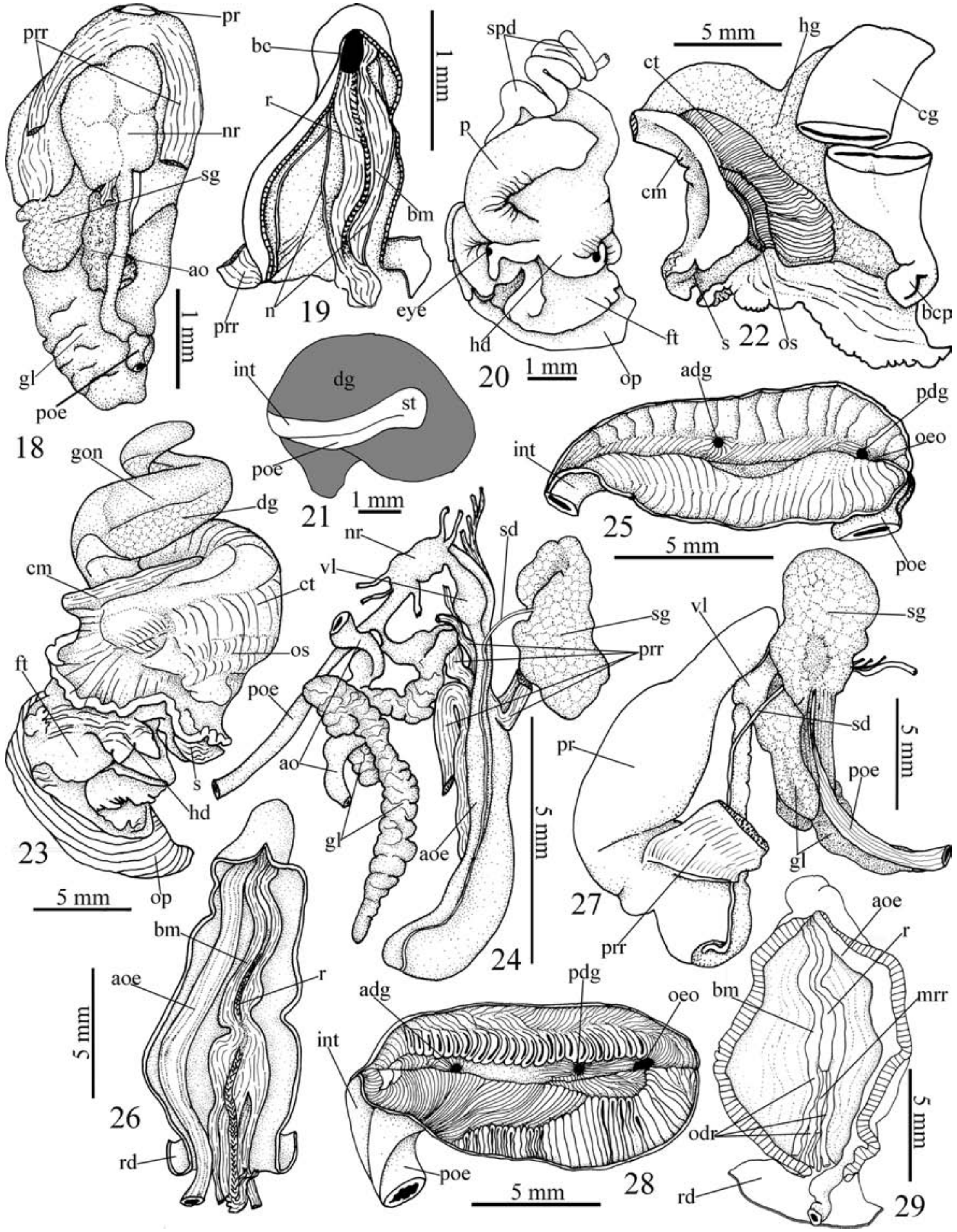
DIGESTIVE SYSTEM: Proboscis extremely long, narrow (Figures 4–5, **pr**), compactly folded within rhynchodeum (Figure 4, **rd**). Buccal mass occupies ~ ¼ proboscis length. Radula equal in length to odontophore, with structure similar to that illustrated in Bouchet and Warén (1985: 184, fig. 485). Proboscis attached to bottom of body haemocoel by proboscis retractors (Figures 4–5, **prr**) emerging from its base, consisting ~ 6 multiple



Figures 1–7. Anatomy of *Troschelia berniciensis*. **1.** Cephalopodium, visceral mass removed. **2.** section of esophagus with duct of gland of Leiblein. **3.** Mantle. **4.** Foregut. **5.** Proboscis. **6.** Stomach. **7.** Internal structure of the stomach. Abbreviations: **ao,** anterior aorta, **aoe,** anterior esophagus; **cm,** columella muscle; **ct,** ctenidium; **ft,** foot; **gl,** gland of Leiblein; **hd,** head; **ht,** heart; **kd,** kidney; **nr** = nerve ring; **op,** operculum; **os,** osphradium; **p,** penis; **pdg,** posterior duct of digestive gland; **poe,** posterior esophagus; **pr,** proboscis; **prpg,** propodial groove; **prr,** proboscis retractors; **rd,** rhynchodeum; **re,** rectum; **s,** siphon; **sg,** salivary gland; **st,** stomach; **vl,** valve of Leiblein.



Figures 8–17. Anatomy of Fasciolariidae. **8–10.** *Peristernia ustulata.* **8.** Cephalopodium, visceral mass removed. **9.** Foregut, ventral view. **10.** Foregut, lateral view. **11.** Foregut of *Pustulatirus mediamericanus*, ventral view. **12.** Proboscis of *P. ustulata*. **13–15.** *Pustulatirus mediamericanus.* **13.** Mantle. **14.** Proboscis. **15.** Internal structure of the stomach. **16.** Stomach of *Latirus polygonus*. **17.** Stomach of *P. ustulata*. Abbreviations: **aoe,** anterior esophagus; **bc,** buccal cavity; **bm,** buccal mass; **cm,** columellar muscle; **ct,** ctenidium; **dg,** digestive gland; **dgl,** duct of gland of Leiblein; **eye,** eye; **ft,** foot; **gl,** gland of Leiblein; **gon,** gonad; **hd,** head; **int,** intestine; **mo,** mouth opening; **mrr,** medial retractor of radula; **nr,** nerve ring; **odn,** odontophore nerves; **odr,** odontophore retractors; **op,** operculum; **os,** osphradium; **poe,** posterior esophagus; **pr,** proboscis; **prg,** prostate gland; **prr,** proboscis retractors; **r,** radula; **rd,** rhynchodeum; **re,** rectum; **s,** siphon; **sd,** salivary duct; **sg,** salivary gland; **st,** stomach; **vl,** valve of Leiblein.



Figures 18–29. Anatomy of Fasciolariidae. **18–21.** *Fusinus tenerifensis.* **18.** Foregut. **19.** Proboscis. **20.** Cephalopodium. **21.** Stomach. **22–24.** *Opeatostoma pseudodon.* **22.** Mantle. **23.** Soft parts. **24.** Foregut. **25.** Internal structure of the stomach. **26.** Proboscis. **27–29.** *Fasciolaria lignaria.* **27.** Foregut. **28.** Internal structure of the stomach. **29.** Proboscis. Abbreviations: **adg,** anterior duct of digestive gland; **ao,** anterior aorta; **aoe,** anterior esophagus; **bc,** buccal cavity; **bcp,** bursa copulatrix; **bm,** buccal mass; **cg,** capsule gland; **cm,** columella muscle; **ct,** ctenidium; **dg,** digestive gland; **eye,** eye; **ft,** foot; **gl,** gland of Leiblein; **gon,** gonad; **hd,** head; **hg,** hypobranchial gland; **int,** intestine; **mrr,** medial retractor of radula; **n,** nerves; **nr,** nerve ring; **odr,** odontophore retractors; **oeo,** oesophageal opening; **op,** operculum; **os,** osphradium; **p,** penis; **pdg,** posterior duct of digestive gland; **poe,** posterior esophagus; **pr,** proboscis; **prr,** proboscis retractors; **r,** radula; **rd,** rhynchodeum; **s,** siphon; **sd,** salivary duct; **sg,** salivary gland; **spd,** spermoduct; **st,** stomach; **vl,** valve of Leiblein.

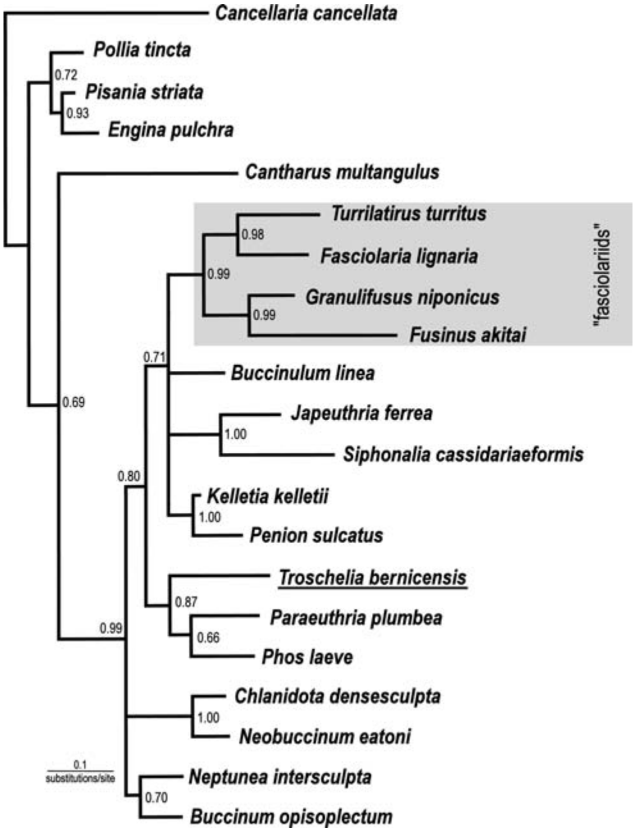


Figure 30. Bayesian topology obtained for the molecular dataset. Numbers at nodes are the Bayesian Posterior Probabilities.

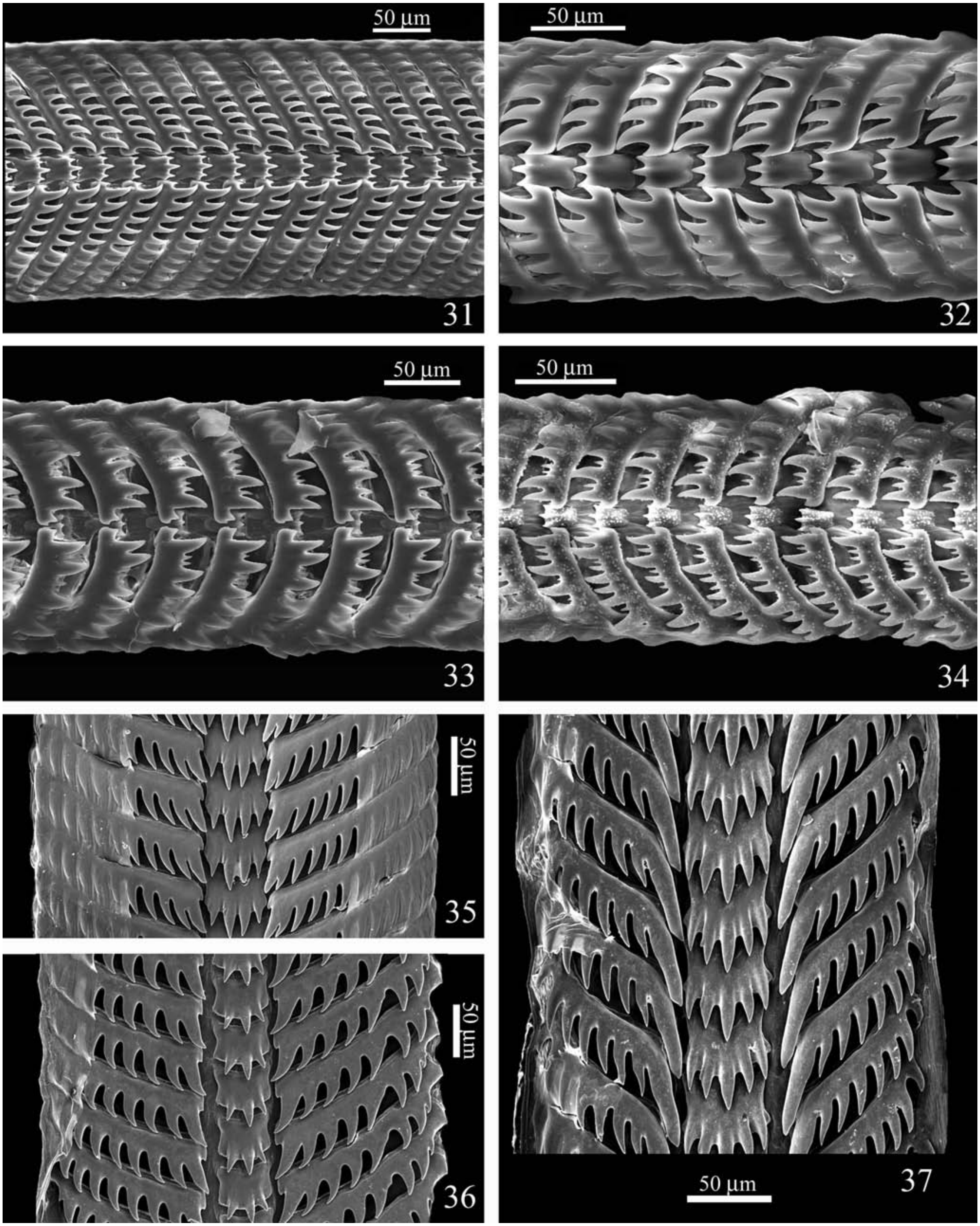
muscle tufts. Anterior esophagus wide, convoluted. Valve of Leiblein small, pyriform, situated immediately anterior to nerve ring. Salivary glands of medium size (Figures 2, 4, **sg**), tightly packed with nerve ring (Figure 4, **nr**) by connective tissue. Anterior aorta (Figure 4, **ao**) passes through nerve ring, runs parallel to posterior esophagus. Stomach narrow, occupying ~ 1/3 whorl (Figure 6, **st**). Posterior mixing area is absent, stomach walls lined with high, transverse folds of epithelium. Opening of posterior duct of digestive gland large, situated above the oesophageal opening (Figure 7, **pdg**).

Anatomy of Fasciolariid Species: Eight species of Fasciolariidae, representing seven genera from three subfamilies, were studied anatomically (Table 1). Main external morphological features included a folded, large muscular foot (Figures 8, 20, 23, **ft**), a broad head with relatively short tentacles (Figures 8, 20, 23, **hd**), and an operculum with a terminal nucleus (Figures 8, 23, **op**). The mantle (Figures 13, 22) has a moderately large, muscular siphon, a ctenidium occupying ¼–1/3 of the mantle width, and an asymmetrical osphradium that may be large (*Fusinus tenerifensis* Hadorn and Rolán, 1999, *Latirus polygonus* (Gmelin, 1791), *Pustulatirus mediamericanus* (Hertlein and Strong, 1951a), *Fasciolaria lignaria* (Linnaeus, 1758), *Peristernia nassatula* (Lamarck, 1822), *P. ustulata* (Reeve, 1847) – Figure 13, **os**) or small (*Opeatostoma pseudodon* (Burrow, 1815) – Figure 22, **os**).

Digestive System: The proboscis is straight and never coiled within the rhynchodeum. It may be rather short in *Pustulatirus mediamericanus* (Figure 14), *Latirus polygonus*, *Opeatostoma pseudodon* (Figure 26), *Fusinus tenerifensis* (Figure 19), and *Fasciolaria lignaria* (Figure 29), with the length of the buccal mass and radula equal to the length of proboscis (Figures 14, 19, 26, 29, **bm**). The proboscis of *Peristernia nassatula, P. ustulata* and *Turrilatirus turritus* (Gmelin, 1791) is very long, spanning one whorl (Figures 8–10, **pr**). The buccal mass and radula are about half of the proboscis length (Figure 12, **bm**) in these taxa. The radula of *Pustulatirus mediamericanus* has a small, 4-cuspid rachidian tooth and broad lateral teeth with 11 cusps of equal size on the left and 12 cusps on the right of the rachidian (Figure 35). The radula of *Latirus polygonus* has a small, 3-cuspid rachidian tooth, with the median cusp slightly longer than marginal cusps; lateral teeth have 11 and 12 equal cusps on left and right longitudinal rows, respectively (Figure 31). The radula of *Turrilatirus turritus* has a similar rachidian tooth and lateral teeth with only 7 equal cusps in each row (Figure 32). *Peristernia nassatula* (Figure 34) and *P. ustulata* (Figure 33) possess very similar radulae, with very small 3-cuspid rachidian teeth and lateral teeth with multiple alternating smaller and larger cusps. The radula of *Opeatostoma pseudodon* (Figure 37) has a 5-cuspid rachidian that is unusually large for fasciolariids, and lateral teeth with 8 equal cusps in each longitudinal row. The radula of *Fasciolaria lignaria* (Figure 36) corresponds to previously published figures (see Bandel, 1984).

There is only one, long and powerful proboscis retractor muscle in *Peristernia nassatula, P. ustulata,* and *Turrilatirus turritus*; it emerges from the middle part of the proboscis, runs ventrally, and attaches to the columellar muscle (Figure 10, **prr**). The single proboscis retractor of *Fasciolaria lignaria* is very wide and short, starting from the posterior section of the rhynchodeum (Figure 27, **prr**). In *Pustulatirus mediamericanus* one powerful ventral proboscis retractor is supplied by two smaller and thinner muscles, situated in the upper part of the rhynchodeum (Figure 11, **prr**). In *Opeatostoma pseudodon* there are two main lateral proboscis retractor muscles as well as several additional thin retractor muscles, situated more anteriorly (Figure 24, **prr**). *Fusinus tenerifensis* (Figure 18, **prr**) and *Latirus polygonus* possess two lateral proboscis retractor muscles.

The anterior esophagus is wide, dorso-ventrally flattened, flanked by two salivary ducts that are not embedded in its wall in all studied species (Figures 9, 11, 24, 27, **aoe, sd**) except for *Latirus polygonus*. The salivary glands (Figures 8, 10, 11, 18, 24, 27, **sg**) are large, separate in *Fusinus tenerifensis*, *Fasciolaria lignaria,* and *P. mediamericanus*, and fused beneath the nerve ring in *Turrilatirus turritus, Peristernia nassatula, P. ustulata* and *Opeatostoma pseudodon*. The



Figures 31–37. Radulae of Fasciolariidae. **31.** *Latirus polygonus*. **32.** *Turrilatirus turritus*. **33.** *Peristernia ustulata*. **34.** *Peristernia nassatula*. **35.** *Pustulatirus mediamericanus*. **36.** *Fasciolaria lignaria*. **37.** *Opeatostoma pseudodon*.

Table 3. Distinguishing anatomical features of the buccinids and fasciolariids examined in this study.

Feature Species	Proboscis	Proboscis retractors	Salivary glands	Salivary ducts	Stomach	Radula
Troschelia berniciensis	Long, coiled, buccal mass is 1/6 of pr. length	Multiple	Fused	?	Narrow, **pma** absent	5:1:5, lateral cusps of equal size
Pustulatirus mediamericanus	Short, straight, buccal mass is equal to pr. length	Single powerful and two thin additional	Fused	Free, twisting	Broad, with several medium high folds, **pma** absent	7:3:8, lateral cusps of equal size
Latirus polygonus	Short, straight, buccal mass is equal to pr. length	Paired	?	Embedded	Narrow, **pma** absent	11:3:12, lateral cusps of equal size
Turrilatirus turritus	Long, straight, buccal mass is ½ of pr. length	Single, long and narrow	Fused	Free, straight	Medium broad, **pma** absent	7:3:7, lateral cusps of equal size
Peristernia nassatula, Peristernia ustulata	Long, straight, buccal mass is ½ of pr. length	Single, long and narrow	Fused	Free, straight	Medium broad, **pma** absent	12:3:12, lateral cusps of different size
Opeatostoma pseudodon	Short, straight, buccal mass is equal to pr. length	Two powerful and several additional	Fused	Free, straight	Medium broad, with several medium high folds, **pma** absent	8:5:8, lateral cusps of equal size
Fusinus tenerifensis	Short, straight, buccal mass is equal to pr. length	Paired	Separate	?	Narrow, **pma** absent	?
Fasciolaria lignaria	Short, straight, buccal mass is 2/3 of pr. length	Single, short and broad	Separate	Free, straight	Broad, with multiple low folds, **pma** absent	12:3:12, lateral cusps of equal size

valve of Leiblein is moderately large and pyriform. The gland of Leiblein is very large in *Fusinus tenerifensis, Turrilatirus turritus,* and *Peristernia* species, and of medium size in *O. pseudodon, P. mediamericanus,* and *F. lignaria* (Figures 9–11, 18, 24, 27, **vl, gl**). The anterior aorta (**ao**) is very large and thick-walled. The stomach of all fasciolariids examined lack a posterior mixing area. They are narrow and long, with well-developed inner epithelial folds, high in *O. pseudodon* and *P. mediamericanus* (Figures 15, 25), and low in *F. lignaria* (Figure 28). The stomachs of *O. pseudodon* and *F. lignaria* possess two openings of ducts of the digestive gland, situated a short distance from each other, A longitudinal fold (Figures 25, 28, **lfl**) is present on the inner stomach wall, as are multiple transverse folds on the outer wall. The internal stomach structure of the remaining species was not studied due to poor preservation (outer view on Figures 16, 17, 21). Anatomical features of the studied fasciolariid species are summerized in the Table 3.

Molecular Analysis: A total of 9 new, partial 16S ribosomal DNA sequences were obtained, each 487–493 bp long (including the outgroup *Cancellaria cancellata*), and analyzed together with 12 previously published buccinoidean 16S sequences (Hayashi, 2005) (See Table 2). The aligned dataset comprised 514 nucleotide positions.

A χ^2 test of base homogeneity, uncorrected for phylogeny, indicated that base composition was not significantly different across all sites ($P = 0.999$). The model used for Bayesian analysis was HKY+I+G, as selected by the Akaike Information Criterion in MrModeltest 2.2. In the resulting tree, *Troschelia berniciensis* occupies a basal position in a clade with *Paraeuthria plumbea* and *Phos laeve* (Figure 30) ,with a bayesian posterior probability (bpp) of 0.87. This clade is the sister group to a larger, unresolved grouping, comprising several buccinid species and all the Fasciolariidae included in the analysis. The fasciolariids in this study form a well-supported monophyletic group (bpp=0.99). However, the placement of the fasciolariid clade among the buccinid taxa suggest that the Buccinidae is paraphyletic in our analysis, and that the Fasciolariidae may be a stem group within Buccinidae.

DISCUSSION

Troschelia berniciensis, though differing in radular structure, is very similar to other boreal representatives of the family Buccinidae in the morphology of its foregut, especially to Atlantic species of *Colus* (Kosyan, pers.

observ.), and to *Ancistrolepis* (Kantor, 1988). All have a long, coiled proboscis, proboscis retractors consisting of multiple tufts of muscular fibers that attach to the base of the proboscis, and a stomach without a posterior mixing area. The last feature has been considered to be typical for Fasciolariidae (Kantor, 2003), as is a radula with a small rachidian tooth and multi-cuspidate lateral teeth.

Although Ponder (1970) concluded that there are no reliable anatomical differences readily distinguishing the families included in Buccinoidea, Kantor (2003), and later Fraussen et al. (2007), reported that a combination of features, including a characteristic stomach morphology, together with multicuspid lateral radular teeth, a very small central tooth, single or paired proboscis retractor muscles, and salivary ducts passing within the esophagus walls, allows for the confident diagnosis of the family Fasciolariidae.

Our data confirm that the anatomy of the fasciolariids we studied is, in general, very similar to that of Buccinidae. Fasciolariid stomachs lack the posterior mixing area, and vary in internal structure from buccinid-like (*Pustulatirus mediamericanus, Opeatostoma pseudodon*) to fasciolariid–like (*Fasciolaria lignaria*). The salivary ducts, when it was possible to follow them, passed freely along the esophagus, or were bound with it by connective tissue, but in no case were embedded into the esophagus walls. A single morphological character was common to all fasciolariids and was never found in buccinids. This character is the structure of the proboscis retractor muscles, represented in fasciolariids by single or paired tufts of muscle fibers. In contrast, all buccinids studied have retractor muscles consisting of multiple muscle tufts, sometimes packed into two secondary tufts by connective tissue (Kosyan and Kantor, 2009). Thus, from a morphological perspective, Fasciolariidae constitute a derived group within Buccinidae. It is noteworthy that this pattern also emerged from our preliminary molecular analysis.

In the phylogenetic hypothesis derived from the molecular dataset (Figure 30), including 16 buccinid and 4 fasciolariid taxa, *Troschelia* is in the same clade with the tropical buccinids *Pareuthria* and *Phos.* Although the relationships among several buccinid clades are still not clearly resolved in our topology (possibly due to both a significantly incomplete taxonomic coverage, and the use of a suboptimal marker), we recovered a strong signal of close relationship between Fasciolariidae and Buccinidae.

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