

MORPHOLOGY AND SYSTEMATIC RELATIONSHIPS OF *BROCCHINIA CLENCHI* PETIT, 1986 (MOLLUSCA, CAENOGASTROPODA, CANCELLARIIDAE) FROM THE AZORES

M.G. Harasewych¹ & Richard E. Petit²

¹Dept. of Invertebrate Zoology, MRC-163, National Museum of Natural History, Smithsonian Institution, PO Box 37012, Washington, D.C. 20013-7012 USA e-mail: Harasewych@si.edu
²806 St. Charles Road, North Myrtle Beach, SC 29582 USA

ABSTRACT

Data on the shell morphology, microstructure, anatomy, and DNA sequences of two mitochondrial genes (COI and 16S rDNA) are used to infer the phylogenetic relationships of *Brocchinia clenchi* Petit, 1986 within the morphologically diverse and taxonomically complex deep-water genus *Brocchinia*. Initial molecular data show that this small, narrow-shelled species from the Atlantic is more closely related to a morphologically and ecologically similar species from the Indo-Pacific than either is to a representative of a broad-shelled group within the genus that is also present in the Atlantic and Indo-Pacific Oceans. While the thick shell, prominent columellar folds and apertural lirae of many *Brocchinia* species are features shared with members of the subfamily Cancellariinae, the absence of a radula is a characteristic of some, though not all Admetinae. Sequence data for the COI gene places *Brocchinia* within the Cancellariinae, while 16S rDNA data analyzed separately or together with COI places *Brocchinia* in a clade with *Mirandaphera*. This clade falls within Admetinae (16S only), or is separate from both Admetinae and Cancellariinae (COI + 16S), but not with strong support. Neither morphological characters nor molecular data have yet been able to unambiguously place the genus *Brocchinia* within the family Cancellariidae.

RESUMO

Utilizaram-se dados sobre a morfologia da concha, microestrutura, anatomia e seqüências de ADN de dois genes mitocondriais (COI e 16S rDNA) para inferir das relações filogenéticas de *Brocchinia clenchi* Petit, 1986 dentro do morfologicamente diverso e taxonomicamente complexo gênero de águas profundas *Brocchinia*. Dados moleculares preliminares mostram que esta pequena espécie de concha estreita do Atlântico é mais relacionada com uma espécie do Indo-Pacífico morfológica e ecologicamente similar do que ambas o são de um representante de um grupo de conchas largas dentro do gênero, também presente nos oceanos Atlântico e Indo-Pacífico. Enquanto que a concha espessa, as dobras columelares proeminentes e as liras aperturais de muitas espécies de *Brocchinia* são características partilhadas com membros da subfamília Cancellariinae, a ausência de rádula é uma característica de alguns, embora não todos, Admetinae. Dados de seqüência para o gene COI colocam *Brocchinia* com os Cancellariinae, enquanto que dados de 16S rDNA analisados separadamente ou juntamente com COI colocam *Brocchinia* num clado com *Mirandaphera*. Este clado está incluído em Admetinae (apenas 16S), ou é separado de Admetinae e de Cancellariinae (COI + 16S), mas não com forte suporte. Nem os caracteres morfológicos nem os dados moleculares têm até agora sido capazes de colocar sem ambiguidade o gênero *Brocchinia* na família Cancellariidae.

INTRODUCTION

The genus *Brocchinia* was introduced by Jousseume (1887: 221) to contain two small species of cancellariids with elongated shells, one, *Brocchinia mitraeformis* (Brocchi, 1814), a fossil from the Pliocene of Italy, the other, *Brocchinia pusilla* (H. Adams, 1869), a

Recent species from bathyal depths off the Canary Islands. The fossil species, *Brocchinia mitraeformis*, was subsequently designated as the type species of the genus by Sacco (1894: 68) (Figures 1-3). As both taxa included by Jousseume are junior homonyms, their nomenclature was clarified by Petit (1986).

While the family Cancellariidae

comprises a morphologically distinctive and easily recognized clade, the allocation of species among the more than 120 described genera (Petit & Harasewych, 2005) has often been difficult and provisional, particularly when based solely on shell morphology, as is necessary in cases when the type species are fossils. Thirteen Recent species are currently attributed to *Brocchinia* (Table 1), eight from the Atlantic, five from the Indo-Western Pacific, nearly all from bathyal depths. Many had originally been described in or subsequently assigned to other genera, among them *Admete* Krøyer in Möller, 1842, *Anapepta* Finlay, 1930, *Cancellaria* Lamarck, 1799, *Gergovia* Cossmann, 1899, *Inglisella* Finlay, 1924, *Merica* H. & A. Adams, 1854, and *Solutosveltia* Habe, 1961.

The anatomy of *Brocchinia* remains largely unknown. Verhecken (2007: Table 1) reported that radulae are absent in *B. nodosa* and *B. decapensis*. In their description of *B. canariensis*, Rolán & Hernández (2009: 105, fig. 7) described the animal as being white, with black eyes, a flattened and elongated penis, a gill of 9 thick lamellae, and without a radula or operculum. Martins *et al.* (2009: pl. 13, fig. 213) illustrated a living specimen of *B. clenchi* in color.

In their landmark paper on the molecular phylogeny of Cancellariidae, Modica *et al.* (2011) included two species of Indo-Pacific *Brocchinia*. These authors noted that this genus had traditionally been classified in the Cancellariinae (their table 1), but their results indicated that these two *Brocchinia* formed a

TABLE 1. Recent species attributed to the genus *Brocchinia* Jousseaume, 1887 in the World Register of Marine Species (Bouchet & Gofas, 2013) *.

Species	Locality	Depth (m)
ATLANTIC SPECIES		
<i>Brocchinia azorica</i> (Bouchet & Warén, 1985)	Azores	1,220-2,900
<i>Brocchinia canariensis</i> Rolán & Hernández, 2009	Canary Islands	350-400
<i>Brocchinia clenchi</i> Petit, 1986	Azores, Josephine Bank, Canary Islands	15-1,520
<i>Brocchinia decapensis</i> (Barnard, 1960)	Cape Point, South Africa	2,890-2,963
<i>Brocchinia harasewychi</i> Barros & Lima, 2007	Brazil	452-690
<i>Brocchinia nodosa</i> (Verrill & S. Smith, 1885)	Eastern USA	678-1,950
<i>Brocchinia pustulosa</i> Verhecken, 1991	Brasil	637
<i>Brocchinia verheckeni</i> Barros & Lima, 2007	Brasil	365-690
INDO-PACIFIC SPECIES		
<i>Brocchinia exigua</i> (E.A. Smith, 1891)	Australia	200-1,463
<i>Brocchinia fischeri</i> (A. Adams, 1860)	Korea to South Australia	40-1,017
<i>Brocchinia kaiensis</i> Verhecken, 1997	Indonesia	400
<i>Brocchinia septentrionalis</i> (Finlay, 1930)	New Zealand	137
<i>Brocchinia tanimbarensis</i> Verhecken, 1997	Indonesia	300-500

*two additional, unnamed species from Indonesia have been illustrated and discussed by Verhecken (2011).

clade together with two species of *Mirandaphera* (possibly due to long branch attraction). Although this clade was provisionally included in the subfamily Admetinae (their figures 3-5), the authors acknowledged that the relationship is not well supported (Modica *et al.*, 2011: 691; Figs. 3, 4), and noted that this group “may represent an independent lineage ... worthy of more detailed investigation.”

Of the three species of Cancellariidae that occur in the Azores (Verhecken, 2007), two have been referred to the genus *Brocchinia*. *Brocchinia azorica* (Bouchet & Warén, 1985), was originally described in the genus *Admete*, but transferred to *Brocchinia* by Verhecken (1991: 549). It is a wide ranging species (off NW Ireland to the Azores, and possibly to South Africa) that inhabits lower bathyal to abyssal depths (1,220-2,900 m).

In contrast, the range of *Brocchinia clenchi* Petit, 1986, is narrower, and includes the Macaronesian Archipelagos (Azores, Madeira, Canary Islands), the Josephine Bank and adjacent seamounts, and the coast off Western Sahara, at depths of 15-1,520 m. Verhecken, (2007: 313) noted that specimens from shallower waters off the Azores and Canary Islands tend to be smaller and smoother than the more strongly sculptured deeper water samples from the Josephine Banks and the Canary Islands, but provisionally regarded the variants to be conspecific in the absence of additional material.

Brocchinia clenchi has a complex taxonomic history. In his description of *Brocchinia clenchi*, Petit (1986: 25) stated that this species had been previously described as *Cancellaria pusilla* Adams, 1869, but that name is preoccupied by *Cancellaria pusilla* Sowerby, 1832. Although a specimen from the Canary Islands in the collection of The Natural History Museum (NHMUK 1855.4.4.202, ex McAndrew collection) was labeled

as a “possible type” of *Cancellaria pusilla* Adams, Petit concluded that the type of *C. pusilla* Adams could not be positively identified. Instead of designating a *nomen novum* for this species, Petit chose to redescribe the species so that a holotype could be designated, and included the NHMUK specimen as a paratype of *Brocchinia clenchi*. *Cancellaria pusilla* Adams, 1869 (type locality Canary Islands, depth unspecified) was considered to be a synonym of *Brocchinia clenchi* (type locality Josephine Bank, 622-786 m).

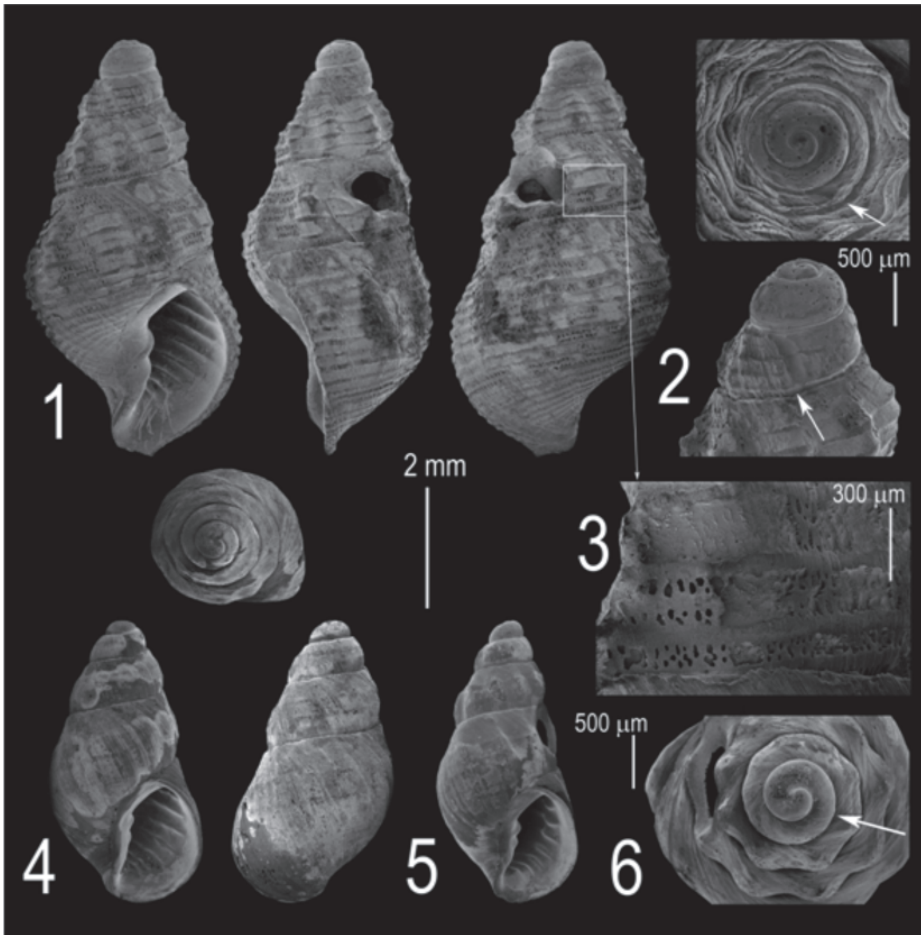
More recently, Rolán & Hernández (2009) described *Brocchinia canariensis* from slightly shallower depths (350-400 m) off the Canary Islands, differentiating it from the type specimens of *B. clenchi* primarily on the basis of it having a proportionally larger protoconch and smaller, narrower shell with a more pronounced suture and a proportionally smaller final whorl. They did not include in their discussion or comparisons the 93 specimens taken off the Canary Islands at depths ranging from 65-1,520 m that were listed by Verhecken (2007: 312). Rolán & Hernández incorrectly referred to the NHMUK specimen as the lectotype of *Cancellaria pusilla* Adams and acknowledged that it could be an abnormal form of *B. canariensis*. However, they (Rolán & Hernández, 2009: 105-106) chose not dispute its status as a paratype of *B. clenchi*. This ambiguity may lead to one of the following interpretations:

1) That two distinct upper bathyal species of *Brocchinia* (*B. clenchi* and *B. canariensis*) both occur in the Canary Islands.

2) That these two species do not co-occur in the Canary Islands, in which case the NHMUK specimen is mis-attributed to *B. clenchi*, and *Cancellaria pusilla* Adams is actually a synonym of *B. canariensis* rather than of *B. clenchi*.

3) That only a single, morphologically variable, upper bathyal species of *Brocchinia* occurs throughout the broad geographic range attributed to *B. clenchi* by

Verhecken (2007: 313), in which case, both *Cancellaria pusilla* and *Brocchinia canariensis* become synonyms of *B. clenchi*.



FIGURES 1-6. *Brocchinia*. 1-3, *Brocchinia mitraeformis* (Brocchi, 1814). Petit collection (#2126), Rio Torsero, Savona, Italy (Lower Pliocene). 1, Apertural, lateral and dorsal views of shell. 2, Apical and lateral views of the protoconch. 3, Detail of shell, showing pitted surface (intritalx?). 4-6. *Brocchinia clenchi* Petit, 1986. 4, Apertural, dorsal and apical views of the holotype, USNM 849002. 5, Apertural, and 6, apical views of paratype, USNM 189694, both from Josephine Bank, due W of Cape St. Vincent, Portugal (~ 37°N, 14°W), in 622-786 m. Arrows indicate transition from protoconch to teleoconch.

The present study documents the shell morphology, anatomy, biology and investigates the phylogenetic relationships (based on morphology and DNA sequences) of *Brocchinia clenchi* from the Sabrina Bank, off São Miguel, Azores, in order to provide a basis for more comprehensive studies of the biogeography and evolutionary relationships within the genus *Brocchinia*.

MATERIALS AND METHODS

Specimens of *Brocchinia clenchi* were collected from dredged sediment samples take off Portinho Velho das Capelas, São Miguel, Azores. A sample from 50-100 m (Sta. SAB2011.DR2, 37°51'N, 25°40'W, July 7, 2011) (USNM 1197432) contained a single living specimen and four dead shells inhabited by sipunculans, as well as several shell fragments. A sample from 200 m in the same area (Sta. SAB2011.DR3, 37°51.280'N, 25°41.971'W, July 7, 2011) (USNM 1197434) contained ten dead shells inhabited by sipunculans and several fragments. A third sample, from 200 m on the Sabrina Bank, off São Miguel, Azores (Sta. SAB2011.DR6, 37°51.547'N, 25°41.706'W, July 8, 2011) (USNM 1197435) contained four living specimens as well as eight dead shells and fragments. Living animals and dead shells populated by sipunculans were preserved in 95% ethanol. Empty shells and fragments were preserved dry.

Measurements and whorl counts for the holotype (USNM 849002) and paratype (USNM 189694) of *Brocchinia clenchi* as well as for ten Azorean shells (including two juveniles) were obtained from enlarged scanning electron micrographs of the apertural and apical views of the specimens using a digital caliper (Table 2). Data for the holotype and paratype of *Brocchinia canariensis* and for *Cancellaria pusilla* Adams were obtained from published figures (Rolán & Hernández,

2009: pls. 1-2). Data for *Brocchinia mitraeformis* was obtained from scanning electron micrographs of a specimen from the Petit collection (PC 2126). The number of protoconch whorls was counted using the method of Jablonski & Lutz (1980: 332) in order to be consistent with a prior study of cancellariid development (Pawlik *et al.*, 1988). This method of measurement results in values that are ½ whorl greater than those produced by the method used by Verhecken (2007: fig. 4). A dendrogram based on these measurements was produced using Dendro-UPGMA (Garcia-Vallve *et al.*, 1999; <http://genomes.urv.cat/UPGMA/>) using the Pearson (r) coefficient.

Shells were cracked for four of the living animals. One male and one female were dissected. Following dissection, the uppermost whorls of the male and female specimens, comprising portions of the digestive gland and gonad, were used for DNA extraction, as were portions of the introvert of two specimens of sipunculans inhabiting empty *Brocchinia clenchi* shells. Using protocols identical to those documented for *Cerion* in Harasewych *et al.* (2011), portions of the mitochondrial cytochrome c oxidase I and 16S rDNA genes were sequenced for all four samples.

A search of the GenBank nucleotide database using the Basic Local Alignment Search Tool (BLAST) identified both sipuncular samples as *Aspidosiphon (Aspidosiphon) muelleri* Dising, 1851, based on a 97% match of the COI sequences and a 99% match of the 16S sequences.

Sequences for the cytochrome c oxidase I and 16S rDNA genes from two specimens of *Brocchinia clenchi* (1♂ + 1♀) and a representative selection of 14 cancellariid taxa (Table 3) spanning the cancellariid phylogenetic tree (Modica *et al.*, 2011: fig. 5) were aligned against those of *Bivetiella cancellata* (Linnaeus, 1767) derived from its complete mitochondrial

TABLE 2. Measurements and whorl counts of *Brocchinia* species. Institutional Acronyms: NHMUK, The Natural History Museum, London; MNCN, Museo Nacional de Ciencias Naturales, Madrid; MNHN, MNHN Muséum National d'Histoire Naturelle, Paris; PC, Petit collection; USNM, National Museum of Natural History, Smithsonian Institution, Washington, DC. Abbreviations: AL, aperture length; LLW, length of last whorl, SL, shell length; SW, shell width.

Species of <i>Brocchinia</i>	Locality	Specimen Repository	SL (mm)	SW (mm)	LWL (mm)	AL (mm)	# whorls teleconch	# whorls protoconch	Protoconch width, initial (µm)	Protoconch width, final (µm)
<i>mitraeformis</i>	Italy	PC 2126	6.90	3.68	4.65	2.84	3.0	3.2	120	1193
<i>pusilla</i>	Canary Islands	NHMUK1855.4.4.202	4.56	2.00	2.98	1.73	3.0	1.5	248	800
<i>clenchi</i> HT	Josephine Banks	USNM849002	3.53	1.88	2.69	1.67	3.0	1.6	269	714
<i>clenchi</i> PT	Josephine Banks	USNM 18964	3.98	2.00	2.78	1.74	3.5	1.5	258	735
<i>clenchi</i> juv1	Azores	USNM1197435	2.24	1.58	1.78	1.22	1.5	1.3	236	637
<i>clenchi</i> juv2	Azores	USNM 1197435	2.92	1.86	2.21	1.44	2.2	1.4	253	654
<i>clenchi</i> adult1	Azores	USNM 1197435	3.35	1.95	2.47	1.52	2.5	1.4	249	763
<i>clenchi</i> adult2	Azores	USNM 1197435	4.35	2.27	3.09	1.96	3.0	1.3	283	793
<i>clenchi</i> adult3	Azores	USNM 1197435	4.57	2.39	3.05	1.89	3.0	1.3	245	729
<i>clenchi</i> adult4	Azores	USNM 1197435	4.11	2.20	2.95	1.78	2.9	1.3	230	704
<i>clenchi</i> adult5	Azores	USNM 1197434	5.17	2.64	3.61	2.01	3.2	1.3	256	768
<i>clenchi</i> adult6	Azores	USNM 1197434	4.45	2.23	2.90	1.70	3.1	1.6	263	789
<i>clenchi</i> adult7	Azores	USNM 1197434	4.38	2.14	3.14	1.84	2.6	1.4	256	729
<i>clenchi</i> adult8	Azores	USNM 1197434	4.49	2.34	3.09	1.89	3.0	1.3	247	740
<i>clenchi</i> adults only (n=8) mean±SD	Azores		4.36 ±0.51	2.27 ±0.20	3.04 ±0.31	1.82 ±0.16	2.9 ±0.2	1.4 ±0.1	254 ±15	752 ±31
<i>clenchi</i> all (n=10) mean±SD	Azores		4.00 ±0.89	2.16 ±0.30	2.83 ±0.53	1.73 ±0.26	2.7 ±0.5	1.4 ±0.1	251 ±15	731 ±53
<i>canariensis</i> HT	Canary Islands	MNCN 15.05/47567	4.20	1.86	2.78	1.83	3.5	1.4	169	487
<i>canariensis</i> PT	Canary Islands	MNHN	3.80	1.95	2.53	1.64	3.5	1.3	203	528

TABLE 3. Cancellariid taxa included in phylogenetic analyses based on molecular data. Taxa in **bold** indicate new sequence data obtained as part of this study. Institutional abbreviations for voucher material: MNHN, Muséum national d'Histoire naturelle, Paris; USNM, National Museum of Natural History, Smithsonian Institution, Washington, DC. Sequences with voucher specimens in MNHN are from Modica *et al.*, 2011.

Taxon	Voucher	Locality	GenBank COI	GenBank 16S
<i>Plesiofrion vivus</i> Habe and Okutani, 1981	MNHN:IM200732105	Pamilean Is., Philippines	FR717513.1	FR718357.1
<i>Admete viridula</i> (Fabricius, 1780)	MNHN:IM200742761	Canada	FR717525.1	FR718364.1
<i>Miranadaphera araffurensis</i> (Verheeken, 1997)	MNHN:IM200732350	Solomon Islands	FR717528.1	FR718370.1
<i>Miranadaphera cayrei</i> Bouchet & Petit, 2002	MNHN:IM200732349	Solomon Islands	FR717531.1	FR718369.1
<i>Brocciniina tanimborensis</i> Verheeken, 1997	MNHN:IM20095624	Mozambique	-----	FR718373.1
<i>Brocciniina fischeri</i> (A. Adams, 1860)	MNHN:IM200732361	Solomon Islands	FR717527.1	FR718366.1
<i>Brocciniina clenchi</i> Petit, 1986 Female	USNM 1197435	São Miguel, Azores	KF824511	KF824508
<i>Brocciniina clenchi</i> Petit, 1986 Male	USNM 1197435	São Miguel, Azores	KF824510	KF824507
<i>Admetula garrardi</i> Petit, 1974	MNHN:IM200742767	Philippines	FR717501.1	FR718338.1
<i>Cancellaria cancellata</i> (Linnaeus, 1767)			NC013241	NC013241
<i>Cancellaria albida</i> Hinds, 1843	MNHN:IM200910393	Perlas Islands, Panama	FR717471.1	FR718319.1
<i>Cancellaria reticulata</i> (Linnaeus, 1767)	USNM 1175024	Mayport, Florida	KF824512	KF824509
<i>Merica sinensis</i> (Reeve, 1856)	MNHN:IM200732354	NE Taiwan	FR717497.1	FR718303.1
<i>Nipponaphera agastor</i> Bouchet and Petit, 2008	MNHN:IM200732357	Solomon Islands	FR717498.1	FR18331.1
<i>Scalptia obliquata</i> (Lamarck, 1822)	MNHN:IM200732399	Tangoa Island, Vanuatu	FR717507.1	FR18347.1
<i>Trigonostoma thysitlon</i> Petit & Harasewych, 1987	MNHN:IM200742768	New Caledonia	FR717511.1	FR718350.1

genome (16,648 bp) (Cunha *et al.*, 2009) using Clustal X 2.1 (Larkin *et al.*, 2007). For the cytochrome *c* oxidase I gene, an alignment of 658 bases, corresponding to positions 39 to 696 of the COI gene in *Bivetiella cancellata*, was produced after primers were trimmed. After trimming primers, the 16S rDNA sequences corresponded to a 514 bp region (positions 806 to 1319) of this gene in *Bivetiella cancellata*, derived from its complete mitochondrial genome. However, due to length variation in this portion of the gene among the studied taxa, the alignment spanned 588 positions.

Using default settings and the Akaike Information Criterion, jModelTest v. 2.1.3 (Daribba *et al.*, 2012) selected the HKY+I+G nucleotide substitution model for the COI data, and the GTR+I+G model for the 16S data. The COI, 16S, and concatenated (COI+16S) data sets were analysed using Mr. Bayes v. 3.2.1 (Ronquist *et al.*, 2012). The concatenated data set was analyzed keeping the two partitions unlinked, using the corresponding model for each partition. Metropolis-coupled Markov Chain Monte Carlo simulations were performed with four chains for 1,000,000 generations, with trees sampled every 500 generations, after a burn-in of 2500 generations, for each of the data sets. The standard deviation of split frequencies was below 0.01 and the potential scale reduction factor was 1.00 for each of the analyses. As only 16S sequences were available for *Brocchinia tanimbarensis*, this species appears only in the analysis based on 16S sequences.

SYSTEMATICS

Class GASTROPODA

Order Neogastropoda Wenz, 1943

Family Cancellariidae Forbes & Hanley, 1851

Genus *Brocchinia* Jousseaume, 1887

Brocchinia clenchi Petit, 1986

Synonymy

Cancellaria pusilla H. Adams, 1869: 274, pl. 19, fig. 12 (non *Cancellaria pusilla* G.B. Sowerby I, 1832: 7, fig. 34).

Cancellaria mitraeformis Brocchi – Jeffreys, 1885: 49.

Cancellaria pusilla H. Adams – Sykes, 1911: 332.

Cancellaria (Brocchinia) pusilla H. Adams – Dautzenberg 1927: 73, 377.

Narona (Brocchinia) pusilla (H. Adams) – Nordsieck, 1968: 151, pl. 25, fig. 87.30.

Narona (Sveltia) pusilla (H. Adams) – Nordsieck & Garcia-Talavera, 1979: 152.

Narona pusilla (H. Adams) – Rolan Mosquera 1983: 236.

Brocchinia clenchi Petit, 1986: 24, figs. 1-4; Verhecken 2002: 510; Verhecken 2007: 311, figs. 28-30.

?*Brocchinia canariensis* Rolán & Hernández, 2009: 104, figs. 1-7; Hernández *et al.* 2011: 221-222, fig. 72 J-M.

DESCRIPTION

Shell morphology: Shell (Figures 4-9, Table 2) small (to 5.2 mm), thick, with tall, conical, spire, and rounded anterior. Protoconch (Figures 6, 10) paucispiral, increasing in diameter from 251 μ m to 731 μ m in 1.4 smooth, evenly rounded, conical, glassy whorls (mean values, see Table 2). Protoconch initially deviated from teleoconch axis by $\sim 15^\circ$. Transition to teleoconch (Figure 10, arrow) distinct, marked by an abrupt change from smooth, glassy surface to matte texture and onset of numerous, very fine prosocline growth lines. Transition may be less conspicuous on dead or eroded specimens. Teleoconch of up to 3.5 very thick, evenly convex to weakly shouldered whorls. Axial sculpture of fine prosocline growth striae and weak, irregularly spaced nodes most evident above shell periphery in whorls 1–2.5, becoming less prominent as shell increases in thickness. Spiral sculpture of 3–5 low cords, one running along shell periphery, 1–2 above and 1–2 below periphery. Suture distinct, weakly impressed, abutting peripheral cord

in early whorls (Figure 8), descending below peripheral cord and becoming more strongly impressed after second whorl (Figures 7, 9). Aperture D-shaped, deflected from coiling axis by 20-22°. Outer lip smooth, rounded anteriorly and posteriorly. Inner surface with 5-8 short ($\leq \frac{1}{4}$ whorl) lirae that expand to form pronounced denticles recessed within the lip, beneath thickened axial node (Figure 12). Parietal callus thin to moderately thick, translucent. Columella thick, straight, axial, forming an angle of 122-136° with parietal region (angle decreases as whorl number increases), with two oblique columellar folds (Figure 11, cf), and a weaker siphonal fold (Figure 11, sf). Posterior columellar fold longer and thicker. Siphonal canal short, broad, not well differentiated. Shell color uniform white. Operculum absent.

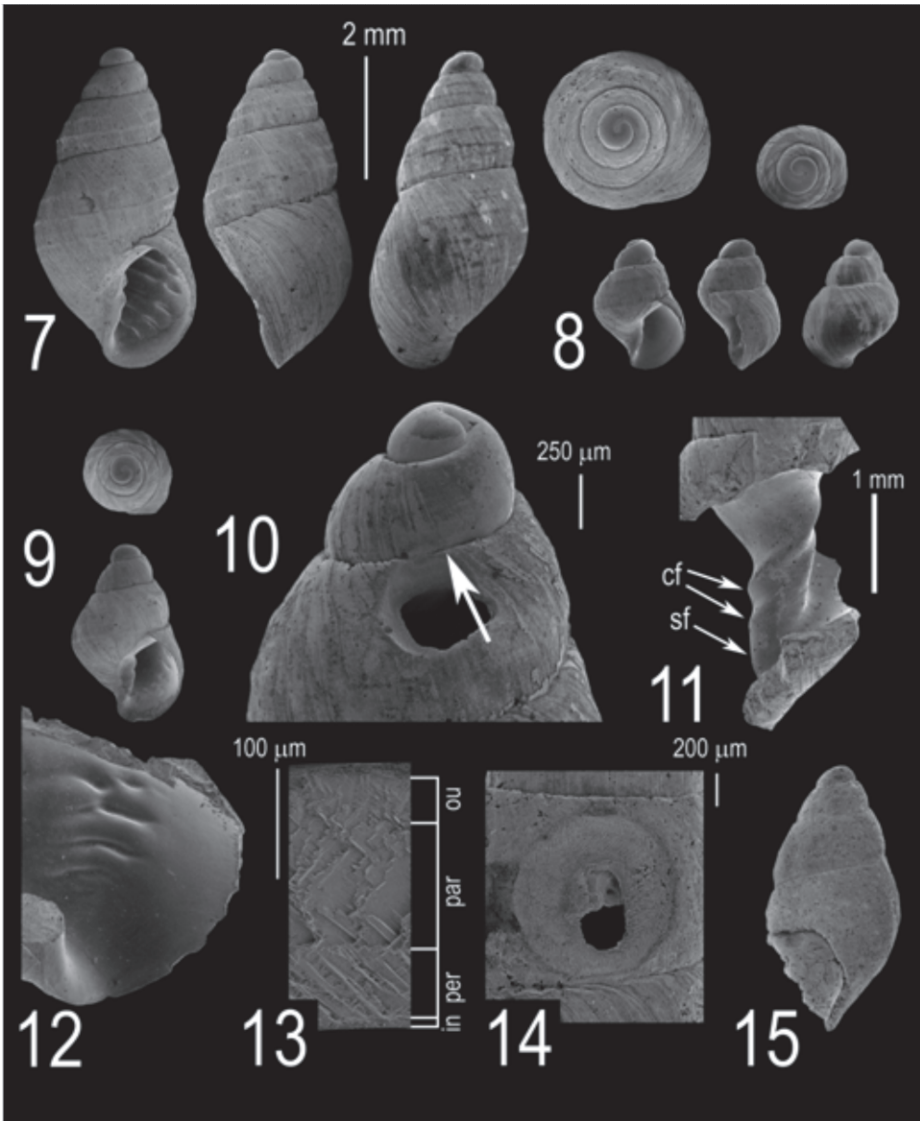
Shell ultrastructure: (Figure 13) Shell thick ($\approx 220 \mu\text{m}$), composed of four distinct crystal layers. The innermost layer (Figure 13, in) ($\approx 10 \mu\text{m}$) comprises the glaze that lines the inner surfaces of the shell. The adjacent layer (Figure 13, per) ($\approx 60 \mu\text{m}$) is composed of cross-lamellar aragonite, with crystal faces oriented roughly perpendicular to the growing edge of the shell. The next layer (Figure 13, in) ($\approx 110 \mu\text{m}$) is also comprised of cross-lamellar aragonite, with crystal faces orthogonally oriented parallel to the growing edge. The outermost layer (Figure 13, in) ($\approx 40 \mu\text{m}$) is prismatic. It lacks the pitted surface (intritalx?) that is present in the Pliocene type species *Broccchinia mitraeformis* (Figures 1, 3), and in some Recent Indo-Pacific species [e.g., *B. fischeri* and *B. kaeiensis* but not *B. tanimbarensis* (see Verhecken, 1997: 300-307)].

External anatomy: Extended animal (Figure 16) spans $3\frac{1}{2}$ whorls, with the mantle cavity covering nearly 1 whorl,

the kidney occupying $\frac{1}{4}$ whorl along its right posterior limit, and the digestive gland and gonad spanning $2\frac{1}{4}$ whorls. The terminal 1- $1\frac{1}{4}$ whorls appears to be primarily gonadal tissue. The foot is twice as long as wide, with a pronounced propodial groove. The head is broad with a pair of long tentacles (contracted when preserved) and large black eyes along their outer margins. Mantle edge thickened, smooth, without a conspicuous siphon. When alive, the animal is white, with brighter white speckles. Preserved animals are a uniform pale ivory.

Mantle cavity: The mantle cavity is broad, with organs situated as in most neogastropods. The osphradium is greatly reduced, the ctenidium consisting of about a dozen lamellae. A thin hypobranchial gland extends across the roof of the mantle cavity. The pericardium is situated at the left posterior margin of the mantle cavity and contains a minute auricle.

Alimentary system: The proboscis is short, broad and pleurembolic (L/W ~ 3), occupying most of the cephalic haemocoel. Mouth small, forming a dorsoventral slit at proboscis tip, leads to a small buccal cavity. Buccal mass minute ($\sim \frac{1}{2}$ proboscis width), ovate, situated posterior to proboscis tip. Radula absent. Neither a cuticularized oral tube nor a jaw were observed in this species. A minute valve of Leiblein is situated posterior to the buccal mass. Salivary glands were large and flanked the nerve ring, with narrow salivary ducts leading to the buccal mass. The mid-esophagus passes through the nerve ring then expands as it runs posteriorly to rear of cephalic hemocoel and to the stomach, which is thin, narrow and runs along the anterior portion of the digestive gland. Intestine leads to rectum that runs along the right



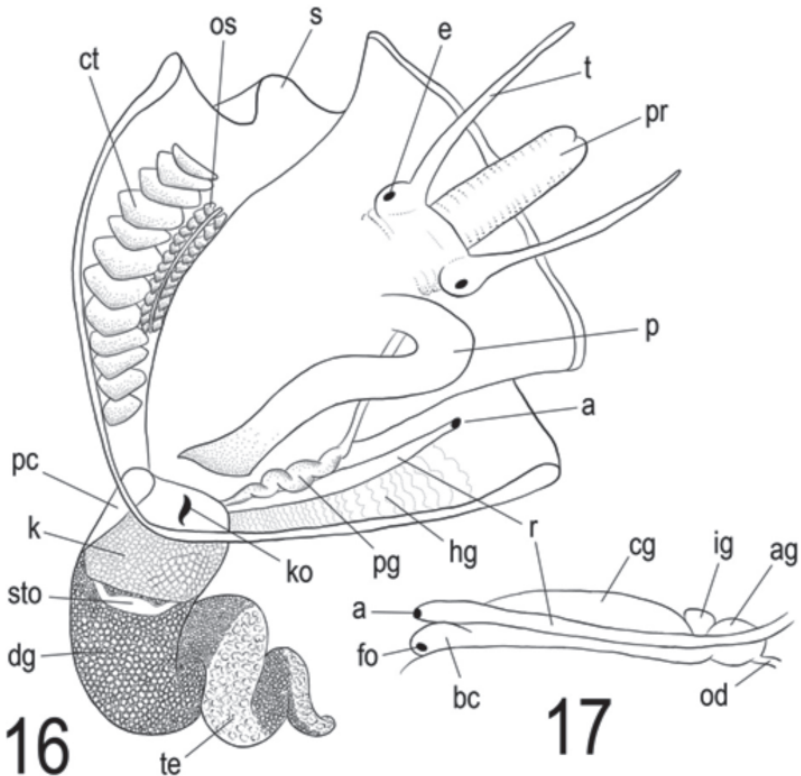
FIGURES 7-15. *Brocchinia clenchi* Petit, 1986. USNM 1197435, Sabrina Bank, São Miguel, Azores, Portugal (37°51.547'N, 25°41.706'W), dredged in 200 m, July 8, 2011 (Sta. SAB2011.DR6). **7**, Apertural, lateral, dorsal, and apical views of adult specimen. **8**, Apertural, lateral, dorsal, and apical views of juvenile specimen. **9**, Apertural and apical views of juvenile specimen. **10**, Lateral view of protoconch, showing transition to teleoconch (arrow). **11**, Shell fractured to reveal columella. **12**, Inner surface of outer lip, showing depth of denticles and lirae. **13**, Shell ultrastructure, surface parallel to growing edge of shell, $\frac{1}{4}$ whorl behind the lip. **14**, Naticid borehole, indicative of drilling predation. **15**, Repaired shell break, indicative of shell peeling predation by a crustacean. **cf**, columellar fold; **in**, innermost shell layer; **ou**, outer, prismatic shell layer; **par**, crossed-lamellar aragonite, crystal faces parallel to growing edge; **per**, crossed-lamellar aragonite, crystal faces perpendicular to growing edge; **sf**, siphonal fold.

side of the mantle cavity. Anus situated posterior to thickened mantle edge.

Male reproductive system: The testis spans the apical whorl of tissue, lining the digestive gland tissue and giving rise to a narrow testicular duct that leads anteriorly to the right wall of the mantle cavity, where it expands to form a slightly convoluted prostate gland and then constricts to form a narrow vas deferens that descends to the floor

of the mantle cavity before entering the base of the penis, situated just behind the right cephalic tentacle. Penis long, dorsoventrally flattened, parallel sided, tapering distally but lacking a distinct papilla.

Female reproductive system: The ovary is large, acinous, occupying most of the terminal whorl of the visceral mass. A thin-walled oviduct runs anteriorly along the columellar side of the digestive



FIGURES 16-17. Anatomical features of *Brocchinia clenchi* Petit, 1986. 16, Male specimen removed from shell, partially uncoiled and mantle cavity opened mid-dorsally. 17, Diagrammatic representation of female pallial gonoduct. a, anus; ag, albumen gland; bc, bursa copulatrix; cg, capsule gland; ct, ctenidium; dg, digestive gland; e, eye; fo, female opening; hg, hypobranchial gland; ig, ingesting gland; k, kidney; ko, kidney opening; od, oviduct; os, osphradium; pc, pericardium; p, penis; pg, prostate gland; pr, proboscis(extended); r, rectum; s, siphon; sto, stomach; t, tentacle; te, testes.

gland, passing alongside the kidney and pericardium before giving rise to the pallial gonoduct (Figure 17), consisting of an albumen gland, ingesting gland, large capsule gland, and a small bursa copulatrix, with the female opening at its tapered, distal end.

Ecology: Published records of *Brocchinia clenchi* in the Azores range in depth from 15-1,250 m (Petit, 1986: 26; Verhecken, 2007: 312), with the deepest record (1,250 m) from between São Jorge and Pico. Martins *et al.* (2009: 64, 98-99, text figure 1) reported this species off the south central coast of São Miguel, living at depths of 58-81 m. In this study, the species was collected at depths ranging from 50-200 m, on sand and rubble bottoms in the vicinity of the Sabrina Bank, off western São Miguel. Most (80%) of the intact shells were dead collected and inhabited by sipunculans [*Aspidosiphon* (*Aspidosiphon*) *muelleri* Diesing, 1851]. Of these, most (63%) had been drilled by naticids (Figures 10, 14). Two specimens had repaired breaks to the outer lip (Figure 15), indicating that they had survived an attack by a crustacean.

Molecular phylogeny: Bayesian analyses of the COI and 16S gene sequences analyzed separately and together each support the monophyly of the genera *Brocchinia*, *Mirandaphera*, *Cancellaria* and the subfamily Cancellariinae (based on very limited taxon sampling). However, inference of the relationships of *Brocchinia* differ depending on the gene. COI sequences place *Brocchinia*, but not *Mirandaphera*, within Cancellariinae (Figure 20). 16S sequence data place *Brocchinia* as sister taxon to *Mirandaphera* in a well-supported clade within the Admetinae (Figure 21), while the concatenated data set (COI + 16S) retain the sister group relationship between

Brocchinia and *Mirandaphera*, but place this clade outside of both Cancellariinae and Admetinae (Figure 23).

DISCUSSION

Species presently allocated to the genus *Brocchinia* conform to one of three basic shell morphotypes. Fossil species, including the type species of the genus (Figures 1-3), tend to have comparatively large (to 13 mm), narrow shells with tall, conical spires and rounded anteriors, with the final whorl occupying about ½ the shell length. The protoconch (Figure 2) spans ~3 whorls, the teleoconch 5-6 whorls. Sculpture consists of thin but distinct spiral cords and low axial nodes that are most conspicuous along the shoulder. The aperture is ovate, with two columellar folds and a prominent siphonal fold that demarcates a short but distinct siphonal canal. The outer lip is thin, with prominent lirae. The shell has a distinctively pitted surface (Figure 3), especially between spiral cords, which is thought to correspond to the intritralx (see Verhecken, 1997: 301 for discussion of this pitted sculpture).

Two different morphotypes occur among the Recent species. One is characterized by a much broader shell, usually <12 mm in length, but may reach 27 mm in one species. These shells have a short, wide spire and a last whorl that spans $\geq\frac{3}{4}$ of the shell length. In this group, protoconchs range from $1\frac{1}{4}$ - $3\frac{1}{4}$ whorls, while teleoconchs span 3- $4\frac{1}{2}$ whorls. Sculpture consists of strong spiral cords and prominent axial ribs that produce a cancellate to nodulose shell surface. The aperture is large, broadest adapically, generally with a single columellar fold and a prominent siphonal fold. The outer lip may be undulating, but lacks lirae. A pitted shell surface is present in some species. Species with this morphotype include: *Brocchinia azorica*, *B. decapensis*, *B.*

nodosa and *B. pustulosa* in the Atlantic and *B. kaiensis*, as well as *B. tanimbarensis* in the Indo-west Pacific.

The remaining Recent species generally have a small (≤ 6 mm, although *B. fischeri* is reported to reach 12.8 mm), narrow shells, with conical spires and rounded anteriors. The last whorl spans $\leq \frac{1}{2}$ the shell length. Protoconchs span $1\frac{1}{4}$ - 2 whorls, while teleoconchs usually comprise $3\frac{3}{4}$ -4 whorls (up to 6 in *B. fischeri*).

Shell sculpture ranges from smooth, with barely discernible spiral cords, to prominent axial ribs and spiral cords that produce a cancellate to weakly nodulose surface. The aperture is D-shaped, with an axial columella bearing two columellar folds and a weak siphonal fold. The outer lip is thick, with prominent lirae in most species. Pitted shell surface (intritricax?) is present in one species. Species that share this morphotype include: *Brocchinia*

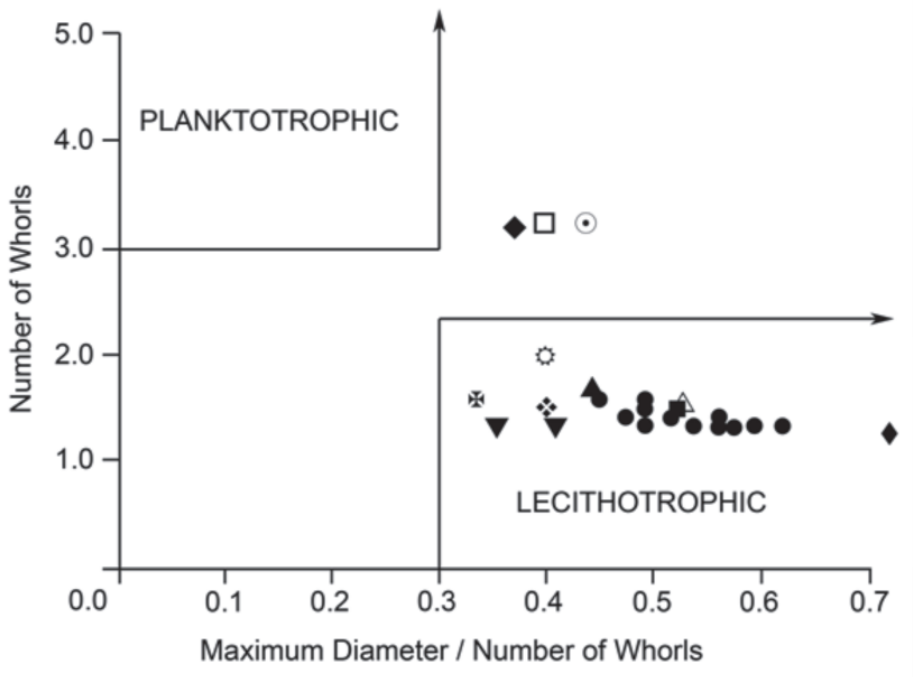


FIGURE 18. Inference of larval development type based on relationship between maximum protoconch diameter and number of protoconch whorls following the model proposed by Shuto (1974) and applied to cancellariids by Pawlik *et al.* (1988).

◆ = *Brocchinia mitraeformis*; ■ = "*B. pusilla*" NHMUK specimen; ● = *B. clenchi*, and ▼ = *B. canariensis* (all based on data in Table 2). □ = *Brocchinia azorica* (data from Verhecken, 1997: 310)¹; ⊙ = *B. nodosa*, Verhecken (1997: 317) reported this data but questioned the identification of the juvenile specimen on which it was based¹; ⊠ = *B. pustulosa* (data from Verhecken, 1991: 548)¹; ◆ = *B. verheckeni*; △ = *B. harasewychi* (data from de Barros & de Lima, 2007); ▲ = *B. fischeri*; ◆ = *B. tanimbarensis*; ⊙ = *B. kaiensis* (data from Verhecken, 1997: 310)¹

¹ The number of protoconch whorls reported in this plot is based on a method of measuring whorls (Jablonski & Lutz, 1980: 332) that results in values $\frac{1}{2}$ whorl greater than those produced by the method shown by Verhecken (2007: fig. 4). Values reported by Verhecken have been increased by $\frac{1}{2}$ whorl.

clenchi in the eastern and mid-Atlantic, *B. harasevychi* and *B. verheckeni* in the western Atlantic and *B. exigua*, *B. fischeri* and *B. septentrionalis* in the Indo-west Pacific.

Although these three morphotypes are easily distinguished, several of the Recent species have features that span two of the morphological groupings. The Indo-Pacific species *Brocchinia fischeri*, for example, has the shell shape and proportions as well as protoconch and aperture morphology that unite it with the small, narrow shelled group of Recent species, yet shares the large shell size, high number of whorls and pitted shell surface of the fossil group. Both *Brocchinia azorica* and *B. nodosa* have the characteristic teleoconch features of the broad-shelled group of Recent species, yet their multispiral protoconch is more similar to that of the fossil group. This may also be true of *B. decapensis*, but its protoconch morphology remains unknown.

In the absence of direct observations, larval development is often inferred from the morphology of the protoconch. The multispiral protoconchs of the fossil *Brocchinia* species have been interpreted as being indicative of a planktonic larval stage, while the paucispiral protoconchs of most of the Recent species were inferred to have lecithotrophic, likely direct, development (Verhecken, 2007; Rolán & Hernández, 2009). The model proposed by Shuto (1974), as applied by Pawlik *et al.*, (1988: fig. 23), supports the interpretation that most Recent *Brocchinia* with paucispiral protoconchs have lecithotrophic larvae (Figure 18). However, the type of larval development of the fossil and Recent species with multispiral protoconchs cannot be inferred unambiguously as planktotrophic using these criteria. The only Recent species with multispiral protoconchs are those members of the broad-shelled group that occur at abyssal depths.

The species *Brocchinia canariensis* was distinguished from *B. clenchi* based on comparisons of measurements of the protoconchs and adult shells of the type material of these two taxa. A somewhat broader analysis (cluster analysis, UPGMA, Pearson (r) coefficient) that included the same type specimens as well as eight adult and 2 juvenile Azorean specimens and used similar measurements (Table 2) did not distinguish *B. canariensis* from *B. clenchi* (Figure 19). Rather, it grouped the holotype of *B. clenchi* together with the paratype of *B. canariensis*, and the holotype of *B. canariensis* with the two paratypes of *B. clenchi*, of which one is the possible syntype of *B. pusilla* from the Canary Islands. This analysis is not sufficiently rigorous to show conclusively that *B. canariensis* is a synonym of *B. clenchi*. However, it does favor Verhecken's (2007: 313) provisional interpretation that only a single, highly-variable, bathyal species of

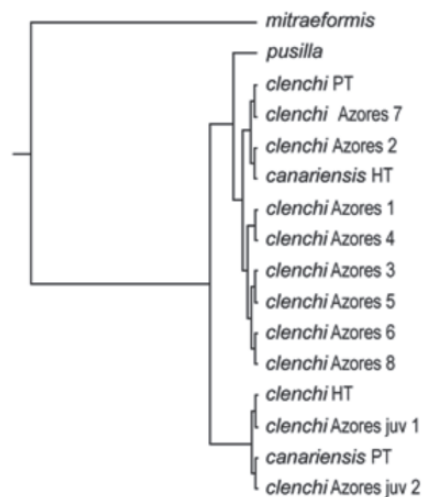
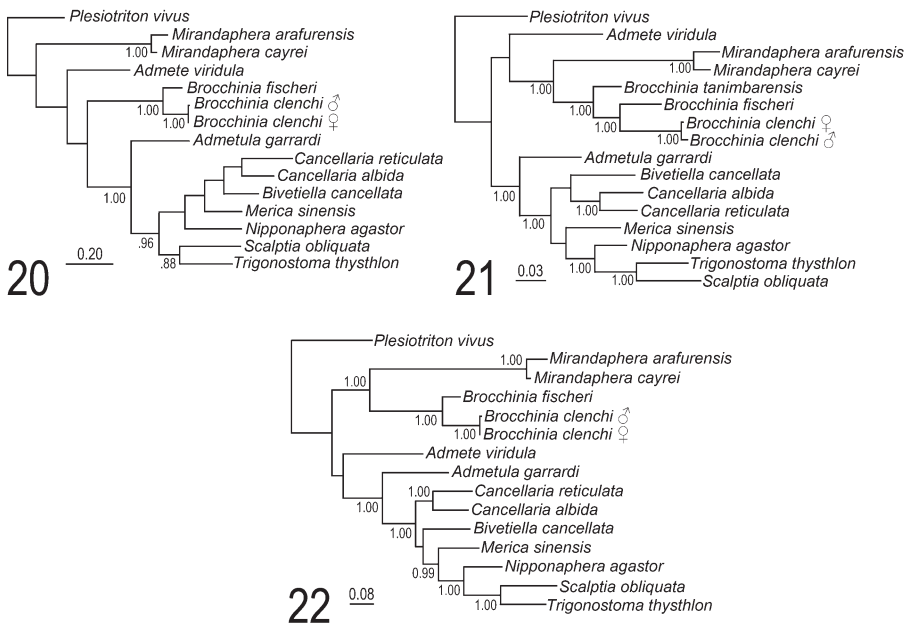


FIGURE 19. UPGMA cluster analysis [Pearson (r) coefficient] based on measurements of protoconch and shell (Table 2). HT= holotype, PT= paratype.

Brocchinia with a broad geographic and bathymetric range inhabits the eastern Atlantic. Clearly, additional analyses, possibly involving the much larger suite of available specimens listed by Verhecken (2007: 312), and/or molecular comparisons are needed to resolve the relationship between these two taxa.

The limited molecular data indicate that the species conforming to the small, thin-shelled morphotype from the Atlantic and Indo-Pacific oceans (*B.*

clenchi and *B. fischeri*) are more closely related to each other than either is to the broad-shelled group (*B. tanimbarensis*) (Figure 21). As recognized by Modica *et al.* (2011), the relationships of *Brocchinia* within the Cancellariidae are as yet not well resolved by molecular data. Analyses using COI sequence data place *Brocchinia* within Cancellariinae, not as sister taxon of *Mirandaphera* (Figure 20). However, sequence data from the 16S rDNA gene analyzed separately (Figure 21) or



FIGURES 20-23. Relationships of *Brocchinia clenchi* within Cancellarioidea based on Bayesian analyses of: **20**, A 658 bp portion of the cytochrome c oxidase I (COI) gene, **21**, a portion of the 16S rDNA gene (alignment length = 588 positions), **22**, concatenated COI and 16S rDNA sequences. Posterior probabilities > 0.85 are indicated at nodes.

concatenated with the COI sequences (Figure 22), supports the sister relationship between *Brocchinia* and *Mirandaphera* and groups these genera with *Admete* (16S only) or in a group separate from Admetinae and Cancellariinae (COI + 16S), although without strong support.

While the thick shell, prominent columellar folds and apertural lirae of many *Brocchinia* species are features shared with members of the Cancellariinae, the absence of a radula is a characteristic of some, though not all Admetinae. Thus far, neither morphological characters nor molecular data have been able to determine unambiguously the relationship of the genus *Brocchinia* within the family Cancellariidae. As suggested by Modica *et al.* (2011: 691), *Brocchinia* and *Mirandaphera* may both be part of an independent lineage that is certainly “worthy of more detailed investigation.”

ACKNOWLEDGEMENTS

We are grateful to Prof. António Frias Martins, Dr. Joana R. Xavier and their staff and students for organizing the Fourth International Workshop of Malacology to study the fauna of Sabrina Bank, in São Miguel, Azores, which the senior author attended.

Support from FLAD (Portuguese-American Foundation for Development) is gratefully acknowledged.

Thanks also to Elizabeth Diamond for assistance with histology, to Yolanda Villacampa for taking the Scanning Electron Micrographs, and to Eléonore Dixon-Roche for preparing the anatomical illustrations.

LITERATURE CITED

- ADAMS, H., 1869. Description of a new genus and fourteen new species of marine shells. *Proceedings of the Zoological Society of London*, [1869]: 272-275, pl. 19.
- BOUCHET, P., & S. GOFAS, 2013. *Brocchinia* Jousseau, 1887. Accessed through: *World Register of Marine Species* at <http://www.marinespecies.org/aphia.php?p=taxdetails&id=137725> on 2013-06-23
- BROCCHI, G.B., 1814. *Conchiologia fossile subapennina con osservazione geologiche sugli Apennini e sul suolo adiacente*, 2 vols. [1: 1-240, 2: 241-712, pls. 1-16.]. Stamperia Reale, Milano.
- CUNHA, R.L., C. GRANDE & R. ZARDOYA, 2009. Neogastropod phylogenetic relationships based on entire mitochondrial genomes. *BMC Evolutionary Biology*, 9: 210.
- DARRIBA, D., G.L. TABOADA, R. DOALLO & D. POSADA, 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods*, 9(8): 772.
- DAUTZENBERG, P., 1927. Mollusques provenant des campagnes scientifiques du Prince Albert I^{er} dans l'Atlantique et dans le Golfe de Gascogne. *Résultats des Campagnes scientifiques Albert I^{er}*, 72: 1-401.
- DE BARROS, J.C.N., & S.F. DE LIMA, 2007. Three new species of Cancellariidae (Gastropoda: Neogastropoda) from northeast Brazil with first record of *Gergovia* for the Atlantic ocean. *Zootaxa*, 1387: 59-68.
- GARCIA-VALLVE, S., J. PALAU & A. ROMEU, 1999. Horizontal gene transfer in glycosyl hydrolases inferred from codon usage in *Escherichia coli* and *Bacillus subtilis*. *Molecular Biology and Evolution*, 9: 1125-1134.
- HARASEWYCH, M.G., M. SIKAROODI & P.M. GILLEVET, 2011. The Delray Beach, Florida colony of *Cerion* (*Paracerion*) *tridentatum* *costellata* Pilsbry, 1946 (Gastropoda: Pulmonata: Cerionidae): Evidence for indirect Cuban origins. *The Nautilus*, 125: 173-181.
- HERNÁNDEZ, J.M., E. ROLÁN & F. SWINNEN, 2011. Part 3. Gastropoda: Prosobranchia. In: ROLAN, E. (ed.), *Molluscos y conchas de Canarias*, pp. 54-269. ConchBooks, Hackenheim & Emelio Rolan, Vigo.
- JABLONSKI, D., & R.A. LUTZ, 1980. Molluscan larval shell morphology: ecological and paleontological applications. In: RHOADS, D.C., & R.A. LUTZ (eds.), *Skeletal Growth of aquatic organisms: biological records of environmental change*, pp. 323-377. Plenum Press, New York.
- JEFFREYS, J.G., 1885. On the Mollusca procured during the 'Lightning' and 'Porcupine'

- Expeditions, 1868-70. (Part IX). *Proceedings of the Zoological Society of London*, 1885: 27-63, pls. 4-6.
- JOUSSEAUME, F.P., 1887. La famille des Cancellariidae (Mollusques gastéropodes). *Le Naturaliste*, Année 9, 2^e Série [Année 1]: 155-157, 192-194, 213-214, 221-223.
- LARKIN, M.A., G. BLACKSHIELDS, N.P. BROWN, R. CHENNA, P.A. McGETTIGAN, H. McWILLIAM, F. VALENTIN, I.M. WALLACE, A. WILM, R. LOPEZ, J.D. THOMPSON, T.J. GIBSON & D.G. HIGGINS, 2007. Clustal W and Clustal X version 2.0. *Bioinformatics*, 23: 2947-2948.
- MARTINS, A.M.F., S. ÁVILA, J.P. BORGES, P. MADEIRA & B. MORTON, 2009. Illustrated checklist of the infralittoral molluscs off Vila Franca do Campo. In: MARTINS, A.M.F. (ed.), *The Marine Fauna and Flora of the Azores. Proceedings of the Third International Workshop of Malacology and Marine Biology, São Miguel 2006. Açoreana*, Supplement 6: 15-103.
- MODICA, M.V., P. BOUCHET, C. CRUAUD, J. UTGE & M. OLIVERIO, 2011. Molecular phylogeny of the nutmeg shells (Neogastropoda, Cancellariidae). *Molecular Phylogenetics and Evolution*, 59(3): 685-697.
- NORDSIECK, F., 1968. *Die Europäischen Meeres-Gehäuseschnecken (Prosobranchia) vom Eismeer bis Kapverden und Mittelmeer*, 273 pp. Gustav Fischer, Stuttgart.
- NORDSIECK, F., & F.G. GARCIA-TALAVERA, 1979. *Moluscos marinos de Canarias y Madeira (Gastropoda)*, 208 pp. Aula de Cultura, Tenerife.
- PAWLIK, J.R., J.B. O'SULLIVAN & M.G. HARASEWYCH, 1988. The egg capsules, embryos and larvae of *Cancellaria cooperi* (Gastropoda: Cancellariidae). *The Nautilus*, 102(2): 47-53.
- PETIT, R.E., 1986. Notes on species of *Broccchinia* (Gastropoda: Cancellariidae). *The Nautilus*, 100(1): 23-26.
- PETIT, R.E., & M.G. HARASEWYCH, 2005. Catalogue of the Superfamily Cancellarioidea Forbes and Hanley, 1851 (Gastropoda: Prosobranchia) – 2nd Edition. *Zootaxa*, 1102: 1-161.
- ROLÁN, E., & J.M. HERNÁNDEZ, 2009. Nueva información sobre el género *Broccchinia* (Gastropoda, Cancellariidae) en Canarias. *Revista de la Academia Canaria de Ciencias*, 20(4): 103-109.
- ROLÁN MOSQUERA, E., 1983. *Moluscos de la Ria de Vigo*. I. *Gasteropodos*, 383 pp. Universidad de Santiago, Colegio universitario de Vigo, Santiago de Compostella.
- RONQUIST, F., M. TESLENKO, P. VANDER MARK, D.L. AYRES, A. DARLING, S. HOHNA, B. LARGET, L. LIU, M.A. SUCHARD & J.P. HUELSENBECK, 2012. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, 61: 539-542.
- SACCO, R., 1894. *I molluschi dei terreni terziarii del Piemonte e della Liguria*. Pt. 16 (Cancellariidae), 78 pp., 3 pls. C. Clausen, Torino.
- SHUTO, T., 1974. Larval ecology of prosobranch gastropods and its bearing on biogeography and paleontology. *Lethaia*, 7: 239-256.
- SOWERBY, G.B., L., 1832-1833. *Cancellaria. The Conchological Illustrations*. Parts 9-13, 5 plates. London.
- SYKES, E.R., 1911. On the mollusca procured during the Porcupine expedition 1869-70. Supplemental notes. *Proceedings of the Malacological Society*, 9: 331-348.
- VERHECKEN, A., 1991. Description of two new species of bathyal Cancellariidae (Mollusca, Gastropoda) from off Brazil. *Bulletin du Muséum national d'Histoire naturelle Paris*, 4^e sér. 12, Sect. A (3-4): 547-553.
- VERHECKEN, A., 1997. Mollusca Gastropoda: Arafura Sea Cancellariidae collected during the KARUBAR Cruise. In: CROSNIER, A., & P. BOUCHET (eds.), *Résultats des campagnes MUSORSTOM*, volume 16. *Mémoires du Muséum national d'Histoire naturelle*, 172: 295-323.
- VERHECKEN, A., 2002. Atlantic bathyal Cancellariidae (Neogastropoda: Cancellarioidea): additional data, and description of a new species. *Journal of Conchology*, 37(5): 505-514.
- VERHECKEN, A., 2007. Revision of the Cancellariidae (Mollusca, Neogastropoda, Cancellarioidea) of the eastern Atlantic (40°N-40°S) and the Mediterranean. *Zoosystema*, 29(2): 281-364.