

# Development, phylogeny, and taxonomy of *Bostrycapulus* (Caenogastropoda: Calyptraeidae), an ancient cryptic radiation

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Calyptraeid gastropods are well known for the taxonomic difficulties caused by their simple, phenotypically variable shells. In this paper I demonstrate that what was previously considered to be a single species, *Crepidula aculeata*, is an ancient (3–15 Myr) cryptic species complex made up of at least eight species, and that this group should be placed in the genus *Bostrycapulus*. Despite the difficulty in finding diagnostic adult shell and anatomical features upon which species can be unambiguously identified, DNA sequences, protoconch morphology, embryonic morphology and developmental characters clearly differentiate these eight species. A single species with direct development and nurse eggs is present in the South Atlantic, and a species with planktotrophic development occurs in the equatorial Pacific. The species from Japan, Australia, Florida, the Pacific coasts of Mexico and Central America, and the Cape Verde Islands all have direct development. Most of these species are separated by >15% divergence in COI sequence data. The fossil record of *Bostrycapulus* goes back to the Miocene, which agrees with genetic estimates of divergences within the genus ranging from 3 to 15 Mya. Surprisingly, these ancient species differ only slightly in morphology from each other and genetic differentiation does not correlate with geographical distance. I revise the genus *Bostrycapulus* on the basis of differences in adult morphology, embryonic morphology, mode of development, protoconch morphology, and DNA sequence data. I also describe four new species (*B. pritzkeri* sp. nov., *B. odites* sp. nov., *B. latebrus* sp. nov. and *B. urraca* sp. nov.) and remove three others (*B. gravispinosus*, *B. calyptraeformis*, and *B. cf. tegulicius*) from synonymy with *B. aculeatus*. © 2005 The Linnean Society of London, *Zoological Journal of the Linnean Society*, 2005, 144, 75–101.

ADDITIONAL KEYWORDS: biogeography – *Crepidula aculeata* – gastropod taxonomy – new species – sibling species.

## INTRODUCTION

The application of molecular methodologies to the systematics of marine invertebrates and fishes has resulted in the discovery of numerous morphologically cryptic species (e.g. Murphy, 1978; Hoagland, 1984; Knowlton, 1993; Collin, 2000a; Colborn *et al.*, 2001; Lessios, Kessing & Pearse, 2001; Muss *et al.*, 2001; Rocha-Olivares, Fleeger & Foltz, 2001; Véliz, Guisado & Winkler, 2001; Wares, 2001; Véliz, Winkler & Guisado, 2003). Cryptic species are not restricted to plastic and morphologically simple groups like sponges and corals, but occur in well-studied, morpho-

logically complex groups like snapping shrimp, starfish, sea urchins and fish (Knowlton, 1993; Williams, 2000; Lessios *et al.*, 2001; Muss *et al.*, 2001). Genetic analysis suggests that they may have been reproductively isolated for many thousands of generations (e.g. Knowlton & Weigt, 1998), and the occurrence of old cryptic species shows that isolation for hundreds of thousands to millions of years does not necessarily result in morphological differentiation.

There are a growing number of recognized 'ancient' cryptic species complexes in freshwater invertebrates (e.g. *Daphnia* up to 50 Myr, Colbourne & Hebert, 1996; Colbourne *et al.*, 1998; *Hyaella* sp. complex in the mid-Miocene, Witt & Hebert, 2000; *Brachionus plicatilis* more than 20 Myr, Gómez *et al.*, 2002), although only a few such radiations have been discovered in the marine environment (e.g. bonefish 4–20 Myr, Colborn

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*et al.*, 2001). Careful examination of morphologically cryptic species often reveals chemical, behavioural, or developmental differences, further supporting their status as distinct species (Knowlton, 1993). Such differences demonstrate that there has been adequate time for divergence to occur, despite the observed morphological stasis.

Calyptraeid gastropods are one such group where morphological characters cannot always be used to discriminate effectively among species. The simplicity and plasticity of calyptraeid shells has led to much taxonomic uncertainty and instability in this family (Hoagland, 1977, 1984, 1986; Collin, 2000a, 2002; Véliz *et al.*, 2001, 2003). Anatomical features can be used to distinguish among major species groups within some genera, but are very conservative and often cannot be used to distinguish between closely related species (Collin, 2003a). Genetic and developmental features appear to diverge more rapidly than do morphological characters. Therefore, it is not surprising that recent work with the genetics and development of *Crepidula* and *Crucibulum* has led to the recognition and formal description of several morphologically cryptic species (e.g. Gallardo, 1979; Hoagland, 1984; Collin, 2000a, 2002; Véliz *et al.*, 2001).

High-level phylogenetic analyses of calyptraeids have led to the discovery of a group of eight cryptic species, all of which were most recently ascribed to *Crepidula aculeata* (Gmelin, 1791) (Hoagland, 1977). *Crepidula aculeata s.l.* has a distinctive shell shape; it is one of the few '*Crepidula*' that retains clear traces of coiling, the shell has a distinctive longitudinal ridge and the shell often has numerous spines (Fig. 1). The shells are distinct enough to have been attributed to a separate subgenus, *Bostrycapulus* Olsson & Harbison, 1953, which has sometimes been raised to genus level. Phylogenetic analyses of calyptraeids based on anatomical (70 species; Collin, 2003a) and molecular (94 species; Collin, 2003b) data show that species in the *aculeata* group fall outside *Crepidula s.s.* and are more closely related to *Crucibulum* and *Crepipatella*. Therefore, they should be referred to *Bostrycapulus*.

In her review of *Crepidula*, Hoagland (1977) synonymized all the named species with shell characteristics that place them in the '*aculeata*' group with *C. aculeata* but noted that it was unusual for a single species without planktonic larvae to have such a wide, disjunct, geographical distribution. After more extensive work with development, she removed samples from Panama, which she called *C. echinus* (Broderip, 1834), from synonymy with *C. aculeata* without explaining the change in nomenclature (Hoagland, 1986). My previous phylogenetic analyses of calyptraeids have included individuals of what appear to be several species in this group (labelled as '*cf.*

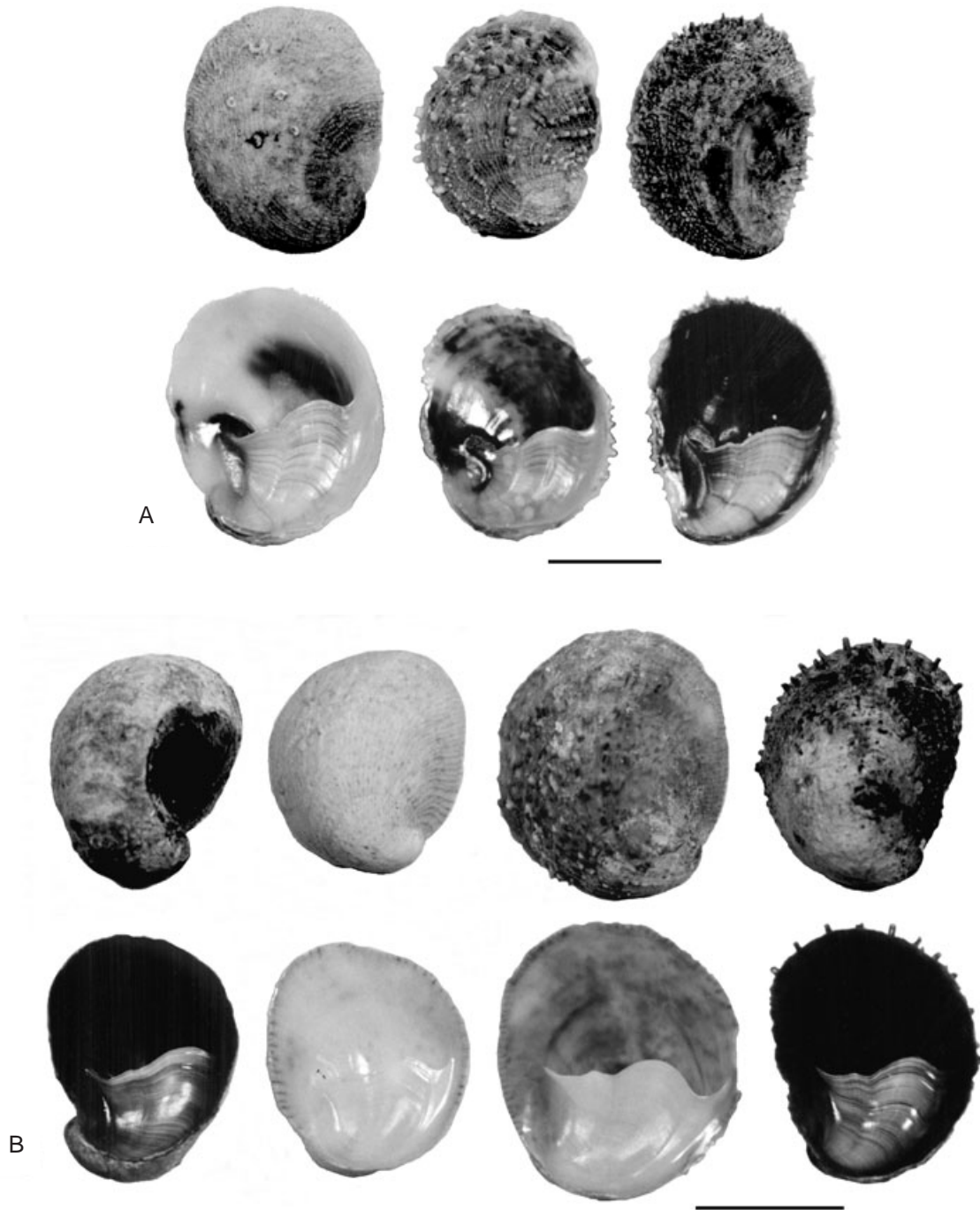
*aculeata*' in Collin, 2003a, b). Here I present morphological, developmental, genetic, and biogeographical data with the following aims: (1) augmenting the description of *Bostrycapulus*; (2) determining the relationships between the different species within *Bostrycapulus*, and (3) clearly delimiting the species within the genus.

## MATERIAL AND METHODS

Locality information for the live-collected specimens that were examined or sequenced for this study are listed in Table 1. Vouchers are deposited at the Field Museum of Natural History, Chicago, USA, The Natural History Museum, London, England, and the Academy of Natural Sciences, Philadelphia, USA. Protoconchs were obtained from the same populations except for the Japanese animals, which were obtained from shells provided by K. Noda from Minabe, Wakayama Prefecture and Cape Verdian animals where protoconchs were obtained from shells provided by Emilio Rolán from Rife de Chaves, Sal Rei Bay, Boa Vista. Numerous museum lots of mostly dry material were examined from FMNH, ANSP, CAS, NMP, NMNZ, USNM, AMS, BMNH and IZUA. Museum abbreviations follow Leviton *et al.* (1985).

A 611 base pair fragment of the mitochondrial cytochrome oxidase I (COI) gene and a fragment of mitochondrial 16S rDNA were sequenced for individuals from each locality and deposited in GenBank. As these data were originally obtained for interspecific phylogenetic analysis, few individuals were sequenced for each locality and in several cases only a single ethanol-preserved individual was available for sequencing (e.g. *B. cf. tegulicius*). DNA was extracted from ethanol-preserved tissue with Puregene (Gentra Systems) or DNeasy (Quiagen) extraction kits, amplified using Ready-To-Go PCR beads (Pharmacia Biotech), and primers and PCR profile of Folmer *et al.* (1994) and 16sar–16sbr of Palumbi (1996). PCR products were sequenced in both directions with dRhodamine (Perkin Elmer) or Big Dyes cycle sequencing dye terminator kits using the amplification primers and an ABI 377 automated sequencer. Sequences were aligned using Sequencher 3.0 and alignments were adjusted by eye.

Phylogenetic analyses were conducted using PAUP\* v. 4b02 (Swofford, 1998). An equal-weighted, unrooted, parsimony analysis was performed with gaps coded as a fifth character, using a heuristic search with TBR branch swapping and 1000 random additions. Bootstrap support for each clade was assessed based on 1000 bootstrap replicates with TBR branch swapping and ten random additions. I included *Crepipatella lingulata*, *C. capensis*, and *Crucibulum auriculum*, three close outgroups of *Bostry-*



**Figure 1.** Photographs of (A) *Bostrycapulus calyptraeformis* from Venado Beach in Panama and (B) *B. odites* sp. nov. from the subtidal of Playa Orenge (the three shells on the right) and the intertidal zone of nearby San Antonio Oeste (the shell on the left), Argentina. Both plates show the variation in shell colour and spine development found in samples collected from the same site. Samples from within a site do not differ in more than three or four base pairs in COI sequences. Scale bars = 10 mm.

**Table 1.** Summary of live-collected material used for observations of development and anatomy and for DNA sequencing. *Abbreviations:* BMSM, Bailey-Matthews Shell Museum; KLLC, K. L. Kaiser Collection

Species	Locality	Mode of development	Voucher nos.	Genbank nos. COI	Genbank nos. 16S
<i>B. aculeatus</i>	Mote Marine Laboratory, Lido Key, Florida, USA, 27°20'N, 82°42'W [ >30 animals]	Direct (observed)	ANSP A19745, FMNH 282365 BM20010455	AY061792, AY618322-3	AY061774
	Harbor Branch Oceanographic Institute, Florida, USA, 28°29'N, 81°20'W [3 animals]	Direct (observed)	FMNH 306481	AY618317	–
	Florida Keys, USA, 24°40'50'N, 82°16'02'W [2 animals]	Direct (observed)	FMNH 282284	AY061777	–
	Abaco, Bahamas, 26°42' 56'N, 77°14' 33'W [5 animals]	Not observed	BMSM 26292 BMSM 26293 BMSM 26294 BMSM 26295	AY618346-7	–
<i>B. gravispinosus</i>	Chijiwa, Nagasaki, Japan [1 animal]	Not observed	FMNH 282336	AY061783	AY061766
<i>B. calyptraeformis</i>	Playa Venado, Panama, 8°55'N, 79°38'W [ >100 animals]	Planktotrophic (observed)	FMNH 282273, ANSP A19740 BM20010452	AY061786, AY618328 AY618334-40, AY618342-5 AY618327	AY061777, AY616674
	Isla Raya, Azuero Peninsula, Panama, 7°23.64'N 80°16.02'W Kaneohe Bay, Hawaii, USA [5 animals]	Direct (observed)	FMNH 306482	AY618327	AY616673
	USS Machinist, Guam [3 animals] Zorritos, Peru, 3°45'S, 80°40'W [20 animals]	Planktotrophic (Bell, 1993) Not observed Planktotrophic (observed)	FMNH 282171-2	AY061790	AY061769
<i>B. cf. tegulicius</i>	Los Aubaz, Paita, Peru, 5°10'S, 81°10'W [20 animals]	Planktotrophic (observed)	ANSP A19746, FMNH 282351 BM20010454	AY061785, AY618329-32	AY061772
	Calheta Funda, Sal Island, Cape Verde, 16°40'N, 22°03'W [1 animal]	Direct (from protoconch)	FMNH 282359	AY061776	AY061775

<b><i>B. pritzkeri</i></b>	Edwards Beach, Sydney, Australia, 33°51'S, 151°13'E [14 animals] TYPE LOCALITY	Direct (observed)	FMNH 282302 ANM C400000	AY061793, AY618348	AY061767
<b><i>B. odites</i></b>	Playa Orengo, near San Antonio Oeste, Argentina, 40°53'S, 64°29'W [ >25 animals] Woolley's Pool, Muizenberg, South Africa, 34°04'S, 18°20'E [15 animals] TYPE LOCALITY Port Elizabeth, South Africa [2 animals] Gois Beach, Santos Bay, São Paulo, Brazil, 24°00'S, 46°21'W [5 animals] El Comitán, near La Paz, BCS, Mexico, 24°07'N, 110°24'W [10 animals] TYPE LOCALITY Isla Parida, Gulf of Chiriqui, Panama, 8°5.458'N, 82°18.671'W [ >50 animals] TYPE LOCALITY Isla Raya, Azuero Peninsula, Panama, 7°23.64'N 80°16.02'W Isla Jacaron, Parque National Coiba, Panama, 7°19.10'N, 81°43.83'W [3 animals] El Salvador, Gulf of Fonseca, 13°26.4 N, 89°48.04 W [3 animals] 13°17.23 N, 89°18.07 W [1 animal]	Direct, with nurse eggs (observed) Direct, with nurse eggs (observed) Not observed Direct, with nurse eggs (from protoconch) Direct (observed) Direct (observed) Direct (observed) Direct (observed) Not observed	FMNH 282297, ANSP A19744, BM20010456 FMNH 282277 V9447/ T1783 BM20010453 FMNH 282368 FMNH 282350 FMNH 282193-4 ANSP 412178-9, FMNH 306483 FMNH 306484 KLKC ICP-03-069	AY061782, AY061784, AY061794 AY061780, AY061788 AY061787 AY061779, AY061781 AY061789, AY618333 AY618319, AY618325-6 AY618321 AY618318, AY618320, AY618324	AY061764 - AY061765 AY061763 AY061768 AY616667-9 - -
<b><i>B. latebrus</i></b>		Not observed	ANSP 412180-1	AY618341, AY618349	AY616670-2
<b><i>B. urraca</i></b>		Not observed			

*capulus* (see Collin, 2003b), and used them to root the analysis. Genetic distances were calculated using Kimura 2-parameter distances.

DNA sequence data were also analysed using a Bayesian approach. The appropriate model and starting parameters for Bayesian analysis were chosen for each of the datasets using the likelihood ratio test implemented in ModelTest 3.06 (Posada & Crandell, 1998, 2001) with the default settings and an  $\alpha$ -level of 0.01. Bayesian analyses using MrBayes 2.01 (Huelsenbeck, 2000; Huelsenbeck & Ronquist, 2001) were conducted for each dataset (COI, 16S, and COI and 16S combined) using the model obtained from ModelTest 3.06. The Bayesian analysis using one cold and three incrementally heated chains started from a random tree with a uniform prior for branch lengths and for the Gamma shape parameter. Invariant sites were retained in the sequences and their frequency was estimated using the 'invgamma' setting. The Metropolis-coupled Markov chain Monte Carlo (MCMCMC) analysis was run five times for 1000 000 generations for each dataset and the number of trees to be discarded as representing a 'burn-in' period was determined graphically to be either 25 000 or 50 000 generations. Majority-rule consensus trees for every 50th tree after the 'burn-in' period were created using PAUP\*, and consensus phylograms were created in MrBayes.

The morphology and anatomy of ethanol- or formalin-preserved individuals were examined under a Wild M4 dissecting microscope. The anatomy of five to ten animals was examined from most localities. Prior to mounting for scanning electron microscopy, protoconchs and radulae were cleaned briefly in dilute bleach and rinsed in distilled water. All specimens were gold-coated and viewed with an Almay scanning electron microscope. Two to five radulae from each locality were prepared for SEM. To estimate within-individual variation, the number of denticles on each tooth were counted for ten rows of unworn teeth per individual.

Developmental stages were observed live and measured with dissecting and compound microscopes. Broods from more than 30 animals were observed from Argentina and Panama and five to ten broods were observed for populations from Florida, Peru, South Africa, Sydney and Mexico. Developmental stages were not available for animals from Japan, Brazil, or Cape Verde. Larvae of the single species with planktonic development were raised according to the methods of Collin (2000b).

## RESULTS

The animals examined during this study can be attributed to eight species on the basis of protoconch mor-

phology, developmental biology, embryology, and DNA sequence data.

### DNA SEQUENCE DATA

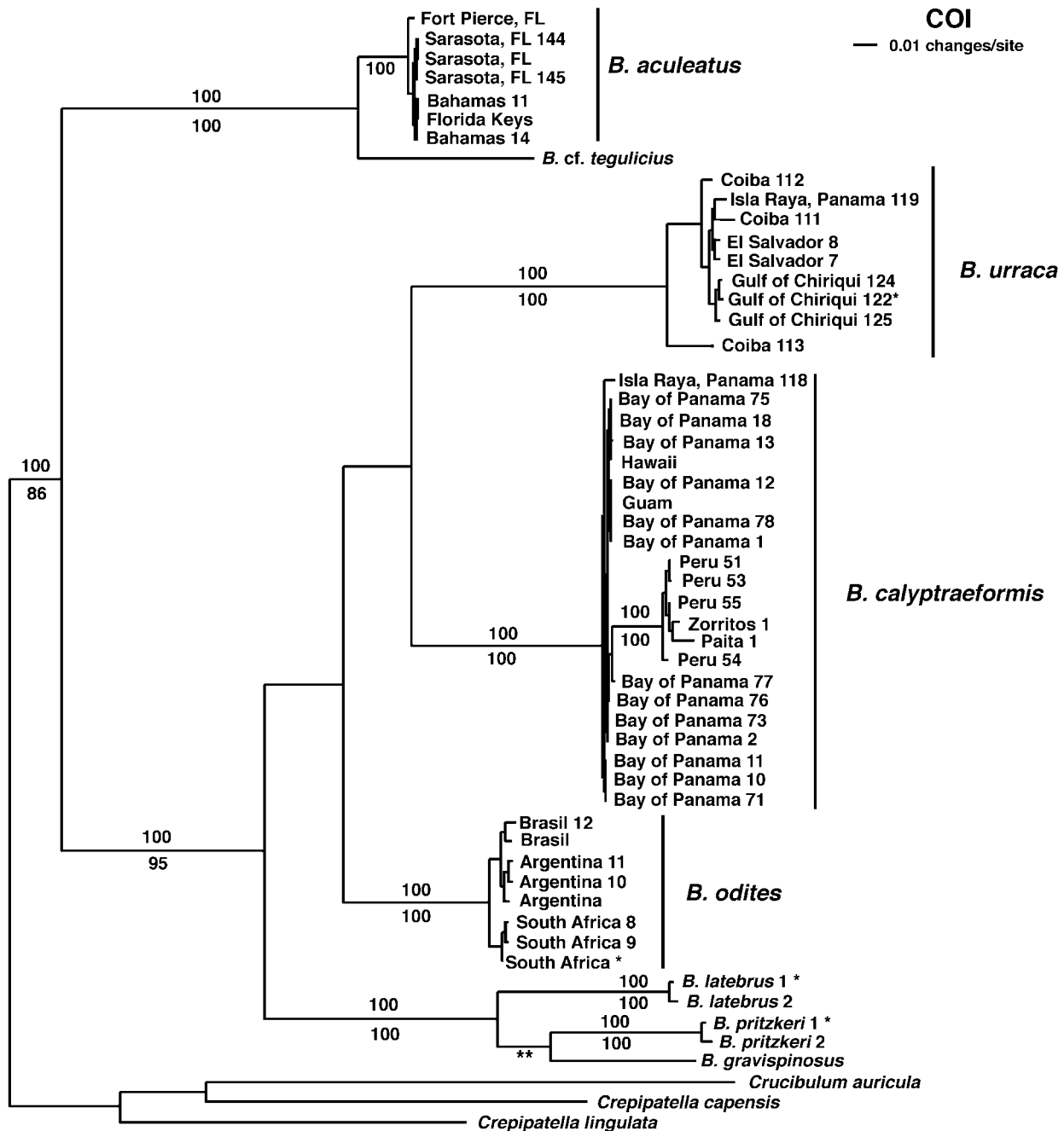
Phylogenetic analysis of COI and 16S DNA sequence data shows little sequence variation within each locality (about 0.5–1% in COI). There are eight distinct clades (species) that differ from each other by 6–21% in COI sequences and by at least 2% in 16S sequences (Fig. 3; Table 2). These groups are supported as monophyletic with bootstrap and Bayesian support above 98 and 100, respectively (Fig. 2). Sequence divergences of such magnitude commonly occur between morphologically well-differentiated species of calyptraeids (Collin, 2003a, b).

Four of these clades include samples for several locations. One clade is composed of sequences from individuals collected from the South Atlantic (Brazil, Argentina, and South Africa); it shows 0.8–1.2% COI divergence between localities. Another clade includes samples from the Bay of Panama, Hawaii, and Guam which are identical to each other and also includes the closely related (4% divergent) material from Peru (Figs 2, 4; Table 2). The third contains samples from both coasts of Florida and the Bahamas. The fourth includes material from the Pacific coast of El Salvador and the western half of Panama. Animals from this clade occur in sympatry with the Panama-Hawaii clade in the Perlas Archipelago and the Azuero Peninsula, Panama. Samples from the remaining localities form their own individual clades.

Analyses of the individual gene sequences and the combined analysis show *Bostrycapulus* to be monophyletic (as did two much larger analyses; Collin, 2003a, b). However, the relationships between the distinct clades within the genus are not well resolved. In all analyses there is high support for the result that the Florida–Bahamas clade is sister to the sample from Cape Verde, and that these two are sister to remaining taxa. The samples from Baja California, Sydney, and Japan group together with 100% bootstrap and Bayesian support, but the relationships between them are unresolved. The relationship of this Pacific clade with the two Panamanian species and the South Atlantic species is unresolved.

### MORPHOLOGY

Shell morphology and colour are variable within each population. Some sympatric individuals have numerous fine spines, some have fewer large spines. Some shells are robust, some are small and gracile (Fig. 1). Shells collected subtidally from Playa Orengo, Argentina are usually completely smooth, although some have numerous large robust spines. Shells from the



**Figure 2.** The Bayesian best estimate topology of the phylogeny of *Bostrycapulus* based on COI. Numbers above the branches represent bootstrap percentages and those below the branches are Bayesian support. Branches are labelled with the collecting locality and the individual code. \*= type individual.

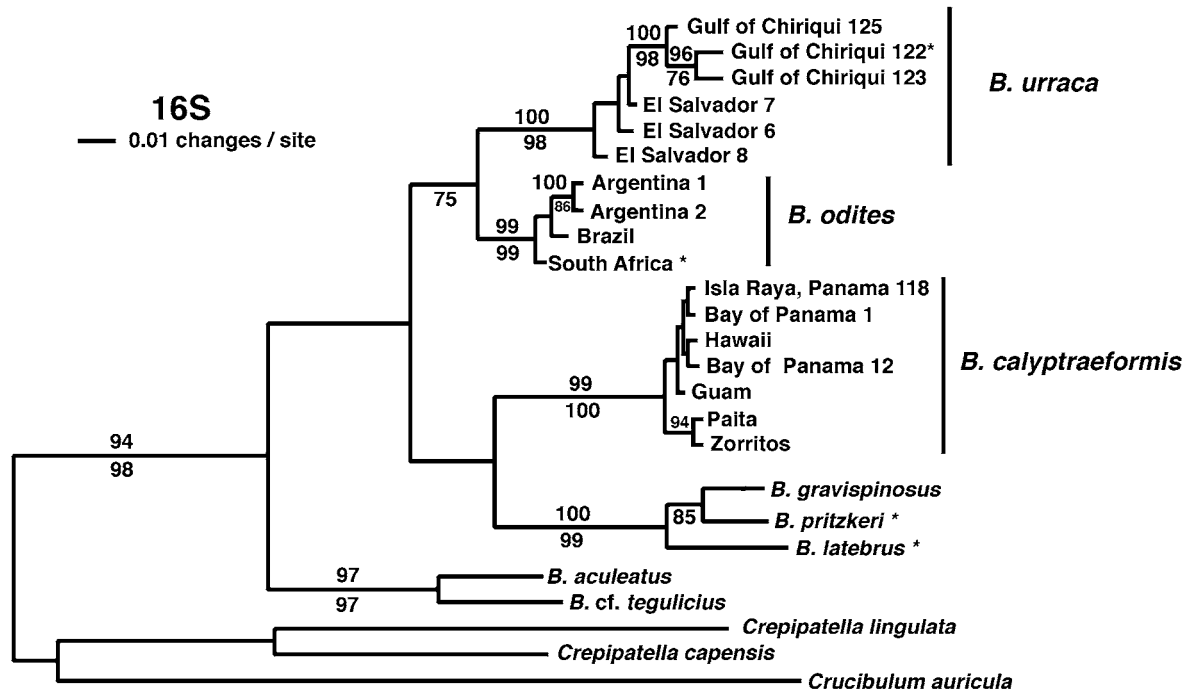
genetically similar animals from Brazil do not share this feature and animals with identical COI sequences collected from the intertidal zone of nearby San Antonio Oeste are smooth and almost black (Fig. 1). Shell colour is also variable. Shells are often dark brown with a wide pale streak running slightly to the right of the midline, although all populations

also contain animals with uniformly pale shells (Fig. 1). Animals with pale shells also have pale bodies. In all populations the shell is usually white but sometimes has brown streaks. All of the ethanol-preserved material available to me from South Africa, Mexico, and Japan is so over-grown with coralline algae and other fouling organisms that the details of

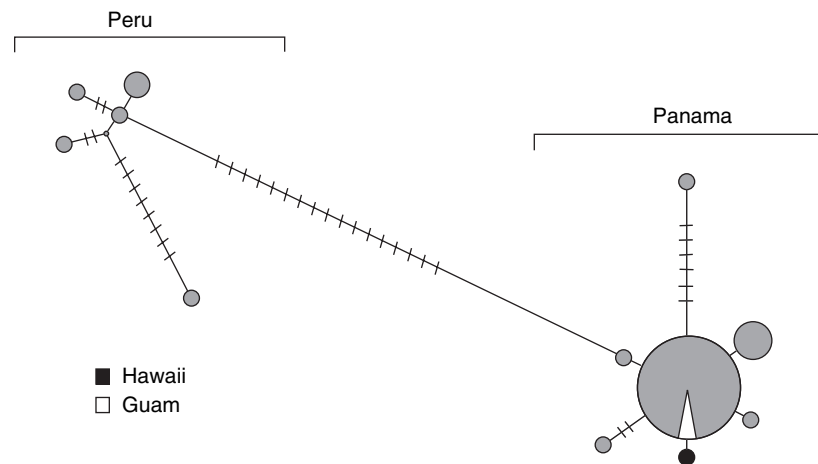
**Table 2.** Pairwise Kimura 2-parameter genetic distances between individuals from each locality. Bold values indicate intraspecific comparisons. *Abbreviations:* Arg, Argentina; Bah, Bahamas; BP, Bay of Panama; CT, Cape Town; CV, Cape Verde; ES, El Salvador; GC, Gulf of Chiriqui

16S COI	CV	Brazil	CT	Arg	Japan	Peru	BP	Hawaii	Guam	Mexico	Florida	Sydney	Bah	ES	GC
CV	–	0.081	0.078	0.083	0.076	0.080	0.081	0.081	0.080	0.074	0.034	0.074	–	0.068	0.075
Brazil	0.168	–	0.062	0.006	0.067	0.051	0.054	0.054	0.054	0.069	0.073	0.070	–	0.041	0.045
CT	0.166	<b>0.012</b>	–	0.008	0.065	0.045	0.047	0.047	0.047	0.067	0.071	0.067	–	0.038	0.043
Arg	0.174	<b>0.008</b>	<b>0.010</b>	–	0.069	0.054	0.056	0.056	0.056	0.072	0.075	0.072	–	0.043	0.047
Japan	0.211	0.158	0.154	0.156	–	0.061	0.056	0.056	0.056	0.034	0.071	0.017	–	0.059	0.068
Peru	0.185	0.126	0.126	0.126	0.174	–	0.006	0.008	0.006	0.066	0.081	0.063	–	0.052	0.054
BP	0.163	0.103	0.103	0.105	0.166	<b>0.044</b>	–	0.002	0.000	0.061	0.081	0.059	–	0.052	0.054
Hawaii	0.166	0.103	0.103	0.105	0.166	<b>0.046</b>	<b>0.002</b>	–	0.002	0.162	0.085	0.059	–	0.052	0.056
Guam	0.163	0.103	0.103	0.105	0.166	<b>0.046</b>	<b>0.000</b>	<b>0.002</b>	–	0.162	0.085	0.059	–	0.053	0.054
Mexico	0.186	0.144	0.140	0.138	0.106	0.164	0.162	0.061	0.061	–	0.069	0.038	–	0.073	0.077
Florida	0.066	0.157	0.155	0.160	0.192	0.169	0.157	0.159	0.157	0.199	–	0.069	–	0.061	0.073
Sydney	0.174	0.133	0.128	0.131	0.094	0.152	0.140	0.140	0.140	0.122	0.174	–	–	0.056	0.072
Bah	0.066	0.155	0.153	0.158	0.190	0.160	0.155	0.157	0.157	0.200	<b>0.002</b>	0.172	–	–	–
ES	0.171	0.167	0.121	0.113	0.123	0.160	0.127	0.129	0.129	0.129	0.155	0.156	0.152	–	0.008
GC	0.161	0.119	0.111	0.121	0.163	0.121	0.123	0.123	0.123	0.167	0.152	0.156	0.151	<b>0.007</b>	–





**Figure 3.** The Bayesian best estimate topology of the phylogeny of *Bostrycapulus* based on 16S. Numbers above the branches represent bootstrap percentages and those below the branches are Bayesian support. Branches are labelled with the collecting locality and the individual code. \*= type individual.



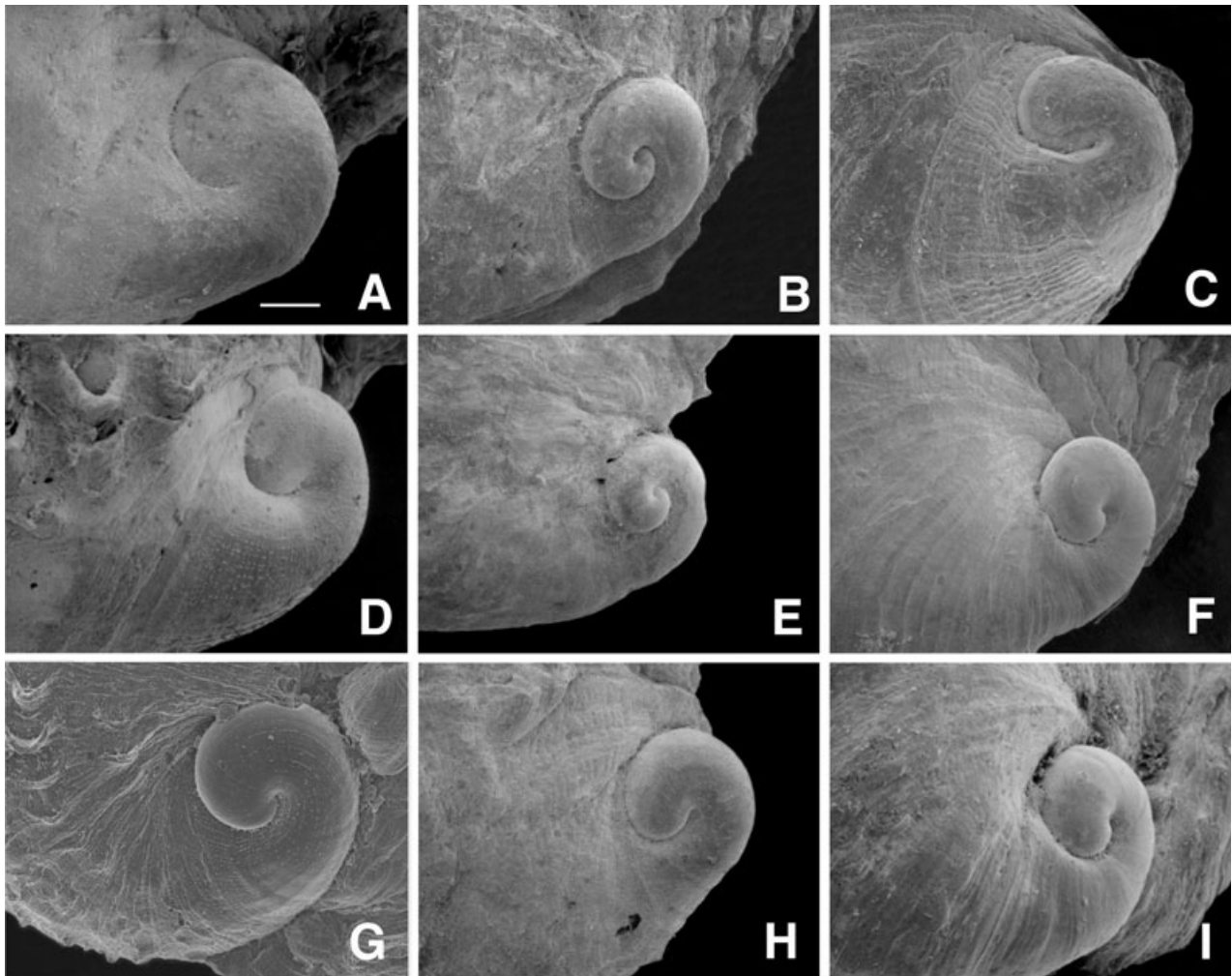
**Figure 4.** Unrooted haplotype network of COI sequences from *Bostrycapulus calyptraeformis*. Slashes on branches show the number of differences between the haplotypes. Branches without slashes have a length of one. Size of the circles represent the number of individuals with that haplotype.

shell sculpture are difficult to distinguish and compare. However, examination of shells in museum collections suggests that such variation in shell shape, colour, and spination is typical of most populations of these species. Such variation can be found in any large lot from a single locality.

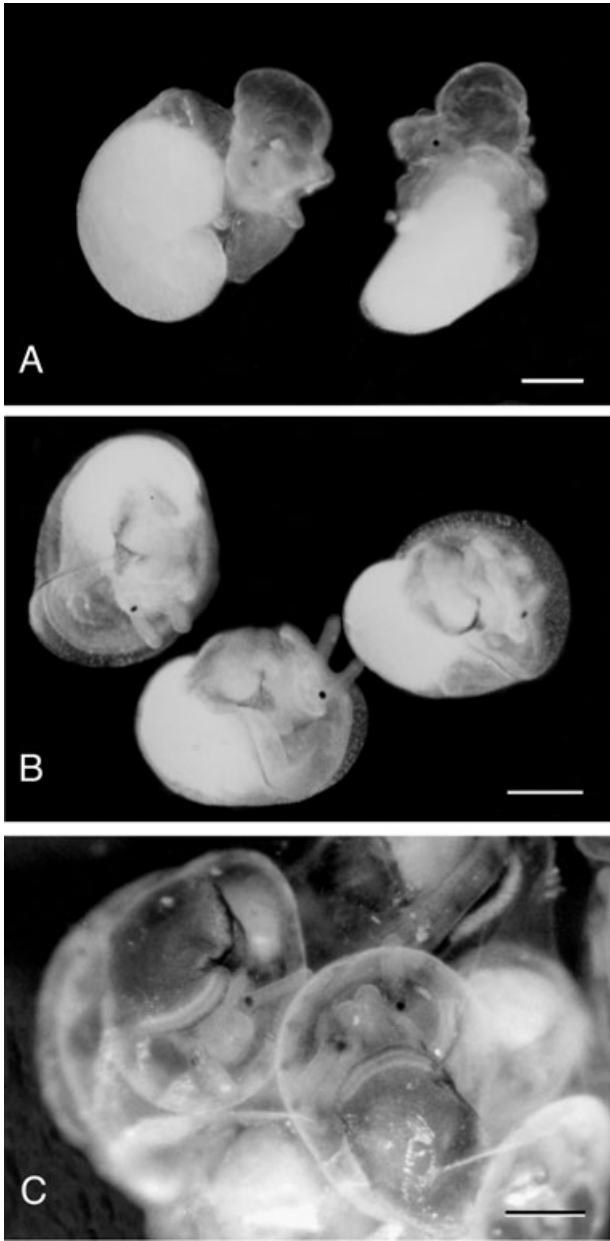
The different species of *Bostrycapulus* can be delimited on the basis of protoconch morphology. Protoconchs do not usually remain intact on the teleoconchs of adult animals and those that are intact often appear worn. SEMs show that juvenile shells from Brazil and Argentina have large protoconchs of

slightly more than a single whorl, with irregular growth lines (Figs 5F, 4I) typical of direct development with nurse eggs. Shells from the Bay of Panama and Peru have smaller more coiled protoconchs, typical of planktotrophic development, that increase regularly in size (Figs 5B, 4E). Protoconchs from Australia, Cape Verde, Japan, the Gulf of Chiriqui and Florida (Figs 5A, 4C, D, G, H, respectively) are more globose than those from the Bay of Panama and Peru and have less than a single whorl. As expected from differences in egg size (see below), the protoconchs from Australia are the most globose. Granular sculpture was evident on the protoconchs from Gulf of Chiriqui and Japan, although no sculpture was evident on those obtained from juvenile or

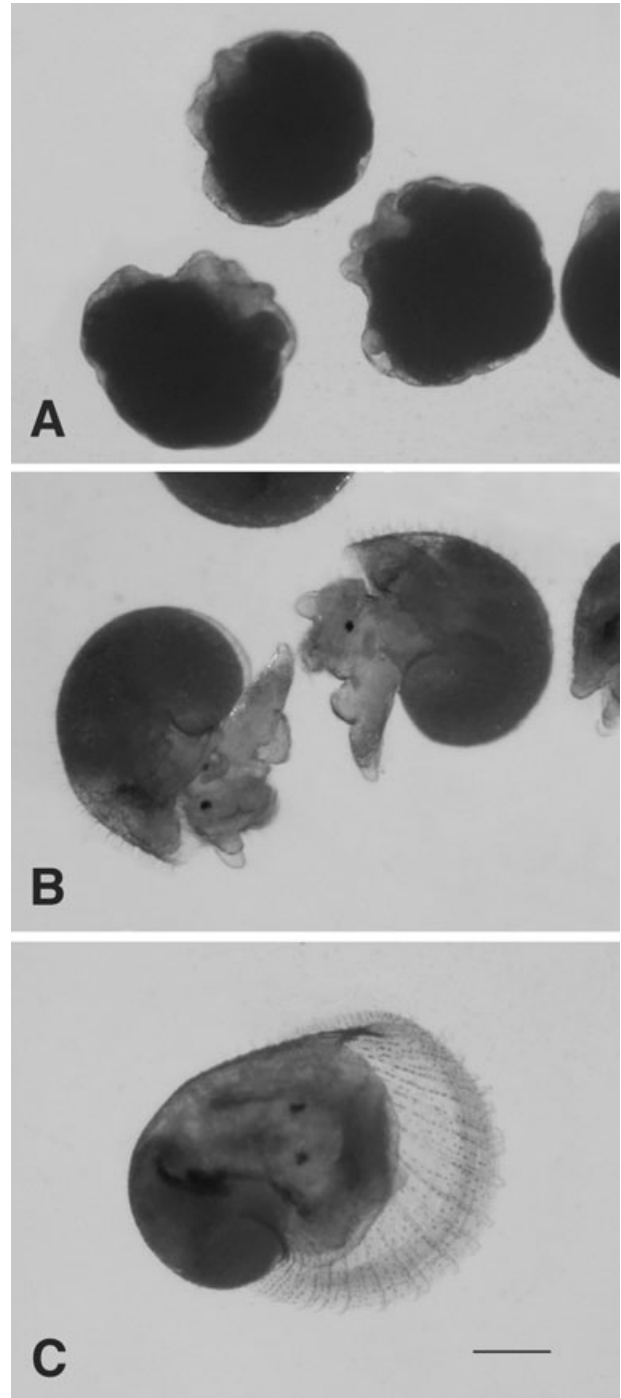
adult shells from the other localities. Shells of direct developing embryos that had been removed from their capsules from both Australia (Fig. 6) and Mexico showed spiral rows of fine granular sculpture when examined under a dissecting microscope, and prehatching stages from the Gulf of Chiriqui showed well-developed spines (Fig. 7). Likewise, large granular sculpture is visible under a compound microscope on the larval shell of 1-week-old and 3-week-old larvae from the Bay of Panama (Fig. 8), but this was not retained on the protoconchs obtained from the same species. It is unknown for how long sculpture is retained after hatching or settlement. However, its presence in early stages appears to be typical of *Bostrycapulus* species.



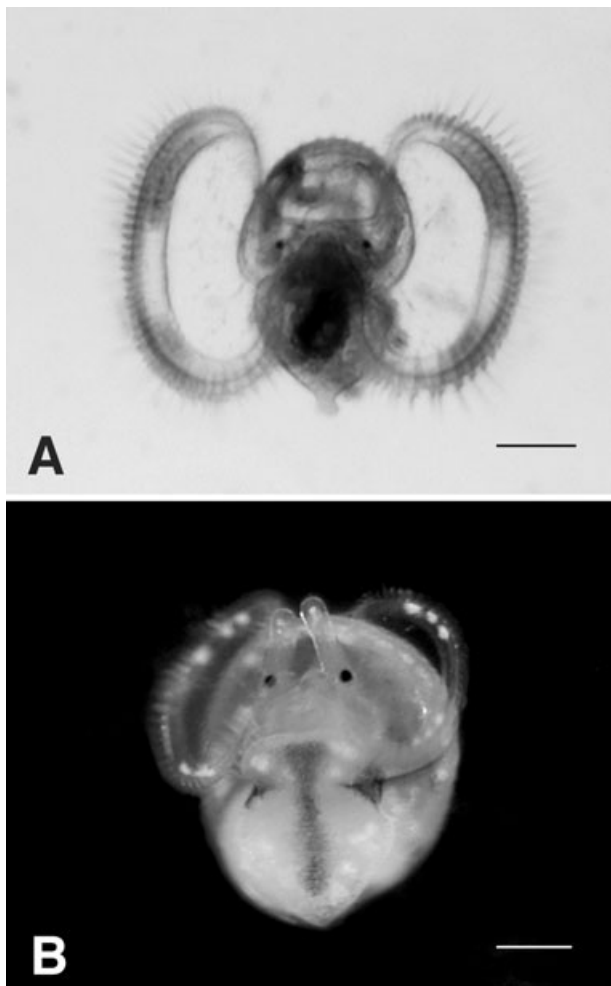
**Figure 5.** Protoconchs. A, *Bostrycapulus pritzkeri* sp. nov. from Sydney. B, *B. calyptraeformis* from the Perlas Islands, Panama. C, *B. cf. tegulicia* from Cape Verde. D, *B. gravispinosus* from Minabe, Wakayama Prefecture, Japan. E, *B. calyptraeformis* from Paita, Peru. F, *B. odities* sp. nov. from Playa Orenge, Argentina. G, *B. urraca* sp. nov. from Isla Parida, Panama. H, *B. aculeatus* from Lido Key, Florida. I, *B. odities* sp. nov. from São Paulo, Brazil. All are to the same scale. Scale bar = 500  $\mu$ m.



**Figure 6.** Embryos of *Bostrycapulus pritzkeri* sp. nov. Note the distinctive granular shell sculpture and the absence of a distinct velum at all stages. A, excapsulated early stage embryos at the beginning of shell formation. Scale bar = 150  $\mu$ m. B, excapsulated embryos with well-developed shells showing granular shell sculpture and the small ridge of the velum at the base of the tentacle. Scale bar = 250  $\mu$ m. C, encapsulated embryos near hatching with fully developed shell and body pigmentation. Scale bar = 250  $\mu$ m.



**Figure 7.** Embryos of *Bostrycapulus urraca* sp. nov. A, early postgastrula stage where the embryo is covered with a thin ciliated epithelium. B, mid-veliger stage, showing the granulated shell sculpture, the operculum behind the well-developed foot, the single embryonic kidneys and the reduced velum. C, Hatching stage, showing the well-developed shell sculpture. Scale bar = 150  $\mu$ m.



**Figure 8.** A, 2-week-old larva of *Bostrycapulus calyptraeformis* showing the velar pigment, shell sculpture (on the top of the shell) and large foot. Scale bar = 300  $\mu$ m. B, intracapsular larva of *B. aculeatus* showing the well-developed velum with pigment spots and body pigmentation. Scale bar = 200  $\mu$ m.

Examination of preserved samples did not show any consistent anatomical differences among samples from the different locations. The anatomy of *Bostrycapulus* is, however, distinct from the other major calyptraeid genera (Fig. 9; Simone, 2002; Collin, 2003a; and see below). The female genital papilla which has proven to be a useful character in distinguishing closely related *Crepidula* species (Collin, 2000a) is absent in *Bostrycapulus*. Radula morphology (Fig. 10; Table 3) does not appear to be useful in differentiating among these groups. There is significant within-individual variation in the number of denticles on each tooth. In addition, individuals collected from the same locality often vary in the frequency of teeth with few or many denticles, as well as in the maximum and minimum number of denticles.

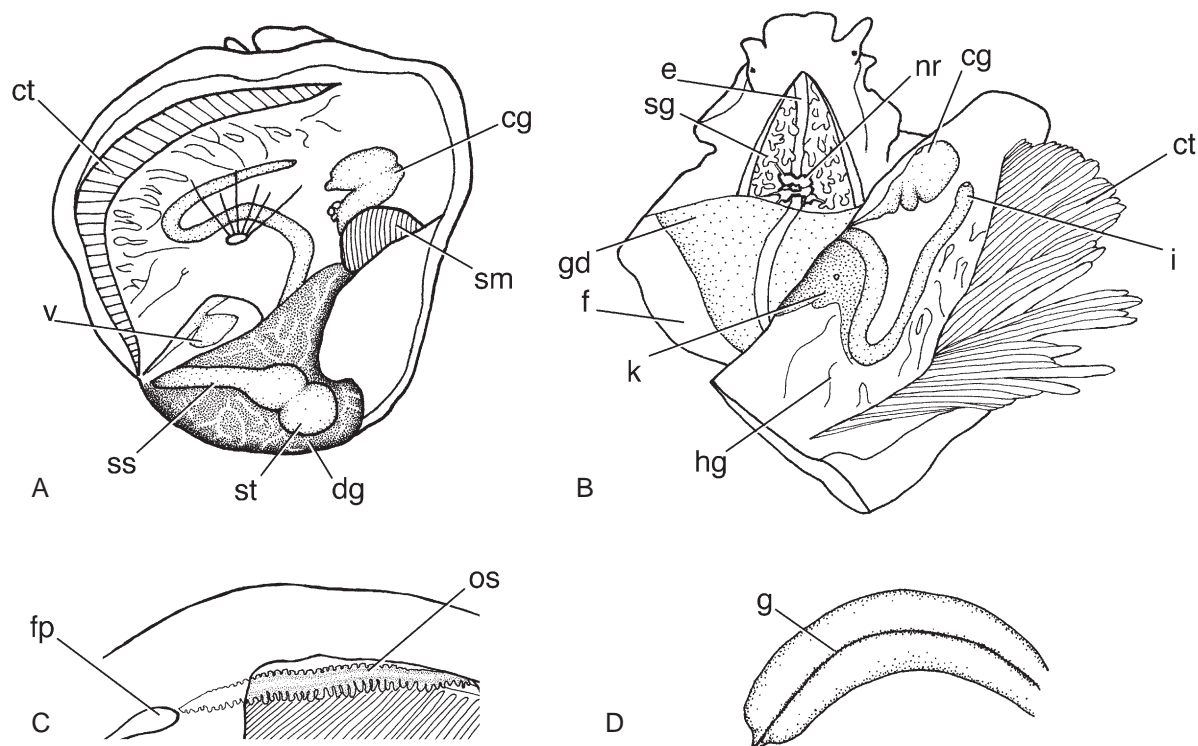
#### DEVELOPMENT

Three different modes of development are observed in the *Bostrycapulus* species examined here: (1) planktotrophic larvae; (2) direct development with large eggs, and (3) direct development from small eggs with nurse eggs (Table 4). These differences in modes of development and smaller differences in the details of development correspond to the same eight clades identified by the DNA sequence analysis and protoconch morphology.

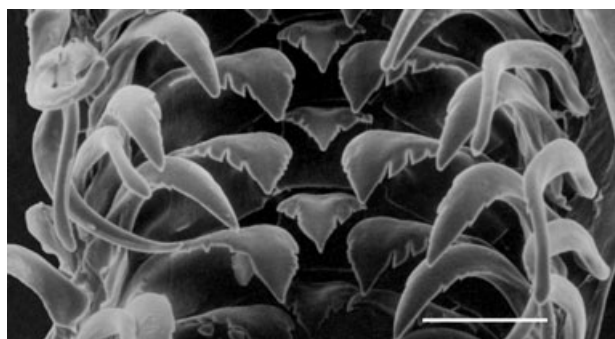
The clade from the South Atlantic has direct development from small eggs which consume nurse eggs and hatch as crawling juveniles. The nurse eggs begin to develop and cannot be distinguished from the embryos until after gastrulation. The clade from the Bay of Panama, Hawaii, and Peru has planktotrophic development. Animals from Australia, Mexico, Florida and western Panama develop directly from large eggs. Direct development without nurse eggs is also reported for animals from Japan (Ishiki, 1936) but the egg size seems too small (Ishiki, 1936; Amio, 1963) to produce such large juveniles. It is unlikely that these

**Table 3.** Variability of radula characteristics of five species of *Bostrycapulus*

Species	<i>B. aculeatus</i>	<i>B. calyptraeformis</i>	<i>B. pritzkeri</i>	<i>B. odites</i>	<i>B. latebrus</i>
Number of animals (ten rows each)	3	2	2	5	2
Number of denticles					
Rachidian	2–3	2–4	2–4	2–5	2–3
Inner side of lateral	1–3	1–3	1	1–3	1
Outer side of lateral	5–8	5–10	6–12	5–11	4–7
Inner side of inner marginal	2–7	2–8	6–10	3–11	2–5
Outer side of inner marginal	0–6	4–6	4–8	0–3	1–4
Inner side of outer marginal	0–3	0–2	3–8	1–7	0–4
Outer side of outer marginal	0	0	0	0	0



**Figure 9.** Illustrations of anatomy of *Bostrycapulus*, drawn from observations of several animals of *B. odites* sp. nov. from Argentina. There are no differences among species in the characters depicted here. A, dorsal view of the animal subsequent to removal from the shell. B, dorsal view of the animal with the mantle reflected. C, osphradium. D, penis. *Abbreviations:* cg, capsule gland; ct, ctenidia; dg, digestive gland; e, oesophagus; f, foot; fp, food pouch; g, seminal groove; gd, gonad; hg, hypobranchial gland; i, intestine; k, kidney; nr, nerve ring; os, osphradium; sg, salivary gland; sm, shell muscle; ss, style sac; st, stomach; v, ventricle.



**Figure 10.** Radula of *Bostrycapulus aculeatus* collected from Mote, Florida. Scale bar = 100  $\mu$ m.

differences in development are the result of interspecific variation, as poecilogony is not known in caenogastropods (Hoagland & Robertson, 1988; Bouchet, 1989) and no variation in development was observed among individuals from a single locality. More details of embryology are given below with the descriptions of each species.

## DISCUSSION

Although the populations examined here cannot be easily distinguished on the basis of shell morphology or easily visible anatomical features, the available data show that at least eight distinctly different mitochondrial haplotype lineages are present in *Bostrycapulus*. The levels of intraspecific DNA sequence divergence reported for other calyptraeid species (Collin, 2000a, 2001) are similar to the divergences between sequences reported here for individuals belonging to the South Atlantic clade or to the equatorial Pacific clade. Genetic divergences between each of the eight clades are considerably greater than divergences between cryptic sibling species of other calyptraeids (Collin, 2000a, 2001) and they are, in fact, often much larger than divergences between many clearly defined species of *Crepidula* (Collin, 2003a, b). The only other anatomical work that examined and compared several of these clades (animals from Spain, Brazil, Hawaii and Sydney; Simone, 2002) also found no consistent morphological differences among populations. Such cryptic differentiation

**Table 4.** Summary of *Bostrycapulus* species. Diagnostic features are highlighted in bold text. Abbreviations: ss, spiral sculpture

	<i>B. aculeatus</i>	<i>B. gravispinosus</i>	<i>B. calyptraeformis</i>	<i>B. tegulicius</i>	<i>B. pritzkeri</i>	<i>B. odites</i>	<i>B. latebrus</i>	<i>B. urraca</i>
Authority	(Gmelin, 1791)	(Kuroda & Habe, 1950)	(Deshayes, 1830)	(Rochebrune, 1883)	sp. nov.	sp. nov.	sp. nov.	sp. nov.
Fate of type	St. Petersburg	unknown	Paris Museum	Paris Museum	Australian Museum	Natal Museum	Field Museum	Field Museum
Type locality	Middle American Islands	Hirado Is., Nagasaki Pref. Japan	Peru (dubious)	Senegal	Edwards Reef, Sydney, Australia	Woolley's Pool, Cape Town, SA	La Paz, BCS, Mexico	Gulf of Chiriqui, Panama
Development	direct	direct	<b>planktotrophic</b>	direct (from protoconch)	direct	<b>direct with nurse eggs</b>	direct	direct
Egg size (µm)	380 (Hoagland, 1986)	200 (questionable) (Amio, 1963)	180	–	<b>530–560</b>	198	<b>488</b>	370
Hatching size (µm)	840 (Hoagland, 1986)	1000–1200 (Ishiki, 1936)	380	–	–	–	–	888
Embryonic operculum	present	–	present	–	<b>absent</b>	present	present	present
Distinct velum with food groove	medium, spotted with food groove	–	large, pigmented	–	<b>absent</b>	small, unpigmented,	small, unpigmented,	absent
Embryonic shell sculpture	granular ss	ss at hatching (Amio)	fine spines over entire larval shell	–	granular ss	<b>smooth with irregular growth lines</b>	granular ss	granular ss
Protoconch	1 whorl	1 whorl	1.5 whorls	less than 1 whorl	not available	1.25 whorls	less than 1 whorl	less than 1 whorl
Localities	Florida, Yucatan, Bahamas	Japan	Peru, Panama, Hawaii, Guam	West Africa, Cape Verde Is.	Australia	South Africa, Patagonia, Brazil	La Paz, Mexico	Panama, El Salvador

is not unusual or unexpected among calyptraeid species (e.g. Gallardo, 1979; Collin, 2000a, 2001; Véliz *et al.*, 2001, 2003), but the large number of cryptic species is unusual.

The results presented here suggest that *Bostrycapulus* shows as much among-species genetic divergence in the Pacific as in the Atlantic (e.g. 16.2% COI divergence between Panama and Mexico and 16.8% between Cape Verde and Brazil; Table 2). The maximum levels of genetic divergence (21%) between *Bostrycapulus* species are similar to or somewhat greater than those reported for other widespread marine genera: 4–6% in cytochrome *b* from trumpet fish species (Bowen *et al.*, 2001); 2–19% in ATPase and COI from *Diadema* species (Lessios *et al.*, 2001); 8–20% in COI from *Eucidaris* (Lessios *et al.*, 1999); up to 16% in cytochrome *b* from *Ophioblennius* fish (Muss *et al.*, 2001); up to 23% in COI from *Chthamalus* barnacles (Wares, 2001). In most of these cases, however, the species can be distinguished on morphological grounds and have been historically recognized as distinct. The higher levels of genetic divergence and almost complete absence of morphological differentiation among *Bostrycapulus* species suggest that the rate of morphological evolution relative to genetic change is considerably slower in calyptraeids than it is in these other groups.

There is ample evidence that the radiation of *Bostrycapulus* is an ancient cryptic radiation like that documented for bonefish (Colborn *et al.*, 2001). Museum records place *Bostrycapulus* as far back as the Miocene in Florida and California (Hoagland, 1977). Application of two separately derived molecular clock rate estimates to the divergences listed in Table 2 provides similar rough estimates of the age of the group and also places it well into the Miocene. Application of a rate calibration of 0.88%/Myr for COI of cowries (C. P. Meyer, unpubl. data) gives the divergence times among the eight *Bostrycapulus* lineages as 3.7–12.0 Myr. Application of Marko's (2004) rate of 2.2 substitutions per base per year for mitochondrial third positions in *Nucella* dates the divergences at 3.7–15 Myr. Because the fossil record of *Bostrycapulus* is poor and because none of the sister-species pairs discovered here are separated by well-dated barriers like the Isthmus of Panama, it was not possible to calibrate the *Bostrycapulus* sequences.

The geographical range of marine invertebrates is usually assumed to be related to mode of development. Species with direct development are presumed to have higher levels of population structure and smaller geographical ranges than those with planktotrophic development. These expectations do not appear to be borne out in the case of *Bostrycapulus*. The direct developing species in the South Atlantic show very little genetic differentiation over a large geographical

range. COI sequences show less differentiation between these South African and South American populations than is present over hundreds of kilometres along the east coast of North America in other direct developing *Crepidula* species (Collin, 2001). It is unlikely that the genetic similarity of populations in Argentina, Brazil, and South Africa is due to recent unrecorded introductions. Fossil '*C. aculeata*' have been collected from the Pliocene and Pleistocene of Argentina (Hoagland, 1977), and the Pleistocene of South Africa (R. Kilburn, pers. comm.). The placement of the South African populations as sister to the other two suggests that the trans-Atlantic dispersal event predates the COI coalescence of the Argentine and Brazilian populations. It is possible that animals could be transported between South America and South Africa on the holdfasts of drifting *Ecklonia* spp., *Durvillaea antarctica*, and *Marcrocystis pyrifera* kelp (Smith, 2002). Individuals of a *Bostrycapulus* species have been found attached to holdfasts of such kelp (R. Kilburn, pers. comm.), as have the brooding bivalve *Gaimardia trapesina* (Lamarck, 1819) (Helmuth, Veit & Holberton, 1994). Widely dispersed marine species are not uncommon in the southern hemisphere (e.g. Waters & Roy, 2004).

The clade in the equatorial Pacific shows genetic differentiation between Peru and Panama, but not over the thousands of kilometres between Hawaii, Guam and Panama (Figs 2, 4). The Bayesian estimate of COI phylogeny (Fig. 2) shows the clade from Peru nested within the Panama haplotypes, while the estimate based on 16S shows the clades as sisters, suggesting that the root of the Peru clade has been misplaced in the phylogeny. The unrooted haplotype network (Fig. 4) shows that the two clades are reciprocally monophyletic and that the Hawaiian and Guam haplotypes nest firmly within the Panamanian clade.

It is probable that the genetic similarity between the geographically distant populations in Guam, Hawaii and Panama is the result of human-mediated introductions. For example, the samples from Guam that were used in this study were obtained from a dry dock after its arrival from Hawaii. Because *Bostrycapulus* has not historically been present in Guam (G. Paulay, pers. comm.) these animals may represent the founders of a new biological invasion. Likewise, *B. aculeatus* is often listed as an introduced species in Hawaii (Coles *et al.*, 2000), and the earliest recent material appears to have been collected in Hawaii in 1915. However, Sowerby (1883) and Reeve (1859) both list '*Crepidula aculeata*' as occurring in the 'Sandwich Islands' (presumably the Hawaiian Islands and not South Sandwich). Pleistocene material that is possibly attributable to *Bostrycapulus* from Hawaii is deposited at ANSP (ANSP 116536) but is in such poor condition that it is not possible to identify it with

certainty. Animals identified as *B. aculeatus* have also been collected in Alicante, Spain (Simone, 2002), an area outside their historical range. Unfortunately, the phylogenetic affinity of these animals within *Bostrycapulus* is unclear and diagnostic material is not currently available for study. The possible and realized potential for *Bostrycapulus* species to become established invaders makes the documentation of natural ranges and clarification of species identifications of pressing concern.

### TAXONOMIC DESCRIPTIONS

I feel that it is necessary to formally recognize each of the clades recovered in this study as distinct species, despite the difficulty in finding diagnostic features in adult morphology. There is no theoretical reason to expect that mechanisms of speciation should always result in species that can be distinguished visually. I believe that the high levels of genetic differentiation among the samples examined here, the clear differences in development, and the large geographical separations strongly support the status of these different clades as separate species. Continued application of the *B. aculeatus* s.l. concept would only further obscure data that could possibly be used to distinguish these species as they come to light, as well as limiting our ability to identify species introductions and extinctions (e.g. Geller *et al.*, 1997; Geller, 1999). Application of the available names for the species from Japan, the equatorial Pacific, and West Africa, without formally naming the other clades would leave a poly- and paraphyletic *B. aculeatus*, a clearly undesirable situation. Therefore, I remove the available names from synonymy with *B. aculeatus* and formally describe four new species. I take a conservative approach and describe new species only if a putative species differs from other groups in development and forms a topologically well-defined monophyletic clade in the mitochondrial gene trees. This approach discounts the possibility that the low levels of genetic differentiation within the South Atlantic and the equatorial Pacific clades reflect additional poorly differentiated species. Further study and greater geographical sampling is necessary to determine the status of these populations.

Hoagland (1977) synonymized a number of species with *B. aculeatus* (Gmelin, 1791). However, *C. tomentosa*, *C. maculata* and *C. foliacea* need to be removed from this synonymy and should not be placed in *Bostrycapulus*. Examination of the original descriptions and type material shows that *C. tomentosa* Quoy & Gaimard, 1832–33 (see Hoagland, 1983) and *C. maculata* Quoy & Gaimard, 1832–33 are both more similar to *Calyptraea* or *Sigapatella* than they are to *Bostrycapulus*. They have a cap-shaped shell with a central apex and obvious coiling. The thick shaggy

periostracum gives the impression that the shells are spiny. The figure with the original description, and the type material of *C. foliacea* (Broderip, 1834) are more similar to *Crepidatella fecunda* or *Crepidatella dilatata* and are also clearly not allied with *Bostrycapulus*. Broderip (1834) placed this species in *Crepidatella*, which appears to be a more appropriate designation.

The following eight species are recognized here as members of *Bostrycapulus*: *B. aculeatus* (Gmelin, 1791), *B. gravispinosus* (Kuroda & Habe, 1950), *B. calyptraeformis* (Deshayes, 1830), *B. cf. tegulicius*, *B. pritzkeri* sp. nov., *B. odites* sp. nov., *B. latebrus* sp. nov. and *B. urraca* sp. nov.

*Crepidula holiotoidea* Fischer von Waldheim, 1807 (non *Crepidula haliotoidea* Marwick, 1926) is also clearly a *Bostrycapulus* species (not a synonym of *C. dilatata* (Ivanov *et al.*, 1993)) but I consider it a *nomen dubium* because the type locality is unknown (Ivanov *et al.*, 1993) and the lack of diagnostic shell characters in any of the species in this complex make it impossible to assign material other than the lectotype to *C. holiotoidea* with any confidence. The name *C. californica* Tryon, 1886 also refers to an animal in this group, but it is a *nomen nudum*. Neither of these names will be considered further.

According to museum records, shells fitting the description of *Bostrycapulus* species have been collected from the Galapagos Islands, the Marquesas, Venezuela, Cuba, Chile, Senegal, India, and Korea, although no observations of development or molecular data are available for samples from these places. Despite recent concerted efforts, no live animals have been collected from Chile (pers. observ.; D. Véliz & O. Chaparro, pers. comm.) or southern Peru (pers. observ.; A. Indacochea, pers. comm.), despite material at the ANSP listing a locality of 'Callao, Peru'. Therefore, the occurrence of these animals in Chile and southern Peru may be episodic. Clearly, further sampling of these taxa, including developmental and molecular characters, would contribute significantly to our understanding of their evolution, biogeography, and taxonomy.

### *BOSTRYCAPULUS* OLSSON & HARBISON, 1953

*Type species: Bostrycapulus aculeatus* (Gmelin) by original designation.

#### *Original description*

'Shell widely slipper-shaped, with a strongly eccentric apex, closely appressed and spirally coiled towards the left side (viewed dorsally). Surface with strong, radial riblets or threads, the primary ones often becoming scabrous or spiniform. Diaphragm as in *Crepidula* s.s.,



its edge nearly straight, the muscle scar below small but distinct'.

*Morphological description*

*Shell*: externally, the shell is relatively flattened and more coiled, but generally similar to that of *Crepidula* species. The internal septum extends about half the length of the shell, and the anterior margin is indented medially and notched on the animal's left side. A distinct but small medial ridge or crease extends from the medial indentation to the posterior shell margin near the apex. The small lunar muscle scar on the animal's right side anterior to the shelf is often more deeply indented than in *Crepidula* species. The shell is distinctly coiled with about one single whorl after the protoconch–teleoconch boundary. The apex is appressed, usually occurring slightly above the posterior shell margin on the right; it is not excavated. External shell sculpture ranges from widely spaced large scale-like plicate spines to tightly packed pointed granular bumps along fine spiral ribs. Shell colour ranges from overall cream with scattered brown markings to solid chocolate brown, sometimes with a pale streak and occasionally solid tan. The markings are sometimes speckled and often streaky. No teleoconch characters have been found to unambiguously diagnose species in the genus.

*Protoconch*: the size of the protoconch varies between species depending on the mode of development but is less than two whorls and is often eroded in adult specimens. Hatchlings and embryos show a linear pattern of fine, widely spaced granules on the protoconch. Protoconch characters can be used to diagnose several species.

*Pigmentation*: the head, neck, foot and mantle are cream, but there is a matt black marbled area along the edge of the foot. Large yellow or orange splotches are scattered along the neck lappets and concentrated on the lips and tentacles. Black pigment also occurs on the dorsal side of the head and neck. The intensity of all pigmentation varies, with some animals showing almost no black pigment. The black pigment is retained in preserved or fixed material, although the yellow and orange markings are lost. There are no diagnostic differences in pigmentation among the species described here.

*Anatomy*: the overall anatomy of *Bostrycapulus* spp. is similar to that of other calyptraeids (Kleinsteuber, 1913; Werner & Grell, 1950; *B. aculeatus* s.l. described by Simone (2002) (Fig. 9)). The foot is round with a rectangular propodium and extends slightly more than half the length of the shell. There are no mesopodial flaps. The corners of the propodium are not

extended laterally and cannot extend free of the rest of the foot. The neck is dorsoventrally flattened with lappets along each side and with a narrow food groove travelling forward to the tentacle on the right side. Tentacles are stubby, with a simple black eye on the lateral side about a third of the way to the distal end. The lips are equal in size with small, thin jaws embedded in the dorsal side. Tentacles narrow suddenly, immediately distal to the eye. The food pouch at the anterior medial edge of the mantle is surrounded by thick flaps. The tissue connection between the mantle margin and the foot extends anterior to the foot and to the shelf on the animal's left side. The osphradium is a dark, tightly packed strip of bipectinate filaments at the base of the gill filaments. The anterior filaments are smaller than the posterior filaments. The osphradium extends from the food pouch to slightly within the mantle cavity. The long, narrow gill filaments are somewhat thickened at their base. The salivary glands are huge, filling the entire neck and extending from the buccal mass, externally past the nerve ring, to the anterior margin of the visceral mass. They are intricately branched along their entire length.

When removed from the shell the distal third of the viscera curves to the animal's right. The tapered mantle cavity and gills extend about two thirds of the way to the tip of the viscera on the dorsal left side. The crescent-shaped shell muscle extends dorsally from the foot to the shell roof on the right side. A small, dorsal attachment muscle runs from within the dorsal mantle tissue above the intestine to the medial shell roof just anterior to the shelf.

The stomach is visible dorsally to the right of the posterior end of the mantle cavity. The oesophagus runs ventrally in the viscera and enters the stomach posteroventrally. The short style sac runs laterally from the stomach to the left margin of the visceral mass in the dorsal viscera posterior to the mantle cavity. The distal end of the style sac narrows to connect with the intestine, which runs directly to the right side in the ventral visceral mass. The distal loop of the intestine is visible in the dorsal wall of the mantle cavity. This arrangement of the digestive system with respect to the mantle cavity is distinct from the arrangement in *Crepidula*, where the mantle cavity extends to the end of the visceral mass and the style sac is ventral to the mantle cavity. The brown digestive gland surrounds the stomach and extends to the end of the visceral mass. In fresh and ethanol-preserved material a network of thick white vessels running through the digestive gland is clearly visible. These vessels are not visible in formalin-fixed material.

The heart and kidney are similar to *Crepidula* species. The heart and pericardial cavity are visible in the dorsal side of the viscera. The pericardial cavity is at

an angle to the antero-posterior axis and extends along the posterior margin of the mantle cavity. In *Crepidula* species the pericardial cavity is orientated anterior-posteriorly. The hollow kidney is located in the roof of the mantle cavity anterior to the pericardial cavity and posterior to the distal loop of the intestine. The nephrostome opens into the mantle cavity midway between the pericardial cavity and the distal loop of the intestine.

The cream or yellow gonad is somewhat external to the digestive gland and covers almost the entire ventral side of the visceral mass in females and the anterior ventral side in males. The seminal vesicle is a convoluted, narrow tube in the right anterior dorsal margin of the viscera below the mantle cavity and opens into the open-grooved vas deferens. The vas deferens runs to the base of the penis where an open sperm groove runs medially on the ventral side to its distal end. The thick flattened penis ends bluntly with a very small papilla. The penis is usually considerably longer than the tentacles and often exceeds the animal's body length in small males. In females the visceral oviduct and gonopericardial duct join at the right anterior dorsal margin of the visceral mass where the albumen gland extends up into the roof of the mantle cavity. Several seminal receptacles connect to the albumen gland. Distal to the seminal receptacles, the two lobes of the capsule gland converge and open directly into the mantle cavity through the genital pore. The female genital papilla is absent. All species described here show evidence of protandry.

The nerve ring is located at the posterior margin of the neck just anterior to the visceral mass and completely embedded in the salivary glands. The nerve ring is the same as in *C. fornicata* (Werner & Grell, 1950). A pair of buccal ganglia are located against the dorsal medial margin of the buccal mass.

*Radula*: the taenioioglossate radula (Fig. 10) is similar to that of other calyptraeids. In *Crepidula* the major cusps are straight-sided (e.g. Collin, 2000a), producing a dagger-shaped or triangular cusps. In *Bostrycapulus* the sides of the major cusps on the rachidian and lateral teeth are sinuous. The minor cusps on all teeth are more appressed to the body of the tooth than in other species. The number of denticles on each tooth varies significantly among rows within an individual and among individuals (Table 3).

*Development*: the transparent, thin-walled egg capsules of *Bostrycapulus* species are typical of all calyptraeids. The stalks are wide, flattened ribbons and not thread-like as in some species. The female broods the capsules between the neck and substrate and propodium until hatching. Differences in development are diagnostic among species.

There are currently eight recognized species in *Bostrycapulus* (see Table 4 for summary).

#### *BOSTRYCAPULUS ACULEATUS* (GMELIN, 1791)

##### *Synonymy*

*Patella aculeata* Gmelin, 1791: 3693.

*Crepidula aculeata* – Lamarck, 1822: 25. Reeve, 1859.

Sowerby, 1883 [in part]: 67, sp. 9., figs 124, 125;

Sowerby, 1887 [in part]: 67, figs 39, 40. Parodiz,

1939 [in part]: 695. Hoagland, 1977 [in part]: 364.

Collin, 2003a: 541–593. Collin, 2003b: 618–640.

*C. intorta* var. Say, 1822: 227 [in part].

*C. costata* Morton, 1829: 115, pl. 7, figs 2, 3. Maryland Tertiary [non *C. costata* Sowerby, 1824 nec *C. costata* Deshayes, 1830].

*C. spinosa* Conrad, 1843: 307. Miocene Virginia.

*C. ponderosa* H. C. Lea, 1846: 249. Virginia Tertiary.

*Crypta aculeata* – Mörch, 1877: 93–123.

*Bostrycapulus aculeatus* – Olsson & Harbison, 1953 [in part]: 280. Simone, 2002 [in part]: 18.

*Original description*: '*Patella aculeata*. Shell oval, brown, with prickly striae: crown recurved. Chemn. Conch. 10, tab. 168, 624, 1625. Da Costa Conch. tab. 6, fig. 1, Elements t 2, f 2. Favann. Conch. 1, tab. 4, fig. 3. Walch. Naturs. 10 tab. 1, fig. 5. 2. Inhabits American Islands. resembles the last shell small, chestnut or white with longitudinal striae, lip white dividing the cavity into equal parts'.

*Fate of original type material*: the types of *B. aculeatus* have previously been referred to as 'lost' (Hoagland, 1977). Fates of most of the shells figured in the works referred to by Gmelin are unknown. However, the material Chemnitz cited as 'Ex Museo Nostro' was sold at public auction and the catalogue 'Enumeratio Systematica Conchyliorum beat J. H. Chemnitzii' by Havniae 1802 lists *Patella aculeata* as number 1144 (Martynov, 2002). A shell with the number 1144 attached to it and matching the figure in Chemnitz is housed in the Zoological Museum in St. Petersburg, Russia. There are two other shells in the lot with the figured specimen, and notes in the margin of the auction catalogue in St. Petersburg mention 1144 as containing three shells (Martynov, 2002). Specimens of *Patella aculeata* described by Favanne from the Cabinet Royal cannot be found in the Museum National d'Histoire Naturelle (P. Bouchet pers. comm.), and *C. aculeata* attributable to da Costa are not in the Natural History London (pers. observ. and D. Reid pers. comm.). Finally, inquiries about material of *C. aculeata* that may be attributable to any of these four authors suggests that possible types do not exist in London, Paris, Leiden, Berlin, Hamburg, Vienna, Copenhagen, Frankfurt, or Stockholm. It is therefore probable that the shell in St. Petersburg fig-

ured by Chemnitz is the only remaining type of *B. aculeatus*.

*Original type locality:* Gmelin states the habitat of *B. aculeatus* to be 'Islands of the Americas'. This is most likely following 'Westindischen' from Chemnitz.

*Diagnosis:* This species can be distinguished from other *Bostrycapulus* species by features of development and mitochondrial DNA sequences. Development is direct from large, 380 µm eggs. Embryos develop characteristic larval features but reabsorb them prior to hatching. The globose protoconch is 900 µm in diameter and has less than a single whorl. Diagnostic DNA sequence differences, distinguishing *B. aculeatus* from all other *Bostrycapulus* species are in the following positions in the COI sequences submitted to GenBank (position 1 = position 1537 of the *Drosophila yakuba* mitochondrial genome, GenBank #X03240): 28 (c), 33 (g), 186 (g), 282 (t), 468 (g), 511(c).

*Distribution:* the known distribution of this species includes both coasts of Florida, the Florida Keys, Yucatan, the Bahamas, and probably the northern Caribbean Sea. Shells from as far north as North Carolina also probably belong to this species, although this has not been verified by examination of development or DNA sequence data. It is common on rocks and debris in the shallow subtidal zone, and can also be found on the carapaces of horseshoe crabs. Ranges to a depth of at least 60 m.

#### Description

*Shell:* as described for all *Bostrycapulus* species above. Maximum length = 30 mm

*Protoconch:* globose, comprising a single whorl, c. 900 µm across. No sculpture is retained in material available from juvenile shells. The protoconch–teleoconch boundary is not distinct (Fig. 5H).

*Anatomy:* as described for all *Bostrycapulus* spp. above.

*Radula:* as described for all *Bostrycapulus* spp. above (Fig. 10).

*Development:* the egg capsules of *B. aculeatus* are typical of all calyptraeids. The stalks are wide, flattened ribbons and not thread-like as in some other species. The 20–30 large yolky eggs per capsule all develop directly into crawling juveniles. Embryos develop a small but distinct velum, an operculum (Fig. 8B), a small round yolk-free head vesicle, and a single round embryonic kidney on each side. The velum and tentacles have a few cream spots and a dark stripe develops along the mid-line of the foot late in development

(Fig. 8B). Prior to hatching, the shell begins to turn brown and the granular sculpture can be seen clearly with a dissecting microscope. Despite the relatively large velum, excapsulated embryos are never able to swim free of the bottom of the container. Hoagland (1986) reports an egg size of 380 µm and a hatching size of 840 µm. I found an egg diameter of 378 µm (SD = 9 µm; *N* = 11) for a single female from Lido Key, Florida.

*Notes:* the type locality, 'islands of the Americas' is somewhat vague but most likely refers to a locality in the northern Caribbean. It is possible that *Bostrycapulus* from the southern Caribbean is a distinct species from the species described here as *B. aculeatus* (Gmelin, 1791). I have been unable to find *Bostrycapulus* in the Caribbean surrounding Panama, Cayman Islands, or Trinidad, despite finding ostensibly appropriate habitat. If an additional Caribbean species is discovered, nomenclatural stability would benefit from the description of the southern species as new.

Observations of embryos are limited because virtually all egg capsules collected in Lido Key, Florida in 1997 contained nothing but bacterially infected fluid. However, many of those collected in 2003 developed normally. Animals are often solitary or form pairs; they do not form large stacks. Fossil shells with this morphology date from the Miocene in Florida (Hoagland, 1977).

#### *BOSTRYCAPULUS GRAVISPINOSUS* (KURODA & HABA, 1950)

##### Synonymy

*Crepidula gravispinosa* Kuroda & Habe, 1950: 30. Collin, 2003a: 541–593. Collin, 2003b: 618–640.

*Crepidula aculeata* – Taki, 1938 [in part]: 145. Parodiz, 1939 [in part]: 695. Hoagland, 1977 [in part]: 364.

*Original description:* '*C. gravispinosa* n. sp. for *Crepidula aculeata* (not Gmelin), Illust. Encyclop. Fauna Japan, Rev. Edit., p. 1140, textfig. 239 1947.' The figured referred to is the same as that in the 1927 edition of the *Illustrated Encyclopedia of Japanese Fauna*, but the text differs.

*Fate of original type material:* material illustrated in the *Illustrated Encyclopedia of Japanese Fauna* generally belonged to Kuroda's personal collection, which is currently housed in Nishinomiya. No shell matching the figure can be found in this collection (P. Callomon, pers. comm.), although it does contain two shells of *B. gravispinosus* collected from Akune in 1949 (P. Callomon, pers. comm.). It is also possible that the figured shell was from Shintaro Hirase's col-

lection, or that of his father, in which case it was either taken to Tokyo University or may have remained in the main Hirase collection which is now in the Kyoto University Museum (P. Callomon, pers. comm.). Much of the former collection was destroyed during World War II and the figured shell cannot be found there (R. Ueshima, pers. comm.). It is therefore likely that the type material figured in the encyclopedia is lost.

*Type locality:* Hirado Is., Nagasaki Prefecture, Western Kyushu.

*Diagnosis and description:* the shell morphology and anatomy of *B. gravispinosus* are the same as other species of *Bostrycapulus*. All specimens available to me are smaller (maximum length is 20 mm) than *B. aculeatus*. The protoconchs retain radial rows of fine granular sculpture (Fig. 5D). Development is direct, apparently without nurse eggs (Ishiki, 1936; Amio, 1963), although the egg size of 200 µm cited by Amio is too small to produce large juveniles without some kind of extraembryonic nutrition. The protoconchs from Minabe, Wakayama Prefecture show a morphology typical of direct development from large eggs and an apparent hatching size of about 1 mm. Hatching size is given as 1000 µm by Ishiki (1936) and as 1200 µm by Amio (1963). Since only a single COI sequence is available, it is difficult to evaluate the diagnostic differences. However, apparent diagnostic DNA sequence differences, distinguishing *B. gravispinosus* from all other *Bostrycapulus* species are in the following positions in the COI sequences submitted to GenBank (position 1 = position 1537 of the *D. yakuba* mitochondrial genome, GenBank #X03240): 18(g), 51 (g), 138 (g), 175 (c), 296 (c), 340 (t)

*Distribution:* Japan. South of Boso Peninsula and west of Noto Peninsula to the Amami Islands (Taki, 1938).

*Notes:* fossils occur in Japan in the Tertiary (Hoagland, 1977). The apparent conflict between the egg size and hatching size data reported by Ishiki (1936) and Amio (1963) could be explained if there were two Japanese species that differed in development. Unfortunately, only a single broken specimen was available for DNA sequencing, and the protoconchs were not preserved on most of the available shells, so this possibility cannot be ruled out. If multiple species are present in Japan, either historically or due to recent introductions, the nomenclatural stability of *B. gravispinosus* would benefit from the designation of a neotype that retains either developmental or DNA characters that can be used to unambiguously distinguish it from the other species in the *Bostrycapulus*.

*BOSTRYCAPULUS CALYPTRAEFORMIS*  
(DESHAYES, 1830)

*Synonymy*

*Calyptraea echinus* Broderip, 1834: 39. Broderip, 1835: 203, pl. 29, fig. 1. Isla Lobos, Peru. 3 syntypes BMNH 1975113. Hoagland, 1986: 173–183.

*Calyptraea hystrix* Broderip, 1834: 39. Broderip, 1835: 203, pl. 29, fig. 2., Isla Lobos Peru. 3 syntypes BMNH 1966629.

*Crepidula aculeata* – Parodiz, 1939 [in part]: 695. Hoagland, 1977 [in part]: 364.

*Bostrycapulus aculeatus* – Olsson & Harbison, 1953 [in part]: 280. Simone, 2002 [in part]: 18.

*Crepidula* cf. *aculeata* – sp. 2. Collin, 2003b: 618–640.

*C. cf. aculeata* – Panama. Collin, 2003a: 541–593.

*Original description:* ‘C. testâ ovato-rotundatâ, gibbosâ, rufescente, longitudinaliter striatâ; strius rugosis, ad marginem evanescentibus; apice obliquo, spirato’.

*Type material:* two syntypes in the Paris museum (Hoagland, 1983; P. Bouchet 2001 pers. comm.). One is figured in Hoagland (1983).

*Type locality:* Peru (?). Deshayes (1830) supposed that the types came from Peru because they were bought with shells of other Peruvian species.

*Diagnosis:* the shell morphology and anatomy of *B. calyptraeformis* do not differ from those of *B. aculeatus* as described above. *B. calyptraeformis* can be distinguished from the other species of *Bostrycapulus* by the presence of planktotrophic development and a smooth protoconch with 1.5 whorls (Fig. 5). Diagnostic DNA sequence differences distinguishing *B. calyptraeformis* from all other *Bostrycapulus* species are in the following positions in the COI sequences submitted to GenBank (position 1 = position 1537 of the *D. yakuba* mitochondrial genome, GenBank #X03240): 39 (g), 42 (c), 57 (g), 69 (a), 75 (c), 171 (c), 259 (t), 282 (g), 321 (a), 354 (g), 387 (c), 402 (c), 441 (c), 462 (g), 486 (c), 582 (c).

*Distribution:* northern Peru to the Pacific coast of eastern Panama and the Perlas Islands but not extending into the Gulf of Chiriqui. This species also occurs in Hawaii where it is probably introduced and it may have been recently introduced into Guam. This species can reach densities of greater than 1000 individuals per square meter in the intertidal zone of Panama (unpubl. data) and occurs to depths of at least 50 m.

*Description:* shell morphology and anatomy of this species differ from *B. aculeatus* only in protoconch morphology (Fig. 5B, E). Development is plank-

trophic. Animals from Panama produce small 180 µm eggs (Hoagland, 1986) and hatch at a length of 345 µm (Collin, 2003c). At hatching, the larval shell is covered with fine spines or periostracal hairs that are visible under a compound microscope. Animals from Hawaii hatch at 320 µm (Bell, 1993). The veliger larvae have a black intestine, and two small red stripes in the food groove along the anteriolateral and posteriolateral corners of the velum (Fig. 8B). There are no pigment spots on the foot or velum. Larvae from Panama settle in culture starting at a shell length of 700–800 µm (pers. observ.; Collin, 2003c). Animals from northern Peru (Mancora, Zorritos, and Paita) also have planktotrophic development, but more detailed observations are not available. This is the only known species of *Bostrycapulus* with planktonic larvae.

*Notes:* the type of this species is from Peru and the description presented here is based on material from the north coast of mainland Peru, supplemented with observations of material from Panama and Hawaii. As diagnostic material from Isla Lobos is not currently available, the synonymy of *C. calyptraeformis* with *C. echinus* and *C. hystrix* has yet to be rigorously demonstrated. The genetic differentiation between the samples from Peru, and Panama and Hawaii is almost as great as the differentiation among other sibling *Crepidula* species (e.g. Collin, 2000a, 2001) suggesting that these may be two distinct species. Because the available data are insufficient to unambiguously determine the status of these populations and I am aware of no differentiation other than the DNA sequences, I have chosen to conservatively place them all in *B. calyptraeformis* until more information is available.

#### *BOSTRYCAPULUS* CF. *TEGULICIUS*

##### *Synonymy*

*Crepidula aculeata* – Hoagland, 1977 [in part]: 364. Hoagland, 1983 [in part].

*Crepidula* cf. *tegulicia* – Collin, 2003a: 541–593. Collin, 2003b: 618–640.

*Original description* (of *Crypta tegulicia* Rochebrune, 1883): ‘Testa subovata, crassiuscula, irregulari, oblique curvata, extus albida, concentric striata, et squamis minutis teguliformibus, subdistantibus ornata; intus nitide castaneo violacea; lamella opalina, ad medio et ad latus submarginata. Long 0.019, Lat 0.014’.

*Type:* two syntypes of *B. tegulicius* are in the Paris museum (Hoagland, 1983; P. Bouchet 2001 pers. comm.). One is figured in Hoagland (1983).

*Type locality:* ‘Dakar, Joalles, Pointe de Cap Vert’, Senegal.

*Diagnosis and description:* *B. cf. tegulicius* can be distinguished from other species in the *B. aculeatus* species complex by the large globose protoconch and distinct COI sequence. Material with other potentially diagnostic features is not currently available. Diagnostic DNA sequence differences are difficult to determine, but the single available sequence distinguishing *B. cf. tegulicius* from all other *Bostrycapulus* species is in the following positions in the COI sequences submitted to GenBank (position 1 = position 1537 of the *D. yakuba* mitochondrial genome, GenBank #X03240): 178 (a), 268 (t), 282 (c), 339 (g), 492 (a), 583 (a).

*Distribution:* Cape Verde Islands. The extent of the distribution along the west coast of Africa is unknown.

*Notes:* *B. tegulicius* was originally described from Senegal. As diagnostic material from this country is not currently available, the identity of the Cape Verdian material described here cannot be unambiguously assigned to a new species. It is quite possible that they are different species, since the Cape Verdian animals have direct development (and therefore, presumably limited dispersal) and many Cape Verdian species are endemic to these islands. If animals from Senegal and Cape Verde are demonstrated to belong to different species, the name *B. tegulicius* should be applied to material from mainland Africa while the species from Cape Verde should be given a new name.

#### *BOSTRYCAPULUS PRITZKERI* SP. NOV.

##### *Synonymy*

*Crepidula aculeata* – Parodiz, 1939 [in part]: 695.

Hoagland, 1977 [in part]: 364.

*Bostrycapulus aculeatus* – Olsson & Harbison, 1953: 280. Simone, 2002 [in part]: 18.

*Crepidula* cf. *aculeata* – Australia. Collin, 2003a: 541–593.

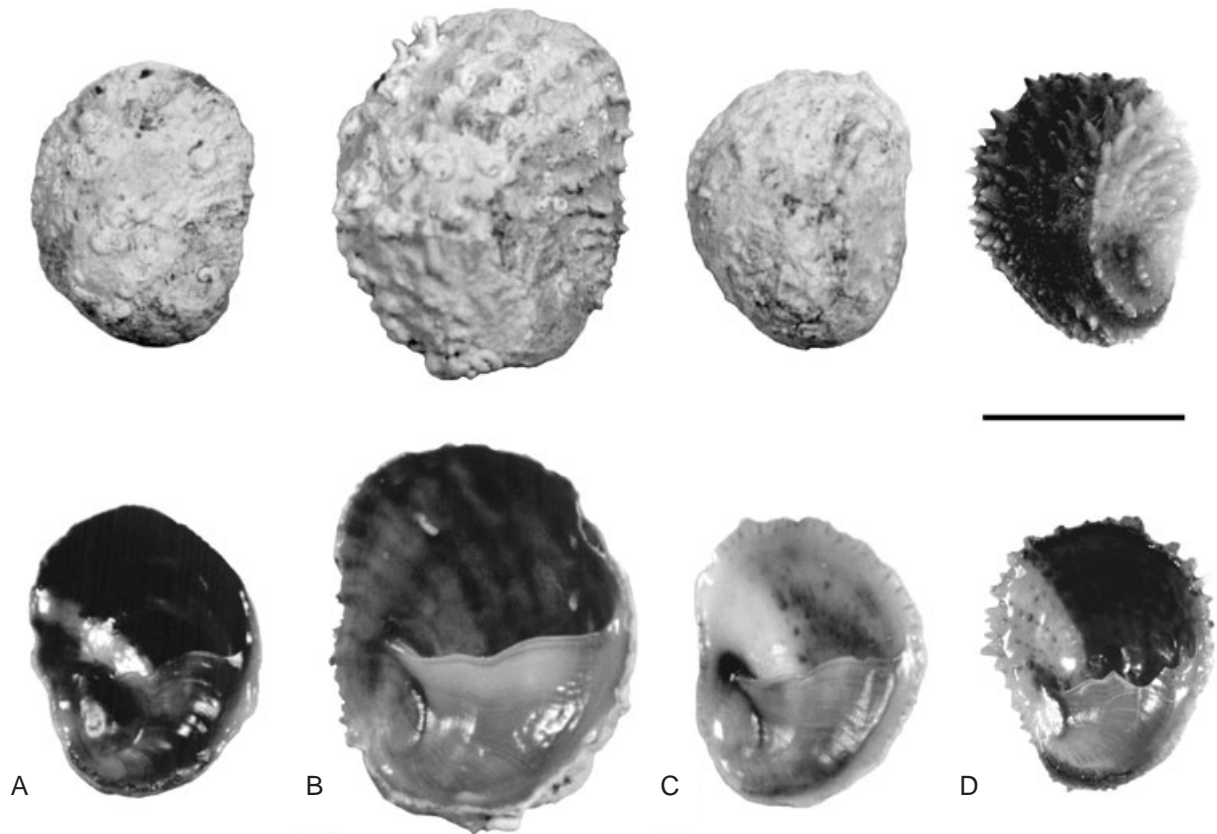
*Crepidula* cf. *aculeata* – Sydney. Collin, 2003b: 618–640.

*Holotype:* Australian Museum #C400000, shell and ethanol-preserved soft parts. Shell illustrated in Figure 11; length = 14.8 mm; width = 11.8 mm; height = 4.1 mm. Frozen tissue of this specimen: FMNH 282361.

*Type locality:* Edwards Reef, Sydney, Australia. 33°51’S, 151°13’E. Low intertidal zone on rocks.

*Other material from type locality:* FMNH 282302 (paratypes).

*Diagnosis:* *B. pritzkeri* can be distinguished from the other species in *Bostrycapulus* by its large, globose protoconch, and direct development from large eggs that produce embryos lacking the larval features present in other direct developing species of *Bostry-*



**Figure 11.** The shells of the holotypes of the four new species. A, *Bostrycapulus latebrus* (FMNH 282358). B, *B. odites* (Natal Museum V9447/T1783). C, *B. pritzkeri* (Australian Museum #C400000). D, *B. urraca* (ANSP 412178). Scale bar = 10 mm.

*capulus*. Diagnostic DNA sequence differences distinguishing *B. pritzkeri* from all other *Bostrycapulus* species are in the following positions in the COI sequences submitted to GenBank (position 1 = position 1537 of the *D. yakuba* mitochondrial genome, GenBank #X03240): 183 (c), 256 (c), 315 (c), 360 (c), 395 (c), 417 (g), 444 (g), 471 (g), 477 (c).

**Distribution:** south-eastern Australia. The Australian National Museum contains shells with this morphology from the coast of New South Wales and Queensland, but the species identity of the latter material needs to be verified with additional observations of live material and genetic data.

**Description:** shell morphology and anatomy of *B. pritzkeri* are the same as *B. aculeatus* except that the yellow or cream pigment makes a pattern of radial stripes or narrow triangles extending from the edge of the foot to the mantle edge. Direct development proceeds from large, ~545 µm, eggs. Intracapsular embryos retain few larval characters. The velum is reduced to a simple ridge at the base of the tentacle

(Fig. 6) and the food groove is absent. There is no operculum and the head vesicle is medium-sized and free of yolk. The embryonic shell has widely spaced rows of granular sculpture which are not visible on the protoconchs examined with SEM. The very large egg size results in the most globose and least coiled of any *Bostrycapulus* protoconch (Fig. 5A).

**Etymology:** the name *pritzkeri* is in honour of R. Pritzker, president of the Pritzker Foundation. The Foundation's support of the Pritzker Laboratory of Molecular Systematics and Evolution at the Field Museum made this work possible.

#### ***BOSTRYCAPULUS ODITES* SP. NOV.**

##### *Synonymy*

*Crepidula aculeata* – Parodiz, 1939 [in part]: 695.

Hoagland, 1977 [in part]: 364.

*Crepidula aculeata* var. *fortis* – Parodiz, 1939: 696.

*Bostrycapulus aculeatus* – Olsson & Harbison, 1953 [in part]: 280. Simone, 2002 [in part]: 18.

*Crepidula cf. aculeata* – sp. 1, Collin, 2003b: 618–640.

*Crepidula cf. aculeata* – South Africa. Collin, 2003a: 541–593.

*Crepidula cf. aculeata* – Argentina. Collin, 2003a: 541–593.

*Crepidula cf. aculeata* – Brazil. Collin, 2003a: 541–593.

*Holotype*: Natal Museum V9447/T1783, shell and ethanol-preserved soft parts. Shell illustrated in Figure 11; length 19.3 mm, width 15.6 mm, height 7.2 mm. Frozen tissue of this specimen: FMNH 282360.

*Other live-collected material from the type locality*: FMNH 282277 (paratype), BM20010453.

*Other live-collected material examined*: FMNH 282297, ANSP A19744, BM20010456, FMNH 282350, FMNH 282368

*Type locality*: Wooleys Pool, Muizenburg, Cape Province, South Africa. Low intertidal zone in rock crevices, co-occurring with *Crepidatella capensis*.

*Diagnosis*: *B. odites* differs from the other species in the *B. aculeatus* species complex in exhibiting direct development from small eggs which consume nurse eggs. The protoconch is unsculptured and retains irregular growth lines (Figs 5F, 4I). Adult morphological characters are as described above for *B. aculeatus*. Diagnostic DNA sequence differences distinguishing *B. odites* from all other *Bostrycapulus* species are in the following positions in the COI sequences submitted to GenBank (position 1 = position 1537 of the *D. yakuba* mitochondrial genome, GenBank #X03240): 24 (c), 36 (g), 141 (c), 220 (t), 234 (c), 279 (g), 354 (t), 438 (c), 486 (a), 552 (t).

*Distribution and habitat*: the Atlantic coast of South America, from São Paulo, Brazil to Puerto Madryn, Argentina, as well as the south coast of South Africa from Cape Town to Port Elizabeth and north to northern Natal (Natal Museum). Material examined here was collected from rocks intertidally in South Africa and Brazil, and intertidally from rocks and subtidally from the shells of pen-shells and oysters in Argentina. This species occurs to depths of at least 40 m.

*Description*: shell morphology and anatomy are the same as *B. aculeatus*, with the exception of the protoconch. The 1 mm diameter protoconch is smooth with irregular growth lines towards the aperture (Figs 5F, 4I). The indistinct protoconch–teleoconch boundary occurs after slightly more than a single whorl is completed.

Observations of development are available for animals from Argentina and Cape Town, but only the

early stages were collected. Eggs from Argentinian animals are 197 µm in diameter. All eggs in a capsule begin to develop synchronously and gastrulation includes invagination. Gastrulas look similar to those of *C. lingulata* (Collin, 2000b). After gastrulation, the nurse eggs remain spherical with centralized yolk and ciliated epithelium. After consuming the nurse eggs, the remaining embryos form intracapsular veligers with a single embryonic kidney on each side, a distinct operculum, a very small distinct velum with a food groove, a small round head vesicle, and some black pigment on the intestine. The shells of early embryos appear smooth under a dissecting microscope. Embryos near hatching were not observed in October and November in Argentina, or December in South Africa. I observed the development of a few broods of South African animals. It appears that the embryos begin to consume the nurse eggs slightly earlier in development than those from Argentina, and that the larval features are therefore slightly less well developed relative to embryo size. Maximum length = 30 mm.

*Etymology*: *odites* is a Greek noun meaning traveller. This name refers to the large geographical distribution this species has attained despite its direct development.

#### *BOSTRYCAPULUS LATEBRUS* SP. NOV.

##### *Synonymy*

*Crepidula aculeata* – Hoagland, 1977 [in part]: 364.

*Crepidula cf. aculeata* – Mexico. Collin, 2003a: 541–593. Collin, 2003b: 618–640.

*Holotype*: FMNH 282358, shell and ethanol-preserved soft parts. Shell illustrated in Figure 11; length = 15.0 mm, width = 11.9 mm, height = 4.1 mm. Frozen tissue is also deposited at the FMNH under the same lot number.

*Type locality*: just north of La Paz, Baja California Sur, Mexico, along the coast of Ensenada La Paz near El Comitán. Collected from rocks in the low intertidal zone.

*Other material from the type locality*: FMNH 282193 (paratype), FMNH 282194.

*Diagnosis*: *B. latebrus* can be distinguished from other species of *Bostrycapulus* by DNA sequence data and by its direct development from large eggs with embryos that retain larval features (unlike *B. pritzkeri*). The shell morphology and anatomy of *B. latebrus* do not differ from that described above for *B. aculeatus*. Diagnostic DNA sequence differences distinguishing *B. latebrus* from all other *Bostrycapulus* species are in the following positions in the COI

sequences submitted to GenBank (position 1 = position 1537 of the *D. yakuba* mitochondrial genome, GenBank #X03240): 3 (g), 108 (c), 144 (g), 192 (g), 243 (a), 270 (c), 306 (g), 327 (g), 423 (c), 522 (t).

*Distribution:* material whose identity can be verified as *B. latebrus* has only been collected near La Paz, Mexico. Shells that may be from this species occur commonly along the Pacific coast of Baja California and have been reported from as far north as southern California. However, observations of development and DNA data are necessary before their identity can be verified.

*Description:* shell morphology and anatomy are the same as for *B. aculeatus*, although the shells of the live-collected material were all smaller than large examples of *B. aculeatus* and *B. odites*. The protoconch distinguishes this species from *B. calyptraeformis* and *B. odites*, and *B. pritzkeri*, but cannot be used to clearly distinguish it from the other species of *Bostrycapulus*. *B. latebrus* has direct development from eggs with a diameter of 488 µm. The embryos develop into intracapsular ‘veligers’ with a very small but distinct un-pigmented velum, a small round head vesicle and a single embryonic kidney on each side. An operculum is present but is lost before hatching. Embryonic shell sculpture consists of widely spaced rows of fine granules similar to the larval sculpture of *C. lingulata* (Collin, 2000b). Embryos hatch as crawling juveniles. Maximum shell length 16 mm ( $N = 20$ ).

*Etymology:* The species name *latebrus* is Latin, meaning ‘hidden’ or ‘obscure’, referring to both the difficulty of distinguishing this from the other species of *Bostrycapulus* and also to the fact that shells are often so encrusted with epibionts that they are effectively hidden in the field.

*Notes:* *C. californica* Tryon, 1886 is a *nomen nudum*. However, it may possibly have been applied to this species in the previous literature. Fossil shells with similar morphology occur in the Pliocene and Pleistocene of California, USA and Baja California, Mexico.

#### ***BOSTRYCAPULUS URRACA* SP. NOV.**

*Holotype:* ANSP 412178. Figure 11D; length = 15.4 mm, width = 13.1 mm, height = 5.7 mm.

*Paratypes:* ANSP 412179 (two animals from the type locality).

*Type locality:* Isla Parida, Gulf of Chiriqui, Panama. 8°5.458'N, 82°18.671'W

*Other live material from type locality:* Isla Parida, Gulf of Chiriqui, Panama; FMNH 306483.

*Diagnosis:* *B. urraca* can be distinguished from other species of *Bostrycapulus* by a combination of the following. It has a large globose protoconch and direct development that retains most of the larval features. Diagnostic DNA sequence differences distinguishing *B. urraca* from all other *Bostrycapulus* species are in the following positions in the COI sequences submitted to GenBank (position 1 = position 1537 of the *D. yakuba* mitochondrial genome, GenBank #X03240): 261 (t), 285 (g), 309 (g), 375 (t), 474 (c), 495 (a), 588 (t).

*Distribution:* material whose identity has been verified as *B. urraca* has been collected in Panama from the Gulf of Chiriqui, Isla Coiba, the Azuero Peninsula, and the Perlas Archipelago. In El Salvador it has been collected from the Gulf of Fonseca. This species occurs from the intertidal zone to at least 50 m and can occur in densities up to several hundred per square meter in the intertidal zone.

*Description:* shell morphology and anatomy are the same as *B. aculeatus*. Although *B. urraca* often has more distinct spination than the other species of *Bostrycapulus*, sequences of particularly spiny and smooth animals from the Gulf of Chiriqui and Bay of Panama showed that this is intraspecific variation and cannot be used to distinguish between these two species. This species has direct development from eggs with a diameter of 360 µm (SD = 7.2;  $N = 57$ ) and with a hatching size of 888 µm (SD = 70,  $N = 75$ ). The embryos develop and coiled shell, an operculum and paired embryonic kidneys (Fig. 7). The velum is limited to a ring of ciliated cells around the head and does not have a well-defined food-groove (Fig. 7).

*Etymology:* The species name *urraca* is a noun in apposition. The name honours the R/V Urraca, the Smithsonian Tropical Research Institute's research vessel, which was used to collect samples of this species. Urraca was the name of a Guaymí chief who fought bravely against the Spanish in Panama.

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