Phylogeny of the *Crepidula plana* (Gastropoda: Calyptraeidae) cryptic species complex in North America

Rachel Collin

Abstract: The taxonomy of *Crepidula* species with flat white shells is particularly difficult. These animals from the east coast of North America have generally been classified as a single species, *Crepidula plana* Say, 1822. Based on allozyme and developmental data, however, Hoagland (K.E. Hoagland. 1984. Malacologia, **25**: 607–628; K.E. Hoagland. 1986. Am. Malacol. Bull. **4**: 173–183) concluded that two species of flat white-shelled *Crepidula* live along the east coast of the United States, but she did not apply any name to the second species. Herein I use molecular techniques to characterize populations of flat white-shelled *Crepidula* species from Texas, Florida, Georgia, North Carolina, and |Massachusetts, and describe their morphology and development. DNA-sequence data support the existence of three species. One species is readily distinguished on the basis of morphology and development, but the other two are very similar. To clarify the nomenclature of these species, I designate neotypes for *C. plana* Say, 1822 and *Crepidula depressa* Say, 1822, and describe ***Crepidula atrasolea*** sp.nov.

Résumé : La taxonomie des espèces de *Crepidula* à coquilles blanches et plates est particulièrement difficile; sur la côte est nord-américaine, ces animaux sont généralement considérés comme appartenant tous à une même espèce, *Crepidula plana* Say 1822. Cependant, d'après les allozymes et les données sur le développement, Hoagland (K.E. Hoagland. 1984. Malacologia, **25**: 607–628; K.E. Hoagland. 1986. Am. Malacol. Bull. **4**: 173–183) a conclu que deux espèces de *Crepidula* plates et blanches habitent sur la côte est des États-Unis, mais elle n'a pas donné de nom à la deuxième espèce. J'utilise ici des techniques moléculaires pour caractériser les populations de *Crepidula* blancs et plats du Texas, de la Floride, de la Georgie, de la Caroline du Nord et du Massachussets et je décris leur morphologie et leur développement. Les séquences d'ADN confirment l'existence de trois espèces. Une espèce se reconnaît facilement à sa morphologie et à son développement mais les deux autres sont très semblables. Pour clarifier la nomenclature de ces espèces, j'ai choisi des néotypes pour *C. plana* Say 1822 et *Crepidula depressa* Say 1822 et je décris une nouvelle espèce, ***Crepidula atrasolea*** sp.nov.

[Traduit par la Rédaction]

Introduction

Slipper shells, gastropods in the genus *Crepidula*, are abundant and diverse in shallow marine habitats along both coasts of North America. These sedentary filter-feeders are commonly used in developmental (e.g., Conklin 1897; Moritz 1939; Pechenik 1980; Lima and Pechenik 1985; Pechenik et al. 1996; Dickinson et al. 1999; Collin 2000), ecological (e.g., Hoagland 1977; Matusiak and Fell 1982; Loomis and VanNieuwenhuyze 1985; Shenk and Karlson 1986; McGee and Targett 1989), and behavioral (Hoagland 1978; Vermeij et al. 1987; Collin 1995) research. *Crepidula* species are also responsible for some of the most profound examples of exotic-species introductions in marine habitats (Carlton 1979 and references therein; Deslous-Paoli 1985; Woodruff et al. 1986; Knudsen 1994; Sauriau et al. 1998). Despite being common, large, and easily accessible, species identification is difficult and the discovery of new species is still common (e.g., Gallardo 1977, 1979; Hoagland 1986; Brown and Olivares 1996).

The taxonomy of the numerous species with pale flattened, concave, or recurved shells is particularly difficult and uncertain (Hoagland 1977; Berry 1955), owing to both the nature of the group and historical accidents. Virtually all *Crepidula* species were described originally from shells alone, many of which were not figured (e.g., Gmelin 1791; Say 1822; Menke 1851). Several types have been lost (see Hoagland 1977) and the type localities are often not clearly identified. The simple limpet-shaped *Crepidula* shell is extraordinarily plastic, growing to fit any hard substrate: *Crepidula* individuals growing on scallops have ribbed shells, those living in shells occupied by hermit crabs have recurved shells, and those living on flat surfaces have smooth convex shells. Because substrate controls shell size, shape, and sculpture, shell morphology is often not diagnostic. Developmental, anatomical, and molecular characters can distinguish among species, but original species descriptions do not generally refer to these characters. As a result, species cannot always be associated unambiguously with an existing name and type specimen.

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Here I report on the morphology, development, and mitochondrial cytochrome oxidase I (COI) sequences for multiple populations of what has previously been considered a single species, *Crepidula plana* Say, 1822. These characters show that there are three distinct taxa. On the basis of these results, I designate neotypes of *Crepidula plana* Say, 1822 and *Crepidula depressa* Say, 1822, and describe *Crepidula atrasolea* sp.nov.

Crepidula plana taxonomy

Say (1822) named two *Crepidula* species with flat white shells from the east coast of North America, *C. plana* and *C. depressa*. The original species descriptions suggest that the shells are similar but that *C. depressa* has a yellowish brown periostracum. He reported that *C. plana* ranges from New Jersey to eastern Florida and that *C. depressa* occurs in the southeastern United States. Neither species is figured and the types are now lost (Hoagland 1977). Hoagland (1977) included *C. depressa* and two southern fossil species *Crepidula lamina* H.C. Lea, 1843 and *Crepidula rhyssema* Olsson and Harbison, 1953 within *C. plana*. This taxonomy has been applied in subsequent studies of animals with flat white shells from the east coast of North America (e.g., Ament 1979; Pechenik 1980; Thiriot-Quiévreux and Scheltema 1982; Hoagland 1983, 1984, 1986; McGee and Targett 1989; Griffin 1998).

Crepidula plana has always been considered distinct from the similar south Atlantic *Crepidula protea* d'Orbigny, 1841, the Mediterranean *Crepidula unguiformis* Lamarck, 1822, as well as the Pacific *Crepidula nivea* Adams, 1852, *Crepidula perforans* Valenciennes, 1846, *Crepidula williamsi* Coe, 1947, *Crepidula fimbriata* Reeve, 1859, *Crepidula explanata* Gould, 1853, and *Crepidula isabellae* Taki, 1938 (Hoagland 1977). Shell characters serve to distinguish only some of these, and among the Pacific species it is unclear how many actual species are represented by the available names. In addition, *Crepidula sinuosa* Turton, 1825 was described from a shell collected from the bottom of a boat in Yorkshire, England. Because slipper shells with this morphology have never subsequently been reported in England, the type specimen may have been transported by boat from the United States (which was the reported origin of the ship). Although *C. sinuosa* may be a junior synonym of *C. plana*, Hoagland (1977) synonymized it with the Mediterranean *C. unguiformis*. Its true identity may never be determined.

A distance analysis of allozyme data showed that *C. plana* individuals from New England form a distinct cluster from that formed by individuals from Florida (Hoagland 1984). This work and her observations of development led Hoagland (1984, 1986) to conclude that there are two cryptic species in eastern North America. She referred to the species in New England with planktotrophic development as *C. plana* and the southern species with direct development as *C.* cf. *plana*.

Materials and methods

I collected live specimens of flat white-shelled *Crepidula* species on both the east and west coasts of Florida, the Florida Keys, and California (Table 1). I have also examined live material from Panacea, Florida, and Woods Hole, Massachusetts, and preserved animals from Port Aransas, Texas, St. Catherine's Island, Georgia, and Core Sound, North Carolina (Fig. 1, Table 1). Voucher specimens are deposited at the Field Museum of Natural History, Chicago, Ill., U.S.A. (Table 1). Museum names are abbreviated following Leviton et al. (1985), with the addition of the Bailey–Matthews Shell Museum (BMSM).

In addition I examined the type material of the similar North American species: *C. sinuosa* Turton, 1825 (USNM 188033), *Crepidula nivea* var. *glottidiarum* Dall, 1905 (USNM 183455), *C. explanata* Gould, 1853 (MCZ 169137), *C. nivea* Adams, 1852 (MCZ 186291, lectotype), *Crepidula nummaria* Gould, 1846 (USNM 5870), and *Crepidula williamsi* Coe, 1947 (CAS 064338). The types of *Crepidula striolata* Menke, 1851 could not be located.

A 614 base-pair sequence of the COI gene was sequenced for several individuals from each locality (Table 1, Appendix Table A1). DNA was extracted from ethanol-preserved tissue with a Puregene® extraction kit (Gentra Systems, Minneapolis, Minn.), amplified using Ready-To-Go™ PCR beads (Pharmacia Biotech, Piscataway, N.J.) and primers, and the PCR profile of Folmer et al. (1994). Both strands were cycle-sequenced with dRhodamine cycle-sequencing-dye terminator kit (PE Biosystems, Foster City, Calif.) using the amplification primers and sequenced using an ABI 377 automated sequencer. Sequences were aligned using Sequencher 3.0 (Gene Codes Corp.).

As part of a larger ongoing analysis of the genus, I sequenced and examined the morphology of a variety of flat white-shelled *Crepidula* species from the Pacific Ocean and the Mediterranean Sea. Preliminary phylogenetic analysis of 50 calyptraeacean species shows a species similar to *C. perforans* from Santa Barbara, California, U.S.A., to be the closest sister-species to the species examined here (R. Collin, unpublished data). I use this species and *C. unguiformis* as outgroups in this analysis.

Phylogenetic analyses were conducted using PAUP version 3.1.1 (Swofford 1993). An equal weighted parsimony analysis was performed using a heuristic search with tree bisection reconnection (TBR) branch-swapping and 100 random additions. Bootstrap support for each clade was assessed based on 1000-bootstrap replicates with TBR branch-swapping and 10 random additions.

Morphological and anatomical characters were examined under a Wild M4 dissecting microscope for ethanol-preserved individuals from populations in Georgia, Florida, Texas, and Massachusetts, and formalin- and gluteraldehyde-preserved animals from Florida and Massachusetts. Prior to mounting for scanning electron microscopy (SEM), female reproductive structures were dehydrated in a graded series of ethanol followed by hexamethyldisalizane, and protoconchs and radulae were bleached and rinsed in distilled water. All specimens were gold-coated and viewed under an Almary scanning electron microscope. Between 7 and 10 radulae per species, from animals with shell lengths of 8–25 mm, were prepared for SEM. To sample within-individual variation, the number of cusps on both lateral teeth and on the outer side of the marginal teeth were counted for up to 10 rows of teeth per individual. Preliminary analysis suggested that the number of cusps on these teeth varied among species. Developmental stages for individuals from populations from Florida and Massachusetts were observed and measured with a dissecting microscope. Descriptions of development and body pigment are based on observations of approximately 100 individuals of each species. At least three males and three females of each species were dissected in detail from each locality.

Results

Three species from eastern North America (*C. plana*, *C. depressa*, and *C. atrasolea* sp.nov.) can be distinguished

Table 1. Summary of individuals sequenced in this study.

	Locality	Material examined	Individual	Museum No.	GenBank No.	Collector
C. unguiformis	Italy	Ethanol	*C. unguiformis*	FMNH 282344	AF178156	M. Oliviero
C. cf. *perforans*	Naples Reef, Santa Barbara, Calif. (34°20′N, 120°01′W)	Live, ethanol, development	*C.* cf. *perforans*	FMNH 282243	AF178155	R. Collin
C. plana	Woods Hole, Mass. (41°30′N, 70°40′W)	Live, ethanol, formalin, development	MBL1*	—	AF178119	MBL Supply Co.
			MBL2	FMNH 282210	AF178120	
			MBL3	FMNH 282214	AF178121	
			MBL4†	ANSP 19186	AF178122	
	Core Sound, N.C. (35°33′N, 76°80′W)	Ethanol	NC1	FMNH 282234	AF178126	E. Sotka
			NC2	FMNH 282235	AF178127	
	St. Catherines Island, Ga. (31°60′N, 81°15′W)	Ethanol, formalin	GA1	FMNH 282230	AF178123	J. Slapcinsky
			GA2	FMNH 282231	AF178124	
			GA3	FMNH 282233	AF178125	
C. depressa	Lake Worth, Fla. (26°48.4′N, 80°02.8′W)	Live, ethanol, development	LW1	—	AF178146	R. Collin
			LW2	FMNH 282202	AF178147	
			LW3	—	AF178148	
			LW4	—	AF178149	
	Sanibel Marina, Fla. (26°27′N, 82°02′W)	Live, ethanol, development	SM1	FMNH 282201	AF178150	R. Collin
			SM2†	ANSP 19187	AF178151	
			SM3	FMNH 282211	AF178152	
	Panacea, Fla. (30°00′N, 84°30′W)	Live, ethanol, development	Pan1	FMNH 282228	AF178153	Gulf specimens
			Pan2	FMNH 282229	AF178154	
	Port Aransas, Tex. (27°55′N, 97°08′W)	Ethanol	TX1	FMNH 282222	AF178141	J. Wise
			TX2	FMNH 282223	AF178142	
			TX3	FMNH 282224	AF178143	
			TX4	FMNH 282225	AF178144	
			TX5	FMNH 282226	AF178145	
C. atrasolea	Wulfert Point, Sanibel Island, Fla. (26°29′N, 82°10′W)	Live, ethanol, development	WP1†	ANSP 19188	AF178133	R. Collin
			WP2	FMNH 282217	AF178134	
			WP3	FMNH 282203	AF178135	
			WP4	FMNH 282206	AF178136	
	Harbor Branch Oceanographic Institute, Fla. (28°30′N, 81°20′W)	Live, ethanol, development	HB1	FMNH 282209	AF178128	R. Collin
			HB2	FMNH 282213	AF178129	
			HB3	FMNH 282218	AF178130	
			HB4	FMNH 282204	AF178131	
			HB5	—	AF178132	
	Florida Keys, Fla. (24°40′51′′N, 82°16′02′′W)	TBE-ethanol	Keys	FMNH 282242	AF178140	
	Core Sound, N.C. (35°33′N, 76°80′W)	Ethanol	NC3	FMNH 282237	AF178137	E. Sotka
			NC4	FMNH 282238	AF178138	
			NC5	FMNH 282239	AF178139	

*Some specimens were so small that the entire specimen was used to extract DNA.
†Type specimen.

on the basis of the morphological, developmental, and biochemical characters examined here (Table 2).

Molecular analysis

Cladistic analysis of 614 base pairs (91 parsimony informative sites) of COI-sequence data produces one island of 267 trees (length = 233, consistency index (CI) = 0.81, retention index (RI) = 0.94), all of which show three distinct groups each with 99–100% bootstrap support (Fig. 2). The southern black-footed *C. atrasolea* sp.nov. is sister to the southern *C. depressa*, with the northern *C. plana* sister to the clade they form. All topographic variation amongst the most parsimonious trees is due to the rearrangements of individuals within these three groups. There is little sequence

Fig. 1. Map showing collection localities and inferred geographic range of *Crepidula plana*, *Crepidula depressa*, and *Crepidula atrasolea*. ?? indicates ambiguity of the range boundaries.

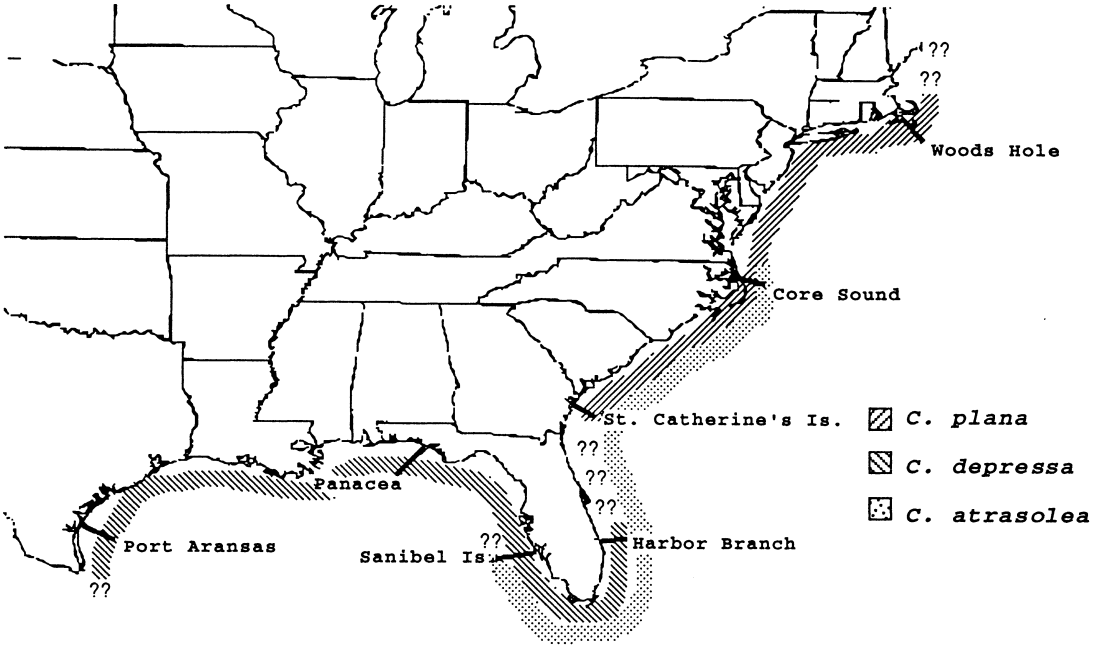


Table 2. Summary of differences among *C. plana*, *C. depressa*, and *C. atrasolea*.

	C. plana Say, 1822	*C. depressa* Say, 1822	*C. atrasolea* sp.nov.
Localities	Woods Hole, Mass.; Core Sound, N.C.; St. Catherines Island, Ga.	Sanibel Marina, Fla.; Lake Worth, Fla.; Panacea, Fla.; Port Aransas, Tex.	Wolfert Point, Fla.; Harbour Branch, Fla.; Teatable Key and near Key West, Fla.; Core Sound, N.C.
Foot color	White	White	Sooty black
No. of cusps on inner side of outer marginal[a]	6.5±1.9 (n = 55)	2.7±1.4 (n = 54)	2.3±1.4 (n = 52)
No. of cusps on inner side of inner marginal[a]	14.5±3.4 (n = 75)	9.5±1.9 (n = 59)	8.5±1.7 (n = 63)
No. of cusps on outer side of inner marginal[a]	5.3±1.5 (n = 67)	2.9±1.2 (n = 48)	3.0±1.0 (n = 50)
No. of cusps on outer side of lateral tooth[a]	8.8±1.7 (n = 81)	7.7±1.3 (n = 78)	7.7±1.5 (n = 72)
No. of protoconch whorls	1	1	0.5
Egg diameter	136 μm (Hoagland 1986)	?	335 μm
Development type	Planktotrophic	Planktotrophic	Direct
Length at hatching	~300 μm[b]	255 μm (n = 20)	1.02 mm (n = 4)
Female genital papilla	Blunt, deep, wide groove	Blunt, shallow, narrow groove	Pointed, deep, wide groove

[a]Values are given as the mean ± SD.
[b]See Pechenik et al. (1996).

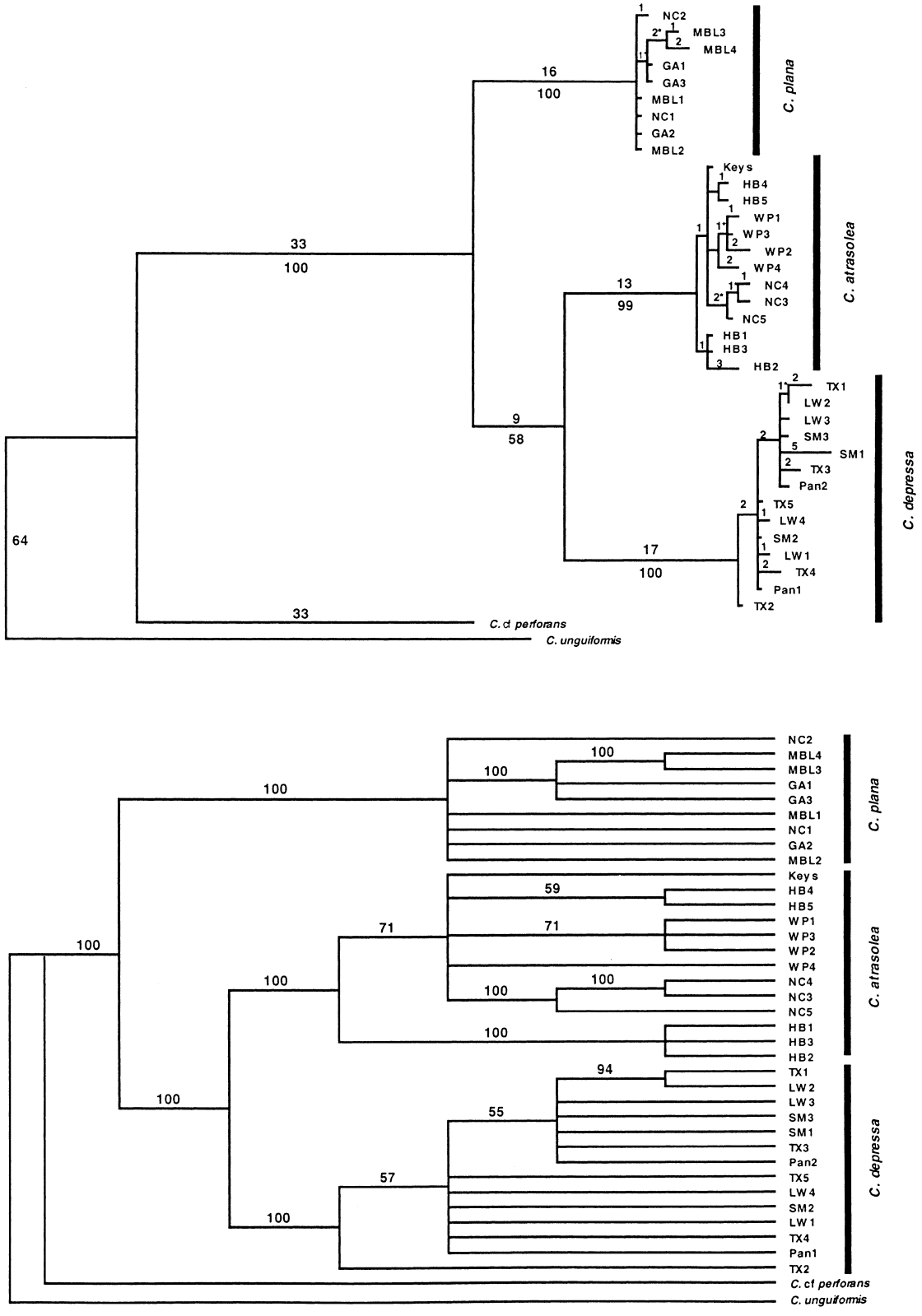
variation within any of these clades, but there is a 4.6% sequence divergence between the southern clades, and the northern group is 6.2–6.9% different from either of them. Translation using the *Drosophila* mitochondrial genetic code, which applies to other gastropods (Yamazaki et al. 1997; Wilding et al. 1999), shows that all but one of these substitutions are synonymous. There is one autapomorphic non-synonymous substitution in a *C. atrasolea* from Harbor Branch and two non-synonymous substitutions between the *C. plana* complex and *C.* cf. *perforans*.

Several individuals with brownish periostracum from Texas were included in this analysis. They are not genetically distinct from the other *C. depressa*.

Morphology

There is morphological differentiation coincident with the genetic divergences described above. Examination of superficial body pigmentation clearly distinguishes *C. atrasolea* from the other two groups. All *C. atrasolea* sequenced in this study and all animals collected at the same localities in Florida had superficial sooty black pigment on the foot and mantle. All individuals of *C. plana* and *C. depressa* sequenced in this study and all animals collected in Massachusetts, Georgia, and Texas and in Panacea, Lake Worth, and Sanibel Marina, Florida, had no pigmentation on the foot, mantle, or neck, although the dark color of the digestive gland can be seen through the foot. Both morphotypes were collected in Core Sound, North Carolina, and their sequences further confirm that the sooty black morphotype is genetically distinct. Although the darkness of the pigmentation in the black- footed animals varies, close examination of the foot shows a dusting of pigment even in the smallest live animals. Dried bodies in museum collections retain this black pigment, as do recently (at least up to 3 years) preserved

Fig. 2. (*a*) Phylogram of 38 individuals of *Crepidula* with flat white shells representing one of 267 most parsimonious (mp) trees from an equal weighted parsimony analysis of 614 base pairs (91 parsimony informative characters) of the COI sequence data (length = 233, CI = 0.81, RI = 0.94). Branch lengths, represented as the number of base-pair changes, are the numbers above the branches and vary slightly among different mp trees. Bootstrap values of over 50% for branches are indicated below the branch. (*b*) Majority-rule consensus tree of all 267 most parsimonious trees. The proportion of trees supporting that branch is represented by the numbers above the branches.



specimens. The one available preserved specimen from the Florida Keys had a pale foot and was genetically identical to the black-footed animals. The pale foot is probably a preservation artifact because this animal was preserved with several specimens of *Crepidula aculeata* Gmelin, 1791, a darkly pigmented species, which now also show no black pigment.

Analyses of radula cusp number (Figs. 3 and 4) show that *C. plana* generally has more cusps on both the inside and outside of the inner marginal teeth (hereinafter referred to as marginals), the inside of the outer marginals, and the outside of the lateral teeth (hereinafter referred to as laterals) (Kolmogorov–Smirnov test, $p < 0.005$) than does *C. depressa*. *Crepidula plana* also has more cusps (Kolmogorov–Smirnov test, $p < 0.005$) than *C. atrasolea* in all characters other than the outside of the lateral teeth (Kolmogorov–Smirnov test, $p > 0.2$). *Crepidula depressa* and *C. atrasolea* differ only in the number of cusps on the inside of the outer marginals (Kolmogorov–Smirnov test, $p < 0.05$). There was no effect of size (measured as radula width) for the range examined (width from 100 to 300 μm, corresponding to shell lengths from 8 to 28 mm). The rachidian and the inner side of the lateral tooth have between three and five cusps in all three species. The outer side of the outer marginal has no cusps. Teeth in different rows in one individual commonly varied by three cusps, but sometimes by as many as five or six cusps. This variation among radula rows and the slight variation among individuals make it difficult to distinguish among the species solely on the basis of radular characters (Fig. 4). Caution should be used in interpreting these data on radula morphologies until the possibility of phenotypic plasticity has been examined (e.g., Padilla 1998).

In *Crepidula* the distal end of the anterior pallial oviduct extends into the mantle cavity. The female genital papilla varies among the three species (Fig. 5) in the shape of the distal end and in the depth and width of the groove in the longitudinal epithelial fold. In both *C. plana* and *C. atrasolea* the groove is wide and deep, making it an obvious feature visible with a dissecting microscope. In *C. depressa* the groove is shallow and narrow and the longitudinal epithelial fold is more closely appressed. In both *C. plana* and *C. depressa* the distal end of the female genital papilla is blunt. In *C. plana* it is bulbous at the distal end and thicker than in *C. depressa*. In *C. atrasolea* the end of the female genital papilla is pointed. The size of the female genital opening varies relative to the rest of the structure in *C. atrasolea*. However, the absolute size of the opening does not appear to vary greatly (Fig. 5). These features of the female genital papilla were often indistinct in individuals with trematode infections.

The osphradium, which has previously been suggested to be a useful taxonomic character (Brown and Olivares 1996), could not be used to distinguish among these three species. The number of monopectinate leaflets increased with size, from a minimum of 3 leaflets in tiny juveniles (2–3 mm in length) to 8–12 leaflets in animals about 20 mm in length. The leaflets are clustered tightly together slightly to the left of the food pouch.

Individuals with a thick brownish periostracum (presumably corresponding to the original description of *C. depressa*) did not make up more than 1 or 2% of any population that I examined (one from Lake Worth and six from Port Aransas). All of these individuals had white feet. Animals with thick brownish periostracum did not appear to be present in populations from Massachusetts and North Carolina and are uncommon in museum collections.

Developmental characters

Crepidula atrasolea is also developmentally distinct from the other two species (Table 2). *Crepidula plana* and *C. depressa* both have planktonic larvae matching the description of Thiriot-Quiévreux and Scheltema (1982). The small eggs of *C. depressa* produce well-developed veligers with smooth, 255 μm long shells at hatching. Larval pigmentation is the same in both species: the number of yellow spots on the foot and velum varies, the gut is never darkly pigmented, and there is a dark spot on the mantle roof.

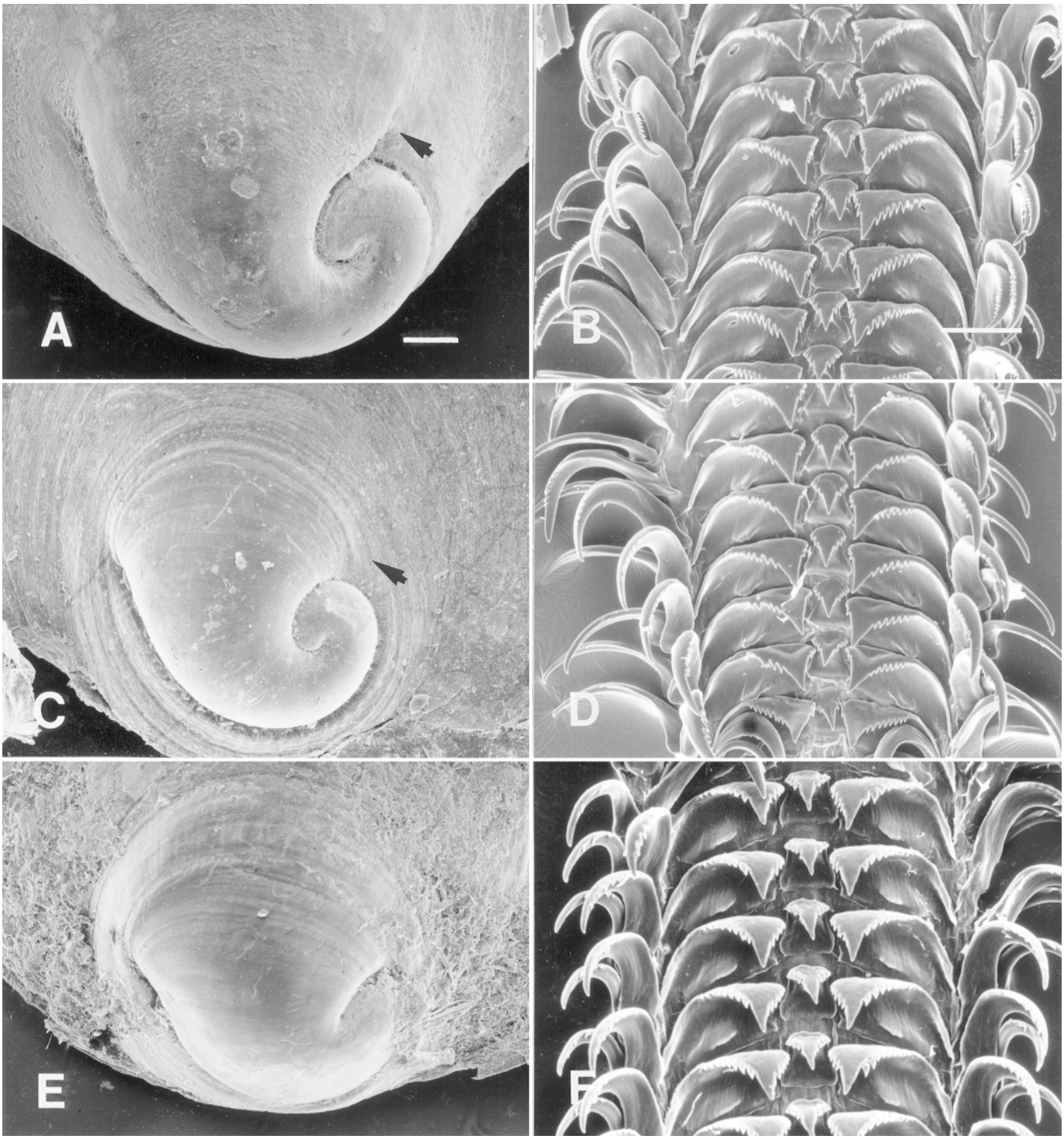
Black-footed animals produce large (335 μm) eggs with direct development. The embryo develops into an intracapsular veliger with a small but distinctive velum. The embryonic stomach and intestine are black, there are yellow pigment spots on the velum, and there are often a pair of cream or yellow spots on the lower lip and another pair on the anterior edge of the propodium. The embryos develop a single pair of embryonic kidneys. At no stage is the head vesicle large and the embryos do not develop an operculum. All embryos in a capsule develop synchronously and there is no evidence of nurse eggs. At hatching the crawling juveniles are about 1 mm long.

These developmental differences result in different protoconch morphologies (Fig. 3). The direct developers have a protoconch with half a whorl, measuring approximately 55 μm in diameter. The planktotrophs produce smaller protoconchs with a single whorl. The inner whorl of the protoconch is more gracile in *C. depressa* than it is in *C. plana*, which may be due to the slight difference in egg size. The subsequent whorl expands more quickly, bulging slightly around the embryonic shell in *C. plana* (compare arrows in Fig. 3). Unfortunately the protoconchs are seldom preserved on shells more than a few millimetres long and are often eroded or encrusted with epibionts. The fewer than five protoconchs available for each species meant I could not assess within-species variability.

Discussion

In the absence of data on reproductive isolation, recognition of species is generally based on one of two criteria. Evolutionary biologists ideally recognize species as genetically discrete groups of individuals, arguing that such genetic differentiation is the result of reproductive isolation. More traditional taxonomists rely on morphological distinction to identify different species. Morphological differences are thought to reflect underlying genetic differences and have the added advantage of often allowing identification of individuals in the field. However, it is not clear how much morphological or molecular differentiation should be expected between species. The genetic data presented here supports the species status of all three groups. The genetic distances between the species are far greater than the within-species variation, they are greater than the genetic distances between some other traditionally recognized *Crepidula* species (R. Collin, unpublished data), and the mitochondrial haplotypes have clearly coalesced within each species. The absolute

Fig. 3. SEMs of protoconchs and radulae from *C. plana* (A, B), *C. depressa* (C, D), and *C. atrasolea* (E, F). The arrows highlight the bulge of the expanding shell whorl that is more prominent in *C. plana*. Protoconchs are all to the same scale, as are the radulae. Scale bars = 100 μm.



congruence of morphological and molecular differentiation in areas of sympatry also suggests that hybridization does not occur. The morphological differentiation among these species appears to be rather limited. This pattern of low morphological differentiation may not be unusual in this genus because, although the genus *Crepidula* appears to have originated in the Cretaceaous (Hoagland 1977), the extant species are highly plastic and show very little morphological differentiation (Owen 1834; Hoagland 1986; R. Collin, personal observation). However, morphological differentiation of the female genital papillae is also indicative of reproductive isolation.

Recent flat white-shelled *Crepidula* species have been described from both coasts of North and South America,

Fig. 4. Histogram of cusp numbers for outer side of lateral, outer side of inner marginal, inner side of outer marginal, and inner side of inner marginal, in all three species. The considerable overlap shows that cusp number cannot be definitively used to identify individuals.

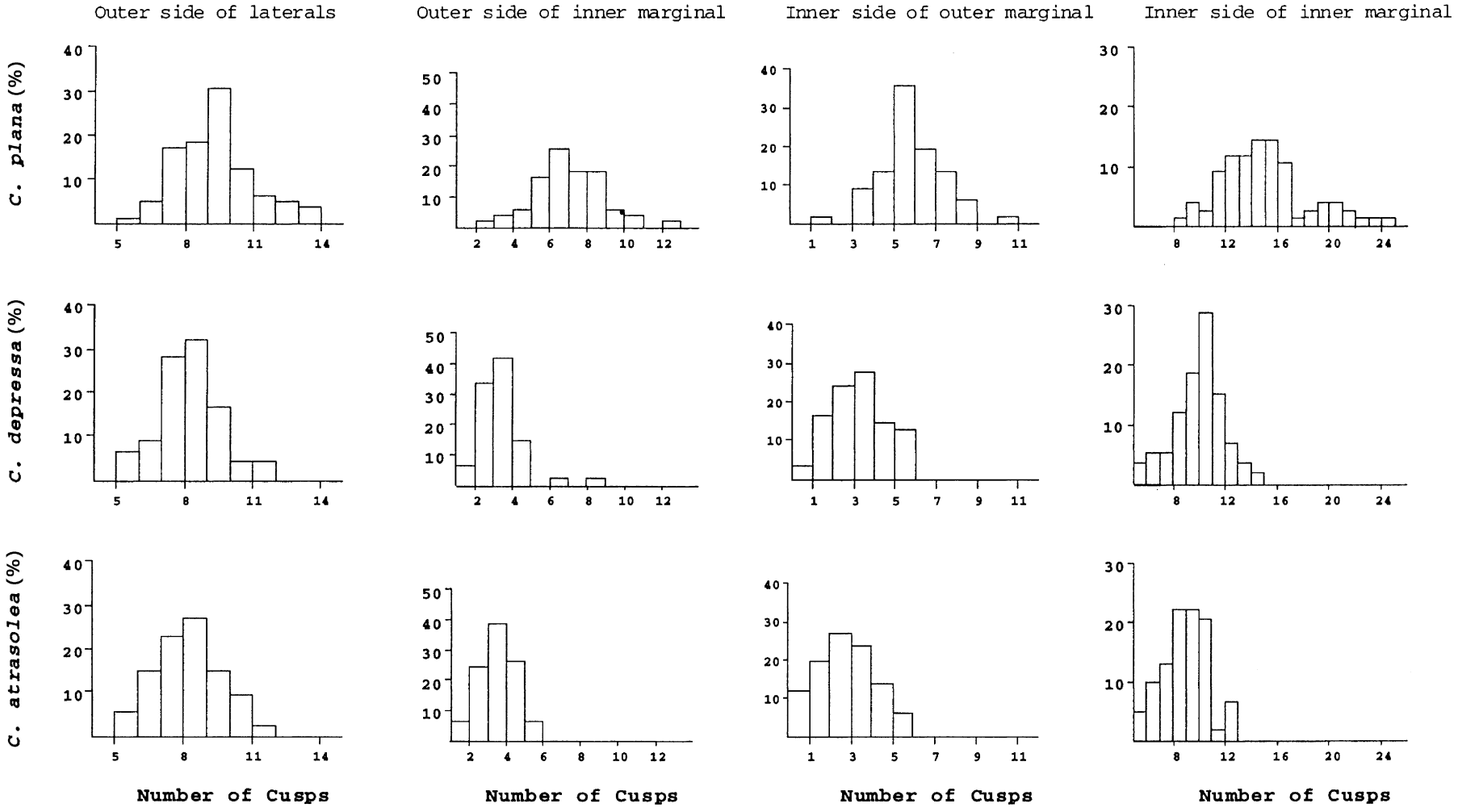
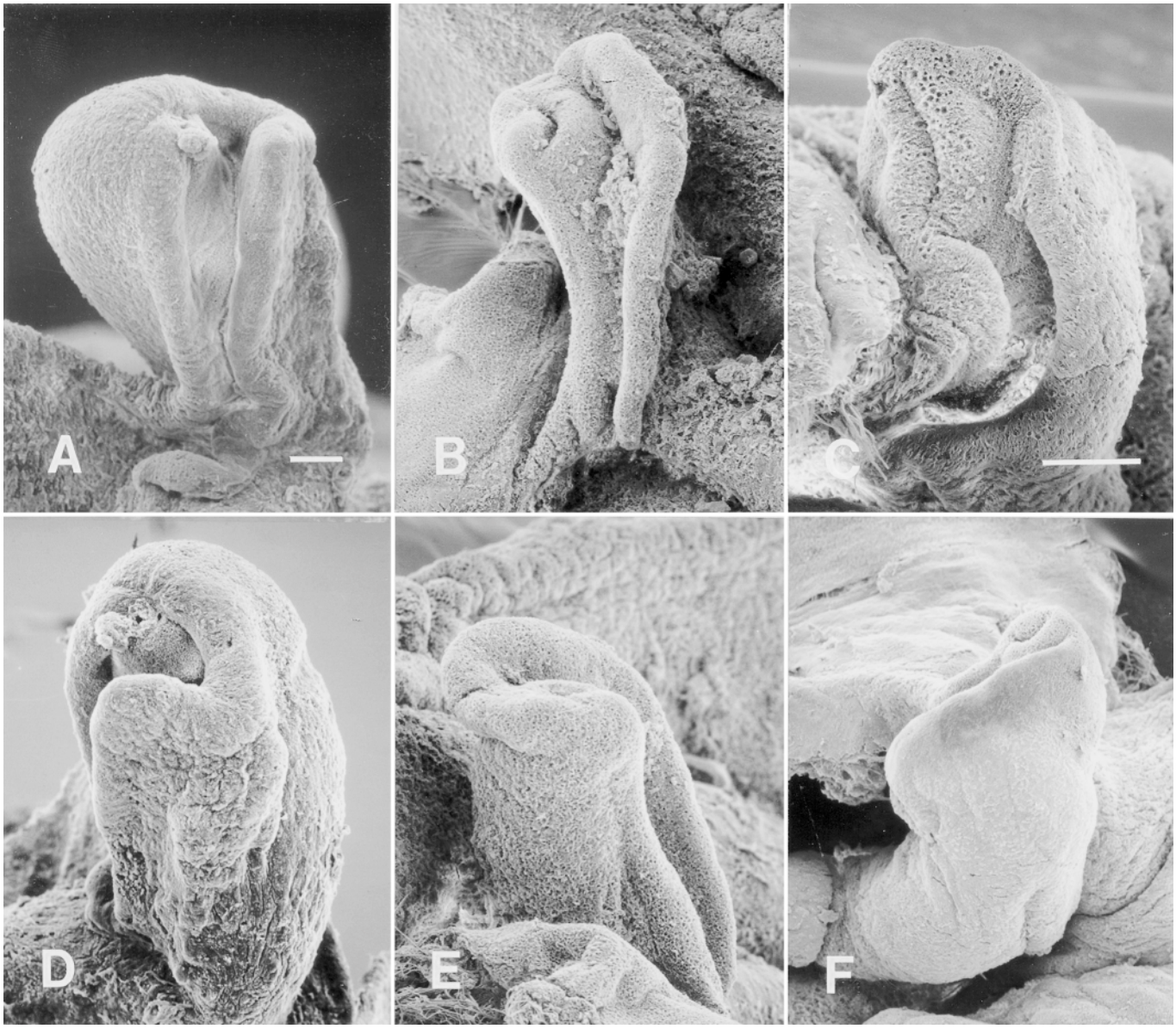


Fig. 5. Scanning electron micrographs of the female genital papilla of *C. plana* (A, D), *C. depressa* (B, E), and *C. atrasolea* (C, F). A view facing towards the groove, which is medial in life (A, B, C) and a view showing the profile (D, E, F). Note the deep and wide groove in *C. plana* and *C. atrasolea* and the narrow groove in *C. depressa*, and the pointed end of the female genital papilla in *C. atrasolea*. A and D and B and E both represent different views of the same individual. C and F are from different individuals and show the variation in the relative size of the female genital opening. A, B, D, E, and F are all to the same scale; scale bars = 100 μm.



Japan, and the Mediterranean (Hoagland 1977 and references therein; Gallardo 1979; Brown and Olivares 1996). Because only shell characters have been described in many cases, many species are distinguished based solely on their geographic locality or substrate type of the type specimen. The three genetically distinct species examined here are also indistinguishable on the basis of shells alone (except in the rare cases when the protoconch is preserved). Two species are virtually indistinguishable based on classical morphological characters. They can be distinguished using a suite of developmental, radular, and anatomical characters. Such morphological similarity may account for these species having remained undescribed for so long, despite occurring in well-studied areas. I have not found both southern species co-occurring at any site and the two morphologically similar species have non-overlapping ranges, while the range of the distinct *C. atrasolea* overlaps with both of them (Fig. 1). The morphologically and developmentally distinct *C. atrasolea* nests within a clade of three distinct genetic species, *C. plana*, *C. depressa*, and *C.* cf. *perforans*, that are extraordinarily morphologically similar. The morphological and developmental divergence of *C. atrasolea* is not associated with increased molecular divergence or increased population structure (Fig. 2). Application of molecular techniques better suited to detect population structure may show such differences.

There are various reports of introductions of *C. plana* into bays in the northeastern Pacific (Carlton 1979 and references

therein). Numerous native Pacific species with shell morphologies and habits indistinguishable from those of the *C. plana* species complex make it difficult to assess the extent of these introductions. Further work on the anatomy and development of these Pacific species may facilitate discrimination among species. If intraspecific variation is as high in these species as in the *C. plana* species complex, it may be necessary to use molecular methods to confidently identify individual specimens.

The biogeographic patterns of the *C. plana* complex in the southern U.S.A. are roughly congruent with the patterns found in other marine invertebrates. Avise and colleagues (reviewed in Avise 1992; Bert 1986; Reeb and Avise 1990) have demonstrated major genetic breaks along the Atlantic coast of Florida in a variety of marine animals. This is roughly concordant with the eastern range boundary of *C. depressa* and the southern boundary of *C. plana*. Interestingly these studies have not detected major genetic breaks in the vicinity of Cape Hatteras, North Carolina, a well-known biogeographic break for molluscs (Fisher 1960). However Cape Hatteras does appear to be the northern range boundary of *C. atrasolea*. The complex biogeographic patterns of marine animals in the southeast United States reflects a long history of sea-level change, vicariance, and variation in current patterns, and are not easily explained by a single event (Bert 1986; Avise 1992).

The geographic ranges reported here are necessarily uncertain, owing to the scarcity of material available for study. *Crepidula plana* is reported to extend north to Prince Edward Island (Hoagland 1977) but preserved material should be examined to verify that Canadian animals are the same as *C. plana* as defined here. Species ranges probably also extend farther south than reported here because species of marine invertebrates commonly range throughout the Gulf of Mexico to the Yucatan Peninsula. In addition on the basis of shell collections, Hoagland (1977, 1983) stated that *C. plana* extends south to Brazil or Uruguay and Parodiz (1939) states that *C. plana* occurs in Argentina. Because flat white-shelled *Crepidula* species cannot be identified by shells alone, the actual identity of these animals remains uncertain. It is unlikely that they are *C. plana* (as defined here) because *C. plana* does not appear to range farther south than Georgia. It is also unlikely to be *C. atrasolea* because Hoagland (1983) did not refer to south American animals as *C.* cf. *plana*, suggesting that they were not obviously distinct from *C. plana* or *C. depressa*. In addition the few specimens of *C. protea* and *C. plana* from Brazil that I have examined do not have sooty black pigmentation. It is likely that further systematic work on flat white-shelled *Crepidula* in the Atlantic will bring to light additional new species.

Taxonomic descriptions

Genus *Crepidula* Lamarck, 1822

Crepidula plana Say, 1822

Synonymy:

C. plana Say, 1822

C. plana Say, 1822 (Hoagland 1977, 1984, 1986) in part

Hoagland (1977) synonymized *C. lamina* H.C. Lea, 1843 (nomen nudum) from the Tertiary Period of Petersburg, Virginia, and *C. rhyssema* Olsson and Harbison, 1953 from the Pliocene Epoch of St. Petersburg, Florida, with *C. plana*. However, it is not possible to determine the systematic status of this material on the basis of shells alone.

FATE OF ORIGINAL TYPE MATERIAL: Say's types were originally deposited in the ANSP. They were removed in 1825, many were subsequently destroyed in a fire, and what remained was returned to the ANSP after his death (V. Maes cited in Mikkelsen and Mikkelsen 1984). The type of *C. plana* was considered lost by Hoagland (1977) and it cannot be located in the ANSP collection.

NEOTYPE: ANSP 19186 (ethanol-preserved female; 24.8 mm in length; Fig. 6).

OTHER MATERIAL FROM SAME LOCALITY: FMNH 282207, 282210, 282214; BMNH 20000007; BMSM (ethanol preserved).

NEOTYPE LOCALITY: Woods Hole, Massachusetts (41°30′N, 70°40′W). Subtidally in shells occupied by hermit crabs.

Original description

"Shell depressed, flat, oblong oval, transversely wrinkled, lateral margins abruptly deflected; apex not prominent and constituting a mere terminal angle, obsolete in the old shells; within white; diaphragm occupying half the length of the shell, convex, contracted in the middle and at one side. Length 1 and 1–10 of an inch. Inhabits the coast of the United States…. I have found it on the coasts of Maryland, Carolina, Georgia and East Florida and my brother, Mr. Benjamin Say, discovered it on the shores of New Jersey" (Say 1822). The type was not figured.

Diagnosis

Crepidula plana can be distinguished from other species of *Crepidula* by the following suite of characters. Shell flat and white, body white. Development includes small planktotrophic larvae, with no black pigment on the intestine. More cusps on the marginal teeth than *C. depressa* and *C. atrasolea* (Table 1). Female genital papilla bulbous, blunt-ended, and with a distinct groove. Cytochrome-oxidase sequence shows synapomorphies (with CI = 1 in this analysis) corresponding to *Drosophilia yakuba* COI positions 39 (T), 40 (T), 87 (C), 150 (A), 222 (G), 237 (C), 324 (C), 390 (G), 520 (C), and 538 (C).

Distribution

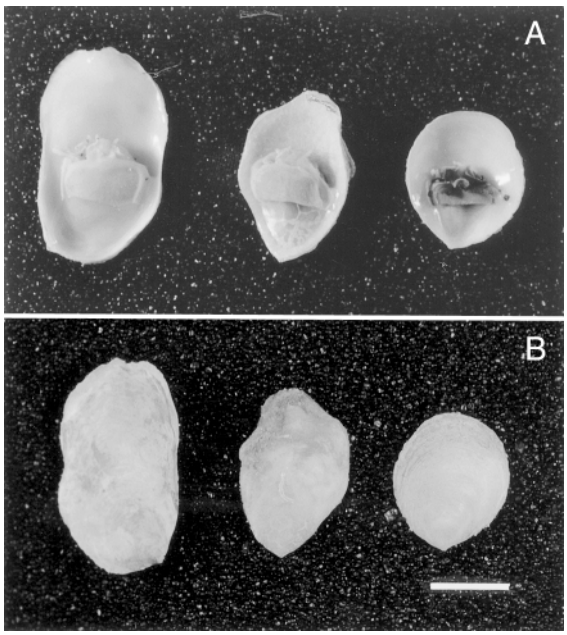
East coast of North America from New England to Georgia. Low intertidal and subtidal. Living on shells and inside shells inhabited by hermit crabs and occasionally on a variety of other hard substrates. Shells from as far north as Prince Edward Island.

Description

Shell

The shell is generally flat and white, ranging from extremely recurved to somewhat convex, depending on the habitat of the individual. Animals from the inside of shells inhabited by hermit crabs are often spatulate, with the shell widening anteriorly. Animals from exposed substrates are often oval and convex. The shelf is flat in convex shells and

Fig. 6. Photographs of the ethanol-preserved types; from left to right: *C. plana* neotype, *C. depressa* neotype, and *C. atrasolea* holotype. (A) Ventral view showing body pigmentation. Note that the posterior portion of foot has been removed for DNA extraction. (B) Dorsal view of the shells. Scale bar = 10 mm.



convex in recurved shells, with a notch on the right side where it attaches to the shell and also a depression in the center of the shelf margin. There are no muscle scars. The small apex is sometimes inclined slightly to the right but usually at the posterior shell margin. The shell is white inside and out. Sometimes there is a thin, parchment-like periostracum, usually limited to the margins of the shell and often absent. The smooth protoconch, without an obvious protoconch–teleoconch boundary (Fig. 5), is usually eroded. Early shell expands quickly, bulging past the protoconch. There is no sculpture other than growth lines. Length up to 47 mm.

Morphology

External body color is translucent white, with opaque white on the tips of the tentacles and the lips. There are white granulations in the mantle and neck lappets. General morphology is typical of all *Crepidula* species and is similar to that described in detail for *C. fornicata* (Werner and Grell, 1950). There are no shell muscles running from the foot to the shell roof. The slightly yellowish osphradium is a small tightly packed cluster of monopectinate leaflets with 3–10 leaflets. The mesopodial flaps and propodium are especially well-developed in the females. The long and narrow food pouch lies at the mantle margin directly above the head. The thin tubular salivary glands extend from the buccal mass halfway to the nerve ring. The female genital papilla, with a bulbous blunt end and a distinct groove, extends well into mantle cavity. In females there are 5–9 sperm receptacles. Males have a slightly flattened penis that tapers suddenly to a thin filament at the distal end.

Development

The small eggs (Hoagland 1986) produce typical planktotrophic veliger larvae with a smooth shell that is almost planospiral in shape at hatching. There are usually more than 100 eggs/capsule, but the number of eggs per capsule increases with female size. Unlike the larvael gut of most other *Crepidula* species the larval gut of *C. plana* is not pigmented. The velum has some yellow spots and there is a dark pigment spot on the dorsal mantle. Extensive literature on the development of this species includes Pechenik et al. (1996), Thiriot-Quiévreux and Scheltema (1982), and Conklin (1897).

Notes

Although Say's original species description could easily be applied to any of the three *Crepidula* species discussed here, in the interests of nomenclatural stability I have chosen to apply it to the northern species. The extensive literature on "*C. plana*" is based almost exclusively on specimens from New England (e.g., Conklin 1897; Hoagland 1978, 1984, 1986; Ament 1979; Pechenik 1980; Thiriot-Quiévreux and Scheltema 1982; Lima and Pechenik 1985; McGee and Targett 1989; Zimmermann and Pechenik 1991; Pechenik et al. 1996), and thus refers to *C. plana* as defined here.

Crepidula depressa Say, 1822

FATE OF ORIGINAL TYPE MATERIAL: Say's types were originally deposited in the ANSP. They were removed in 1825, many were subsequently destroyed in a fire, and what remained was returned to the ANSP after his death (V. Maes cited in Mikkelsen and Mikkelsen 1984). The type of *C. depressa* was considered lost by Hoagland (1977) and it cannot be located in the ANSP collection.

NEOTYPE: ANSP 19187 (ethanol-preserved female; 19.4 mm in length; Fig. 6).

OTHER MATERIAL FROM SAME LOCALITY: FMNH 282201, 282211, 282240; BMNH 20000006 BMSM 15001 (ethanol-preserved animals).

NEOTYPE LOCALITY: Sanibel Marina, Sanibel Island, Florida (26°27′N, 82°02′W). On oyster shells and in shells occupied by hermit crabs in 3–5 m of water.

Original description

"Shell very much depressed, transversely wrinkled, nearly equilateral; epidermis pale yellowish brown; apex not curved, forming a simple acute terminal angle upon the margin of the aperture; aperture subovate; within white; diaphragm convex, edge contracted in the middle and at one side. Length: four-fifths of an inch. Inhabits the southern coast of the United States" (Say 1822). The type was not figured.

Diagnosis

Crepidula depressa can be distinguished from other species of *Crepidula* by the following suite of characters. Shell flat and white, body white. Development with small planktotrophic larvae with no black pigment on the intestine. Fewer cusps on the marginal teeth than *C. plana* (Table 1). The groove in the blunt cylindrical female genital papilla is shallow and relatively indistinct. Cytochrome-oxidase sequence shows synapomorphies (with CI = 1 in this analysis) corresponding

to *D. yakuba* COI positions 3 (C), 42 (T), 243 (G), 291 (G), 345 (C), and 420 (T).

Distribution

Gulf coast of Texas, both Gulf and Atlantic coasts of Florida. Low intertidal to subtidal. Living on shells and inside shells inhabited by hermit crabs.

Description

Shell

The shell is generally flat and white, ranging from extremely recurved to somewhat convex, depending on the habitat of the individual. Shells of animals from exposed substrates are often oval and convex. Large spatulate shells like those common in *C. plana* from New England are uncommon. The shelf is flat in convex shells and convex in recurved shells, with a notch on the right side where it attaches to the shell. There is also a depression in the center of the shelf margin. There are no muscle scars. The small apex lies at the level of shell margin and sometimes curves slightly to the right. The shell is white inside and out, rarely with brown streaks. Periostracum is usually absent, but sometimes there is a covering of thick yellowish brown periostracum or thin, parchment-like periostracum limited to the margins of the shell. The smooth protoconch, without an obvious protoconch–teleoconch boundary (Fig. 4), is usually eroded. The early shell expands more slowly past the protoconch than in *C. plana*. There is no sculpture other than growth lines. Length up to 40 mm.

Morphology

The translucent white body has opaque white on the tips of the tentacles and the lips and some white spots in the mantle and neck lappets. The general morphology is the same as other *Crepidula* species and as described in detail for *C. fornicata* (Werner and Grell, 1950). There are no shell muscles running from the foot to the external shell. The yellowish osphradium is a tight cluster of 3–12 monopectinate leaflets, with the occasional bipectinate leaflet. The mesopodial flaps and propodium are especially well-developed in females. The long narrow food pouch lies at the mantle margin directly above the head. The simple tubular salivary glands extend from the buccal mass halfway to the nerve ring. The female genital papilla, with a blunt end and a narrow shallow groove, extends well into mantle cavity. Males have a slightly flattened penis that tapers suddenly to a thin filament at the distal end.

Development

The small eggs produce typical planktotrophic veliger larvae with a smooth, almost planospiral shell at hatching. There are usually at least 100 eggs/capsule, but the number of eggs increases with female size. Unlike the larvae of most *Crepidula* species the larval gut is not pigmented. The velum has some yellow spots and there is a dark pigment spot on the dorsal mantle. Average shell length at hatching is 255 μm (n = 20).

Notes

Crepidula depressa Say, 1822 has precedence over *C. depressa* Deshayes, 1830 and *C. depressa* Lesson, 1830, both of which are probably synonyms of *C. dilitata* Lamarck, 1822 (Hoagland 1977, 1983).

***Crepidula atrasolea* sp.nov.**

Synonymy:

C. cf. *plana* (Hoagland 1984, 1986)

HOLOTYPE: ANSP 19188 (ethanol-preserved female; 15.8 mm in length; Fig. 6).

PARATYPES FROM TYPE LOCALITY: FMNH 282203, 282206, 282217 (ethanol preserved).

OTHER MATERIAL: FMNH 282241, BMNH 20000005, BMSM 15002 (ethanol preserved).

TYPE LOCALITY: Wolfert Point, Sanibel Island, Florida (26°29′N, 82°10′W). On oyster shells near mangrove roots in less than 1 m of water.

Diagnosis

Crepidula atrasolea can be distinguished from other *Crepidula* species by the following suite of characters. Shell flat and white. Development is direct. Foot, mantle, and neck with diffuse to intense sooty black pigmentation. Faint yellowish blotches on the mantle edge in some live animals. Distal end of distinctly grooved female genital papilla is pointed. Protoconch with less than one whorl. Cytochrome-oxidase sequence shows synapomorphies (with CI = 1 in this analysis) corresponding to *D. yakuba* COI positions 327 (C), 342 (G), 438 (C), 465 (G), 510 (A), 543 (G).

Etymology

From the Latin *atra* for black and *solea*, which most commonly means sandal or slipper but was used by Virgil (M. McGrade, personal communication) to refer to the soles of animals' feet. This name describes the sooty black color of the bottom of the foot, which appears to be unique to this species amongst the *Crepidula* species with depressed white shells.

Distribution

Gulf and Atlantic coasts of Florida, extending north to North Carolina and south through the Florida Keys (Table 1). Low intertidal to at least 20 m depth. Often in oyster shells in the shallow subtidal. Also subtidally in shells inhabited by hermit crabs in the keys.

Description

Shell

The shell is flat and white, ranging from recurved to somewhat convex depending on the habitat of the individual. Animals from exposed substrates are often oval and convex, with a more robust shell. Large spatulate shells like those common in *C. plana* from New England are uncommon. The shelf is flat in convex shells and convex in recurved shells, with a notch on the right side where it attaches to the shell and also a depression in the center of the shelf margin. There are no muscle scars. Apex at the shell margin usually directly posterior and sometimes slightly recurved to the right. The shell is white inside and out. Periostracum is generally absent. The smooth protoconch comprises half a whorl. There is no obvious protoconch–teleoconch boundary

(Fig. 4) and the protoconch is usually eroded. There is no sculpture other than growth lines.

Morphology

There is diffuse to intense sooty black pigmentation over the foot, mantle, and neck. There are faint yellowish blotches on the mantle edge in some live animals. The sooty black pigmentation is retained in recently preserved animals, but the yellow color is lost. The general morphology is the same as described in detail for *C. fornicata* (Werner and Grell, 1950). There are no muscles running from the foot to the external shell. The yellowish osphradium is a tight cluster of 3–9 monopectinate leaflets. The mesopodial flaps and propodium are especially well-developed in females. The long narrow food pouch lies at the mantle margin directly over the head. The pointed, distinctly grooved female genital papilla extends well into the mantle cavity. The simple tubular salivary glands are tucked up around the buccal mass and do not extend along the neck. Males have a slightly flattened penis that tapers suddenly to a thin filament at the distal end.

Development

Development is direct. Uncleaved eggs average 335 μm in diameter (n = 30, means for each of 3 broods are 315, 325, and 354 μm). Usually fewer than 20 eggs/capsule. The embryo develops into an intracapsular veliger with a small but distinct velum. The embryonic stomach and intestine are black, there are yellow pigment spots on the velum, and there are often a pair of cream or yellow spots on the lower lip and another pair on the anterior edge of the propodium. The embryos develop a single pair of embryonic kidneys. At no stage is the head vesicle large and the embryos never develop an operculum. Hatchlings with mean shell length 1.02 mm crawl away. Nurse eggs are absent.

Notes

Similar species in the Atlantic include *C. plana*, *C. depressa*, and *C. protea*. These species have pale feet, and are thus unlikely to be confused with *C. atrasolea*. Although *C. depressa* has an overlapping range, *C. depressa* and *C. atrasolea* do not seem to co-occur at any given site. *Crepidula atrasolea* is also clearly distinct from the type of *C. sinuosa*, which has a small protoconch with multiple whorls.

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References

Adams, C.B. 1852. Catalogue of shells collected at Panama. Ann. Lyceum Nat. Hist. N.Y. **5**: 5–334.

Ament, A.S. 1979. Geographic variation in relation to life history in three species of the marine gastropod genus *Crepidula*: growth rates of newly hatched larvae and juveniles. *In* Reproductive ecology of marine invertebrates. *Edited by* E. Stancyk. University of South Carolina Press, Columbia.

Avise, J.C. 1992. Molecular population structure and the biogeographic history of a regional fauna: a case history with lessons for conservation biology. Oikos, **63**: 62–76.

Berry, S.S. 1955. The West coast's confused and confusing white slipper shells, *Crepidula*, subgenus *Ianacus*. Am. Malacol. Union Inc. Annu. Rep. 1955: 32.

Bert, T.M. 1986. Speciation in western Atlantic stone crabs (genus *Menippe*): the role of geological patterns and climatic events in the formation and distribution of species. Mar. Biol. (Berl.), **93**: 157–170.

Brown, D.I., and Olivares, C.A. 1996. A new species of *Crepidula* (Mollusca: Mesogastropoda: Calyptraeidae) from Chile: additional characters for the identification of eastern Pacific planar *Crepidula* group. J. Nat. Hist. **30**: 1443–1158.

Carlton, J.T. 1979. History, biogeography, and ecology of the introduced marine and esturine invertebrates of the Pacific coast of North America. Ph.D. dissertation, University of California, Davis.

Coe, W.R. 1947. Biology of *Crepidula williamsi*, a new species of prosobranch gastropod from the Pacific coast. J. Morphol. **81**: 241–248.

Collin, R. 1995. Sex, size, and position: a test of models predicting the size at sex change in the protandrous gastropod *Crepidula fornicata*. Am. Nat. **146**: 815–831.

Collin, R. 2000. Sex change, reproduction, and development of *Crepidula adunca* and *C. lingulata* (Gastropoda: Calyptraeidae) Veliger, **43:** 24–33.

Conklin, E.G. 1897. The embryology of *Crepidula*. J. Morphol. **13**: 3–209.

Dall, W.H. 1905. Notes on a variety of *Crepidula nivea* C.B. Adams, from San Pedro, California. Nautilus, **19**: 26–27.

Deshayes, G.P. 1830. Encyclopedie méthodique des vers. Vol. 2. Part II. pp. 24–28.

Deslous-Paoli, J.M. 1985. *Crepidula fornicata* L. (gastéropode) dans le bassin de Marennes-Oléron: structure, dynamique et production d'une population. Oceanol. Acta, **8**: 453–460.

Dickinson, A.J.G., Nason, J., and Croll, R.P. 1999. Histochemical localization of FMRFamide, serotonin and catecholamines in embryonic *Crepidula fornicata* (Gastropoda, Prosobranchia). Zoomorphology (Berlin), **119**: 49–62.

d'Orbigny, A. 1841. Mollusques. *In* Voyage dans l'Amérique Méridionale, 1826–1833. Vol. 5. Part III. Ministre de l'Instruction Publique, Paris.

Fisher, A.G. 1960. Latitudinal variations in organic diversity. Evolution, **14**: 64–81.

Folmer, O., Black, M., Hoeh, W., Lutz, R., and Vrijenhoek, R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotechnol. **3**: 294–299.

Gallardo, C. 1977. *Crepidula philippiana* n.sp., nuevo gastrópodo Calyptraeidae de Chile con especial referencia al patrón de desarrollo. Stud. Neotrop. Fauna Environ. **12**: 177–185.

Gallardo, C.S. 1979. Especies gemelas del género *Crepidula* (Gastropoda, Calyptraeidae) en la costa de Chile, una redescripción de *C. dilatata* Lamarck y descripción de *C. fecunda* n.sp. Stud. Neotrop. Fauna Environ. **14**: 215–226.

Gmelin, J.F. 1791. Systema naturae. 13 ed. Vol. 1. Part I. pp. 3021–4120.

Gould, A.A. 1846. Descriptions of shells of the exploring expedition. Proc. Boston Soc. Nat. Hist. **2**: 159–162.

Gould, A.A. 1853. Descriptions of shells from the Gulf of California and the Pacific coasts of Mexico and California. Boston J. Nat. Hist. **6**: 374–408.

Griffin, T. 1998. Effects of salinity and diet on growth rate and the timing of sex change in a protandric hermaphroditic gastropod. Am. Zool. **38**: 186A.

Hoagland, K.E. 1977. Systematic review of fossil and recent *Crepidula*. Malacologia, **16**: 363–420.

Hoagland, K.E. 1978. Protandry and the evolution of environmentally-mediated sex change: a study of the mollusca. Malacologia, **17**: 365–391

Hoagland, K.E. 1983. Ecology and larval development of *Crepidula protea* (Prosobranchia: Crepidulidae) from southern Brasil: a new type of egg capsule for the genus. Nautilus, **97**: 105–109.

Hoagland, K.E. 1984. Use of molecular genetics to distinguish species of the gastropod genus *Crepidula* (Prosobranchia: Calyptraeidae). Malacologia, **25**: 607–628.

Hoagland, K.E. 1986. Patterns of encapsulation and brooding in the Calyptraeidae (Prosobranchia: Mesogastropoda). Am. Malacol. Bull. **4**: 173–183.

Knudsen, J. 1994. Further observations on the egg capsules and reproduction of some marine prosobranch molluscs from Hong Kong. *In* The malacofauna of Hong Kong and southern China III. *Edited by* B. Morton. University of Hong Kong Press, Hong Kong.

Lamarck, J.B. 1822. Histoire naturelle des animaux sans vertébres. Vol. 6. Part II. Paris.

Lea, H.C. 1843. Description of some new fossil shells from the Tertiary of Virginia. Proc. Am. Philos. Soc. **3**: 162–165.

Lesson, R.P. 1830. Zoologie de la coquille. *In* Voyage autour du monde. Vol. 2. Part I. Chap. 11. Mollusques. pp. 239–448.

Leviton, A.E., Gibbs, R.H., Heal, E., and Dawson, C.E. 1985. Standards in herpetology and ichthyology: Part I. Standard symbolic codes for institutional resource collections in herpetology and ichthyology. Copeia, 1985: 802–832.

Lima, G.M., and Pechenik, J.A. 1985. The influence of temperature on growth rate and length of larval life of the gastropod *Crepidula plana* Say. J. Exp. Mar. Biol. Ecol. **90**: 55–71.

Loomis, S.H., and VanNieuwenhuyze, W. 1985. Sediment correlates to density of *Crepidula fornicata* Linnaeus in the Pataguanset River, Connecticut. Veliger, **27**: 266–272.

Matusiak, J.P., and Fell, P.E. 1982. Reproductive cycle of *Crepidula convexa* (Say) within a New England eelgrass community. Int. J. Invertebr. Reprod. **5**: 253–260

McGee, B.L., and Targett, N.M. 1989. Larval and habitat selection in *Crepidula* (L.) and its effect on adult distribution patterns. J. Exp. Mar. Biol. Ecol. **131**: 195–214

Menke, K.T. 1851. Conchylien von Mazatlan, mit kritischen Anmerkungen. Z. Malakozool. **8**: 33–38.

Mikkelsen, P.S., and Mikkelsen, P.M. 1984. Comparison of *Acteocina canaliculata* (Say, 1826), *A. candei* (d'Orbigny, 1841), and *A. atrata* spec. nov. (Gastropoda: Cephalaspidae). Veliger, **27**: 164–192.

Moritz, C.E. 1939. Organogenesis in the gastropod *Crepidula adunca* Sowerby. Univ. Calif. Publ. Zool. **43**: 217–248.

Olsson, A.A., and Harbison, A. 1953. Pliocene Mollusca of southern Florida. Acad. Nat. Sci. Phila. Monogr. No. 8.

Owen, R. 1834. On the anatomy of the Calyptraeidae. Trans. Zool. Soc. Lond. **1**: 207–213.

Padilla, D.K. 1998. Inducible phenotyptic plasticity of the radula of *Lacuna* (Gastropoda: Littorinidae). Veliger, **41**: 201–204.

Parodiz, J.J. 1939. Las especies de *Crepidula* de las costas Argentinas. Physis (B. Aires), **17**: 685–709.

Pechenik, J.A. 1980. Growth and energy balance during the larval lives of three prosobranch gastropods. J. Exp. Mar. Biol. Ecol. **44**: 1–28.

Pechenik, J.A., Hilbish, T.J., Eyster, L.S., and Marshall, D. 1996. Relationship between larval and juvenile growth rates in two marine gastropods, *Crepidula plana* and *C. fornicata*. Mar. Biol. (Berl.), **125**: 119–127.

Reeb, C.A., and Avise, J.C. 1990. A genetic discontinuity in a continuously distributed species: mitochondrial DNA in the American oyster, *Crassostrea virginica*. Genetics, **124**: 397–406.

Reeve, L.A. 1859. Monograph of the genus *Crepidula*. Conchologia Iconica No. 11.

Sauriau, P.-G., Pichocki-Seyfried, C., Walker, P., de Montaudouin, X., Palud, C., and Héral, M. 1998. *Crepidula fornicata* L. (mollusque, gastéropode) en baie de Marennes-Oléron: cartographie des fonds par sonar á balayage latéral et estimation du stock. Oceanol. Acta, **21**: 1–10.

Say, T. 1822. An account of the marine shells of the United States. J. Acad. Nat. Sci. Phila. **2**: 221–227.

Shenk, M.A., and Karlson, R.H. 1986. Colonization of a shell resource by calyptraeid gastropods: tests of habitat selection and preemption models. J. Exp. Mar. Biol. Ecol. **99**: 79–89.

Swofford, D.L. 1993. PAUP: Phylogenetic analysis using parsimony, version 3.1.1. Illinois Natural History Survey, Champaign.

Taki, I. 1938. Systematic study of Japanese species of Calyptraeidae. Venus, **8**: 136–147.

Thiriot-Quiévreux, C., and Scheltema, R.S. 1982. Planktonic larvae of new England gastropods. V. *Bittium alternatum, Tiphora nigrocincta, Cerithiopsis emersoni, Lunatia heros* and *Crepidula plana*. Malacologia, **23**: 37–46.

Turton, W. 1825. Description of some new British shells. Zool. J. **2**: 361–367.

Valenciennes, A. 1846. Voyage autour du monde sur la *Vénus*, 1836–1839. Atlas, Mollusques, 24, pls.

Vermeij, G.J., Lowell, R.B., Walters, L.J., and Marks, J.A. 1987. Good hosts and their guests: relations between trochid gastropods and the epizoic limpet *Crepidula adunca*. Nautilus, **101**: 69–74.

Werner, B., and Grell, K.G. 1950. Die amerikanische Pantoffelschnecke *Crepidula fornicata* L.: Eine Anleitung zur Präparation. Verlag Gustav Fischer, Jena.

Wilding, C.S., Mill, P.J., and Grahame, J. 1999. Partial sequence of the mitochondrial genome of *Littorina saxatilis*: relevance to gastropod phylogenetics. J. Mol. Evol. **48**: 348–359.

Woodruff, D.S., McMeekin, L.L., Mulvey, M., and Carpenter M.P. 1986. Population genetics of *Crepidula onyx*: Variation in a Californian slipper snail recently established in China. Veliger, **29**: 53–63.

Yamazaki, N., Ueshima, R., Terrett, J.A., Yokobori, S., Kaifu, M., Segawa, R., Kobayashi, T., Numachi, K., Ueda, T., Nishikawa,

K., Watanabe, K., and Thomas R.H. 1997. Evolution of pulmonate gastropod mitochondrial genomes: Comparisons of gene organizations of *Euhadra*, *Capaea* and *Albinaria* and implications of unusual tRNA secondary structures. Genetics, **145**: 749–758.

Zimmermann, K.M., and Pechenik, J.A. 1991. How do temperature and salinity affect relative rates of growth, morphological differentiation and time to metamorphic competence in larvae of the marine gastropod *Crepidula plana*? Biol. Bull. (Woods Hole), **180**: 372–386.

Appendix

Table A1. The cytochrome-oxidase sequences obtained from the type specimens of each species. Position 1 corresponds to position 1534 of the *Drosophilia yakuba* mitochrondrial genome (GenBank No. X03240).

```
MBL4 GGTATATGATCTGGTCTAGTAGGTACAGCTTTAAGTCTTTTAATCCGAGCTGAACTAGGT 60
WP1  ....................................CC....T.............
SM2  ..C.................G..................CC.T..T.............

MBL4 CAACCAGGAGCTCTTTTAGGGGATGACCAATTATATAATGTAATTGTTACAGCCCATGCT 120
WP1  ..........C...C....A.....T.....G..............C..G.........
SM2  ..........C...C....A.....T.....G........G.....C...........

MBL4 TTTGTTATAATTTTTTTCCTAGTTATACCAATAATAATTGGAGGCTTTGGAAATTGGTTA 180
WP1  ............................T..............T..C.......A...
SM2  ............................T..............T..C..G..C..A...

MBL4 GTTCCATTAATACTGGGAGCCCCTGACATAGCTTTTCCCCGGTTAAATAATATAAGCTTT 240
WP1  ..........GT.A..........................T..A.............T...
SM2  ............A..........T..........T..A.............T...

MBL4 TGATTATTACCTCCAGCATTATTACTTTTACTTTCATCAGCTGCAGTGGAAAGAGGAGTT 300
WP1  ..........................C.T..................T.......G...
SM2  ..G.......................C.T..................T..G....G...

MBL4 GGGACAGGTTGAACTGTTTATCCCCCTCTTGCCGGAAACCTTGCTCACGCAGGAGGATCT 360
WP1  ..A..................C..A..C..........T..C..............G...
SM2  ..A..................C..A.....................C.............

MBL4 GTTGACCTAGCAATTTTCTCTCTTCACTTGGCTGGTGTTTCCTCAATTTTAGGTGCTGTA 420
WP1  ..C.....................TC.A.....................
SM2  ..C..T..G........T.........C.A..........T..................T

MBL4 AATTTTATTACCACTGTTATTAATATACGATGACGAGGAGTTCAATTCGAACGACTTCCT 480
WP1  ..........T.....C.....................G.................
SM2  ....................C.....................................

MBL4 CTTTTCGTATGATCAGTAAAAATTACTGCTATTTTACTTCTACTTTCCTTACCTGTTCTA 540
WP1  .....T.....................A.........T.................T..
SM2  .....T..T.........G.....................T.................T..

MBL4 GCAGGAGCAATTACAATGCTTTTAACTGATCGAAATTTTAATACTGCTTTTTTTGACCCA 600
WP1  ..G........C.....................C.....................
SM2  ....................................................

MBL4 GCTGGTGGTGGTGA
WP1  ..............
SM2  ..............
```