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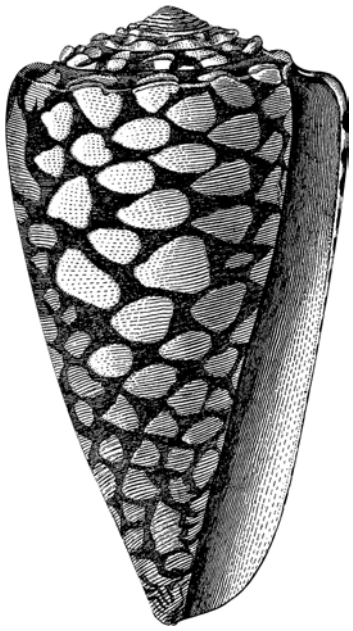

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A new *Littoraria* (Gastropoda : Littorinidae) from northwestern Australia

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Abstract

Littoraria ianthostoma, n. sp. is described from mangrove forests in Joseph Bonaparte Gulf in northwestern Australia. The new species superficially resembles the widespread *Littoraria articulata* (Philippi, 1846), with which it is sympatric, but differs in the closed penial sperm duct and absence of a pseudotrich in the paraspermatozoa. Allozyme analysis confirms a large genetic distance between these two species and fixed allelic differences between sympatric samples. This discovery is surprising because the distribution and taxonomy of the mangrove-associated *Littoraria* of Australia had been considered well known. The anatomy and protoconch indicate that the new species produces pelagic egg capsules and undergoes planktotrophic development. Nevertheless, it has not been found outside Joseph Bonaparte Gulf and so appears to be the most narrowly distributed of the 39 members of the genus. Possible phylogenetic relationships of the new species and apparent isolation of Joseph Bonaparte Gulf are discussed.

Introduction

Recently one of us (MS) had the opportunity to take part in a research cruise to Joseph Bonaparte Gulf in northwestern Australia and made collections of littorinid molluscs as part of a study of molecular genetic variation within species. Among the material were two samples of a small *Littoraria* species from mangrove trees at Forsyth Creek (Northern Territory) and the Berkeley River (Western Australia). Superficially, the shells resembled those of *Littoraria articulata* (Philippi, 1846) and indeed the samples were microsympatric (intermingled on the same mangrove trees) with typical examples of that species. However, the shells were paler in colour, predominantly yellow or pink, and subtly different in shape. Anatomical examination and allozyme electrophoresis have confirmed that the two samples belong to a new species, here formally described.

This discovery is surprising because the genus has been the subject of modern taxonomic study and all 38 species hitherto known worldwide have been documented in detail (Reid 1986, 1999*a*, 2001). In particular, the Australian species had been considered well known because large amounts of material from the field and museum collections have been examined by both of us. The northwestern coast of Australia is one of the most inaccessible and biologically poorly explored regions in the country but, nevertheless, we have seen sufficient material from the Northern Territory and from Western Australia to suggest that the new species may be restricted to Joseph Bonaparte Gulf. This raises interesting questions about the biogeographic history of the area and possible causes of its isolation, and suggests that other narrowly endemic species might be sought there.

Phylogenetic relationships within the genus *Littoraria* have been studied by parsimony analysis of morphological characters (Reid 1986, 1989, 1999*b*). To assess the relationships of the new species we have added it to the dataset of Reid (1999*b*) and repeated the analysis.

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Material and methods

Morphological study

Shell dimensions were measured with vernier calipers to 0.1 mm. Shell height (H) is the maximum dimension parallel to the axis of coiling; shell breadth (B) is the maximum dimension perpendicular to H; and the length of the aperture (LA) is the greatest length from the junction of the outer lip with the penultimate whorl to the anterior lip. Shell shape was quantified as the ratios, H/B and H/LA (relative spire height, SH).

Living animals were fixed in 70% ethanol without prior relaxation. For general accounts of the male and female anatomy of *Littoraria*, see Reid (1986). The relative radular length was calculated as the total radular length divided by shell height. Radulae were cleaned by soaking in a hypochlorite bleaching solution at room temperature for about 5 minutes, rinsing in distilled water, mounting on a film of polyvinyl acetate glue on glass, allowing to dry in air and coating with gold and palladium before examination in a scanning electron microscope. Unworn portions of radulae were viewed in three orientations: in standard flat view from vertically above the radula (to show shapes of teeth); at an angle of 45° from the front end of the radula (to show shapes of tooth cusps); and at an angle of 45° from the side of the radula (to show relief). The shape of the rachidian tooth was quantified as the ratio of the total length (in flat view) to the maximum basal width.

Allozyme analysis

Specimens of the new species were compared with *Littoraria articulata* using allozyme electrophoresis to examine variation at 12 loci. This comparison was performed because of the apparent morphological resemblance between the two species and because *L. articulata* is the only other *Littoraria* with which the new species occurs in sympatry and syntopy (intermingled on the same mangrove trees). In retrospect, our phylogenetic analysis suggests that these two species are not closely related, but the electrophoretic comparison provides an independent test of this conclusion.

Samples of 26 to 48 individuals of *L. articulata* were collected from six sites (Table 1), spanning the geographic range of the species within Australia. These included the two sites at which the new species was found. The new species was sampled from a single site, Forsyth Creek, and specimens included two morphs, one with a brown pattern on a white shell (n = 14) and the other lacking pattern (n = 4).

Samples were frozen alive in liquid nitrogen and stored at –80°C. Enzymes were extracted from both hepatopancreas and foot by grinding one volume of tissue with two volumes of grinding buffer (0.02M Tris-HCL pH 8, 0.25M sucrose, 0.1% (v/v) mercaptoethanol, 0.02% (w/v) bromophenol blue). Genetic variation of enzymes was examined by standard horizontal starch-gel electrophoresis using Tris-EDTA-borate (TEB), Tris-HCL, pH 8 (TC8) and Tris-maleate (TM) buffers and histochemical staining (Richardson *et al.* 1986; Murphy *et al.* 1996). Twelve enzymes representing twelve gene loci were examined: glucose-6-phosphate isomerase (EC 5.3.1.9; TC8 buffer; *Gpi* locus); glutamate oxaloacetate transaminase (EC 2.6.1.1; TM buffer; *Got*); hexokinase (EC 2.7.1.1; TM buffer; *Hk*); isocitrate dehydrogenase (EC 1.1.1.42, TC8 buffer; *Idh–2*); mannose-6-phosphate isomerase (EC 5.3.1.8; TEB buffer; *Mpi*); nucleoside phosphorylase (EC 2.4.2.1; TEB buffer; *Np*); peptidase (EC 3.4.11, EC 3.4.13; buffers TEB, TC8 and TEB; *Pep-A*, *Pep-D*, *Pep-E*); 6-phosphogluconate dehydrogenase (EC 1.1.1.44; TM buffer; *6Pgd*); phosphoglucomutase (EC 2.7.5.1; TM buffer; *Pgm–2*); and sorbitol dehydrogenase (EC 1.1.1.14; TC8 buffer; *Sdh*). Peptidases were detected using the following substrates: *Pep-A* with valyl leucine; *Pep-D* with leucyl proline; and *Pep-E* with leucine napthylamide. Alleles at each locus were labelled numerically in order of electrophoretic mobility of their corresponding allozyme (proportional to the most common allozyme, with a designated value of 100). For enzymes encoded by two loci, these were numbered in order of decreasing mobility. Genetic similarities between all populations and across all 12 loci were quantified using Nei's (1978) unbiased genetic identity, which corrects for small sample sizes. The matrix of identities was summarised with a UPGMA phenogram.

Phylogenetic analysis

The morphological characters of the new species were coded and added to the dataset of Reid (1999*b*). Maximum-parsimony analysis of the data was performed with PAUP version 3.1.1 (Swofford 1993), using the same settings as Reid (1999*b*). The distribution of character states on the trees was examined using MacClade 4.0 (Maddison and Maddison 2000), with polychotomies interpreted as uncertainties in resolution.

Institutional abbreviations

AMS, Australian Museum, Sydney
BMNH, Natural History Museum, London
NTM, Museum and Art Gallery of the Northern Territory, Darwin
WAM, Western Australian Museum, Perth

Systematics

Family **LITTORINIDAE** Anon., 1834
Genus ***Littoraria*** Griffith & Pidgeon, 1834
Subgenus ***Littoraria*** Griffith & Pidgeon, 1834

For diagnosis see Reid (1986, 1989) and for phylogeny, subgeneric classification and fossil history see Reid (1999*b*).

Littoraria* (*Littoraria*) *ianthostoma, n. sp.

(Figs 1*A–F*, 2*A–F*, *I*, *J*, 3*A–F*, 4)

Material examined

Holotype. AMS C.204871.

Paratypes. 1 dry specimen, AMS C.204872; 5 dry specimens, BMNH 20010109; 25 specimens in ethanol BMNH 20010110; 2 dry specimens, NTM P18782; 2 dry specimens, WAM S12711.

Type locality: Forsyth Ck, east coast Joseph Bonaparte Gulf, Northern Territory, Australia (14°56.5′S 129°23.5′E).

Other material examined. 32 preserved specimens, type locality; 3 preserved specimens, Reveley Island, near mouth of Berkeley R., west coast Joseph Bonaparte Gulf, Western Australia, 14°22.7′S 127°45.5′E, BMNH 20010111; total of 5 penes; 2 sperm samples; 6 pallial oviducts; 2 radulae examined.

Diagnosis

Shell small; columella excavated; usually a narrow pseudoumbilical area; 8–9 equally spaced primary spiral grooves on spire whorls; 16–19 low spiral ribs on last whorl, separated by deep narrow grooves. Shell colour polymorphic, yellow or orange-pink, with or without brown dashes on ribs; columella violet. Penis not bifurcate, filament half total length, glandular disc incorporated into base, sperm duct closed. Paraspermatozoa without pseudotrich. Development oviparous and planktotrophic.

Description

Shell (Fig. 1*A–F*). Adult size range 8.4–11.8 mm. Shape high-turbinate (H/B = 1.43–1.87; SH = 1.58–1.90); whorls rounded, suture impressed, periphery of last whorl slightly or not at all angled; moderate thickness. Sexually dimorphic; male shells a little smaller, slightly enlarged last whorl, more elongate aperture. Mature lip not flared; columella pillar relatively short, pinched and slightly excavated at base, producing basal knob; usually a narrow pseudoumbilical area between inner lip of aperture and columella pillar. Sculpture of 8–9 primary spiral grooves on spire whorls, almost equally spaced, posterior rib slightly narrower; most ribs flattened and undivided, separated by deeply impressed lines, but peripheral rib may become slightly raised and rounded on last whorl and adjacent grooves may reach one third of the rib width; 1–5 ribs at and below periphery of last whorl may be divided by a central line; total 16–19 ribs on last whorl; occasionally almost all ribs may be divided, producing up to 32 ribs on last whorl. Microsculpture of

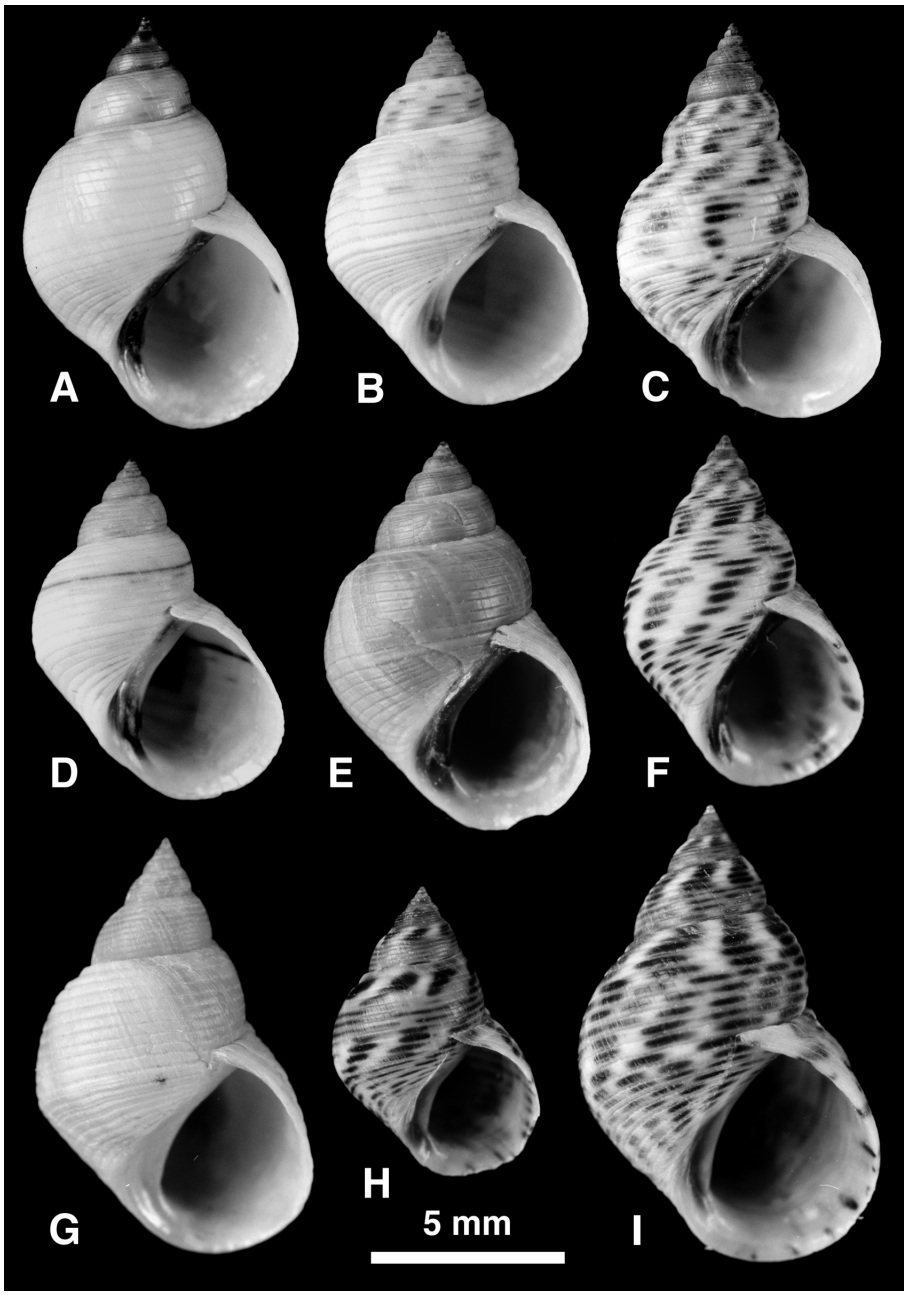

Fig. 1. *Littoraria ianthostoma*, n. sp. (*A–F*) and *Littoraria articulata* (Philippi, 1846) (*G–I*). *A–F*, Forsyth Creek, east coast Joseph Bonaparte Gulf, Northern Territory, Australia. *A*, *C*, *D*, *E*, Paratypes (BMNH 20010109). *B*, Holotype (AMS C.204871). *F*, Paratype (AMS C.204872). *A–C*, *E*, Females. *D*, *F*, Males. *G*, Female, Karratha Beach, Karratha, Western Australia (BMNH 20010113). *H*, *I*, Males, Forsyth Creek, east coast Joseph Bonaparte Gulf, Northern Territory, Australia (BMNH 20010112).

faint spiral striae over glossy rib surface, with axial microstriae in grooves. Protoconch diameter, 0.42 mm; about three whorls, with four spiral ribs and sinusigera notch. Colour polymorphic; ground colour white to pale yellow or rarely (one shell of 34) orange-pink; additional pigmentation usually faint and sparse; traces of brown pattern visible on most shells, but often restricted to apex or all spire whorls, leaving last whorl unpatterned; darkest shells with alternating brown and opaque white dashes on ribs, aligned in 8–10 axial series on last whorl; rarely (two shells of 34) a continuous brown line on shoulder of otherwise unpatterned last whorl. Columella purple-brown to dull violet; aperture yellow (or orange in single orange-pink shell) with traces of pattern showing through.

Animal. Head and sides of foot pale to dark grey; tentacles with dense narrow grey to black bands, unpigmented patch at inside of tentacle base. Operculum paucispiral, thin. Penis (Fig. 2*A–D*) long, not bifurcate; filament smooth, pointed at tip, half total length of penis, separated from wrinkled base by constriction; penial glandular disc incorporated into base just below junction with filament; penial vas deferens a closed tube to filament tip; penis unpigmented; vas deferens across head also closed (leading to closed prostate as in all *Littoraria* species). Paraspermatozoa (Fig. 2*I*, *J*) (observed after preservation in ethanol and therefore probably shrunk by up to 20%; Reid 1996: 6) 26–33 μm, with single large elongate fusiform rod body projecting from cell. Pallial oviduct (Fig. 2*E*, *F*) multispiral, 4.5 whorls, opaque capsule gland extending for 1.5 whorls; copulatory bursa in posterior position (beneath spiral portion of pallial oviduct). Egg capsules not seen, but pelagic capsules inferred from presence of large capsule gland (Reid 1986, 1989); development inferred planktotrophic from protoconch (Reid 1986, 1989). Radula (Fig. 3*A–F*) with relative radular length 0.85–0.94; rachidian length/width 1.00–1.13, base flared posteriorly, central cusp shield-shaped, flanked on either side by smaller pointed cusp and small or vestigial denticle; rachidian hood (additional anterior cutting edge) well developed; lateral with five cusps, central largest and bluntly rounded; inner marginal with four cusps; outer marginal with 3–4 cusps.

Habitat and distribution

Occurs commonly on the lower trunk and lower branches of *Avicennia marina* and occasionally *Rhizophora stylosa* trees on the seaward fringes of mangrove forests. The type locality, situated along the west bank of Forsyth Creek, is an area of intertidal mudflats approximately 50 m wide with scattered *A. marina* saplings and a few larger trees near the seaward edge. At this site, *L. articulata* was also common on the same mangrove trees as *L. ianthostoma* and barnacles covered the bases of the trunks. The only other arboreal gastropods found were scarce *Littoraria filosa* (Sowerby, 1832). Mangrove growth at the second collection site on Reveley Island was similarly sparse with scattered, stunted *A. marina* and *R. stylosa* trees growing on a stony beach. Again *L. articulata* was common and *L. filosa* uncommon on the same trees. So far *L. ianthostoma* has been recorded only from these two sites in Joseph Bonaparte Gulf (Fig. 4), approximately 200 km apart.

Etymology

Latin for ‘violet-mouthed’, in reference to the distinctive colouration of the columella.

Allozyme analysis

In the phenogram of genetic identities, the six populations of *L. articulata* form a group well separated from the sample of *L. ianthostoma* (Fig. 5). The genetic identity between the two species is 0.491, much lower than the identities among *L. articulata* populations

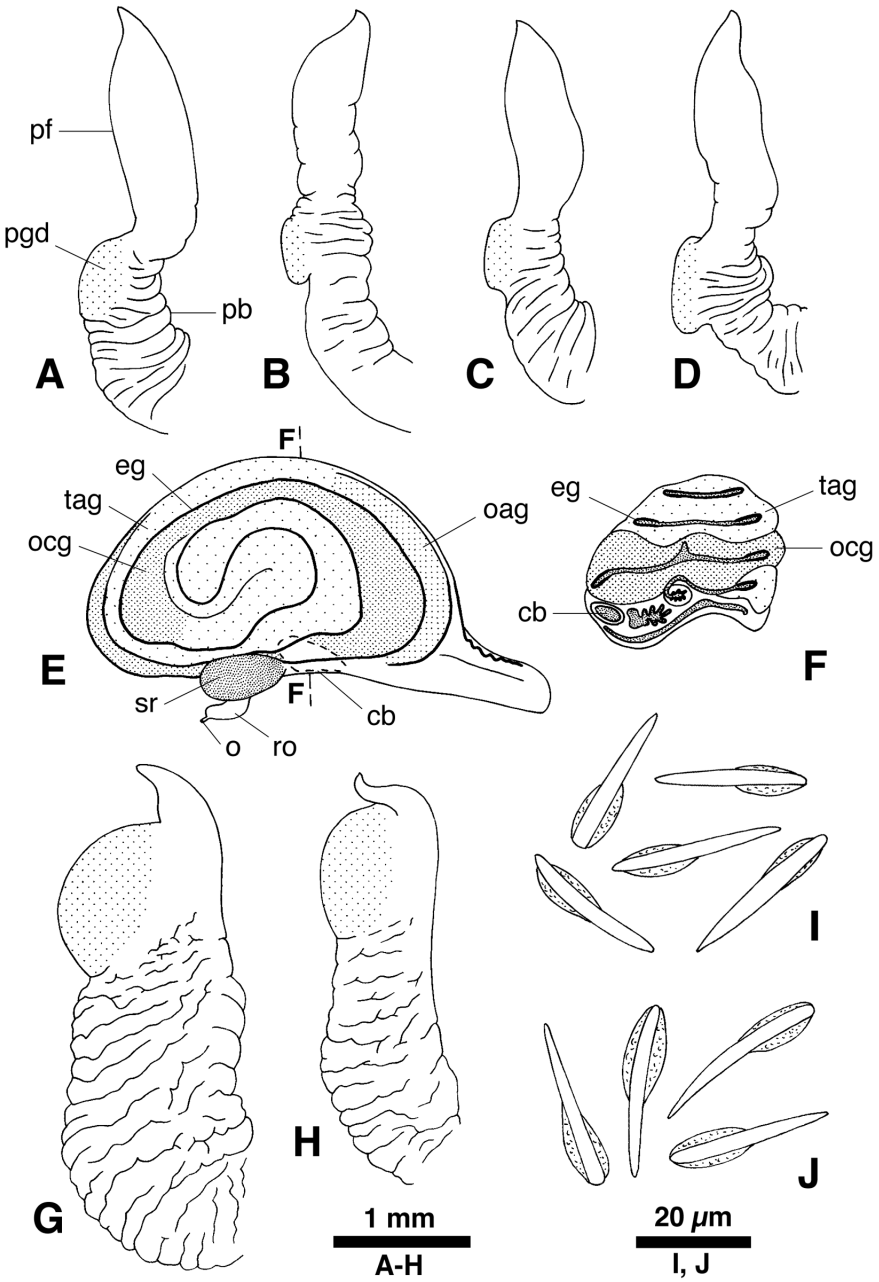

Fig. 2. Anatomy of *Littoraria ianthostoma*, n. sp. (*A–F*, *I*, *J*) and *Littoraria articulata* (*G*, *H*), Forsyth Creek, east coast Joseph Bonaparte Gulf, Northern Territory, Australia (*A–F*, *I*, *J*, paratypes BMNH 20010109, 20010110; *G*, *H*, BMNH 20010112). *A–D*, *G*, *H*, Penes. *E*, Pallial oviduct (with plane of transverse section *F* indicated). *I*, *J*, Paraspermatozoa from two specimens. Abbreviations: cb, copulatory burse (dashed outline, visible only by dissection or in section); eg, egg groove (heavy black line, visible by transparency due to black pigment); o, ovarian oviduct (leading from ovary); oag, opaque albumen gland (mid stipple); ocg, opaque capsule gland (dense stipple); pb, penial base (wrinkled); pf, penial filament (smooth); pgd, penial glandular disc (stipple); ro, renal oviduct; sr, receptacle (darkest stipple); tag, translucent albumen gland (light stipple).

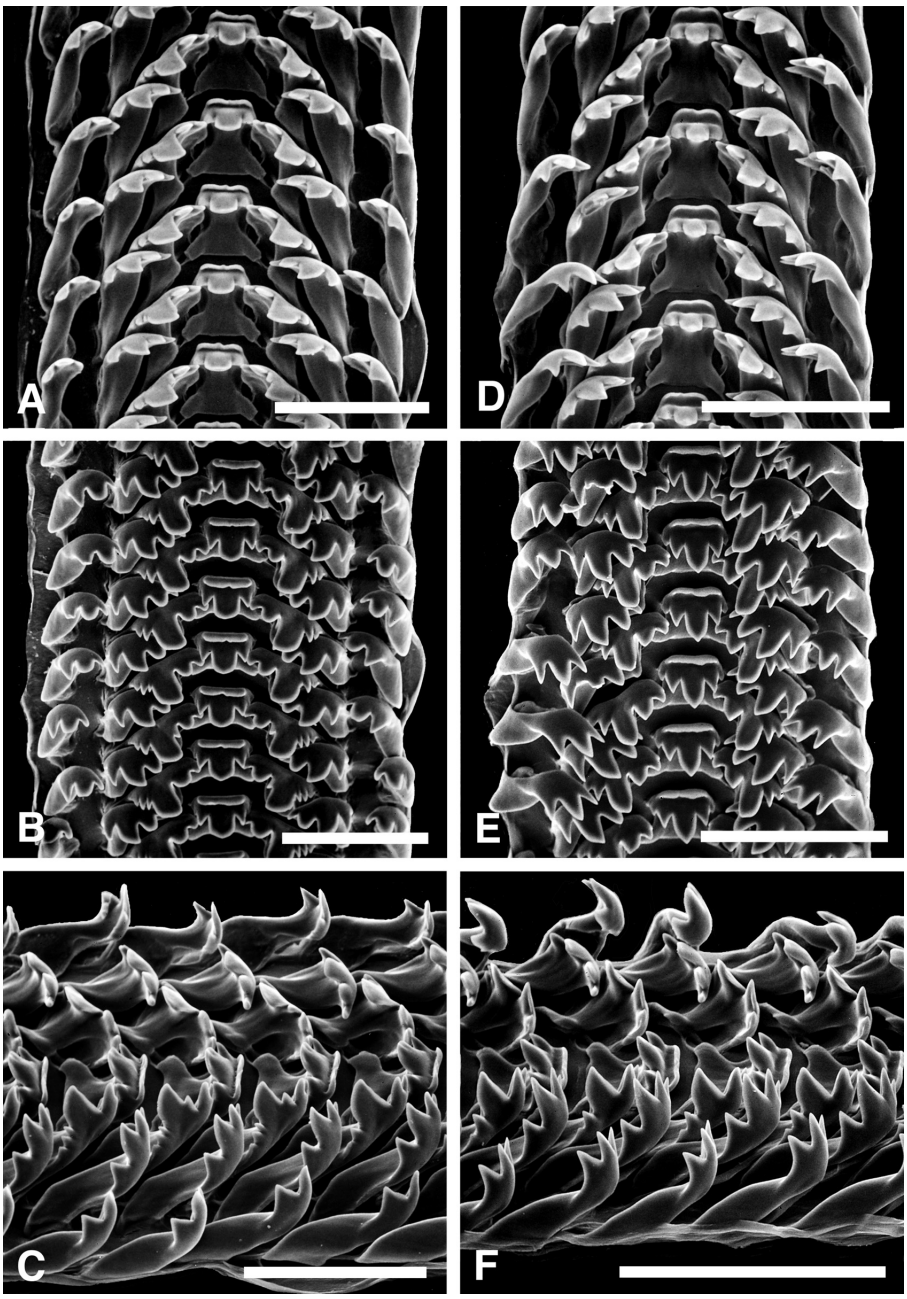

Fig. 3. Radulae of *Littoraria ianthostoma*, n. sp., Forsyth Creek, east coast Joseph Bonaparte Gulf, Northern Territory, Australia (BMNH 20010110). *A–C*, Female, shell H = 11.7 mm, three views: flat (*A*); 45° from anterior (*B*); 45° from side (*C*). *D–F*, Male, shell H = 9.0 mm, three views: flat (*D*); 45° from anterior (*E*); 45° from side (*F*). Scale bars: 100 μm.

(0.974–0.997), even when these originate from opposite sides of the continent. There are fixed differences between the two species at four loci (*Hk*, *Pep*-E, *Np*, *6Pgd*) (Table 2). There are also differences at a further two loci with less than 10% overlap in allelic

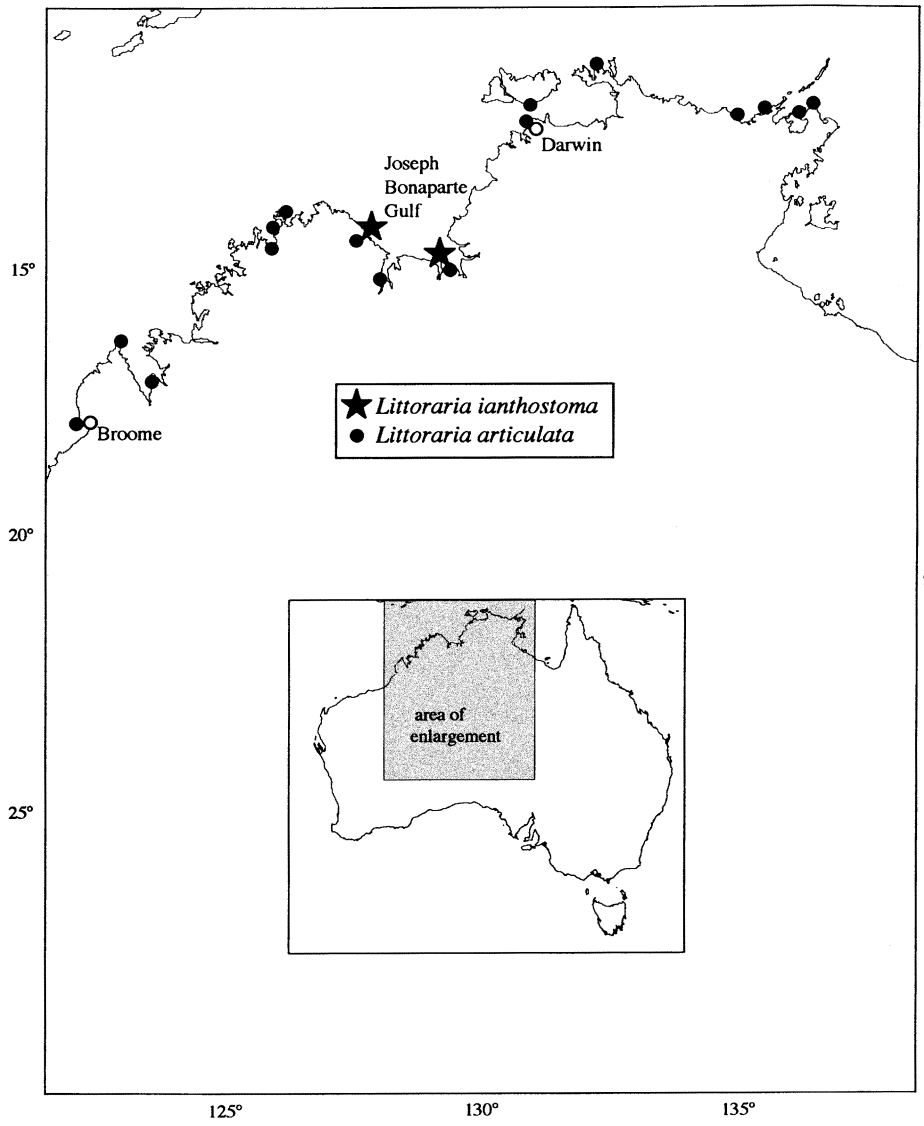

Fig. 4. Distribution of *Littoraria ianthostoma*, n. sp. For comparison, localities of examined samples of *L. articulata* from the vicinity are shown (museum collections in BMNH, AMS, WAM, details available from DGR on request). *Littoraria articulata* also occurs more widely throughout tropical Australia and Indo-Malaya (Reid 1986).

frequencies (*Pgm–2*, *Pep-A*) and, of the remaining six loci, both *Gpi* and *Idh-2* show alleles unique to one or the other species.

Discussion

In its shell characters, the new species closely resembles *Littoraria* (*Palustorina*) *articulata* (Philippi, 1846) (Fig. 1*G–I*), which is abundant on mangrove trees and sheltered rocky shores throughout the tropical coastline of Australia (Reid 1986). At both recorded sites for

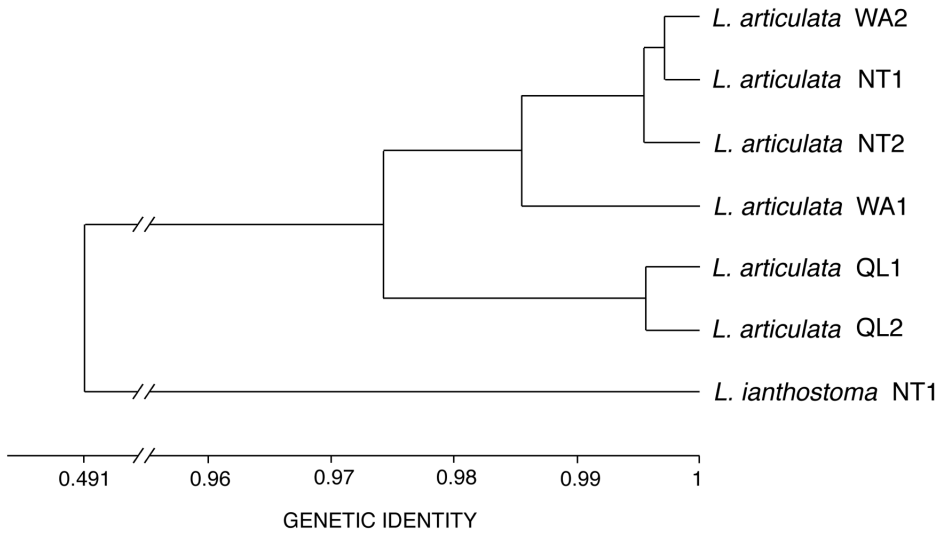

Fig. 5. Phenogram (UPGMA) of genetic identities (Nei 1978) of samples of *Littoraria articulata* and *L. ianthostoma*, n. sp. See Table 1 for locality abbreviations.

L. ianthostoma, the species occurs intermingled with *L. articulata* on the same mangrove trees. However, the similarity in shells is superficial, for there are anatomical differences sufficiently significant that, as discussed below, the two are classified in different subgenera. The allozyme analysis reveals a large genetic distance between these species and four loci at which there are fixed differences, even at the one site where both species were sampled. Even a single fixed allelic difference between sympatric samples provides strong evidence that they belong to different species, because it confirms the absence of interbreeding (Richardson *et al.* 1986).

The differences between *L. ianthostoma* and *L. articulata* are summarised in Table 3. Within Australia, confusion should not occur with any other *Littoraria* species (see Reid 1986). Shell outline, sculpture, microsculpture and aperture are almost identical in these two species. The most obvious shell character is the colouration, which, in the two available samples of *L. ianthostoma*, is strikingly polymorphic, most shells being yellow or faintly

Table 1. Localities of samples of *Littoraria articulata* and *L. ianthostoma*, n. sp. used in allozyme analysis

Species	Locality	Latitude °S	Longitude °E	Code
L. articulata	Karratha Beach, Western Australia	20°34′	116°48′	WA1
L. articulata	Reveley Island, Berkeley River, Joseph Bonaparte Gulf, Western Australia	14°23′	127°46′	WA2
L. articulata	Forsyth Creek, Joseph Bonaparte Gulf, Northern Territory	14°57′	129°24′	NT1
L. articulata	Ludmilla Creek, Darwin, Northern Territory	12°24′	130°52′	NT2
L. articulata	Rowes Bay, Townsville, Queensland	19°14′	146°47′	QL1
L. articulata	Town of 1770, Queensland	24°10′	151°53′	QL2
L. ianthostoma, n. sp.	Forsyth Creek, Joseph Bonaparte Gulf, Northern Territory	14°57′	129°24′	NT1

Table 2. Allele frequencies at 12 loci in samples of *Littoraria articulata* and *L. ianthostoma*, n. sp.
Localities coded as in Table 1. Mean sample sizes shown in parentheses.

Locus	Allele	*L. articulata*						*L. ianthostoma*
		WA1 (47)	WA2 (42)	NT1 (26)	NT2 (46)	QL1 (47)	QL2 (45)	NT1 (17)
Got	*130*	0.052	–	–	–	0.042	–	–
	100	0.948	0.976	1.000	1.000	0.958	0.979	1.000
	70	–	0.024	–	–	–	0.011	–
	45	–	–	–	–	–	0.011	–
Gpi	*105*	0.012	–	0.019	0.011	0.097	0.129	–
	100	0.988	0.988	0.962	0.979	0.764	0.871	0.750
	95	–	0.012	0.019	0.011	0.139	–	0.167
	88	–	–	–	–	–	–	0.083
Hk	*110*	–	–	–	–	0.010	–	–
	100	0.990	0.988	1.000	1.000	0.990	1.000	–
	95	–	–	–	–	–	–	1.000
	80	0.010	0.012	–	–	–	–	–
Idh-2	*145*	0.106	0.024	0.058	0.053	0.042	0.053	0.083
	117	–	–	–	–	–	–	0.167
	115	0.021	–	–	–	–	–	–
	100	0.872	0.952	0.942	0.936	0.958	0.936	0.708
	85	–	–	–	–	–	–	0.042
	70	–	0.024	–	0.011	–	0.011	–
Mpi	*110*	–	0.012	–	0.051	–	–	–
	100	1.000	0.988	0.981	0.949	1.000	1.000	1.000
	90	–	–	0.019	–	–	–	–
Np	*170*	0.010	–	0.135	0.011	0.094	0.043	–
	160	–	–	–	–	–	–	1.000
	100	0.990	0.988	0.808	0.979	0.781	0.840	–
	30	–	0.012	0.058	0.011	0.125	0.117	–
Pep-A	*110*	–	0.060	–	–	–	–	–
	100	0.969	0.810	0.846	0.957	0.948	0.968	–
	85	0.021	0.131	0.154	0.043	0.052	0.011	0.583
	80	0.010	–	–	–	–	0.021	0.389
	70	–	–	–	–	–	–	0.028
Pep-D	*150*	–	–	–	–	–	0.022	–
	145	–	0.095	0.058	–	0.078	0.043	0.059
	125	0.138	0.238	0.327	0.272	0.089	0.261	0.529
	110	0.191	0.202	0.154	0.283	0.300	0.326	0.412
	100	0.617	0.381	0.346	0.402	0.500	0.272	–
	85	0.032	0.083	0.115	0.022	0.033	0.043	–
	75	0.021	–	–	0.022	–	0.033	–
Pep-E	*130*	–	–	–	–	–	–	0.393
	120	–	–	0.019	0.011	–	–	–
	112	0.074	0.061	–	0.032	0.031	0.064	–
	114	–	–	–	–	–	–	0.607
	108	–	–	–	0.021	–	0.021	–
	100	0.809	0.878	0.885	0.926	0.875	0.862	–
	86	0.117	0.061	0.096	0.011	0.083	0.043	–
	78	–	–	–	–	0.010	0.011	–

(*continued next page*)

Table 2. (*continued*)

Locus	Allele	*L. articulata*						*L. ianthostoma*
		WA1 (47)	WA2 (42)	NT1 (26)	NT2 (46)	QL1 (47)	QL2 (45)	NT1 (17)
6Pgd	*170*	–	–	–	–	–	0.011	–
	130	0.021	0.131	0.231	0.074	0.094	0.053	–
	125	–	–	–	–	–	–	1.000
	100	0.917	0.833	0.769	0.883	0.906	0.926	–
	85	0.031	0.036	–	0.021	–	0.011	–
	65	0.031	–	–	0.011	–	–	–
	45	–	–	–	0.011	–	–	–
Pgm-2	*120*	–	–	–	0.011	0.043	0.023	–
	115	0.229	0.107	0.096	0.191	0.032	0.068	–
	110	0.510	0.274	0.308	0.362	0.032	0.023	–
	100	0.229	0.619	0.462	0.426	0.883	0.875	0.056
	90	0.031	–	0.135	0.011	0.011	0.011	0.333
	85	–	–	–	–	–	–	0.611
Sdh	*200*	0.010	–	–	–	0.021	–	–
	150	0.042	–	–	0.043	0.021	–	0.031
	100	0.896	0.988	0.942	0.957	0.958	0.979	0.938
	60	0.052	0.012	0.058	–	–	0.021	0.031

patterned, others with brown dashes aligned in axial series and one of the available specimens (3%) is orange-pink. Shells of *L. articulata* are sometimes variable, but the great majority show a dark brown to black pattern of axially aligned dashes on a cream ground (the axial alignment often interrupted at the shoulder, Fig. 1*H*); yellow and lightly patterned shells are uncommon and the orange-pink colour is extremely rare (Reid 1986: 208). Apertural colouration shows subtle differences; in sympatric samples *L. articulata* has a pink or purple-brown columella, whereas that of *L. ianthostoma* is purple to violet, even in yellow shells lacking dark pattern on the outside of the shell. Further to the south west in its Australian range, *L. articulata* generally has a white columella (Fig. 1*G*). No anatomical characters have been found for the separation of females of *L. ianthostoma* and *L. articulata*. However, in males the form of the penis is diagnostic. The wrinkled base with

Table 3. Comparison of *Littoraria ianthostoma*, n. sp. and *L. articulata*

Character	*L. ianthostoma*	*L. articulata*
Shell colour	Polymorphic (yellow, orange-pink or brown-patterned)	Cream to yellow, with varying degrees of brown patterning, but very rarely plain yellow or orange-pink
Columella colour	Violet to purple	Pinkish- or purplish-brown, often white in NW Australia
Penis	Filament equal in size to wrinkled base, penial vas deferens a closed duct	Filament a small terminal appendage, penial vas deferens an open groove
Paraspermatozoa	Simple, lacking pseudotrich, rod bodies long and projecting	Pseudotrich present, oval rod bodies do not project from cell
Geographical distribution	Joseph Bonaparte Gulf	Indo-Malaya and throughout Australian tropics

integral glandular disc is similar in both, but in *L. articulata* (Fig. 2*G*, *H*) the filament is a small, pointed, terminal appendage, whereas in *L. ianthostoma* (Fig. 2*A–D*) it is equal in size to the entire penial base. In *L. articulata* the penial vas deferens is an open groove, but this is closed as a tube in *L. ianthostoma*. Paraspermatozoa are unlikely to be useful for identification purposes, but are of considerable phylogenetic significance, as discussed below. In *L. articulata*, the cells are 30–42 μm long, contain one or several oval rod bodies and bear a flagellum-like structure that is 170 μm long, called the pseudotrich (Reid 1986; Healy and Jamieson 1993; Buckland-Nicks *et al.* 2000).

Confusion is possible with several of the species of *Littoraria* known from other parts of the world. *Littoraria strigata* (Philippi, 1846) from South East Asia (see Reid 1986) and *L. sinensis* (Philippi, 1847) from China (see Reid 2001) are almost identical in shell characters to *L. articulata* and are separated from that species mainly by the relatively greater lengths of their penial filaments. Except for that one character, the differences listed in Table 3 therefore serve to distinguish *L. ianthostoma* from *L. strigata* and *L. sinensis*. Another similar species is *L. vespacea* Reid, 1986 from South East Asia; in that species the penis is of similar shape but with an open sperm groove, the rod bodies of the paraspermatozoa are short, the copulatory bursa is anterior and the shell is broader, distinctively patterned and not polymorphic (see Reid 1986). In the eastern Pacific Ocean, *L. rosewateri* Reid, 1999 has a polymorphic shell but the shape is narrower, the penis has a closed sperm duct like that of *L. ianthostoma* but the glandular disc is borne on a bifurcation of the base, and the bursa is anterior (see Reid 1999*a*).

The presence of shell colour polymorphism in *L. ianthostoma* is noteworthy. Elsewhere in the genus this character state has been recorded in three clades (Reid 1999*b*) and only in species found on the visually diverse backgrounds of mangrove foliage or marsh grass, not in species found on visually uniform mangrove trunks or rocks (Reid 1986, 1987). From this association it has been suggested that polymorphism may be adaptive in relation to visual selection by unknown predators. The available samples of *L. ianthostoma* are small and habitat details have been recorded at only two sites, so it is not known whether polymorphism is typical of the species and whether its usual habitat fits the trend observed in other species. An additional consideration is that in some bark-dwelling *Littoraria* species, shell colour is paler on some species of mangrove tree (*Avicennia* and *Sonneratia*, for example) than on *Rhizophora*, perhaps implying a direct ecophenotypic influence of substrate (Reid 1986).

The two radulae of *L. ianthostoma* that were examined show differences in cusp shape. This is common in the genus, in which both ontogenetic change and ecophenotypic plasticity have been reported (Reid 1999*a*; Reid and Mak 1999).

The phylogenetic relationships of *Littoraria* species remain poorly understood. Three phylogenetic analyses have been performed using an increasingly refined suite of morphological characters and resulting in a cladogram of the 36 species then recognised (Reid 1986, 1989, 1999*b*). All analyses agree that the genus is monophyletic, as supported by the synapomorphies: closed prostate gland; penial glandular disc; and lack of mamilliform penial glands (although none of these are unique in the family). Within the genus there are three moderately well-supported clades that have been given subgeneric status, *Protolittoraria*, *Littorinopsis* and *Palustorina*; the remainder form a paraphyletic and poorly resolved assemblage, the subgenus *Littoraria* (Reid 1999*b*). Of these subgenera, the most strongly supported is *Palustorina*, defined by the unique synapomorphy of the pseudotrich of the paraspermatozoa. The absence of this character state in *L. ianthostoma* excludes it from this clade.

In order to determine the phylogenetic relationships of *L. ianthostoma*, we added this new species to the morphological dataset used by Reid (1999*b*) and repeated the parsimony analysis. In the strict consensus tree (of more than 16000 trees, length 82 steps, consistency index = 0.524), *L. ianthostoma* appeared in the unresolved group of 14 members of the subgenus *Littoraria* (within clade three in Reid 1999*b*: fig. 4). In the 50% majority-rule tree it appeared (in 74% of the trees) as the sister-species of *L.* (*Littoraria*) *rosewateri*. This pair fell in a clade of western Atlantic and eastern Pacific species (clade five of Reid 1999*b*: fig. 4), united by the closed penial sperm duct. *Littoraria ianthostoma* also shares the synapomorphy of shell colour polymorphism with *L. rosewateri*. However, neither of these characters is unique within the genus, so this phylogenetic placement is not well supported.

Based on the weak criteria of overall resemblance and biogeographic proximity, another possible sister-species of *L. ianthostoma* is *L.* (*Littoraria*) *vespacea* from South East Asia. Similarities (possible synapomorphies) include the non-bifurcate shape of the penis (although the sperm channel is an open groove in *L. vespacea*) and the fusiform rod bodies of the paraspermatozoa (although these are not projecting in *L. vespacea*). At present there is insufficient information to speculate further on the phylogenetic relationships of the new species.

The geographic distribution of *L. ianthostoma* is interesting. It is well known that northwestern Australia is a region of marine endemicity (Wells 1980, 1997). Among the Littorinidae alone, there are two other endemic *Littoraria* species (and two 'geographical forms', Reid 1986) and an endemic *Tectarius* (Rosewater, 1972). Nevertheless, the apparent restriction of the new species to Joseph Bonaparte Gulf is surprising. This might be a collecting artefact, since the region is relatively remote. However, we have examined abundant museum material of the morphologically and ecologically similar *L. articulata* from the vicinities of Broome and Darwin, and seven collections (89 specimens; BMNH, AMS, WAM) from between Cape Leveque and Vansittart Bay (Fig. 4) without discovering additional specimens of *L. ianthostoma*. This suggests that its distribution on the northwestern coast of Australia might indeed be restricted. If confirmed, this distribution would be the most restricted of any species in the genus. Nothing is known about the actual dispersal of *Littoraria* species. It is known (or inferred from protoconch characters) that all but one of the species have planktotrophic development and that most of these (excluding the ovoviviparous subgenus, *Littorinopsis*) produce pelagic egg capsules (Reid 1986, 1999*a*, 1999*b*, 2001). One species, *L. angulifera* (Lamarck, 1822), has an amphi-Atlantic distribution, implying dispersal in ocean currents over some 2000 km (Rosewater and Vermeij 1972; Merkt and Ellison 1998). On the other hand, Janson (1985) found some evidence for genetic isolation by distance among populations of *L. angulifera* from southern Florida. These species, including *L. ianthostoma*, would appear to have the potential for high dispersal and wide distribution, although whether this potential is realised may depend on other factors such as spawning behaviour, current patterns and coastal landforms.

It is not known whether the apparently restricted distribution of *L. ianthostoma* might reflect a general pattern of endemism and isolation of Joseph Bonaparte Gulf, or whether the species represents an isolated case of a relictual distribution. Reviews of oceanographic conditions (Bunt 1987) and of distribution patterns of marine fauna (Wilson and Allen 1987; Glasby *et al*. 2000) in northern Australia have not noted any peculiarity of the Gulf. However, a wider study of allozyme variation within *L. articulata* by one of us (MS, unpublished) hints at possible historical genetic isolation of the Gulf. Comparison of allelic frequencies among Australian populations has shown that the Forsyth Creek population

displays an unusual loss of rare alleles (18% compared with an average of 38%). The distribution of alleles can provide evidence of historical bottlenecks; populations at equilibrium accumulate rare alleles whereas those subject to bottlenecks lose them (Luikart *et al.*1998). The 50% reduction of rare alleles in *L. articulata* from Forsyth Creek therefore suggests an historical reduction in gene flow in the area. Further suggestion of the uniqueness and isolation of the Joseph Bonaparte Gulf region has been provided by studies on fish assemblages, which have found that communities in the area are distinct from those in the nearby Arafura Sea (Saenger and Bucher 1989).

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References

Anon. (1834). [General collection of shells]. In 'Synopsis of the Contents of the British Museum'. Edition 28. pp. 106–118. (British Museum: London.)

Buckland-Nicks, J. A., Healy, J. M., Jamieson, B. G. M., and O'Leary, S. (2000). Paraspermatogenesis in *Littoraria* (*Palustorina*) *articulata*, with reference to other Littorinidae (Littorinoidea, Caenogastropoda). *Invertebrate Biology* **119**, 254–264.

Bunt, J. S. (1987). The Australian marine environment. In 'Fauna of Australia. General Articles. Vol. 1'. (Eds G. R. Dyne and D. W. Walton.) pp. 17–42. (Australian Government Publishing Service: Canberra.)

Glasby, C. J., Hutchings, P. A., and Wilson, R. S. (2000). Biogeography. In 'Polychaetes & Allies: The Southern Synthesis. Fauna of Australia. Volume 4A'. (Eds P. L. Beesley, G. J. B. Ross and C. J. Glasby.) pp. 39–43. (CSIRO Publishing: Melbourne.)

Griffith, E., and Pidgeon, E. (1834). 'The Mollusca and Radiata. The Animal Kingdom by the Baron Cuvier.' Volume 12. (Whittaker & Co.: London.)

Healy, J. M., and Jamieson, B. G. M. (1993). Euspermatozoa, paraspermatozoa and spermatozeugmata of *Littoraria* (*Palustorina*) *articulata* (Prosobranchia: Caenogastropoda) with special reference to the pseudotrich. *Acta Zoologica* **74**, 321–330.

Janson, K. (1985). Genetic and morphological variation within and between populations of *Littorina angulifera* from Florida. *Ophelia* **24**, 125–134.

Luikart, G., Allendorf, F. W., Cornuet, J-M., and Sherwin, W. B. (1998). Distortion of allele frequency distributions provides a test for recent population bottlenecks. *Journal of Heredity* **89**, 238–247.

Maddison, W. P., and Maddison, D. R. (2000). 'MacClade. Analysis of Phylogeny and Character Evolution. Version 4.0.' (Sinauer Associates: Sunderland, Massachusetts.)

Merkt, R. E., and Ellison, A. M. (1998). Geographic and habitat-specific morphological variation of *Littoraria* (*Littorinopsis*) *angulifera* (Lamarck, 1822). *Malacologia* **40**, 279–295.

Murphy, R. W., Sites, J. W., Buth, D. G., and Haufler, C. H. (1996). Proteins: isozyme electrophoresis. In 'Molecular Systematics. Edition 2'. (Eds D. M. Hillis, C. Moritz and B. K. Mable.) pp. 51–120. (Sinauer Associates: Sunderland, Massachusetts.)

Nei, M. (1978). Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* **89**, 583–590.

Reid, D. G. (1986). 'The Littorinid Molluscs of Mangrove Forests in the Indo-Pacific Region: The Genus *Littoraria*.' (British Museum (Natural History): London.)

Reid, D. G. (1987). Natural selection for apostasy and crypsis acting on the shell colour polymorphism of a mangrove snail, *Littoraria filosa* (Sowerby) (Gastropoda: Littorinidae). *Biological Journal of the Linnean Society* **30**, 1–24.

Reid, D. G. (1989). The comparative morphology, phylogeny and evolution of the gastropod family Littorinidae. *Philosophical Transactions of the Royal Society of London*, *Series B* **324**, 1–110.
Reid, D. G. (1996). 'Systematics and Evolution of *Littorina*.' (The Ray Society: London.)
Reid, D. G. (1999*a*). The genus *Littoraria* Griffith & Pidgeon, 1834 (Gastropoda: Littorinidae) in the tropical eastern Pacific. *Veliger* **42**, 21–53.
Reid, D. G. (1999*b*). The phylogeny of *Littoraria* (Gastropoda: Littorinidae): an example of the practice and application of cladistic analysis. *Phuket Marine Biological Center Special Publication* **19**, 283–322.
Reid, D. G. (2001). New data on the taxonomy and distribution of the genus *Littoraria* Griffith & Pidgeon, 1834 (Gastropoda: Littorinidae) in the Indo-West Pacific Ocean. *Nautilus* **115**, 115–139.
Reid, D. G., and Mak, Y-M. (1999). Indirect evidence for ecophenotypic plasticity in radular dentition of *Littoraria* species (Gastropoda: Littorinidae). *Journal of Molluscan Studies* **65**, 355–370.
Richardson, B. J., Baverstock, P. R., and Adams, M. (1986). 'Allozyme Electrophoresis, a Handbook for Animal Systematics and Population Studies.' (Academic Press: Sydney.)
Rosewater, J. (1972). The family Littorinidae in the Indo-Pacific. Part II. The subfamilies Tectariinae and Echininae. *Indo-Pacific Mollusca* **2**, 507–534.
Rosewater, J., and Vermeij, G. J. (1972). The amphi-Atlantic distribution of *Littorina meleagris*. *Nautilus* **86**, 67–69.
Saenger, P., and Bucher, D. (1989). 'An Inventory of Australian Estuaries and Enclosed Marine Waters.' (Australian National Parks and Wildlife Service, Unpublished Consultancy Report and Database: Lismore, New South Wales.)
Swofford, D. L. (1993). 'PAUP: Phylogenetic Analysis Using Parsimony.' (Illinois Natural History Survey: Champaign, Illinois.)
Wells, F. E. (1980). The distribution of shallow-water marine prosobranch gastropod molluscs along the coastline of Western Australia. *Veliger* **22**, 232–247.
Wells, F. E. (1997). Shallow water marine gastropods endemic to Western Australia. *Haliotis* **26**, 73–80.
Wilson, B. R., and Allen, G. R. (1987). Major components and distribution of marine fauna. In 'Fauna of Australia. General Articles. Vol. 1'. (Eds G. R. Dyne and D. W. Walton.) pp. 46–68. (Australian Government Publishing Service: Canberra.)