



Rediscovery of one of the very few ‘unequivocally extinct’ species of marine molluscs: *Littoraria flammea* (Philippi, 1847) lost, found—and lost again?

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ABSTRACT

The littorinid gastropod *Littoraria flammea* has been widely quoted as one of only three or four marine molluscs believed to have become extinct in historic times. It is easily recognized by its delicate, elongate, striped shell. We review the literature and available museum material to show that its only recorded locality was Shanghai and that it has not been collected since about 1855. We report the rediscovery of this species in salt marshes near Shanghai, where the habitat is under threat from invasive *Spartina*. For the first time we describe the penis, pallial oviduct and radula. These are the same as those of *L. melanostoma*, previously recorded only from Fujian province and southwards, where its shell is different from that of *L. flammea*. We now report a population of *L. aff. melanostoma* from between Fujian and Shanghai, with shells of intermediate form and colour. Analyses of sequences of COI, 12S and 28S genes do not support separation of these two *Littoraria* species, in terms of either reciprocal monophyly or genetic distance. Present evidence suggests that *L. flammea* could be only a distinctive morphological form or extreme of a cline, at the northern limit of the range of *L. melanostoma*.

INTRODUCTION

Marine organisms have long been considered to be less vulnerable than terrestrial ones to extinction over evolutionary and historic time scales, by virtue of biological attributes that frequently include high fecundity, wide dispersal and broad geographical range. Nevertheless, now that the potential threats of global climatic change, habitat destruction, exploitation and pollution are becoming apparent, there is growing concern about historic and present-day extinction in the marine realm (Carlton *et al.*, 1999; Dulvy, Sadovy & Reynolds, 2003; del Monte-Luna *et al.*, 2007; Dulvy, Pinnegar & Reynolds, 2009). Against the background of the perceived biodiversity crisis, it comes as a surprise that the first example of a post-Pleistocene extinction of any marine invertebrate, that of the patellogastropod limpet *Lottia alveus alveus*, was only reported in 1991 (Carlton *et al.*, 1991). Since then there have been several reviews of historic extinctions of marine molluscs, but only five species or subspecies have been mentioned as possible candidates (Carlton *et al.*, 1991, 1999; Carlton, 1993; Roberts & Hawkins, 1999; Dulvy *et al.*, 2003, 2009; del Monte-Luna *et al.*, 2007; IUCN, 2014).

Lottia alveus alveus remains the most well known case of possible extinction of a marine mollusc (Carlton *et al.*, 1991) and is an illustrative example. This small limpet lived and fed exclusively on the blades of the eelgrass *Zostera* and was found in a limited area of the northwestern Atlantic from Labrador to New York. As the result of a devastating disease in the early 1930s, the

eelgrass was restricted to refugia in brackish water, where the limpet could not survive. No examples of *L. alveus alveus* have been collected since 1929. Two other subspecies of *L. alveus* were believed to remain in the North Pacific (Carlton *et al.*, 1991), but an unconfirmed report has indicated that at least one of these is not the sister species of the Atlantic form (Holm, 2002), so that *L. alveus* may indeed be globally extinct. This first case demonstrates some of the features that are associated with high extinction risk (Roberts & Hawkins, 1999; Dulvy *et al.*, 2003; del Monte-Luna *et al.*, 2007)—narrow range, stenotopic habit and specialized host association. Anthropogenic disturbance is another common factor in many extinctions, although whether it was involved in the decline of the eelgrass is not known (Carlton, 1993). The case also indicates the importance of correct systematics in the assessment of global extinction of a species.

Among the other possible extinctions of marine molluscs is another patellogastropod, ‘*Collisella edmittchelli*’. This is known as a common Pleistocene fossil in southern California, but a single living specimen was collected in about 1861 and no others have been seen since (Lindberg, 1984; Carlton, 1993), although its extinct status has been questioned (del Monte-Luna *et al.*, 2007). The potamidid snail *Cerithideopsis fuscata* was last collected from mudflats in San Diego Bay, California, in 1935, where its habitat was destroyed by human activity, but its taxonomic status within this variable and complex genus is doubtful (Carlton *et al.*, 1991; Carlton, 1993; see Miura, Torchin & Bermingham, 2010). Common

attributes in all three cases are a restricted geographical range (although this is not so for the more numerous examples of extinctions of marine vertebrates; Vermeij, 1993) and a restricted or otherwise vulnerable, intertidal, habitat. Only one species, *Haliotis sorenseni*, has been driven to near-extinction by human exploitation of its small, subtidal populations, also in southern California (Hobday, Tegner & Haaker, 2001). This once-commercial abalone now exists in such low numbers that recruitment may be impossible (but see del Monte-Luna *et al.*, 2007). A few other extinctions of marine molluscs have been claimed, but have been excluded for reasons of taxonomic uncertainty, poor documentation or indeed have been rediscovered (Carlton, 1993; Carlton *et al.*, 1999). An assimineid snail endemic to Mauritius, *Omphalotropis plicosa*, is currently included as a marine extinction in the IUCN Red List (2014), but is neither marine nor extinct (Florens & Baider, 2007). An additional gastropod species, regarded as a strong case of global extinction by most reviewers (Carlton, 1993, Carlton *et al.*, 1999; Dulvy *et al.*, 2003, 2009; del Monte-Luna *et al.*, 2007; IUCN, 2014), is *Littoraria flammea* (Philippi, 1847), the subject of this paper.

The small number of apparently well-documented cases of historic extinction of marine invertebrates (and of marine organisms in general; Dulvy *et al.*, 2003, 2009; del Monte-Luna *et al.*, 2007) is a cause for concern. Does this reflect reality or ignorance? For obvious reasons, marine species are less well recorded than terrestrial ones, even in the relatively accessible intertidal zone. Knowledge of taxonomy and distribution is limited in many parts of the world, so it is no coincidence that four of the examples listed above are of species indigenous to the well-studied shores of North America. Most marine species remain undescribed and the taxonomy of even familiar shelled gastropods is often revised when molecular techniques are applied. The taxonomic status of rare material collected in the nineteenth and early twentieth centuries is frequently critical in assessing global extinction and requires rigorous examination. The most common means of detecting marine extinctions are indirect, i.e. through archaeological or historical comparison, rather than by direct monitoring of declining populations (Dulvy *et al.*, 2003). All these reasons contribute to the considerable delay between most marine extinction events and their recognition as such; the median time lag has been estimated as 53 years by Dulvy *et al.* (2003). These authors also pointed to the need for critical evaluation of the few ‘unequivocal’ marine extinctions. A subsequent review of the cases listed by Dulvy *et al.* (2003) concluded the number had been overestimated by up to 50% (del Monte-Luna *et al.*, 2007), emphasizing the level of uncertainty about the extent of marine extinction.

Littoraria flammea was first named by Philippi (1847), who recorded its locality simply as ‘China’. In the most recent taxonomic revision of the Indo-Pacific species of *Littoraria*, Reid (1986) noted that specimens were rare in museum collections, that all known material consisted of dry shells dating from the early nineteenth century and that none was localized with any greater precision. He speculated that since it was unknown in the relatively well-studied faunas of Indo-China, Hong Kong and Japan, it might occur on the shores of the East China Sea or Yellow Sea. He found consistent differences from the most similar congener, *L. melanostoma* (Gray, 1839), a well-known species of constant shell form that occurs from Fujian and Taiwan in southern China to India, and concluded that the two species were distinct. From this evidence, and arguing that a snail of 20 mm length was unlikely to have been overlooked on the Chinese coast, Carlton (1993) listed *L. flammea* as a ‘possible’ marine extinction. Since then, no further information has emerged, but the species has repeatedly been included as one of the few recognized marine extinctions. During this repetition, the qualification ‘possible’ was first dropped (Carlton *et al.*,

1999) and the case then became ‘unequivocal’ (Dulvy *et al.*, 2003). Over the course of 30 years one of us (DGR) has sought information on this species from field biologists, malacologists and museums in China—without success. The species is large for a littorinid and instantly recognizable. All Littorininae occupy accessible intertidal habitats and almost always occur at high density. Systematic malacology and field studies have greatly advanced in China in recent years (e.g. Cai & Huang, 1989; Fan, 1989; Ma, 2004; Li & Huang, 2012). The continuing silence on *L. flammea* has therefore argued strongly for its genuine extinction.

While identification of littorinid morphospecies can usually be accomplished from dry shells, the initial morphological delimitation of biological species depended heavily on species-specific characters of the penis (Reid, 1986; Hollander *et al.*, 2013). Both male and female reproductive anatomy was crucial in the first phylogenetic classification scheme for littorinids (Reid, 1986, 1989). With only dry shells available, Reid (1986) placed *L. flammea* in the genus *Littoraria*, subgenus *Palustorina*, on the basis of the obvious similarity of its shell to that of *L. (P.) melanostoma*. The phylogenetic relationships of littorinids (subfamily Littorininae) have recently been reconstructed in a molecular analysis based on three genes, sampled from 97% of the 152 recognized species (Reid, Dyal & Williams, 2012) and are largely consistent with the established generic classification. In a molecular analysis of 37 of the 39 known species of *Littoraria* by Reid, Dyal & Williams (2010), the previous morphological discrimination of species (Reid, 1986, 2001) was largely confirmed. These authors suggested that the unsampled *L. flammea* was the likely sister species of *L. melanostoma*, but also showed that the latter included two divergent mitochondrial lineages, possibly representing cryptic species. Speciation in tropical, planktonic littorinids is predominantly an allopatric process requiring geographical isolation of at least 1200 km, although the geographical signal in the distribution of sister-species pairs of *Littoraria* is weaker than in other genera (Williams & Reid, 2004; Reid *et al.*, 2010, 2012). Members of the subgenus *Palustorina* occur mainly in mangrove and salt-marsh habitats; in addition they are restricted to continental margins and are almost entirely absent from oceanic islands (Reid, 1986, 2001; Reid *et al.*, 2010). The recorded northern limits of *L. melanostoma* are Taiwan (Reid, 1986) and Ningde, Fujian province (300 km north of Xiamen; Chen & Song, 1988). Based on these facts, and if *L. flammea* was the sister of *L. melanostoma*, it can be predicted that it may have occurred on the coast of China some distance to the north of the range limits of the latter species, possibly in salt marshes well beyond the natural northern limit of mangrove vegetation in Fujian (Lin & Wei, 1983).

We aimed to test these predictions. First, we review the available literature records and museum specimens of *L. flammea* and conclude that early material originated from Shanghai. We report that it is still living in this area and discuss possible threats to its continued existence. We describe its reproductive anatomy and radula for the first time and use molecular analysis to investigate its phylogenetic relationships. Based on this evidence, we consider its taxonomic status.

METHODS

All relevant literature was searched for references to the occurrence of *Littoraria flammea*. The collections of most major museums were examined in order to document the known specimens of *L. flammea*, and their recorded localities and dates of collection were evaluated. The institutions with material of the species are: Natural History Museum, London (NHMUK); Muséum National d’Histoire Naturelle, Paris (MNHN); Muséum d’Histoire Naturelle de Rouen (MHNR); Royal Belgian Institute of Natural Sciences, Brussels (RBINS); Senckenberg

Forschungsinstitut und Naturmuseum, Frankfurt (SMF); Löbbecke-Museum, Dusseldorf (LMD); Academy of Natural Sciences of Drexel University, Philadelphia (ANSP) and Museum of Comparative Zoology, Harvard University (MCZ).

Based on our conclusion that *L. flammea* originated from Shanghai, salt marshes in the Yangtze River estuary were searched. Twelve living specimens were collected and preserved in 100% ethanol. Six were deposited in the State Key Laboratory of Marine Environmental Science, Xiamen University, China (registration numbers MELLF 1–6) and six in NHMUK (registration numbers NHMUK 20140805 and 20140806). One male and one female were dissected to examine the reproductive anatomy and the corresponding radulae were prepared, mounted and examined by SEM as described by Reid (1986, 1996). Shells were compared with those of *L. melanostoma* from Hong Kong, from Xiamen (300 km south of the recorded northern limit of its range; Reid, 1986; Chen & Song, 1988) and from a new locality 600 km north of Xiamen. Three specimens of *L. flammea* (MELLF 1–3; corresponding to *L. flammea* 1–3 in Fig. 6) were sequenced for the two mitochondrial genes cytochrome oxidase I (COI) and 12S rRNA, and the nuclear gene 28S rRNA, using the methodology described by Reid *et al.* (2010). These sequences were added to the database of 37 *Littoraria* species published by Reid *et al.* (2010) and Bayesian phylogenetic analyses were performed in the same way, using the same outgroup, *Cenchritis muricatus*.

RESULTS

Literature review

Philippi (1847) noted simply that an unspecified number of specimens of his new species '*Littorina flammea*' were received from Largilliert and that they originated from China. Louis Largilliert was a noted shell collector in Rouen, whose collection still exists in the MHNR. He in turn received specimens from various parts of the world from his friend Admiral Jean-Baptiste Cécille (Lepage & Buffetaut, 2014). Much of this material was from China and Japan (e.g. *Echinolittorina cecillei*, named by Philippi, 1851, in honour of the admiral) and presumably included *Littoraria flammea*. Cécille was the commander of the French navy in Indo-China from 1842 to 1847, so he could have visited various localities in China during that time.

Reeve (1857: sp. 46) included *L. flammea* in his monograph of '*Littorina*' and gave the locality "Shanghai, China; Fortune". Strangely, he also described a slightly more slender shell (sp. 42) as *Littorina fortunei* from "China". Presumably both samples were collected by the plant hunter Robert Fortune, who first visited Shanghai in 1843 (Fortune, 1847) and for the last time in 1855 (Fortune, 1857). The synonymy of *Littorina fortunei* with *L. flammea* was first recognized by Weinkauff (1882). Tryon (1887: 245) also gave 'China' as the locality of *L. flammea*, but suggested that the Australian *L. luteola* was another synonym. He also mentioned that "our museum possesses specimens from Port Natal, South Africa, probably referable to this species", which is likely to be a reference to the superficially similar *L. subvittata*, a species found in Natal. Casto de Elera (1896) included *L. flammea* in a catalogue of shells from the Philippines, but his identifications are unreliable. The name was apparently not mentioned again until listed in the synonymy of '*Littorina scabra*' by Rosewater (1970).

Museum specimens

The known museum material of *L. flammea* is listed in Supplementary material, Table S1. This consists of 14 samples and a total of at least 42 specimens. The earliest possible date of collection is 1847, as indicated by the publication of the name *L. flammea* by Philippi (1847). The latest possible date of

collection is 1935, when the Dautzenberg Collection was acquired by the RBINS, but the majority of dates suggest collection around the mid-nineteenth century. All the labels indicate the locality as China, but only one (RBINS Dautzenberg Collection) is more precise, stating 'Shanghai'. A label stating 'Society & Philippine [Ids]' (NHMUK 1968309–1968311) is believed to be a later, unreliable, addition.

Collection of living material of *L. flammea* and *L. melanostoma*

On 22 August 2014, living animals of *L. flammea* were found at two sites to the north of Shanghai and the mouths of the Yangtze River. Both sites were on the open coast to the northwest of Rudong (Jiangsu province; Fig. 1), about 41 km apart: site 1 (32°32.353'N, 121°06.912'E) and site 2 (32°52.360'N, 120°55.452'E). These were broad salt marshes of native *Suaeda salsa* behind dense beds of invasive *Spartina alterniflora* (Fig. 2). The snails were found only among the stems of *Suaeda*. In total 12 specimens were collected. Other gastropods observed in the same habitat were *L. sinensis*, *Assiminea* sp. and *Ellobium chinensis*.

On 11 November 2014, six *L. aff. melanostoma* were collected in an artificially planted *Kandelia* mangrove at Ximendao, Wenzhou, Zhejiang province (28°21.071'N, 121°11.250'E). This is a new locality for the species, further north than the previously known limit at Ningde, Fujian (Chen & Song, 1988).

Shell description and comparison with *L. melanostoma*

The shells of *L. flammea* and *L. melanostoma* have been described in detail by Reid (1986: 174–182, figs 73, 74, 77), whose diagnoses are summarized here. The shell of *L. flammea* (Fig. 3A–D) is up to 20 mm tall, thin-shelled, relatively narrow and tall-spired, with convex spire profile and lightly rounded whorls. The aperture is elongate-oval and up to four varices (i.e. previous apertural lips; Fig. 3D) may be present. The sculpture consists of 16–20 slightly raised spiral ribs on the final whorl. The colour pattern is of 9–10 brown, oblique, axial stripes on the last whorl

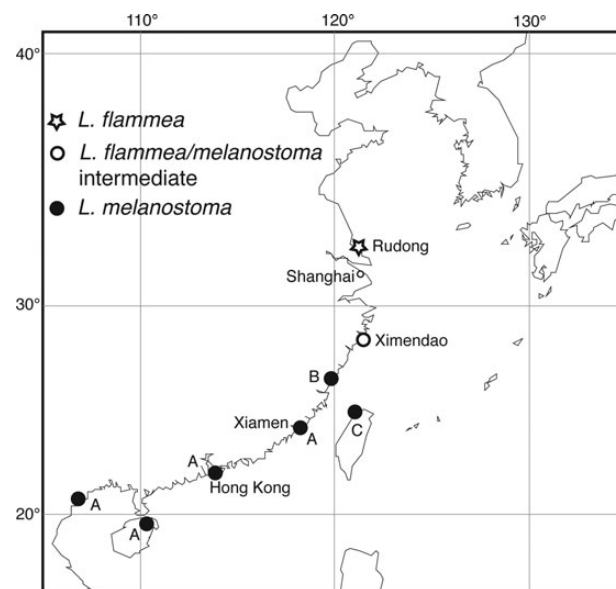


Figure 1. Distribution map showing northern part of range of *Littoraria melanostoma* (closed circles), site of rediscovery of *L. flammea* (open asterisk) and site of intermediate population (open circle). Source of records: A, Reid (1986); B, Chen & Song (1988); C, D.G. Reid (unpubl.: Bali, Taiwan).



Figure 2. Habitat of *Littoraria flammea*, showing plants of *Suaeda salsa* (foreground) and invasive *Spartina alterniflora*. Site 2, Rudong, Jiangsu province (32°52.360'N, 120°55.452'E).

and the parietal callus is pinkish brown. The newly collected material conforms to this description.

In contrast, the shell of *L. melanostoma* (Fig. 3H–K) is up to 31 mm tall, solid, with almost straight sides to the spire and almost flat whorls, slightly angled at the periphery. The aperture is relatively large and quadrangular, and varices are absent. The sculpture consists of 15–17 flat spiral ribs on the final whorl. The colour pattern consists of brown dots aligned to form narrow axial series (27–40 on the last whorl) and the parietal callus is dark purplish brown. Shells from Xiamen and Hong Kong conform to this description.

The new material of *L. aff. melanostoma* from Ximendao is intermediate in appearance (Fig. 3E–G). The shells are up to 20 mm tall, with the straighter sides, flatter whorls, slightly angled periphery, larger aperture and dark callus of *L. melanostoma*. However, the slightly raised ribs number 17–19, the shape is relatively narrow and the colour pattern is of axial stripes, all recalling *L. flammea*.

Anatomical description and comparison with *L. melanostoma*

Only a single male specimen of *L. flammea* was available and showed a penis (Fig. 4) without significant differences from that of *L. melanostoma* (Reid, 1986; fig. 75a–g). The single examined oviduct was the same as that described and illustrated by Reid (1986; figs 7, 8), with 6.5 whorls in a spiral, including albumen and large capsule glands, indicating oviparous development. The radula too was similar to that of *L. melanostoma* (Fig. 5; cf. Reid, 1986; fig. 74b, allowing for different cusp orientation).

Molecular phylogeny

Two specimens of *L. flammea* were successfully sequenced for COI, 12S and 28S, and a third for the two mitochondrial genes only (GenBank accession numbers: COI, KP219882–KP219884; 12S, KP219877–KP219879; 28S, KP219880, KP219881). In the analysis of COI sequences, the three of *L. flammea* formed a highly supported clade (posterior probability PP = 1) together with a single sequence of *L. melanostoma* from northern Vietnam (Fig. 6A), from which they differed in 0, 1 and 2 base substitutions (average Kimura 2-parameter K2P distance = 0.2%). This clade was nested within the monophyletic (PP = 1) subgenus *Palustorina*. Analysis of 12S sequences showed a similar result, *L. flammea* grouping closely with *L. melanostoma* from northern Vietnam (PP = 0.98; 0–2 base substitutions; Fig. 6B). However, as in the corresponding analysis by Reid et al. (2010) there was no

support for a clade of all *Palustorina* species. In the 28S analysis (Fig. 6C), the two sequences of *L. flammea* formed a well-supported clade (PP = 1) with two sequences of *L. melanostoma*, from northern Vietnam and from Singapore, respectively, but clustered most closely with the latter (PP = 0.96). There were six fixed base differences between the two 28S sequences of *L. melanostoma* and those of *L. flammea*, while the latter differed in two positions.

DISCUSSION

Historical records

In total, 42 specimens of *Littoraria flammea* have been located in museums in Europe and the United States, of which the latest possible date of collection is 1935 (Supplementary material, Table S1). The only material associated with a known collector is that in the Largillier collection received from Cécille between 1842 and 1847, and that in the Cuming collection sent by Fortune between 1843 and 1855. The only reliable locality is Shanghai, which has been overlooked since it was recorded by Reeve (1857). The same locality is repeated on a single museum sample; all other records in museums and the literature refer simply to 'China'. Shanghai hardly qualifies as a remote location and, as one of the greatest commercial and shipping centres, it has undoubtedly suffered environmental damage, supporting the supposition of Carlton (1993) that the species was extinct. Nevertheless, it is still extant.

Rediscovery and conservation concern

The sites of collection lie about 100 km north of the centre of Shanghai and at the northern edge of the extensive mud flats of the Yangtze estuary. Only 12 specimens were collected in total, at two sites 41 km apart, so the abundance, typical habitat and conservation status of the population are unknown. The pallial oviduct indicates that *L. flammea* produces egg capsules, which (as in all other oviparous members of the genus; Reid, 1986) are likely to be pelagic. Also by analogy with its congeners (Reid, 1986; Reid et al., 2010), a long planktotrophic larval life of 3 to 10 weeks is likely. Despite this potential for wide dispersal, some *Littoraria* species do have narrow distributions. For example, the morphologically defined subspecies *L. cingulata pristissimi* is restricted to Shark Bay in Western Australia, perhaps limited by local availability of its mangrove and salt-marsh habitat (Reid, 1986). In tropical northwestern Australia, mangrove-dwelling *L. ianthostoma* is restricted to Joseph Bonaparte Gulf, for unknown reasons (Stuckey & Reid, 2002). It is important to establish the geographical extent of the *L. flammea* population or populations.

Meanwhile, there is cause for concern. Mangrove and salt-marsh habitats are under global threat, especially from land reclamation and pollution in the vicinity of large cities (Valiela, Bowen & York 2001; Bromberg Gedan, Silliman & Bertness, 2009). A more specific threat is faced by salt marshes in China, where the highly invasive grass *Spartina alterniflora* is colonizing bare mudflats and replacing the endemic *Phragmites* and *Scirpus* (Li et al., 2009). In the southeastern USA, native *S. alterniflora* salt marshes are the typical habitat of another littorinid, *L. irrorata*, which grazes on senescent plant material and associated fungi (Silliman & Newell, 2003). Furthermore, *L. melanostoma* is found on *Fimbristylis* grass as well as on mangrove trees in Malaysia (Berry & Chew, 1973) and has been recorded on both *Spartina* and on native mangroves in Fujian province (Feng et al., 2014). Nevertheless, no *L. flammea* were observed on *Spartina* at the two sites near Rudong. There have been several studies of the effects of the invasion by *Spartina* on the native salt-marsh invertebrates of the Yangtze River (e.g. Xie et al., 2007) but none has mentioned *L. flammea*. We have found one record of

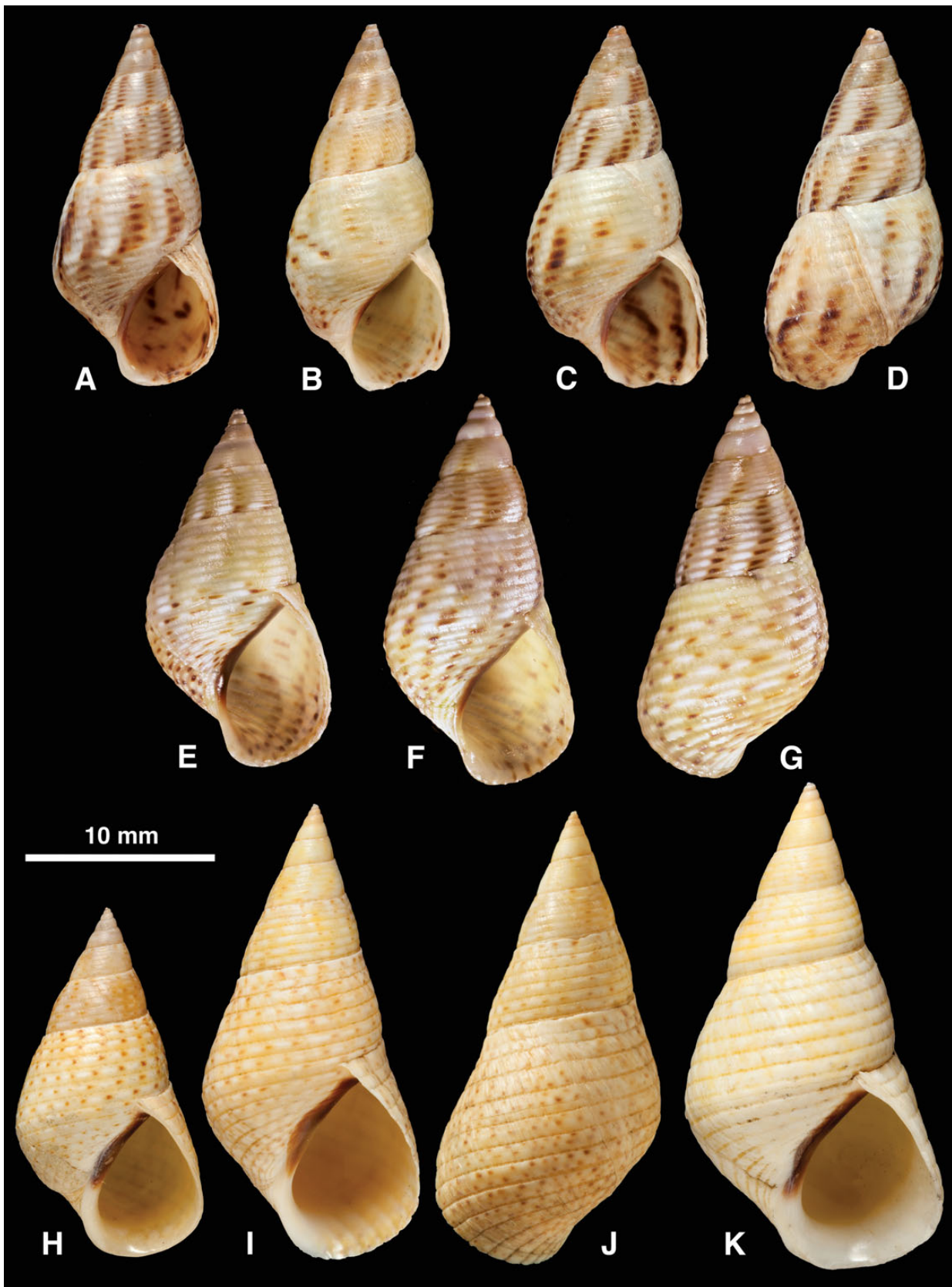


Figure 3. Shells of *Littoraria flammea* (A–D), *L. melanostoma* (H–K) and intermediates (E–G). **A.** Site 2, Rudong, Jiangsu province (NHMUK 20140806). **B–D.** Site 1, Rudong, Jiangsu province (NHMUK 20140805). **E–G.** Ximendao, Wenzhou, Zhejiang province (MELLF). **H.** Xiamen, Fujian province (NHMUK 20140807). **I–K.** Three Fathoms Cove, Hong Kong (NHMUK 20140808).

L. melanostoma from the Yangtze estuary (Chongming I., 121.5°E, 31.5°N; An, 2011), which we suggest probably refers to *L. flammea*. A study of the fauna associated with *Spartina* in Jiangsu province recorded only *L. 'scabra'* (probably a misidentification of *L. sinensis*) (Xie & Gao, 2009). This suggests that *L. flammea* could be scarce or localized, and could indicate that *Spartina* does not provide a suitable habitat.

Phylogeny and species status

The characters of the newly reported penis, pallial oviduct and radula of *L. flammea* do not differ from those of *L. melanostoma*, supporting the suggestion that these are closely related species

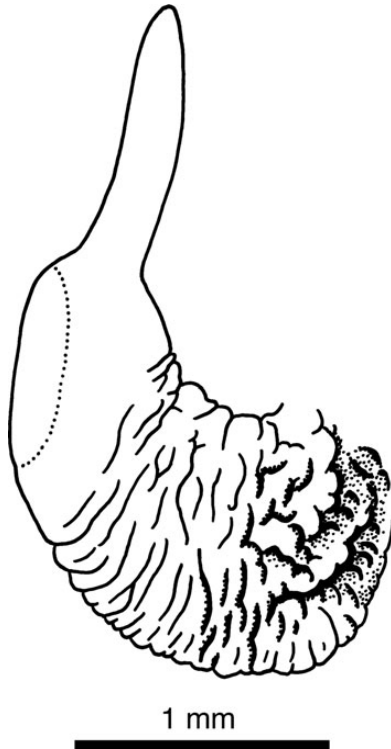


Figure 4. Penis of *Littoraria flammea*, site 1, Rudong, Jiangsu province (NHMUK 20140783). Shell height = 16.1 mm.

in the subgenus *Palustorina* (Reid, 1986). Previously, separation of the two species was based on their distinctive shells; *L. melanostoma* was then known from as far north as Xiamen (formerly Amoy), where shells were “entirely typical”, so that despite some similarities “it does not at present seem possible that *L. flammea* could be a form of *L. melanostoma* . . . [which] is a species of constant characters throughout its rather wide range” (Reid, 1986: 182). The new discovery of *L. aff. melanostoma* from a locality between Xiamen and Shanghai, which is also intermediate between typical shells of *L. melanostoma* and *L. flammea*, casts doubt on this statement.

A molecular phylogenetic analysis of 37 of the 39 known *Littoraria* species revealed two deeply divergent mitochondrial lineages within *L. melanostoma* (K2P for COI = 10.48%, cf. 4.15% for the closest pair of sister species), suggesting the presence of two cryptic species (Reid et al., 2010). In that study the two lineages were each represented by a single individual. Sequencing of additional specimens has confirmed the separation of a mitochondrial clade from China and northern Vietnam from another in the southern South China Sea and Andaman Sea, but this separation was not supported by morphological characters or analysis of 28S sequences (T.P.T. Ng & D.G. Reid, unpublished data). Against this background, the negligible mitochondrial differentiation between *L. flammea* and an individual of *L. melanostoma* from northern Vietnam (K2P for COI = 0.2%) provides no evidence for their taxonomic separation. The 28S analysis, on the other hand, clustered *L. flammea* with *L. melanostoma* from Singapore. Since 28S is a slowly-evolving gene (e.g. Reid et al., 2010) and exhibits numerous but only slightly different genotypes in *L. melanostoma* (T.P.T. Ng & D.G. Reid, unpublished data), the significance of this clustering is doubtful. It should also be noted that at least in one littorinid sister-species pair (*Echinolittorina millegrana* and *E. reticulata*), 28S gave a more reliable phylogenetic separation than COI (Reid et al., 2006). The available molecular evidence, therefore, is equivocal on the species status of *L. flammea* and further investigation, using additional genetic markers, is required.

If *L. flammea* does prove to be a distinct species (or even an isolated population), its geographical location is of interest. The prevailing mode of speciation in tropical littorinids is allopatric (Williams & Reid, 2004; Reid et al., 2010, 2012), so geographical separation from its undoubted closest relative, *L. melanostoma*, would be expected. The coast of China is recognized as an area of high endemism, especially among gastropods of mangrove

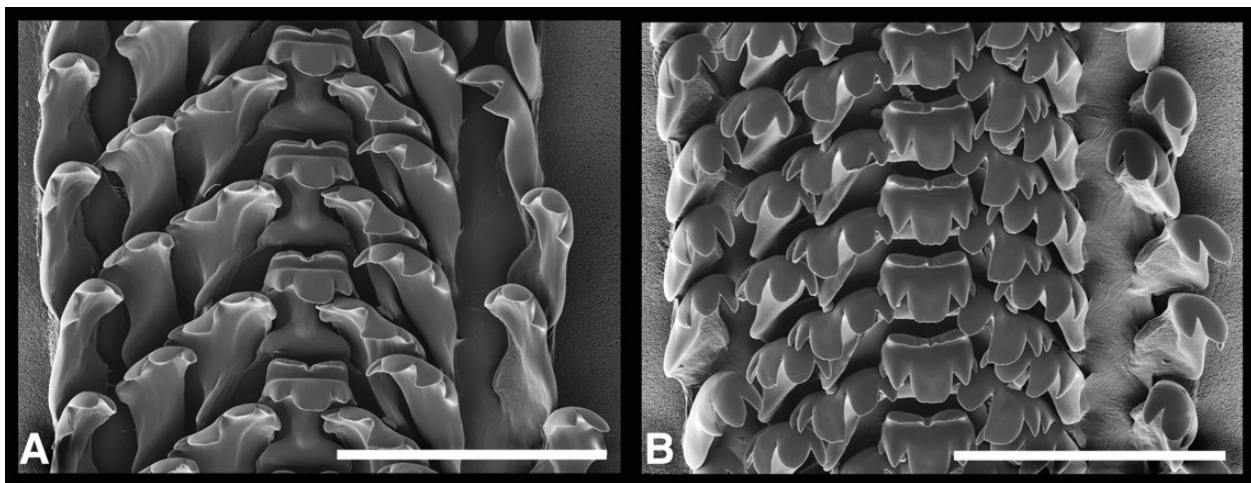


Figure 5. SEM of radula of *Littoraria flammea*, site 2, Rudong, Jiangsu province. Shell height = 15.4 mm (NHMUK 20140806). **A.** Flat view. **B.** 45° view of same specimen.

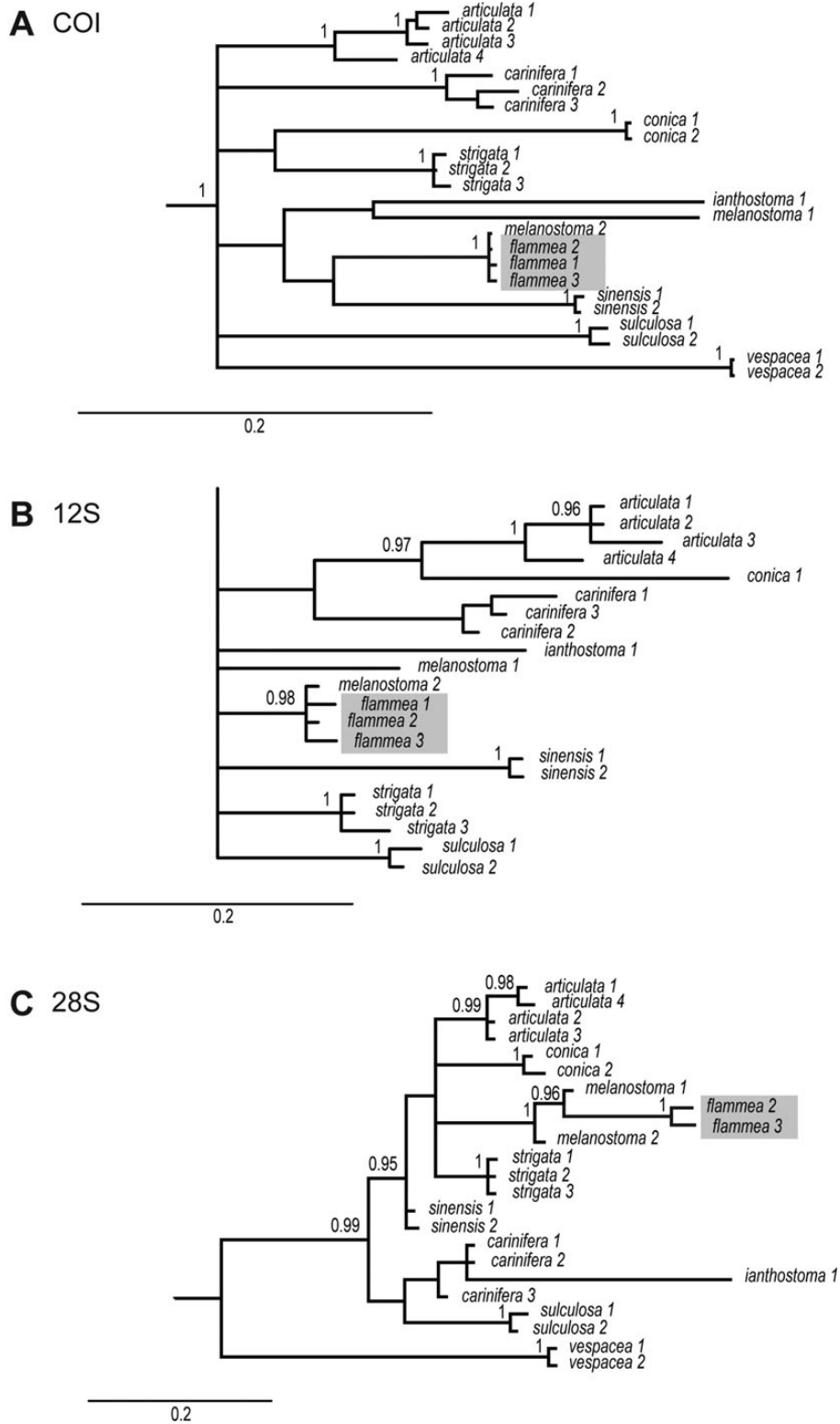


Figure 6. Molecular phylogeny of *Littoraria* species produced by Bayesian analysis of sequences of the mitochondrial COI (**A**), mitochondrial 12S (**B**) and nuclear 28S rRNA (**C**) genes, using dataset of Reid *et al.* (2010) with addition of new sequences from *Littoraria flammea*. Only the subgenus *Palustorina* is shown (tree topologies otherwise as in Reid *et al.*, 2010: fig. 1). Support values are Bayesian posterior probabilities. *L. melanostoma* 1 is from Singapore and *L. melanostoma* 2 from Ha Long Bay, northern Vietnam.

and mudflat habitats (Reid *et al.*, 2013; Reid & Claremont, 2014; Ozawa *et al.*, 2015) On the other hand, the finding of a geographically and morphologically intermediate population of

L. aff. melanostoma at Ximendao suggests that *L. melanostoma* (in Fujian and southwards) and *L. flammea* (near Shanghai) could be the extremes of a cline. Clines in shell form are known in

other *Littoraria* species (Reid, 1986, 2001; Reid et al., 2010), but their genetic structure remains to be investigated.

In conclusion, *L. flammea* is unquestionably no longer to be listed as an 'unequivocal' extinction (Dulvy et al., 2003). However, its habitat appears to be threatened and its status as a distinct species is unconfirmed. It was lost, is now found and is probably threatened—but perhaps it is not a distinct species after all.

There may, however, yet be a final twist in this story. The type locality of *L. melanostoma* (Gray, 1839) is Penang, on the Andaman Sea coast of Malaysia (Reid, 1986). If further molecular studies confirm that its two divergent mitochondrial clades are distinct species, the name *L. melanostoma* will be applied to the clade in the southern South China Sea and Andaman Sea. Then, if *L. flammea* and the clade of *L. 'melanostoma'* from southern China and northern Vietnam prove to be conspecific, the valid name for this species will be *L. flammea* (Philippi, 1847)—so a taxonomic resurrection could await.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *Journal of Molluscan Studies* online.

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REFERENCES

- AN, C. 2011. *The ecological investigation of macrobenthic communities in the intertidal zone of the Yangtze River estuary*. PhD thesis, Normal University of East China, Shanghai.
- BERRY, A.J. & CHEW, E. 1973. Reproductive systems and cyclic release of eggs in *Littorina melanostoma* from Malayan mangrove swamps (Mollusca: Gastropoda). *Journal of Zoology*, **171**: 333–344.
- BROMBERG GEDAN, K., SILLIMAN, B.R. & BERTNESS, M.D. 2009. Centuries of human-driven change in salt marsh ecosystems. *Annual Review of Marine Science*, **1**: 117–141.
- CAI, R. & HUANG, W. (eds) 1989. *Fauna of Zhejiang: mollusks*. Zhejiang Science and Technology Publishing House, Hangzhou.
- CARLTON, J.T. 1993. Neoeinctions of marine invertebrates. *American Zoologist*, **33**: 499–509.
- CARLTON, J.T., GELLER, J.B., REAKA-KUDLA, M.L. & NORSE, E.A. 1999. Historical extinctions in the sea. *Annual Review of Ecology and Systematics*, **30**: 515–538.
- CARLTON, J.T., VERMEIJ, G.J., LINDBERG, D.R., CARLTON, D.A. & DUDLEY, E.C. 1991. The first historical extinction of a marine invertebrate in an ocean basin: the demise of the eelgrass limpet *Lottia alveus*. *Biological Bulletin*, **180**: 72–80.
- CASTO DE ELERA, R.P. 1896. *Catálogo Sistemático de Toda la Fauna de Filipinas, vol. 3. Moluscos y radiados*. Colegio de Santo Tomás, Manila.
- CHEN, P. & SONG, Z. 1988. Preliminary investigation on ecology of molluscs in the intertidal zone north to Minjiang River, Fujian. *Journal of Oceanography in Taiwan Strait*, **7**: 180–188.
- DULVY, N.K., PINNEGAR, J.K. & REYNOLDS, J.D. 2009. Holocene extinctions in the sea. In: *Holocene extinctions* (S.T. Turvey, ed.), pp. 129–150. Oxford University Press, Oxford.
- DULVY, N.K., SADOVY, Y. & REYNOLDS, J.D. 2003. Extinction vulnerability in marine populations. *Fish and Fisheries*, **4**: 25–64.
- FAN, C. (ed.) 1989. *Mollusca of Huanghai and Bohai*. Nongye Chubanshe, Agricultural Publishing House, Beijing.
- FENG, J., GUO, J., QIAN, H., JIANG, J., HUANG, G., YANG, Z. & LIN, G. 2014. Changes in the community structure and diet of benthic macrofauna in invasive *Spartina alterniflora* wetlands following restoration with native mangroves. *Wetlands*, **34**: 673–683.
- FLORENS, F.B.V. & BAIDER, C. 2007. Relocation of *Omphalotropis plicosa* (Pfeiffer, 1852), a Mauritian landsnail believed extinct. *Journal of Molluscan Studies*, **73**: 205–206.
- FORTUNE, R. 1847. *Three years' wanderings in the northern provinces of China*. John Murray, London.
- FORTUNE, R. 1857. *A residence among the Chinese: inland, on the coast, and at sea*. John Murray, London.
- HOBDDAY, A.J., TEGNER, M.J. & HAAKER, P.L. 2001. Over-exploitation of a broadcast spawning marine invertebrate: decline of the white abalone. *Reviews in Fish and Fisheries*, **10**: 493–514.
- HOLLANDER, J., SMADJA, C.M., BUTLIN, R.K. & REID, D.G. 2013. Genital divergence in sympatric sister snails. *Journal of Evolutionary Biology*, **26**: 210–215.
- HOLM, G. 2002. Collecting *Lottia alveus* (Conrad, 1831) in Boundary Bay, British Columbia. *Dredgings*, **42**: 1–4. Also see footnote: www.bily.com/pnwsc/web-content/Articles Accessed 13 February 2015.
- IUCN. 2014. The IUCN Red List of threatened species. Version 2014.3. www.iucnredlist.org. Accessed 27 November 2014.
- LEPAGE, Y. & BUFFETAUT, E. 2014. *Echinolittorina cecillei* (Philippi, 1851): l'histoire d'une petite coquille marine et ses protagonistes. *Bulletin Sciences et Géologie Normandes*, **7**: 41–61.
- LI, B., LIAO, C., ZHANG, X., CHEN, H., WANG, Q., CHEN, Z., GAN, X., WU, J., ZHAO, B., MA, Z., CHENG, X., JIANG, L. & CHEN, J. 2009. *Spartina alterniflora* invasions in the Yangtze River estuary, China: an overview of current status and ecosystem effects. *Ecological Engineering*, **35**: 511–520.
- LI, R. & HUANG, Z. 2012. Class Gastropoda. In: *The living species and their illustrations in China's seas (part II). An illustrated guide to species in China's sea*. Vol. 4 (Z. Huang & M. Liu, eds), pp. 102–279. Ocean Press, Beijing.
- LIN, P. & WEI, X. 1983. Ecological notes on the mangroves of Fujian, China. *Tasks for Vegetation Science*, **8**: 31–36.
- LINDBERG, D.R. 1984. A recent specimen of *Collisella edmitchelli* from San Pedro, California (Mollusca: Acmaeidae). *Bulletin of the Southern California Academy of Sciences*, **83**: 148–151.
- MA, X. 2004. Order Mesogastropoda. In: *Seashells of China* (Z. Qi, ed.), pp. 31–81. China Ocean Press, Beijing.
- MIURA, O., TORCHIN, M.E. & BERMINGHAM, E. 2010. Molecular phylogenetics reveals differential divergence of coastal snails separated by the Isthmus of Panama. *Molecular Phylogenetics and Evolution*, **56**: 40–48.
- MONTE-LUNA, P. DEL, LLUCH-BELDA, D., SERVIERE-ZARAGOZA, E., CARMONA, R., REYES-BONILLA, H., AURIOLES-GAMBOA, D., CASTRO-AGUIRRE, J.L., GUZMÁN DEL PRÓO, S.A., TRUJILLO-MILLÁN, O. & BROOK, B.W. 2007. Marine extinctions revisited. *Fish and Fisheries*, **8**: 107–122.
- OZAWA, T., YIN, W., FU, C., CLAREMONT, M., SMITH, L. & REID, D.G. 2015. Allopatry and overlap in a clade of snails from mangroves and mud flats in the Indo-West Pacific and Mediterranean (Gastropoda: Potamididae: *Cerithiideopsis*). *Biological Journal of the Linnean Society*, **114**: 212–228.
- PHILIPPI, R.A. 1847–1850. *Abbildungen und Beschreibungen neuer oder wenig gekannter Conchylien*. Vol. 3. Theodor Fischer, Cassel.
- PHILIPPI, R.A. 1851. Centuria quinta testaceorum novorum. *Zeitschrift für Malakozoologie*, **8**: 74–96, 123–126.

- REEVE, L.A. 1857–1858. Monograph of the genus *Littorina*. In: *Conchologia iconica*, Vol. 10 (L.A. Reeve, ed.), pls 1–18. L.A. Reeve, London.
- 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. 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. 2001. New data on the taxonomy and distribution of the genus *Littoraria* Griffith and Pidgeon, 1834 (Gastropoda: Littorinidae) in Indo-West Pacific mangrove forests. *Nautilus*, **115**: 115–139.
- REID, D.G. 1996. *Systematics and evolution of Littorina*. Ray Society, London.
- REID, D.G. & CLAREMONT, M. 2014. The genus *Cerithideopsis* Thiele, 1929 (Gastropoda: Potamididae) in the Indo-West Pacific region. *Zootaxa*, **3779**: 61–80.
- REID, D.G., CLAREMONT, M., SMITH, L., SHAMOTO, M., GLAUBRECHT, M. & OZAWA, T. 2013. Mosaics in the mangroves: allopatric diversification of tree-climbing mudwhelks (Gastropoda: Potamididae: *Cerithidea*) in the Indo-West Pacific. *Biological Journal of the Linnean Society*, **110**: 564–580.
- REID, D.G., DYAL, P. & WILLIAMS, S.T. 2010. Global diversification of mangrove fauna: a molecular phylogeny of *Littoraria* (Gastropoda: Littorinidae). *Molecular Phylogenetics and Evolution*, **55**: 185–201.
- REID, D.G., DYAL, P. & WILLIAMS, S.T. 2012. A global molecular phylogeny of 147 periwinkle species (Gastropoda, Littorininae). *Zoologica Scripta*, **41**: 125–136.
- REID, D.G., LAL, K., MACKENZIE-DODDS, J., KALIGIS, F., LITTLEWOOD, D.T.J. & WILLIAMS, S.T. 2006. Comparative phylogeography and species boundaries in *Echinolittorina* snails in the central Indo-West Pacific. *Journal of Biogeography*, **33**: 990–1006.
- ROBERTS, C.M. & HAWKINS, J.P. 1999. Extinction risk in the sea. *TREE*, **14**: 241–245.
- ROSEWATER, J. 1970. The family Littorinidae in the Indo-Pacific. Part I. The subfamily Littorininae. *Indo-Pacific Mollusca*, **2**: 417–506.
- SILLIMAN, B.R. & NEWELL, S.Y. 2003. Fungal farming in a snail. *Proceedings of the National Academy of Sciences of the USA*, **100**: 15643–15648.
- STUCKEY, M. & REID, D.G. 2002. A new *Littoraria* (Gastropoda: Littorinidae) from northwestern Australia. *Molluscan Research*, **22**: 1–15.
- TRYON, G.W. 1887. *Manual of conchology*, Vol. 9. Philadelphia.
- VALIELA, I., BOWEN, J.L. & YORK, J.K. 2001. Mangrove forests: one of the world's threatened major tropical environments. *BioScience*, **51**: 807–815.
- VERMEIJ, G.J. 1993. Biogeography of recently-extinct marine species: implications for conservation. *Conservation Biology*, **7**: 391–397.
- WEINKAUFF, H.C. 1878, 1882. Die Gattung *Littorina*. In: *Systematisches Conchylien-Cabinet von Martini und Chemnitz* (parts 269, 315, 318) (H.C. Küster, W. Kobelt & H.C. Weinkauff, eds), pp. 25–114. Bauer & Raspe, Nürnberg.
- WILLIAMS, S.T. & REID, D.G. 2004. Speciation and diversity on tropical rocky shores: a global phylogeny of snails of the genus *Echinolittorina*. *Evolution*, **58**: 2227–2251.
- XIE, W. & GAO, S. 2009. The macrobenthos in *Spartina alterniflora* salt marshes of the Wanggang tidal-flat, Jiangsu coast, China. *Ecological Engineering*, **35**: 1158–1166.
- XIE, Z., ZHANG, F., LIU, W. & LU, J. 2007. The community structure of benthic macroinvertebrates associated with *Spartina alterniflora* in the Yangtze Estuary, in China. *Zoological Research*, **28**: 167–171.