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Evaluating morphological species recognition in fossil and modern gastropods (Littorinidae, periwinkles)

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Article

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Abstract

Species recognition is an essential part of biological and paleontological study. In gastropods, although species are genetic entities, shell morphology continues to be used as the primary source of information to recognize most species. While there are few directly tested cases, variations in conchological characters for modern species are expected to reflect underlying genetic differences that define a biological species, an assumption that is also applied to identify species in the fossil record. Additionally, how consistently shell shape differentiates gastropod species remains poorly understood. In this study, shell shape of Recent and Pliocene–Pleistocene fossil specimens of well-known intertidal gastropods (Littorinidae, periwinkles: †*Littorina petricola*, *Littorina keenae*, and the sister-species pair *Littorina plena* and *Littorina scutulata*) from the east Pacific was analyzed using landmark-based morphometrics and compared with published molecular data. For the extant species, there is a general positive relationship between shell shape and genetic differences. Discriminant function analyses indicate distantly related species can be more reliably recognized from their shells, while closely related species have a higher error. Fossils and recent specimens were classified with similar consistency. More work is needed to illuminate whether this case applies more widely.

Non-technical Summary

Recognizing biological species is important—so much information is unlocked when you can find out the name of what you are observing. Part of identifying a fossil to species is using its shape. We assume visible shape differences indicate invisible genetic differences (not knowable for most fossils), which seems to be true for many living animals. Using marine snails (periwinkles), we find that species are indeed different in shell shape and genetics from each other. We also see that fossil and modern snails can be identified with similar consistency using their shells. These results give us more confidence when identifying species using their shapes.

Introduction

Species are fundamental biological units, whose accurate identification underlies much of ecological and evolutionary investigation. However, there is continued discussion of "the species problem," as exemplified by a recent survey that suggested that "two randomly chosen respondents will most likely disagree on the nature of species" and use different concepts, largely depending on their research discipline and whether they focus on the micro- or macroevolutionary scale (Stankowski and Ravinet 2021: R428). There are at least two distinct parts to the issue of "the nature of species": conceptualizing what a species is in theory and the practical challenge of species recognition (Allmon 2013, 2016), both of which are often obscured by the widely applied but frequently undefined term "species" (e.g., Struck et al. 2018; Shin and Allmon 2023). A frequently cited key element in species definition is reproductive isolation (after the biological species concept [Mayr 1942]), which might be defined as "a quantitative measure of the effect of genetic differences on gene flow" (Westram et al. 2022). However, reproductive isolation may be difficult to measure. As a result, generally only taxa suspected or known to have non-morphological distinguishing features (e.g., chemical or behavioral differences [Knowlton 1993; Bickford et al. 2007]) might be investigated in detail for an additional suite of data such as phenotypic, life-history, or ecological aspects that might also be considered to aid species discrimination, although this may often be outside the scope of non-taxonomic work. In this paper, we treat species as genetically and phenotypically distinct independent lineages (after the evolutionary species concept [Simpson 1951; Barraclough 2019]) and concentrate on species recognition, which considers the types of information and methods used to delineate species.

In practice, many extant species have been, and continue to be, described based on morphological characters. For organisms only known from fossils or fossil specimens of extant taxa,

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species recognition is based largely on morphology (such species are sometimes called "morphological species" or "morphospecies"), in addition to considering biogeographic and geological age information. In the study of morphospecies, a certain degree of morphological distinction is assumed to mirror species-level genetic differences ("good species" [Allmon 2016]), and morphological variation and features of living species are assumed to similarly apply to fossils (following uniformitarianism).

The correspondence between reproductively isolated species identified in modern and fossil morphological species has only been explored in a few case studies, notably in the widely cited textbook example of bryozoans by Jackson and Cheetham (1990, 1994; included in Foote and Miller [2006]; see discussion in Allmon [2016] and Shin and Allmon [2023]). In their study, differences in allozyme variation and skeletal features of extant colonies were found to have a statistically significant positive correlation value (~0.7, $p < 0.05$; see Jackson and Cheetham [1994: fig. 4, table 3]). Additionally, from statistical analyses, the morphological classification of Recent and fossil colonies was almost the same as the genetic-based designations (for species with available data, 0.4% error [Jackson and Cheetham 1994: table 2]). Other studies have found that dependence on skeletal morphology generally leads to success in recognizing species in fossil and extant specimens of several taxa, although with variable uncertainty (e.g., gastropods [Michaux 1989]; corals [Budd and Pandolfi 2004]; crinoids [Purens 2016]; brachiopods [López Carranza and Carlson 2019, 2021]; crocodilians [Brochu and Sumrall 2020]).

A major concern with relying on morphology to distinguish between species is that there are potential areas for inaccuracy, such as for genetically distinct species that do not have any diagnostic morphology ("cryptic species" or "cryptic species *sensu stricto*," after Chenuil et al. [2019]; definitions vary, see review by Shin and Allmon [2023]) to be overlooked and not counted or for one phenotypically variable species to be identified as multiple species, leading to more species recognized than are present (taxonomic "oversplitting"). Morphologically identical or similar species are often associated with closely related taxa (e.g., "species complexes") that may have recently diverged, but can also occur for species that are millions of years old due to evolutionary stasis (e.g., Cerca et al. 2019), as well as in distantly related taxa from parallel or convergent evolution (Struck et al. 2018). A lack of morphological differences in diverged species has sometimes been attributed to ecological or environmental influences (e.g., environmental preferences can differentiate sympatric taxa; the same morphology adaptive to a particular environment could occur in separate taxa), or if species may be differentiated based on systems (e.g., behavioral or chemical recognition among individuals, timing of life-history events) that are not reflected in their external morphology. Imprecise species recognition may obscure biodiversity metrics and understanding of evolutionary modes and rates through time (e.g., Bickford et al. 2007; Allmon and Smith 2011; Fišer et al. 2018; Struck et al. 2018; Monro and Mayo 2022).

To address this issue, two main approaches have been taken: reviewing the number or information of nominal, described species compared with evidence for cryptic species at varying taxonomic scales or contrasting genetic with morphological classifications for incongruities, usually among related species or genera (e.g., for particular bryozoans [Jackson and Cheetham 1994]; gastropods [Puillandre et al. 2010]; and corals [Sheets et al. 2018]; few studies have been at a global scale, e.g., planktonic foraminifera [Morard et al. 2024]). Taxonomic reviews may include analyses at a class level (e.g., rotifers, where multiple mechanisms toward reproductive isolation are identified [Kordbacheh et al. 2023]), among orders (e.g., from data on multiple insect orders there may be ~3.1 cryptic species per insect morphospecies [Li and Wiens 2023]), clades (e.g., marine gastropods, with ~2% to 30% of species being cryptic with variable confidence in cryptic species status [Shin and Allmon 2023]), and habitats (e.g., <1% nominal marine metazoan species have cryptic species [Cahill et al. 2024]). The degree of inaccuracy for species recognition does not seem to be consistent and may be group specific, based on a combination of methodological (e.g., sampling, taxonomic practice, scientific history) and biological factors (e.g., reproductive mode, type of fertilization [Pérez-Ponce de León and Poulin 2016; Shin and Allmon 2023; Cahill et al. 2024]).

For gastropods, conchological characters (e.g., shell shape, sculpture, and color pattern) remain the primary source of taxonomic information for species description (e.g., Bieler 1992). Indeed, most available material in collections is dry shell specimens, from which genetic measures may not be possible. For marine gastropods, there are 32,000 to 40,000 described species, a number estimated to only be 23% to 32% of their total diversity (Appeltans et al. 2012). Despite the large number of species, marine gastropods have had relatively few cryptic species reported (Knowlton 1993; Pfenninger and Schwenk 2007; Pérez-Ponce de León and Poulin 2016; Chenuil et al. 2019; Shin and Allmon 2023; Cahill et al. 2024), perhaps even less than expected (as modeled by Cahill et al. 2024). A review of extant marine gastropods with adult shells found that while most studies used genetic analyses to separate species, they did not find very many cryptic species *sensu stricto*, perhaps suggesting a similarly high proportion of these species could be reliably identified from morphology, including fossils (Shin and Allmon 2023).

The present study evaluates the consistency of species identification using shell shape, and examines the relationship between shell shape and genetic differences for a set of closely related marine gastropods (Littorinidae, periwinkles: *Littorina* Férussac, 1822). As part of a well-studied extant genus (e.g., Reid 1989, 1996; McQuaid 1996a, b; Rolán-Alvarez et al. 2015; Johannesson et al. 2024), east Pacific *Littorina* provide an opportunity to test gastropod species recognition on both modern and fossil specimens. While all living *Littorina* species are anatomically distinct, there are some species that can be difficult to differentiate from one another based on their shell morphology alone, leading to potential identification error. There may also not be consistent conchological features to diagnose species (e.g., high phenotypic variability due to ecological differentiation or spatial separation, as reviewed in Johannesson et al. [2024]), and shells may appear similar between species (e.g., between the North Atlantic sister species *L. obtusata* (Linnaeus, 1758) and *L. fabalis* (W. Turton, 1825), which are most reliably differentiated by male reproductive organs), or are highly variable (e.g., between the North Atlantic direct developer *L. saxatilis* (Olivi, 1792) and its egg-laying relative *L. arcana* Hannaford-Ellis, 1978; Reid 1996), contributing to misidentification.

From analyses of *Littorina* species with similar shell morphology, estimates of species misclassification vary widely depending on the species compared (e.g., up to ~15% for *L. saxatilis* and its close relatives [Caley et al. 1995; Conde-Padín et al. 2007]; up to 53% between east Pacific *L. plena* A. Gould, 1849 and *L. scutulata* A. Gould, 1849 [Chow 1987]). Nonetheless, there is concordance when anatomical and shell characters and molecular metrics are used in phylogenetic analyses of extant species (Reid 1990, 1996; Reid et al. 1996, 2012). We therefore expect that *Littorina* species

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will be largely recognizable using shell shape and that these distinguishable shell shapes correspond with genetic differences.

Materials and Methods

Case Study: East Pacific Intertidal Littorinidae

The fossil record of *Littorina* is relatively scarce, with the oldest certain *Littorina* fossil dating from the Oligocene (†*L. sookensis* B. L. Clark & Arnold, 1923, British Columbia, Canada). For extant species with a fossil record, most are known from the Pliocene and Pleistocene (Reid 1996). In this study, four co-occurring east Pacific species were chosen: the extinct †*Littorina petricola* Arnold, 1908, and the extant *L. keenae* Rosewater, 1978, *L. plena*, and *L. scutulata* (Figs. 1, 2). These species were chosen because they have relatively abundant and available fossil material and are sympatrically distributed, and the relationship between shell shape variation and species relatedness can be investigated.

†*Littorina petricola* is only found from the Pliocene of Oregon and California, USA. Although little studied since its description (see Keen and Bentson 1944; Groves and Squires 2021), †*L. petricola* is thought to be more closely related to the north Pacific *L. squalida* Broderip & G. B. Sowerby I, 1829, which is recorded from the Miocene, than to other east Pacific species (Reid 1996). While genetic data are not available for this species, †*L. petricola* is included to compare morphologies with extant species. For the three living species, morphology and molecular analyses have found that *L. keenae* is distantly related to *L. scutulata* and *L. plena*, which are sister species (Murray 1979; Mastro et al. 1982; Reid 1996; Reid et al. 1996, 2012). *Littorina keenae* is the oldest extant species of the genus, with an estimated divergence from other congenerics in the Eocene, although its fossils are only found from the Pleistocene onward, while *L. plena* and *L. scutulata* are a sister-species pair that diverged in the Miocene (Reid 1996; Reid et al. 2012). All three extant species have planktotrophic larvae and overlap in different intertidal zones, although each species may have its preferences (*L. keenae* on exposed high shores, *L. plena* in sheltered habitats, and *L. scutulata* on exposed coasts [Behrens Yamada 1992; Reid 1996; Rugh 1997; Hohenlohe 2002, 2003a, b]). When conchologically compared with these extant species, †*L. petricola* can be distinguished by commonly exhibiting surface reticulation, defined whorls, a thicker shell, and no flattened parietal area, which is distinctive in *L. keenae* (Reid 1996).

While the species status of these extant *Littorina* are clearly established (Fig. 2), there has been documented inaccuracy when sister species *L. plena* and *L. scutulata* are identified based only on their shells, as no single conchological feature, discrete or continuous, can reliably differentiate all individuals of the two species (Supplementary Table 1). Previous work has quantified the percentage of individuals likely misidentified based on shell shape

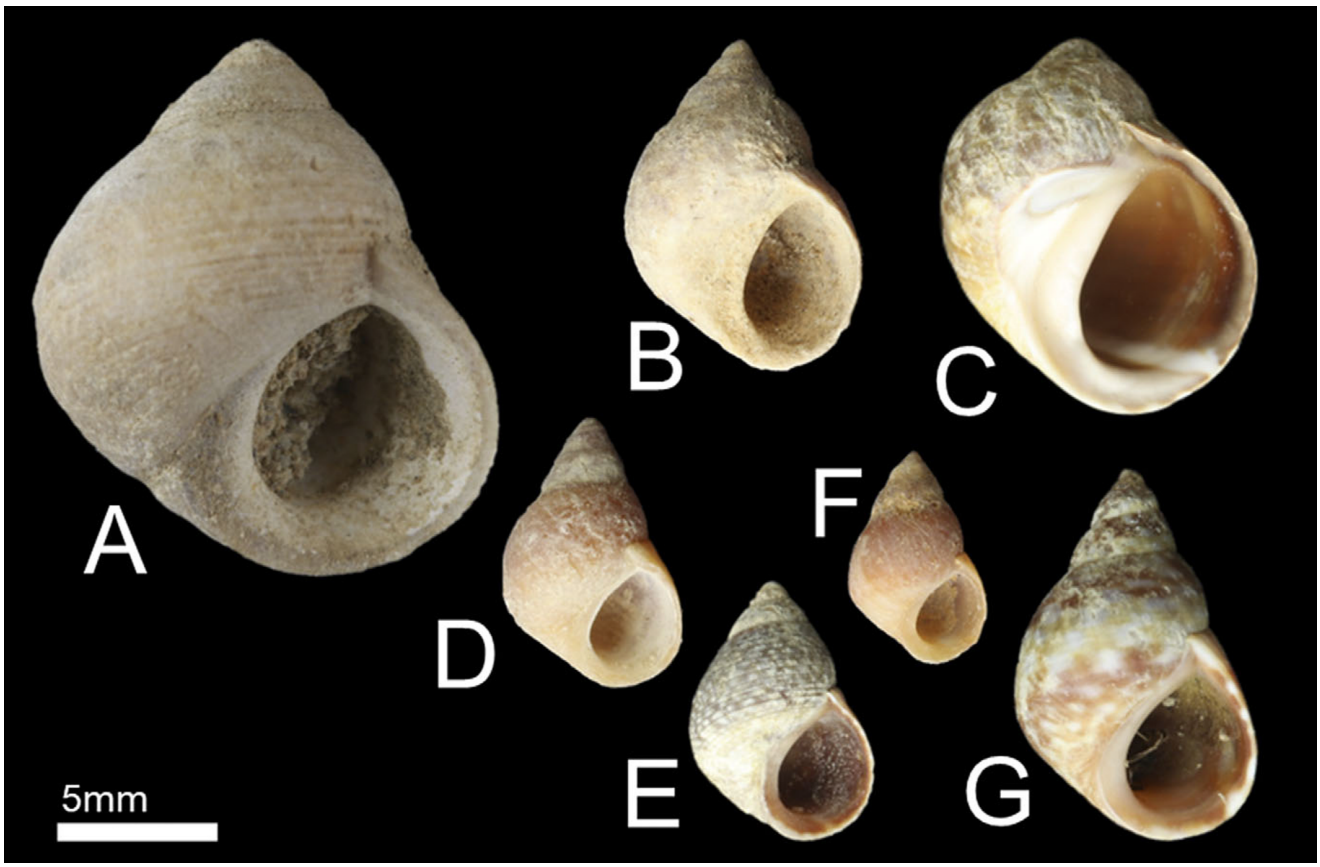

Figure 1. Typical shell forms of well-preserved *Littorina* fossils (**A**, **B**, **D**, **F**) and modern specimens (**C**, **E**, **G**), all from California, USA. **A,** †*Littorina petricola* Arnold, 1908; PRI 76551, Kettleman Hills; Etchegoin Formation, Pliocene. **B,** *Littorina keenae* Rosewater, 1978; LACMIP 5100.71, Point Vincente, Palos Verdes Estates; Quaternary terrace, late Pleistocene. **C,** *Littorina keenae*; LACM 66066, Shell Beach; collected in 1958. **D,** *Littorina plena* A. Gould, 1849; LACMIP 7220.166, Point Loma, San Diego; late Pleistocene. **E,** *Littorina plena*; LACM 1948-39.5, Oyster Cove, Tomales Bay; collected from "sand, mud, and stones," in 1948. **F,** *Littorina scutulata* A. Gould, 1849; LACMIP 5100.80, Point Vincente, Palos Verdes Estates; Quaternary terrace, late Pleistocene. (**G**) *Littorina scutulata*; LACM 1948-37.11, Nick's Cove, Tomales Bay; collected from intertidal "coarse to fine sand and rock," in 1948. See Table 1 for institutional abbreviations.

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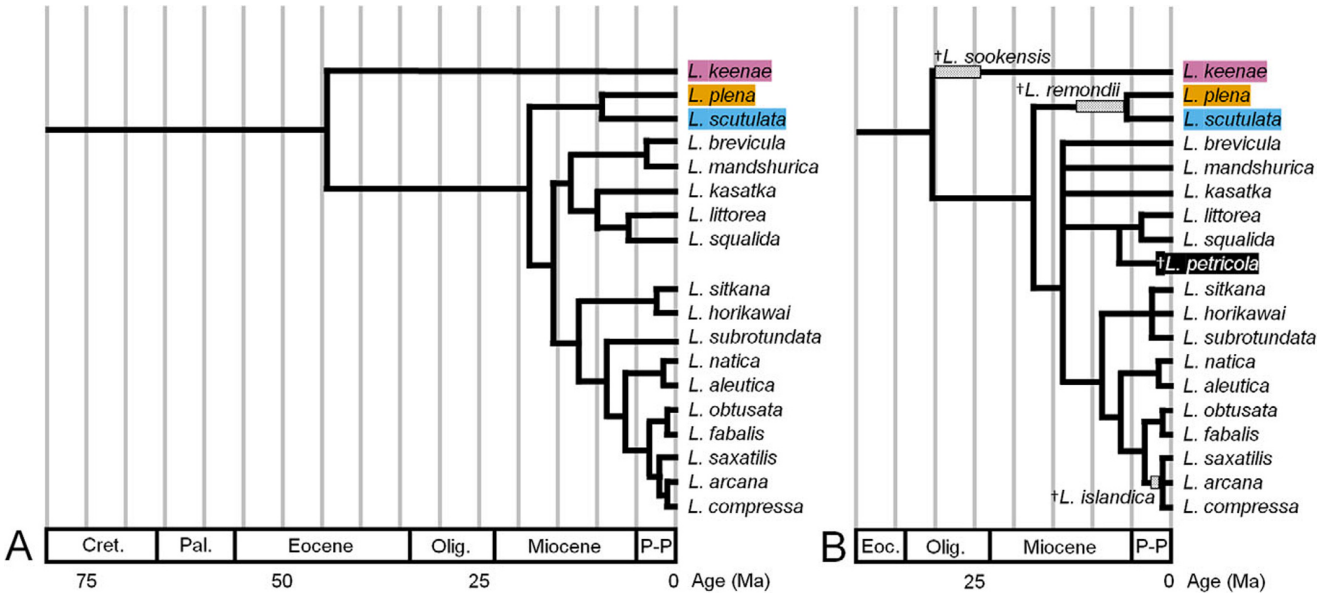

Figure 2. A, Molecular phylogeny of extant *Littorina* (based on 28S rRNA, 12S rRNA, and cytochrome oxidase *c* subunit I [COI] genes with fossil calibrations; approximate divergence times as redrawn from Reid et al. [2012: fig. 2]) and **B,** morphology-based phylogeny of all *Littorina* (from shell and anatomical characters for extant species and shell only for extinct species, with hypothesized branch times; redrawn from Reid [1996: fig. 115]). Species studied are highlighted. Dotted bars indicate possible ancestral species relationships to extant taxa, and their stratigraphic ranges. Ma, millions of years ago.

alone using discriminant function analysis on different conchological aspects (e.g., presence or absence of basal band, shell height, whorl number) of *L. plena* and *L. scutulata*, with variable results (the lowest error of 4% misrecognized specimens [Murray 1982]; the highest error of 53% [Chow 1987]). This study uses similar statistical techniques to quantify the potential error of recognizing *Littorina* species based on shell morphology on a widely sampled fossil and Recent dataset and evaluates the correspondence between morphological and genetic differences for extant species.

Morphological data for three extant and one extinct *Littorina* species are based on specimens from museum collections (Fig. 3). In total, 1020 modern and 388 fossil specimens from 36 modern and 14 fossil samples were analyzed (Table 1, Supplementary Table 2). Pliocene †*L. petricola* were sampled from the Etchegoin

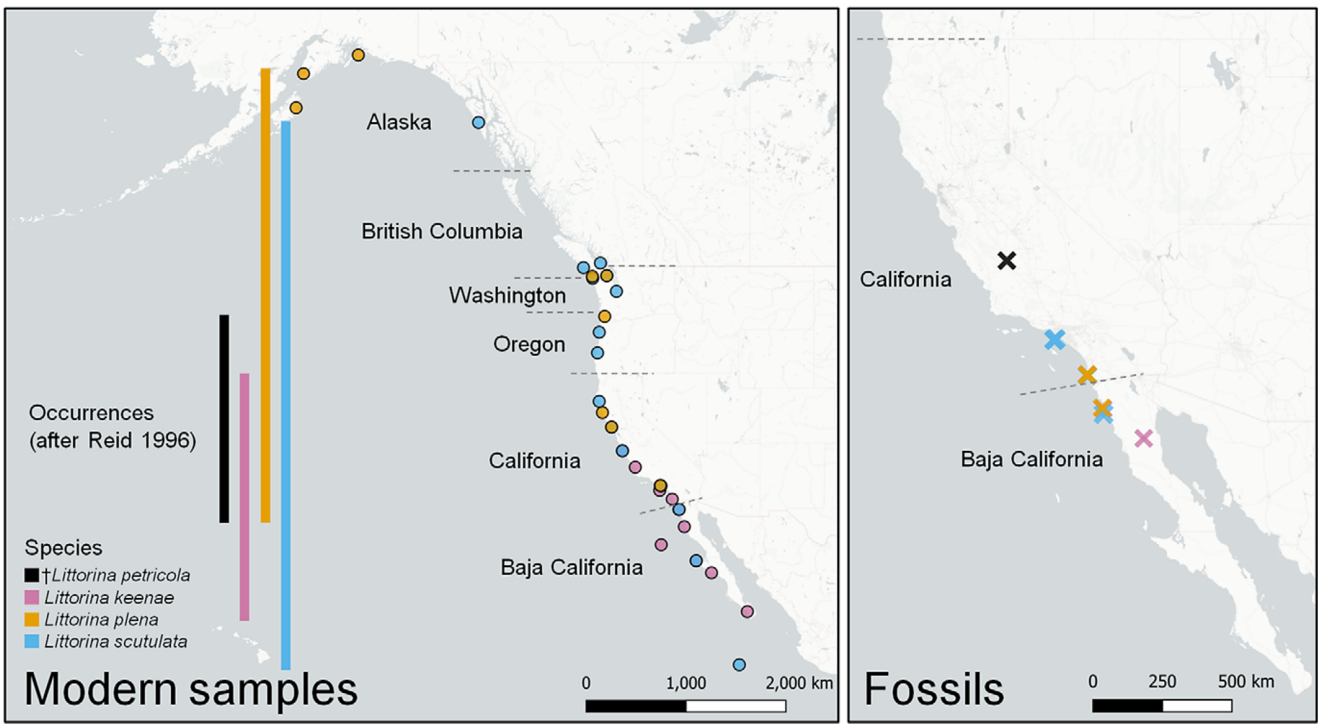

Figure 3. Map of the west coast of North America showing samples, with dotted lines showing state/country boundaries. Species ranges are indicated by the colored bars, based on modern occurrences for extant *Littorina keenae*, *L. petricola*, and *L. scutulata*, and fossil †*L. petricola* records (after Reid 1996). †*Littorina petricola* is only found in the Pliocene, while the extant species are sampled from the Pleistocene.

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Table 1. Summary of studied samples and specimens, listed by species and age. Sample details are listed in Supplemental Table 2. Institutional abbreviations: AMNH, American Museum of Natural History, New York, NY, USA; LACM and LACMIP, Natural History Museum of Los Angeles County, Los Angeles, CA, USA; NHM, Natural History Museum, London, UK; PRI, Paleontological Research Institution, Ithaca, NY, USA; USNM, Smithsonian National Museum of Natural History, Washington, DC, USA

Species	Age	Samples	Specimens	Institution
†*Littorina petricola*	Pliocene, Etchegoin Formation	1 (combined)	21	PRI
Littorina keenae	Late Pleistocene, Quaternary terrace	5	148	LACMIP
	Recent	11	330	LACM, NHM, USNM
Littorina plena	Late Pleistocene, Quaternary terrace	3	90	LACMIP
	Recent	9	233	LACM, NHM, USNM
Littorina scutulata	Pleistocene, Quaternary terrace	1	9	PRI
	Middle Pleistocene, Quaternary terrace	1	30	LACMIP
	Late Pleistocene, Quaternary terrace	3	90	LACMIP
	Recent	16	457	AMNH, LACM, NHM, USNM
	Total fossils	14	388	
	Total Recent	36	1020	

Formation in California, and Pleistocene fossils of *L. keenae*, *L. plena*, and *L. scutulata* came from Quaternary terrace sites in California and Baja California, Mexico. Species identifications from collections' labels were verified by examining shell morphology and recorded biogeographic ranges (after Reid 1996; see Supplementary Table 1), with three samples redesignated from their museum label identifications (two *L. scutulata* samples reassigned as *L. plena* based on locality; one *L. keenae* sample as *L. scutulata* from morphology). As it was not possible to confirm the identity of all *L. scutulata* and *L. plena* specimens based on conchological features alone, the data for these two species may contain a percentage of mixed or misclassified specimens. The majority of fossil samples (from the Natural History Museum of Los Angeles County) had their identification confirmed by A. Hendy (current curator and expert on east Pacific Cenozoic mollusks [e.g., Hendy 2013]). Modern samples from the Natural History Museum, London, had their identification confirmed by D. G. Reid (formerly based at that institution and taxonomic authority on *Littorina* [e.g., Reid 1996]).

Samples were chosen to encompass the breadth of each species distribution (to account for the potential range in shell morphology and habitat occupied) and for larger sample sizes. Relatively large samples (more than 100 specimens from a locality) were available from the Pleistocene and modern collections for all the extant species. No preservational differences were observed between samples assigned to the middle or late Pleistocene, so further discussion treats all Pleistocene samples similarly. A sample size of approximately 30 specimens per sample was selected as sufficient for capturing shape variation after testing sample sizes 15 to 50 at three modern sites in California that included *L. keenae*, *L. plena*, and *L. scutulata*. From principal component analyses of these species at three sites using different sample sizes, average change in the variance explained by components 1 and 2 was <1% and ~2% for component 3, with a total variance of ~0.008. Covariance matrices between all pairwise comparisons of tested sample sizes were similar (matrix permutation test on landmarks, $p < 0.01$). To include samples from every part of the extant species' ranges, smaller samples from underrepresented localities were included (British Columbia, Washington, and Oregon). Recent specimens did not have information on collection date or habitat, so potential sampling effects (e.g., collection from differently exposed environments) could not be controlled for. †*Littorina petricola* did not have a single sample with more than 30 shells, so specimens from multiple samples of the same locality (Kettleman Hills, California) were combined. Both fossil and modern samples are considered "time averaged" in this study, as only broad temporal bins are discussed (Pliocene, Pleistocene, modern), and fossils in each sample are expected to have accumulated over a period of time (most modern samples appear to be live collected). Incomplete specimens were not used. Each specimen was photographed by C.P.S. following a standard apertural view (after Callomon 2019), using a digital camera set in a copy stand for consistent angle and orientation. For digitization of shell shape from photographs, 12 landmarks were placed on each image (after Carvajal-Rodríguez et al. 2005) in tpsDig2 (v. 2.31; Rohlf 2015; Fig. 4). Landmarks have been shown to be adequate in capturing overall shell shape variation in *Littorina* compared with other digitization methods based on shell photographs (e.g., outlines [Doyle et al. 2018]; shape parameters derived from a helicospiral model [Larsson et al. 2020]). Shape changes have been successfully investigated using landmarks, especially among the highly variable *Littorina saxatilis* (e.g., Conde-Padín et al. 2007; Butlin et al. 2014) and when comparing multiple *Littorina* species (e.g., Maltseva et al. 2021). Digitization was conducted solely by C.P.S. Landmarking consistency was tested by digitizing the same sample (20 specimens of *L. keenae*) three times, with no statistically significant differences in means of attempts (T^2 test, all $p > 0.05$; average shape of each attempt shown in Fig. 4), and digitization attempt explained much less of the shape variation (from Procrustes ANOVA on shape, ~17% variance) than the variance contributed by individuals (~83%). The morphometric data were then Procrustes transformed and statistically analyzed in MorphoJ (Generalized Procrustes Analysis; v. 1.07a [Klingenberg 2011]) and R (R Core Team 2023; packages moments [Komsta and Novomestky 2022], ggpubr [Kassambara 2023], and ggplot2 [Wickham 2016] were used). Centroid size, the square root of the sum of squared distances from the center to all landmarks, was used to approximate specimen sizes. Principal component analyses illustrate shape variation among specimens,

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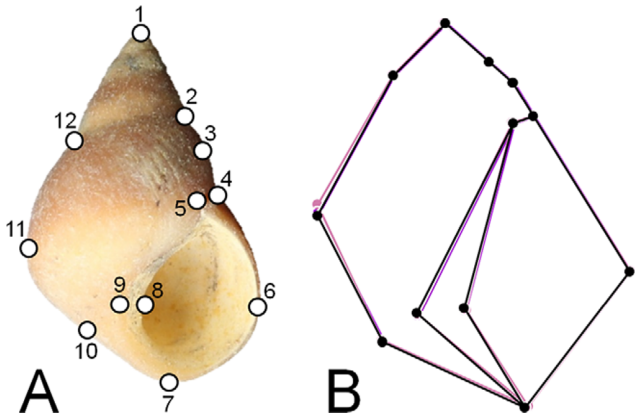

Figure 4. A, Landmarking scheme (after Carvajal-Rodríguez et al. [2005]; landmarks described below) illustrated on an example specimen, *Littorina scutulata.* LACMIP 6153.2 Rancho Palos Verdes, California, Quaternary terrace, middle Pleistocene. Landmark 1 = shell apex; 2 = right upper side of penultimate whorl's suture; 3 = midpoint on curve, between landmarks 2 and 4; 4 = lower suture of penultimate whorl; 5 = end of suture; 6 = rightmost external point on shell lip edge; 7 = lowest point on shell base; 8 = internal border of aperture; 9 = external border of aperture, perpendicular to 8; 10 = midpoint on shell edge, between landmarks 7 and 11; 11 = leftmost external point on penultimate whorl; 12 = left side of penultimate whorl's suture. **B,** Example of landmarking consistency with average configurations of three digitization attempts on the same *L. keenae* sample (black, purple, and pink outlines). LACMIP, Natural History Museum of Los Angeles County, Los Angeles, CA, USA.

and the wireframe diagrams (selected landmarks connected by straight lines for visualization) show the magnitude of shape change among landmarks for each principal component axes. Cross-validated discriminant function analysis between pairs of species was used to estimate the percentage of specimens that would be misclassified based on their shell shape. Mahalanobis distance, the distance between the means of two species shapes while considering their variance and covariance, was also calculated during discriminant function analysis.

For the genetic data, published sequences for the extant species were taken from GenBank (1 specimen per species, for 28S rRNA, 12S rRNA, and cytochrome oxidase *c* subunit I [COI] genes, respectively, as cited in Reid et al. [2012]; Supplementary Table 3). Sequences were aligned with MUSCLE (Edgar 2004; with default parameters of −400 gap opening penalty and unweighted pair group method with arithmetic mean clustering), and pairwise *p* distances, the number of base pair differences per site between two sequences, were calculated in MEGA 11 (Tamura et al. 2021).

Results and Discussion

Fossil and Modern Littorina

Overall, Pleistocene fossils of the extant species were smaller and less variable in size than modern individuals, while Pliocene †*L. petricola* was larger (Fig. 5A). As previously documented, †*L. petricola* and *L. keenae* were commonly larger than *L. plena* or *L. scutulata*, and *L. plena* specimens tended to be smaller than *L. scutulata* (Supplementary Table 1). Centroid sizes were not normally distributed for *L. keenae*, *L. plena*, or *L. scutulata* (Shapiro-Wilk normality test, $p < 0.05$), but were for †*L. petricola* ($p = 0.2$). Among species, centroid sizes were significantly different (Kruskal-Wallis $\chi^2 = 45.1$, df = 3, $p < 0.05$), and pairwise comparisons between all species were statistically significant (Wilcoxon rank-sum test with Bonferroni correction, $p < 0.05$), except between *L. plena* and *L. scutulata* ($p = 0.08$). Although Pleistocene fossil and Recent centroid sizes of all three extant species overlap, they were statistically different for each species (*L. keenae*, Kruskal-Wallis $\chi^2 = 51.9$, df = 1, $p < 0.01$; *L. plena*, Kruskal-Wallis $\chi^2 = 33.1$, df = 1, $p < 0.01$; *L. scutulata*, Kruskal-Wallis $\chi^2 = 15.0$, df = 1, $p < 0.01$), but it is unclear if these size differences reflect time-averaging taphonomic processes or indicate biological change over time. There was no statistically significant difference in sizes for *L. keenae* across sampled regions (Kruskal-Wallis $\chi^2 = 1.2$, df = 1, $p = 0.3$), but there was

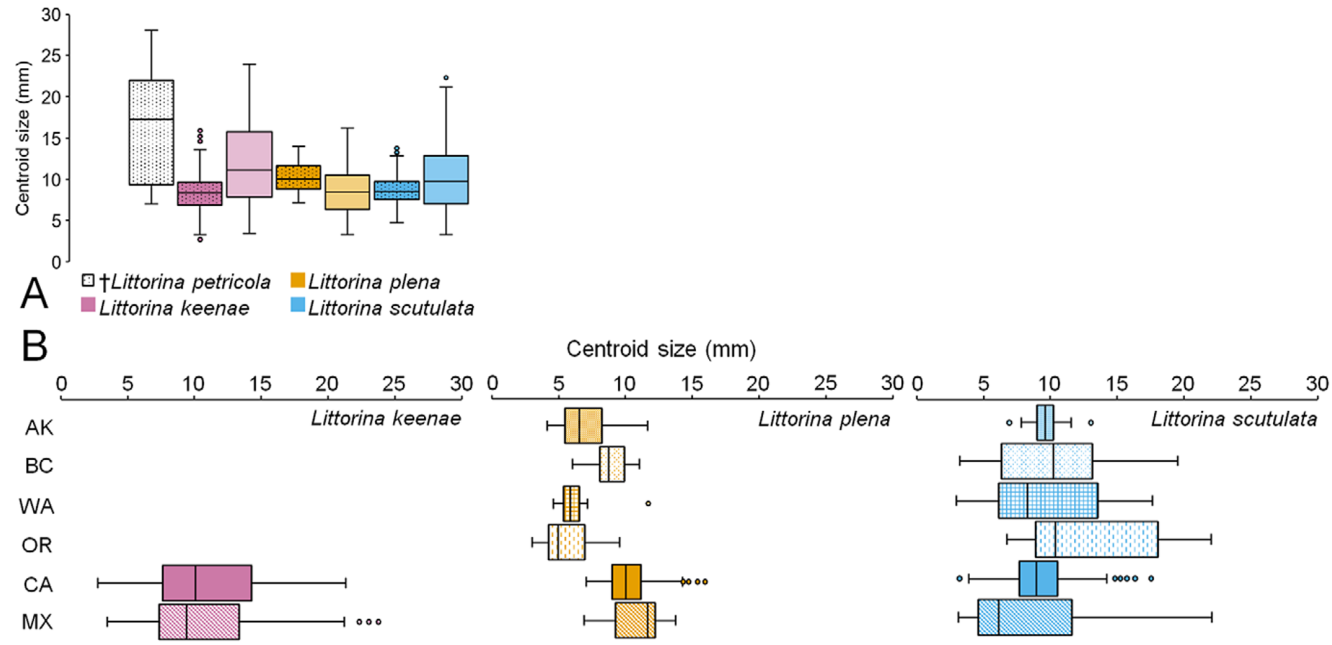

Figure 5. A, Centroid size distribution of studied *Littorina*; dotted plots are sizes of fossil specimens, solid-colored plots are for Recent specimens. **B,** Centroid size distribution of extant *Littorina*, grouped by region, listed from north to south. AK, Alaska; BC, British Columbia; WA, Washington; OR, Oregon; CA, California; MX, Baja California.

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for *L. plena* (Kruskal-Wallis χ^2 = 189.3, df = 5, $p < 0.01$) and *L. scutulata* (Kruskal-Wallis χ^2 = 48.6, df = 5, $p < 0.01$; Fig. 5B). While there were no regions that had statistically significant differences with all others, *L. plena* from Alaska (the northern extent of the species) and *L. scutulata* from Baja California (the southern extent of the species) differed from the most regions when pairwise comparisons were conducted (Wilcoxon rank-sum test with Bonferroni correction, $p < 0.05$).

Principal component analyses of fossil and Recent *Littorina* specimens illustrate groups of shell shapes according to species, particularly separating *L. keenae* from closely overlapping *L. plena* and *L. scutulata* individuals on principal component 1 (PC 1; 52.7% variance), while there was slight differentiation of fossil from modern material on PC 2 (12.0%) and PC 3 (7.8%; Fig. 6). Pliocene †*L. petricola* did not form a discrete morphological cluster, with the landmarked shell shapes plotting among *L. plena* and *L. scutulata* specimens, and showed the least variability compared with the extant species, although this may also be due to the limited sampling. Adding *L. squalida*, the closest extant relative to †*L. petricola* in future analyses could provide another species pair comparison to evaluate this study's approach.

Centroid sizes of *Littorina* species were not correlated with the morphological data in PC 1, PC 2, or PC 3 (when a simple linear regression of principal component axes with each species' sizes are conducted, all adjusted $R^2 < 0.5$; Supplementary Fig. 1). Principal component analyses that considered modern (55.4% variance on PC 1, 12.1% for PC 2, 7.5% for PC 3; Supplementary Fig. 2) or fossil (51.7% variance for PC 1, 10.4% on PC 2, 7.8% on PC 3; Supplementary Fig. 3) material separately exhibit results similar to the analysis that included all specimens. Shell shape variation along the principal component axes could not be attributed to any singular landmark, although for all analyses there is an overall rounder, turbinate shape, and apex height change on PC 1, which is reflective of *L. keenae*, compared with more conical forms of *L. plena* and *L. scutulata* for PC 2 and PC 3 (Fig. 6). It has been suggested that overall shell shape in marine gastropods may be adaptive for temperature regulation in different intertidal zones (e.g., taller spires at higher shore heights [Vermeij 1973]),

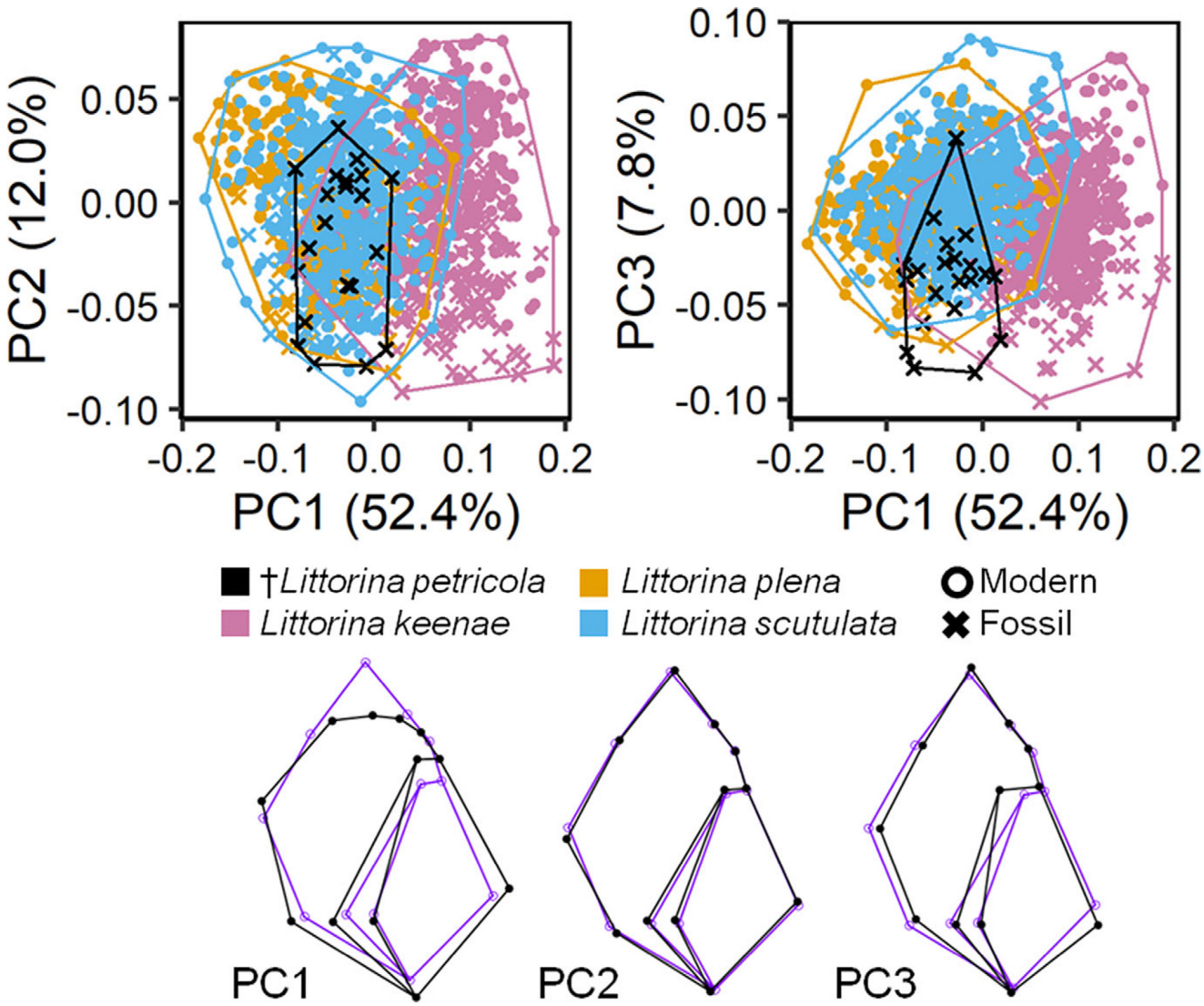

Figure 6. Principal component analysis of landmark data for all specimens of the three extant and one extinct *Littorina* species. Polygons enclose the points for each species. Wireframe diagrams show shape changes for each principal component (PC 1, PC 2, PC 3), from minimum (purple) to maximum values (black) along that axis.

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Table 2. Mahalanobis distances between pairs of *Littorina* species (left quadrant, all pairwise comparisons $p < 0.01$, T^2 test) and percentage of misclassified specimens from cross-validated discriminant function analysis (right quadrant) based on all specimens, modern shells, or fossils only

All specimens	†*L. petricola*	*L. keenae*	*L. plena*	*L. scutulata*
†*L. petricola*	—	<1%	<1%	<1%
L. keenae	6.4	—	4%	4%
L. plena	6.6	3.7	—	37%
L. scutulata	7.0	3.4	0.7	—
Modern only				
L. keenae		—	3%	5%
L. plena		4.0	—	32%
L. scutulata		3.4	1.1	—
Fossils only				
†*L. petricola*	—	1%	1%	1%
L. keenae	6.0	—	5%	4%
L. plena	6.2	4.2	—	33%
L. scutulata	6.0	4.0	1.5	—

although this has not been shown for the extant species in this study (*L. keenae* [Lee and Boulding 2010]; no specific data on *L. scutulata* or *L. plena*). Spire height has been specifically associated with predation risk (e.g., crab predation selects for smaller spires [Seeley 1986]), varying growth rates (e.g., faster growth may result in taller spires [Kemp and Bertness 1984; Boulding and Hay 1993]), and perhaps a larger foot area, which would be advantageous against wave action (for *L. scutulata* and *L. plena* [Hohenlohe 2003a]). It is unclear whether species differences in spire height are adaptive for the studied taxa. Including semilandmarks on certain shell outline sections may complement our current shape-digitization approach.

Little morphological change was observed for extant *Littorina* species, as Pleistocene and Recent specimens had comparable ranges of Mahalanobis distances (Table 2). As expected, the more phylogenetically distant *L. keenae* had larger Mahalanobis distances to *L. plena* and *L. scutulata* (3.4 to 4.2) than between the sister species *L. plena* and *L. scutulata* (1.1 with modern specimens, 1.5 with fossils). Despite a smaller fossil (388 specimens) than Recent dataset (1020 specimens), Pleistocene and modern *L. keenae* display similar overall ranges in shell shape variation, while Pleistocene *L. plena* and *L. scutulata* appear to have less variable shell shapes compared with their Recent specimens (modern data in Supplementary Fig. 2, fossils shown in Supplementary Fig. 3). It is ambiguous whether there are definite biological or environmental factors reducing fossil morphological variation, other than potential sampling effects. However, *L. plena* and *L. scutulata* have analogous ranges of overall shell shape variation, which is slightly different from previous findings (compared with *L. scutulata*, *L. plena* had smaller shell shape variation [Murray 1982]; or *L. plena* has larger shell variation [Reid 1996]). The spread of morphological variation within *L. plena* and *L. scutulata* on PC 1 and to a lesser extent on PC 2 and PC 3 may be attributed to geographic region, although there do not seem to be any strong latitudinal trends or discrete groupings of shapes, as there is overlap in specimens from disjunct areas (e.g., individuals from British Columbia overlay with those from California). As noted by Reid (1996), higher-spired specimens were found at the northern range of *L. plena*, and individuals of *L. plena* from Alaska, British Columbia, and Washington did cluster more toward the maximal values of PC 2 and PC 3, although morphological clusters of both *L. plena* and *L. scutulata* overlapped with each other within every sampled region. Nevertheless, intraspecific variability in shell shape for *L. plena* and *L. scutulata* appears to be continuous, suggesting there may be little effect of time averaging on shape data (e.g., as in other studies, reviewed by Kidwell and Holland [2002]). No consistent shell forms associated with specific environments (ecotypes, defined as "a phenotypically and genotypically distinct form of a species that is adapted to a particular habitat" [Johannesson et al. 2024]) have been described for any of the study species (Reid 1996). Planktotrophic larval development with relatively high dispersal capabilities for all three extant species may explain the continuity in the extent of shell shapes, as populations in the species distribution are generally connected (no spatial population genetic structure was found for *L. keenae*, *L. plena*, or *L. scutulata* [Lee and Boulding 2007, 2009, 2010]).

Evaluating Morphological Species Recognition

Larger Mahalanobis distances between pairwise species comparisons corresponded with higher accuracy in classifying specimens from cross-validated discriminant function analysis, demonstrating that shell shape as captured through landmarking can sufficiently differentiate among species (Table 2). The largest differences between means involved †*L. petricola* and the three extant species (Mahalanobis distances from 6.4 to 7.0), and the smallest between *L. plena* and *L. scutulata* (0.7), with all pairwise comparisons being statistically significant (T^2 test, $p < 0.01$). Nearly all specimens of †*L. petricola* could be distinguished from *L. keenae*, *L. plena*, and *L. scutulata* (<1% misclassified), while the highest error was between *L. plena* and *L. scutulata* (37%). A few individuals of *L. plena* and *L. scutulata* were misclassified when compared with *L. keenae* (4%). A similar trend in Mahalanobis distances and misclassification percentages between species was also found when only fossils or modern specimens were considered. These comparisons of more distantly related species have larger shape differences, and lower identification error is expected, as demonstrated by previous phylogenetic work based on conchological and soft anatomical characters (Fig. 2).

The amount of error in conchologically recognizing *L. plena* and *L. scutulata* (32% to 37%) is generally higher but still in the range of those previously reported using solely modern material, suggesting there is more shape variability captured when sampling widely across the species distribution. However, some error may also be due to some misidentification of species from collections labels. With different data, several studies have used discriminant function analysis to quantitatively compare the accuracy of classifying confirmed *L. plena* and *L. scutulata* (collected live, anatomical features observed) with conchological identification. Murray (1982) reported a 16% error in distinguishing *L. plena* based on its generally smaller size and more frequent appearance of a basal band, although this misclassification percentage could be reduced to 4% if shell length, depth, whorl height, and whorl number are measured (samples from California and Oregon). Misclassification percentages in a similar range were found for the two species based on measured shell length, width, whorl number, and presence of an amber band and tessellations (5% for *L. plena* and 11% for *L. scutulata*, from Bodega Bay, California

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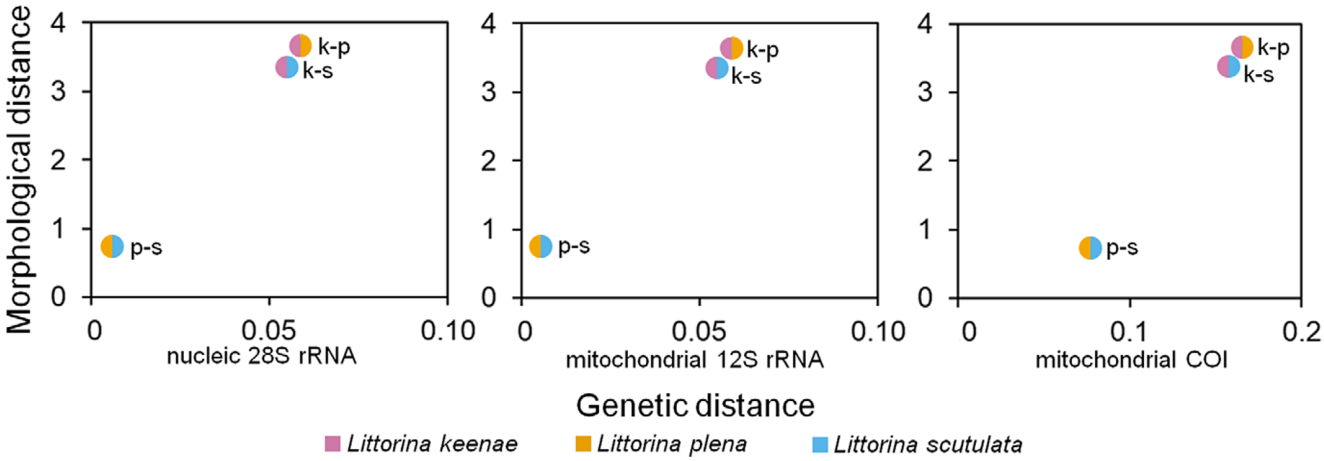

Figure 7. Morphological distance (Mahalanobis distance, the distance of a central point and distribution of landmark data from modern and fossil specimens for that species) and genetic distance (number of base differences per site from published 28S rRNA, 12S rRNA, and cytochrome oxidase *c* subunit 1 [COI] sequences for each species) from pairwise comparisons for *Littorina keenae* (k), *L. plena* (p), and *L. scutulata* (s). Note that morphological distance for all the plots is the same; only the x-axes have changed.

[Chow 1987]). Much higher error percentages were reported for *L. plena* (34%) and *L. scutulata* (31%) from British Columbia and Washington based on measurements of shell height, spire angle, aperture angle, whorl ratio, and aperture shape (Hohenlohe and Boulding 2001). The highest misclassification documented was 53% when classifying *L. plena* between open and protected shores at the same site (Chow 1987). Adding discrete conchological characters such as color (e.g., the presence of a pale basal band and checkered patterning) would increase the accuracy of modern species identification between *L. plena* and *L. scutulata* (Murray 1982; Chow 1987; Rugh 1997; Hohenlohe and Boulding 2001; Supplementary Table 1), although this would not be possible for most fossil specimens.

Species-Level Morphological and Genetic Correlation

Increasing morphological distances (Mahalanobis distance) correspond with genetic *p* distances (from 28S rRNA, 12S rRNA, COI), as seen from pairwise comparisons between *L. keenae*, *L. plena*, and *L. scutulata*, supporting the use of shell shape in recognizing species with modern or fossil material (Fig. 7). This reflects phylogenetic analyses of *Littorina* based on morphological and molecular metrics, which are in close agreement (Reid 1990, 1996; Reid et al. 1996, 2012; Fig. 2).

Our results are similar to the highly correlated morphological (Mahalanobis distance, from fossil and Recent bryozoans) and genetic distances (Nei's unbiased *D*, from allozymes) in the frequently referenced study by Jackson and Cheetham (1994). For the *Littorina* studied here, morphological differences are quantified by Mahalanobis distances, and uncertainty in species identification is gauged by number of misclassified specimens between species, with higher error found to be correlated with how closely related species are. When combined with other approaches to evaluating the potential inaccuracy of relying on morphospecies recognition, such as taxonomic reviews of species status and comparisons of morphospecies and genetic-based taxonomies, it seems that there is a range of potential error that will likely vary and depend on the biology of the taxonomic group and its history of study (e.g., Shin and Allmon 2023; Cahill et al. 2024), emphasizing the importance of evaluating the consistency of species identification for specific groups.

Conclusions

1. Shell shape, as quantified by landmarking, can be used to distinguish among east Pacific *Littorina* species. From cross-validated discriminant function analysis, the number of misclassified specimens between two species is lower when the species are more distantly related (from <1% to 5%) and highest between sister species (32% to 37%).
2. For extant *Littorina*, Pleistocene and Recent specimens are conchologically similar and can be recognized with comparable accuracy. Although Pleistocene *Littorina* specimens were less morphologically variable than modern individuals, the range of fossil variation was contained within the Recent data, and all species had continuous ranges of morphological variation. Although the extinct species from the Pliocene morphologically overlapped with the studied extant species, it could be distinguished with other shell characters.
3. Differences in *Littorina* shell shape are correlated with genetic distances, giving confidence to recognizing *Littorina* based solely on morphology, and this may also apply to other gastropod species. Quantifying the variability in the relationship between morphological and genetic differences for specific taxa may add to our understanding of the potential error when recognizing morphospecies, and elucidate prospective causes in cases where morphological and genetic differentiation are inconsistent.

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Literature Cited

Allmon, W. D. 2013. Species, speciation, and paleontology up to the Modern Synthesis: persistent themes and unanswered questions. *Palaeontology* **56**: 1199–1223.

Allmon, W. D. 2016. Studying species in the fossil record: a review and recommendations for a more unified approach. Pp. 59–120 *in* W. D. Allmon and M. M. Yacobucci, eds., *Species and speciation in the fossil record*. University of Chicago Press, Chicago.

Allmon, W. D., and U. E. Smith. 2011. What, if anything, can we learn from the fossil record about speciation in marine gastropods? Biological and geological considerations. *American Malacological Bulletin* **29**:247–276.

Appeltans, W., S. T. Ahyong, G. Anderson, M. V. Angel, T. Artois, N. Bailly, R. Bamber, et al. 2012. The magnitude of global marine species diversity. *Current Biology* **22**:2189–2202.

Arnold, R. 1908. Descriptions of new Cretaceous and Tertiary fossils from the Santa Cruz Mountains, California. *Proceedings of the United States National Museum* **34**:345–389.

Barraclough, T. G. 2019. *The evolutionary biology of species*, Oxford University Press, Oxford.

Behrens Yamada, S. 1992. Niche relationships in northeastern Pacific Littorines. Pp. 281–291 *in* J. Grahame, P. J. Mill, and D. G. Reid, eds., *Proceedings of the Third International Symposium on Littorinid Biology*. Malacological Society of London, London.

Bickford, D., D. J. Lohman, N. S. Sodhi, P. K. L. Ng, R. Meier, K. Winker, K. K. Ingram, and I. Das. 2007. Cryptic species as a window on diversity and conservation. *Trends in Ecology and Evolution* **22**:148–155.

Bieler, R. 1992. Gastropod phylogeny and systematics. *Annual Review of Ecology, Evolution, and Systematics* **23**:311–338.

Boulding, E. G., and T. K. Hay. 1993. Quantitative genetics of shell form of an intertidal snail: constraints on short-term response to selection. *Evolution* **47**: 576–592.

Brochu, C. A., and C. D. Sumrall. 2020. Modern cryptic species and crocodilian diversity in the fossil record. *Zoological Journal of the Linnean Society* **189**: 700–711.

Broderip, W. J., and G. B. I. Sowerby. 1829. Observations on new or interesting Mollusca contained, for the most part, in the Museum of the Zoological Society. *Zoological Journal* **4**:359–379.

Budd, A. F., and J. M. Pandolfi. 2004. Overlapping species boundaries and hybridization within the *Montastraea "annularis"* reef coral complex in the Pleistocene of the Bahama Islands. *Paleobiology* **30**:396–425.

Butlin, R. K., M. Saura, G. Charrier, B. Jackson, C. André, A. Caballero, J. A. Coyne, et al. 2014. Parallel evolution of local adaptation and reproductive isolation in the face of gene flow. *Evolution* **68**:935–949.

Cahill, A. E., E. Meglécz, and A. Chenuil. 2024. Scientific history, biogeography, and biological traits predict presence of cryptic or overlooked species. *Biological Reviews* **99**:546–561.

Caley, K. J., J. Grahame, and P. J. Mill. 1995. A geographically-based study of shell shape in small rough periwinkles. *Hydrobiologia* **309**:181–193.

Callomon, P. 2019. *Standard views for imaging mollusk shells*. American Malacological Society. https://ams.wildapricot.org/resources/Documents/Standard%20views%20for%20imaging%20mollusk%20shells%20for%20web%202019.pdf.

Carvajal-Rodríguez, A., P. Conde-Padín, and E. Rolán-Alvarez. 2005. Decomposing shell form into size and shape by geometric morphometric methods in two sympatric ecotypes of *Littorina saxatilis*. *Journal of Molluscan Studies* **71**:313–318.

Cerca, J., C. Meyer, D. Stateczny, D. Siemon, J. Wegbrod, G. Purschke, D. Dimitrov, and T. H. Struck. 2019. Deceleration of morphological evolution in a cryptic species complex and its link to paleontological stasis. *Evolution* **74**:116–131.

Chenuil, A., A. E. Cahill, N. Délémontey, E. Du Salliant du Luc, and H. Fanton. 2019. Problems and questions posed by cryptic species. A framework to guide future studies. Pp. 77–106 *in* E. Casetta, J. M. da Silva and D. Vecchi, eds., *From assessing to conserving biodiversity: conceptual and practical challenges*. History, Philosophy and Theory of the Life Sciences 24. SpringerOpen.

Chow, V. 1987. Morphological classification of sibling species of *Littorina* (Gastropoda: Prosobranchia): discretionary use of discriminant analysis. *The Veliger* **29**:359–366.

Clark, B. L., and R. Arnold. 1923. Fauna of the Sooke Formation, Vancouver Island with description of a new coral by T. Wyland Vaughan. Pp. 123–234 *in* A. C. Lawson and G. D. Louderback, eds., *University of California Publications in Geological Sciences*, Vol. **14**. University of California Press, Berkeley.

Conde-Padín, P., A. Carvajal-Rodríguez, M. Carballo, A. Carballero, and E. Rolán-Alvarez. 2007. Genetic variation for shell traits in a direct-developing marine snail involved in a putative sympatric ecological process. *Evolutionary Ecology* **21**:635–650.

Doyle, D., M. P. Gammel, and R. Nash. 2018. Morphometric methods for the analysis and classification of gastropods: a comparison using *Littorina littorea*. *Journal of Molluscan Studies* **84**:190–197.

Edgar, R. C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* **32**:1792–1797.

Férussac, A. E. 1822. Tableaux systématiques des animaux mollusques classés en familles naturelles, dans lesquels on a établi la concordance de tous les systèmes; suivis d'un prodrome général pour tous les mollusques terrestres ou fluviatiles, vivants ou fossils. A. Bertrand. https://www.biodiversitylibrary.org/item/41533.

Fišer, C., C. T. Robinson, and F. Malard. 2018. Cryptic species as a window into the paradigm shift of the species concept. *Molecular Ecology* **27**:613–635.

Foote, M., and A. I. Miller. 2006. *Principles of paleontology*, 3rd ed. Freeman, New York.

Gould, A. 1849. *Proceedings of the Boston Society of Natural History*, Vol. **3**. Boston Society of Natural History. https://www.biodiversitylibrary.org/item/35741.

Groves, L. T., and R. L. Squires. 2021. Checklist of California Paleogene–Neogene marine Mollusca since Keen and Bentson (1944). *PaleoBios* **38**:1–360.

Hannaford-Ellis, C. J. 1978. *Littorina arcana* sp. nov.: a new species of winkle (Gastropoda: Prosobranchia: Littorinidae. *Journal of Conchology* **29**: 304.

Hendy, A. J. W. 2013. Spatial and stratigraphic variation of marine paleoenvironments in the middle-upper Miocene Gatun formation, isthmus of Panama. *Palaios* **28**:210–227.

Hohenlohe, P. A. 2002. Life history of *Littorina scutulata* and *L. plena*, sibling gastropod species with planktotrophic larvae. *Invertebrate Biology* **121**: 25–37.

Hohenlohe, P. A. 2003a. Distribution of sister *Littorina* species, I: tenacity and the wave-exposure gradient. *The Veliger* **46**:162–168.

Hohenlohe, P. A. 2003b. Distribution of sister *Littorina* species, II: geographic and tidal-height pattern do not support sympatric speciation. *The Veliger* **46**: 211–219.

Hohenlohe, P. A., and E. G. Boulding. 2001. A molecular assay identifies morphological characters useful for distinguishing the sibling species *Littorina scutulata* and *L. plena*. *Journal of Shellfish Research* **20**:453–457.

Jackson, J. B. C., and A. H. Cheetham. 1990. Evolutionary significance of morphospecies: a test with cheilostome Bryozoa. *Science* **248**:579–583.

Jackson, J. B. C., and A. H. Cheetham. 1994. Phylogeny reconstruction and the tempo of speciation in cheilostome Bryozoa. *Paleobiology* **20**:407–423.

Johannesson, K., R. Faria, A. Le Moan, M. Rafajlović, A. M. Westram, R. K. Butlin, and S. Stankowski. 2024. Diverse pathways to speciation revealed by marine snails. *Trends in Genetics* **40**:337–351.

Kassambara, A. 2023. ggpubr: 'ggplot2' based publication ready plots. R package version 0.6.0. https://rpkgs.datanovia.com/ggpubr.

Keen, A. M., and H. Bentson. 1944. Checklist of California Tertiary marine Mollusca. *Geological Society of America Special Paper* **56**:1–280.

Kemp, P., and M. D. Bertness. 1984. Snail shape and growth rates: evidence for plastic shell allometry in *Littorina littorea*. *Proceedings of the National Academy of Sciences USA* **81**:811–813.

Kidwell, S. M., and S. M. Holland. 2002. The quality of the fossil record: implications for evolutionary analyses. *Annual Review of Ecology and Systematics* **33**:561–588.

Klingenberg, C. P. 2011. MorphoJ: an integrated software package for geometric morphometrics. *Molecular Ecology Resources* **11**:353–357.

Knowlton, N. 1993. Sibling species in the sea. *Annual Review of Ecology and Systematics* **24**:189–216.

https://doi.org/10.1017/pab.2025.10043 Published online by Cambridge University Press

Komsta, L., and F. Novomestky. 2022. moments: moments, cumulants, skewness, kurtosis and related tests. R package version 0.14.1. http://doi.org/10.32614/CRAN.package.moments.

Kordbacheh, A., H. Rahimian, and D. Fontaneto. 2023. Mechanisms of reproductive isolation among cryptic species in monogonont rotifers. *Hydrobiologia* **850**:4705–4718.

Larsson, J., A. M. Westram, S. Bengmark, T. Lundh, and R. K. Butlin. 2020. A developmentally descriptive method for quantifying shape in gastropod shells. *Journal of the Royal Society Interface* **17**:20190721.

Lee, H. J., and E. G. Boulding. 2007. Mitochondrial DNA variation in space and time in the northeastern Pacific gastropod, *Littorina keenae*. *Molecular Ecology* **16**:3084–3103.

Lee, H. J., and E. G. Boulding. 2009. Spatial and temporal population genetic structure of four northeastern Pacific littorinid gastropods: the effect of mode of larval development on variation at one mitochondrial and two nuclear DNA markers. *Molecular Ecology* **18**:2165–2184.

Lee, H. J., and E. G. Boulding. 2010. Latitudinal clines in body size, but not in thermal tolerance or heat-shock cognate 70 (*HSC70*), in the highly dispersing intertidal gastropod *Littorina keenae* (Gastropoda: Littorinidae). *Biological Journal of the Linnean Society* **100**:494–505.

Li, X., and J. J. Wiens. 2023. Estimating global biodiversity: the role of cryptic insect species. *Systematic Biology* **72**:391–403.

Linnaeus, C. 1758. *Systema Naturae per regna tria naturae, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis*, Vol. **1**. Impensis Direct. Laurentii Salvii. https://www.biodiversitylibrary.org/item/10277.

López Carranza, N., and S. J. Carlson. 2019. Testing species assignments in extant terebratulide brachiopods: a three-dimensional geometric morphometric analysis of long-looped brachidia. *PLoS ONE* **14**:e0225528.

López Carranza, N., and S. J. Carlson. 2021. Quantifying shell outline variability in extant and fossil *Laqueus* (Brachiopoda: Terebratulida): are outlines good proxies for long-looped brachidial morphology and can they help us characterize species? *Paleobiology* **47**:149–170.

Maltseva, A. L., M. A. Varfolomeeva, R. V. Ayanka, E. R. Gafarova, E. A. Repkin, P. A. Pavlova, A. Shavarda, N. A. Mikhailova, and A. I. Granovitch. 2021. Linking ecology, morphology, and metabolism: niche differentiation in sympatric populations of closely related species of the genus *Littorina* (*Neritrema*). *Ecology and Evolution* **11**:11134–11154.

Mastro, E., V. Chow, and D. Hedgecock. 1982. *Littorina scutulata* and *Littorina plena:* sibling species status of two prosobranch gastropod species confirmed by electrophoresis. *The Veliger* **24**:239–246.

Mayr, E. 1942. *Systematics and the origin of species: from a viewpoint of a zoologist*. Columbia University Press, New York.

McQuaid, C. D. 1996a. Biology of the gastropod family Littorinidae. I. Evolutionary aspects. *Oceanography and Marine Biology: An Annual Review* **34**:233–262.

McQuaid, C. D. 1996b. Biology of the gastropod family Littorinidae. II. Role in the ecology of intertidal and shallow marine ecosystems. *Oceanography and Marine Biology: An Annual Review* **34**:263–302.

Michaux, B. 1989. Morphological variation of species through time. *Biological Journal of the Linnean Society* **38**:239–255.

Monro, A. K., and S. J. Mayo, eds. 2022. *Cryptic species: morphological stasis, circumscription, and hidden diversity*. Cambridge University Press, Cambridge.

Morard, R., K. F. Darling, A. K. M. Weiner, C. Hassenrück, C. Vanni, T. Cordier, N. Henry, et al. 2024. The global genetic diversity of planktonic foraminifera reveals the structure of cryptic speciation in plankton. *Biological Reviews* **99**:1218–1241.

Murray, T. 1979. Evidence for an additional *Littorina* species and a summary of the reproductive biology of *Littorina* from California. *The Veliger* **21**: 469–474.

Murray, T. 1982. Morphological characterization of the *Littorina scutulata* species complex. *The Veliger* **24**:233–238.

Olivi, G. 1792. *Zoologia Adriatica, ossia catalogo ragionato degli animali del golfo e della lagune di Venezia*. https://www.biodiversitylibrary.org/item/123881.

Pérez-Ponce de León, G., and R. Poulin. 2016. Taxonomic distribution of cryptic diversity among metazoans: not so homogenous after all. *Biology Letters* **12**:20160371.

Pfenninger, M., and K. Schwenk. 2007. Cryptic animal species are homogenously distributed among taxa and biogeographical regions. *BMC Evolutionary Biology* **7**:121.

Puillandre, N., A. V. Sysoev, B. M. Olivera, A. Couloux, and P. Bouchet. 2010. Loss of planktotrophy and speciation: geographic fragmentation in the deep-water gastropod genus *Bathytoma* (Gastropoda, Conoidea) in the western Pacific. *Systematics and Biodiversity* **8**:371–394.

Purens, K. J. S. 2016. Detecting comatulid crinoid cryptic species in the fossil record. *Palaeogeography, Palaeoclimatology, Palaeoecology* **446**:195–204.

R Core Team. 2023. *R: a language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org.

Reid, D. G. 1989. The comparative morphology, phylogeny and evolution of the gastropod family Littorinidae. *Philosophical Transactions of the Royal Society B* **324**:1–110.

Reid, D. G. 1990. A cladistic phylogeny of the genus *Littorina* (Gastropoda): implications for evolution of reproductive strategies and for classification. Pp. 1–19 *in* K. Johannesson, D. G. Raffaelli, and C. J. Hannaford Ellis, eds., *Progress in littorinid and muricid biology*. Kluwer Academic, Dordrecht, Netherlands.

Reid, D. G. 1996. Systematics and evolution of *Littorina*. Ray Society, London.

Reid, D. G., E. Rumbak, and R. H. Thomas. 1996. DNA, morphology and fossils: phylogeny and evolutionary rates of the gastropod genus *Littorina*. *Philosophical Transactions of the Royal Society B* **351**:877–895.

Reid, D. G., P. Dyal, and S. T. Williams. 2012. A global molecular phylogeny of 147 periwinkle species (Gastropoda: Littorininae). *Zoologica Scripta* **41**: 125–136.

Rohlf, F. J. 2015. The tps series of software. *Hystrix* **26**: 9–12. [Software is distributed by the author at: https://sbmorphometrics.org/index.html.]

Rolán-Alvarez, E., C. J. Austin, and E. G. Boulding. 2015. The contribution of the genus *Littorina* to the field of evolutionary biology. *Oceanography and Marine Biology: An Annual Review* **53**:157–214.

Rosewater, J. 1978. A case of double primary homonymy in eastern Pacific Littorinidae. *The Nautilus* **92**:123–125.

Rugh, N. S. 1997. Differences in shell morphology between sibling species *Littorina scutulata* and *Littorina plena* (Gastropoda: Prosobranchia). *The Veliger* **40**:350–357.

Seeley, R. H. 1986. Intense natural selection caused a rapid morphological transition in a living marine snail. *Proceedings of the Natural Academy of Sciences USA* **83**:6897–6901.

Sheets, E. A., P. A. Warner, and S. R. Palumbi. 2018. Accurate population genetic measurements require cryptic species identification in corals. *Coral Reefs* **37**:549–563.

Shin, C. P., and W. D. Allmon. 2023. How we study cryptic species and their biological implications: a case study from marine shelled gastropods. *Ecology and Evolution* **13**:e10360.

Simpson, G. G. 1951. The species concept. *Evolution* **5**:285–298.

Stankowski, S., and M. Ravinet. 2021. Quantifying the use of species concepts. *Current Biology* **31**:R415–R429.

Struck, T. H., J. L. Feder, M. Bendiksby, S. Birkeland, J. Cerca, V. I. Gusarov, S. Kistenich, et al. 2018. Finding evolutionary processes hidden in cryptic species. *Trends in Ecology and Evolution* **33**:153–163.

Tamura, K., G. Stecher, and S. Kumar. 2021. MEGA 11: Molecular Evolutionary Genetics Analysis version 11. *Molecular Biology and Evolution* **38**: 3022–3027.

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

Vermeij, G. J. 1973. Morphological patterns in high-intertidal gastropods: adaptive strategies and their limitations. *Marine Biology* **20**:319–346.

Westram, A. M., S. Stankowski, P. Surendranadh, and N. Barton. 2022. What is reproductive isolation? *Journal of Evolutionary Biology* **35**:1143–1164.

Wickham, H. 2016. *ggplot2: elegant graphics for data analysis*. Springer-Verlag, New York.

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