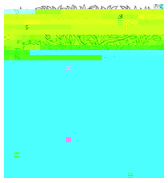

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage:

(

geographical ranges at odds with predictions based on protoconch morphology, for instance [illegible] species have proportionately large protoconchs, implying direct development with limited means of dispersal, but both species within the genus are distributed across vast geographical distances and bathyal depths (Fig. 1b).

We analyse shell morphology using a landmark-based two-dimensional geometric morphometric approach. Geometric morphometric methods are widely seen as superior to traditional morphological measurements as shape can be compared mathematically while controlling for variation in the size, translation (position) and orientation of objects, and they capture information about two- or three-dimensional arrangements as opposed to simple, one-dimensional linear or angular measurements (Webster and Sheets, 2010; Mitteroecker et al., 2013; Monteiro, 2013; Polly et al., 2013). Geometric morphometric methods are multivariate analyses, which are statistically more powerful and robust than uni- or bivariate approaches conducted using linear measurements (Webster and Sheets, 2010; Polly et al., 2013). With the integration of Kendall's 'shape space' (Kendall, 1984), the methods have a strong theoretical underpinning in mathematics and shape theory (Bookstein, 1995). Geometric morphometric analyses can also reveal unexpected variation that is not obvious to human observers (Webster and Sheets, 2010).

We sample all extant species of [illegible], [illegible] and [illegible] and analyse shell shape and size variation using a geometric morphometric method. We compare results of this morphological analysis to a

Table 1
Genomic sequencing of marine snails. Specimens of , and and , and the outgroup genera , and subjected to high-throughput Illumina sequencing, with reads assembled into mitochondrial genome and nuclear ribosomal 45S cassette sequences.

Taxon	GenBank accession

regions of missing sequence (Ns) and ambiguous bases removed. Gblocks 0.91b (Castresana, 2000) was used to remove poorly aligned positions and regions with low homology (Vaux et al., 2017a). SplitsTree 4 (Huson and Bryant, 2006) was used to investigate the unrooted phylogenetic network of sequence alignments. Sequence data were partitioned into protein-encoding, tRNA and rRNA genes. The best fitting nucleotide substitution model for each gene partition was assessed using jModelTest 2.1.6 (Guindon and Gascuel, 2003; Darriba et al., 2012), and were unlinked for phylogenetic inference. The generalised time reversible substitution model (GTR + I + G) (Tavaré, 1986) was found to be the most appropriate substitution model for the mtDNA protein-encoding and nuclear rDNA sequences, whereas the HKY + I + G model (Hasegawa et al., 1985) was most suitable for the mitochondrial tRNA and rRNA regions.

Molecular phylogenies for whole genomic and short-length sequence data were estimated using Bayesian MCMC inference in BEAST 1.8.3 (Drummond et al., 2012). Maximum-likelihood phylogenetic trees were also estimated using RAxML 8.2.8 (Stamatakis, 2014). Posterior statistics for Bayesian MCMC parameters were evaluated using Tracer 1.6 (Rambaut et al., 2014). Tree outputs were viewed and edited in Figtree 1.4.2 (FigTree, 2015), and node support was assessed using posterior probability. All phylogenetic reconstruction was processed using CIPRES Science Gateway (Miller et al., 2010).

Variation in shell morphology was analysed using the same two-dimensional landmark-based geometric morphometric method used to investigate sexual dimorphism in (for detailed method see Vaux et al., 2017b). Shells were photographed with the aperture facing upward (Vaux et al., 2017b), and the positioning and orientation of shells was controlled carefully (see discussion by Webster and Sheets,

2010). Experimental error during photography and digitisation is unlikely to be a confounding source of variation, based on a previous error study for [illegible] (photographic and digitisation error estimated to contribute 1.2% and 0.08% of intraspecific shape variation respectively; Vaux et al., 2017b

mtDNA and nuclear rDNA trees (Fig. 2). Since this di□erence might be due to the shorter sequence length and smaller number of variable sites for the rDNA sequence alignment, we examined phylogenetic signal using as a splits network, which revealed that the mtDNA sequence provides much better resolution than the rDNA data (Supplementary Figs. 4 and 5).

Two statistically significant PCs of shell shape variation were identified (broken-stick test) for the full dataset sampling all shells of and species: PC1 (60.6% of variation), and PC2 (14.5%). In the subsampled dataset, principal components 1 (65.6%) and 2 (14.8%) were also significant. Although the remaining PCs overall account for 24.9% of sample variance (19.6% of subsample) in each dataset respectively, any further PC is unlikely to describe biologically meaningful shape variation (Zelditch et al., 2004). The shape variation represented by PC1 and PC2 in the full and subsampled datasets was almost identical (Fig. 3, Supplementary Fig. 8). Principal component 1 appeared to reflect variation in the width of the shell, with change being most obvious in the aperture, last spire whorl and the siphonal canal, whereas PC2 captured variation in the overall height of the spire and aperture (Fig. 3, Supplementary Fig. 8).

siphonalS70e 3

and aperture, and the width of the aperture and the last whorl. These shape traits are already considered in taxonomy (Ponder, 1973; Powell, 1979; Beu and Maxwell, 1990). Without su□cient ecological or behavioural data, is unclear how di□erences in these features could be adaptive, although in related buccinid snails, the height of the teleoconch spire is likely influenced by water depth and exposure to wave action (Ponder, 1971).

Overall, naïve model-based analysis of shell shape and size identified clusters that reflected generic classification (Fig. 4, Supplementary Fig. 10). Using the full dataset, models with three or four clusters received high BIC support (Supplementary Fig. 6), which frequently grouped specimens classified as [illegible] and [illegible] into unique clusters (Fig. 4). Specimens of [illegible] species were sometimes present in two or more groups, but mostly remained separate from the other genera (Fig. 4). Cluster analysis of the subsampled dataset produced similar results, and models with three clusters often accurately distinguished each genus (Fig. 4, Supplementary Fig. 10). This result suggests that shell shape and size can be used naïvely to identify separate [illegible], [illegible] and [illegible], provided that sampling is approximately even. Unsurprisingly, we resolved the same patterns in shell shape and size identified via the traditional morphological examination of shells, but the cluster analysis accomplished this without reference to location, taxonomy, phylogeny or independent traits such as shell colouration.

Where specimens were classified according to taxonomy, PCA of shell shape indicated that the three genera readily could be distinguished from one another (Fig. 5). Results from the full and subsampled datasets were similar (Fig. 5). Inof1rea

hierarchically nested across models (Fig. 4, Supplementary Fig. 7), but these groupings corresponded with low accuracy to particular subclades or species within (compare Figs. 2 and 4). For example, based on shell shape, the sister species from Australia (and) each cluster with a di erent New Zealand species (and respectively; Fig. 4). Such groupings may highlight ecological similarity and potential evolutionary convergence, as and are both smaller species that can be found on rocky substrates in shallow water (1–50 m), whereas and are larger and are mostly restricted to soft sediments at greater depths (Ponder, 1973; Powell, 1979). Given the sister relationship of and in the genetic data (Fig. 2), and their overlapping extant and fossil ranges (Fig. 1; Ponder, 1973), it is possible that niche partitioning by prey size or water depth could have facilitated ecological sympatric speciation in Australian waters. Cluster analysis of the subsampled dataset did not distinguish species nor subclades within each genus (Supplementary Fig. 10).

The failure to naïvely distinguish any species within and may be due to the limited sampling of these taxa. That said, the consistent placement of in a cluster with (Fig. 4, Supplementary Fig. 10) concords with recent genetic results that indicated a close evolutionary relationship (Vaux et al., 2017a), despite disagreeing with previous morphologically derived taxonomy that identified as a species of (Powell, 1979) A future investigation focussed on shell shape and size variation in alone, the genus with the most abundant fossil record, might yield PCs that can separate species. However, given the conflicting evidence from mitochondrial and nuclear markers, a morphological analysis y9dy779.26tp[re

a n d

2011

Jackson, D.A., 1993. Stopping rules in principal components analysis: a comparison of heuristical and statistical approaches. Ecology 74, 2204–2214.
Kantor, Y.I., 2003. Comparative anatomy of the stomach of Buccinoidea (Neogastropoda). J. Molluscan Stud. 69, 203–220.
Kantor, Y.I., 2013. Deep-water Buccinidae (Gastropoda: Neogastropoda) from sunken wood, vents and seeps: molecular phylogeny and taxonomy. J. Mar. Biol. Assoc. U.K. 93, 2177–2195.
Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Mentjies, P., Drummond, A., 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28, 1647–1649.
Kendall, D.G., 1984. Shape manifolds, procrustean metrics, and complex projective spaces. Bull. London Math. Soc. 16, 81–121.
Klingenberg, C.P., 2011. MorphoJ: an integrated software package for geometric morphometrics. Mol. Ecol. Resour. 11, 353–357.
Kurata, K., Kikuchi, E., 2000. Comparisons of life-history traits and sexual dimorphism between [illegible] and [illegible] shells. Venus 59,6(Molluscan)-341(Stud.)-336.3(69,)-339.8(81)]TJ/T11Tf3.998590Td(-)Tj/T101Tf0.50680Td(220)196idea (Neogastropoda).