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Evolutionary lineages of marine snails identified using molecular phylogenetics and geometric morphometric analysis of shells

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ABSTRACT

The relationship between morphology and inheritance is of perennial interest in evolutionary biology and palaeontology. Using three marine snail genera *Penion, Antarctoneptunea* and *Kelletia*, we investigate whether systematics based on shell morphology accurately reflect evolutionary lineages indicated by molecular phylogenetics. Members of these gastropod genera have been a taxonomic challenge due to substantial variation in shell morphology, conservative radular and soft tissue morphology, few known ecological differences, and geographical overlap between numerous species. Sampling all sixteen putative taxa identified across the three genera, we infer mitochondrial and nuclear ribosomal DNA phylogenetic relationships within the group, and compare this to variation in adult shell shape and size. Results of phylogenetic analysis indicate that each genus is monophyletic, although the status of some phylogenetically derived and likely more recently evolved taxa within *Penion* is uncertain. The recently described species *P. lineatus* is supported by genetic evidence. Morphology, captured using geometric morphometric analysis, distinguishes the genera and matches the molecular phylogeny, although using the same dataset, species and phylogenetic subclades are not identified with high accuracy. Overall, despite abundant variation, we find that shell morphology accurately reflects genus-level classification and the corresponding deep phylogenetic splits identified in this group of marine snails.

1. Introduction

A persistent problem for evolutionary biology and palaeontology is whether morphology accurately reflects phylogenetic relationships in a given set of organisms. Morphological traits are desirable for systematic study because they can be considered across the entire range of living systems from subcellular pathogens (e.g. Roberts and Compans, 1998; Diaz-Avalos et al., 2005), to unicellular (e.g. Siefert and Fox, 1998) and multicellular organisms (e.g. Niklas, 2000; Valentin et al., 2002; Hills et al., 2012; Dowle et al., 2015). Morphology can be considered at numerous levels, including nucleic acid and protein structure (e.g. Ender and Schierwater, 2003; Sakamaki et al., 2015), gametes (e.g. Landry et al., 2003), and body plans (e.g. Niklas, 2000; Valentin et al., 2002), and it includes obvious traits, often likely to be under selection, that are intuitive to observe and measure. Morphology is the predominant evidence preserved in the fossil record, which is our only source of primary data for the majority of evolutionary time and the overwhelming majority of taxa that have ever lived (Marshall, 2017). However, a significant problem for evolutionary analysis is that morphological variation does not necessarily concord with the splitting and divergence of evolutionary lineages (Bapst, 2013; Vaux et al., 2016). Consequently, instances where morphological change is concordant with phylogeny provide the best opportunity to estimate rates of evolution over long periods of time (Hunt, 2013), as well as speciation and changes in diversity.

Molluscan shells have the potential to provide information about both the pattern and process of morphological evolution. Their calcareous shells frequently preserve in good condition, meaning that marine molluscs have some of the best-preserved fossil records of all animals (Wagner, 2001; Crampton et al., 2006). Many lineages are consequently used to investigate speciation and models of evolutionary change (e.g. Michaux, 1989; Wagner, 2001; Monnet et al., 2011; Hills et al., 2012; Collins et al., 2016; Combosch and Giribet, 2016). Shell morphology can capture features of development and growth

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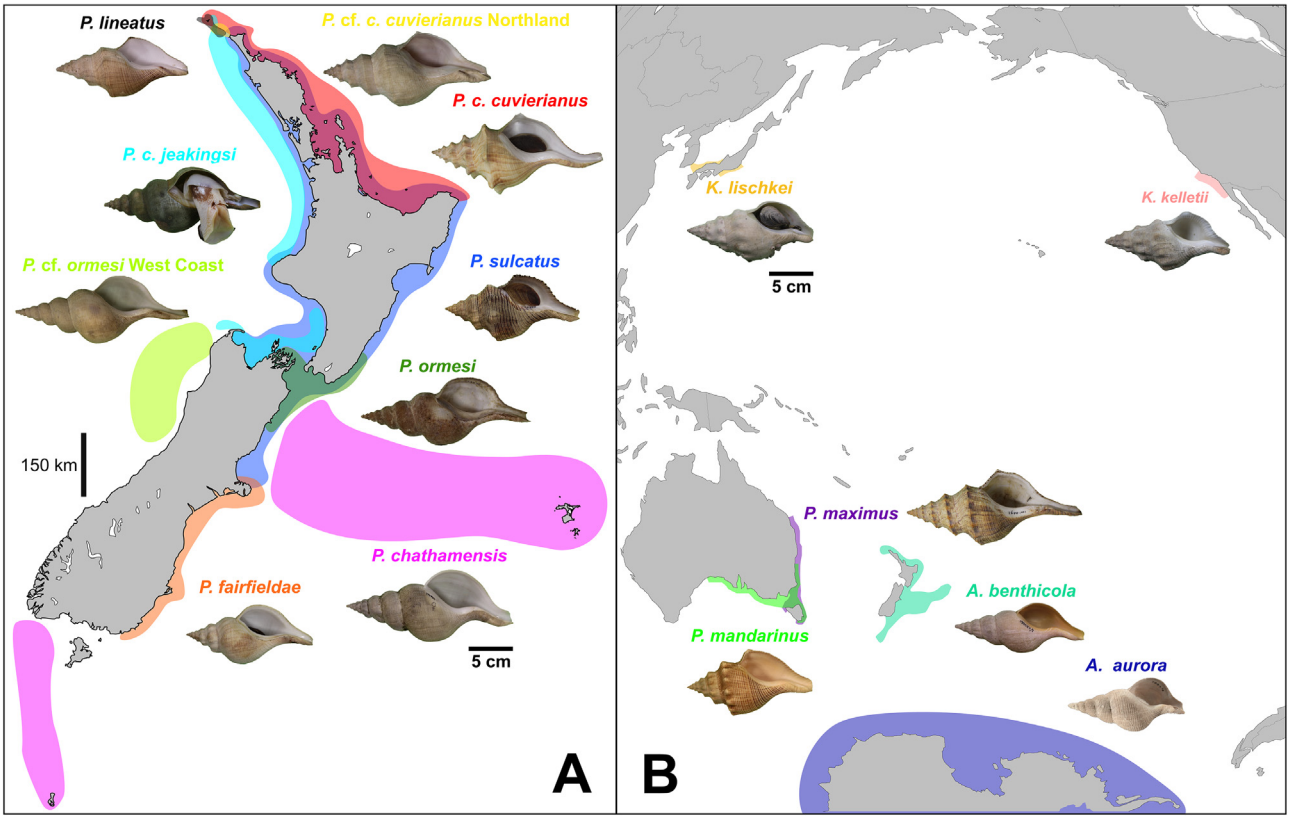

Fig. 1. Geographical distributions of extant *Penion, Antarctoneptunea* and *Kelletia* species, showing sympatry of many taxa. The range of each putative taxon is highlighted in a different colour and an example shell is shown at the same scale (animal included for *P. cuvierianus jeakingsi*). References for geographical distributions are given in the text. (A) The distribution of *Penion* taxa in New Zealand waters. Scale bars shown 150 km for map and 5 cm for shell photos. (B) The distribution of Australian *Penion* species, *Antarctoneptunea* and *Kelletia* throughout the Pacific Ocean. Scale bar shows 5 cm for shell photos.

(Thompson, 1942; Seilacher and Gunji, 1993), reflect habitat and niche adaptation (e.g. Seilacher and Gunji, 1993; Vermeij, 1995), and even indicate the morphology of non-preserved soft tissue (e.g. Runnegar and Bentley, 1983). However, instead of genetic difference, variation in shell morphology can also represent convergent evolution (e.g. Serb et al., 2011), phenotypic plasticity in response to environmental variation (e.g. Palmer, 1990; Trussell, 2000, Hollander et al., 2006; Gemmell, 2017), or sexual dimorphism (e.g. Kurata and Kikuchi, 2000; Avaca et al., 2013).

In this study we investigate whether variation in shell morphology is concordant with phylogenetic relationships among three closely related gastropod genera: *Penion* P. Fischer, 1884, *Antarctoneptunea* Dell, 1972 and *Kelletia* Bayle, 1884 (Neogastropoda, Buccinoidea). All three genera contain medium to large, benthic marine snails that exhibit considerable taxonomic and morphological diversity (Fig. 1). Molecular evidence supports a close relationship between these three genera (Hayashi, 2005; Vaux et al., 2017a), which was predicted by shell morphology (Dell, 1972; Ponder, 1973). As with many other buccinoideans (e.g. Willan, 1978; Kantor, 2003; Walker et al., 2008), soft part radular and opercular morphology is too conservative to distinguish most taxa reliably (e.g. Dell, 1956; Ponder, 1973). Based on traditional morphological measurements, adult (teleoconch) and larval (protoconch) shells vary significantly within and between putative species (e.g. Ponder, 1973; Powell, 1979; Beu and Maxwell, 1990).

Six extant species and one subspecies of *Penion* are currently recognised in New Zealand waters (Powell, 1979; Spencer et al., 2017; Marshall et al., 2018), and a further two species are endemic to southeastern Australia (Ponder, 1973, Fig. 1). There is also a rich fossil record for *Penion* in the South Pacific (e.g. Ponder, 1973; Beu and Maxwell, 1990; Nielsen, 2003; Beu, 2009; Crame et al., 2014). *Kelletia* is represented by only two extant species, endemic to waters off Japan and South Korea (Hayashi, 2005; Hwang et al., 2014), and southern California and Baja Mexico, respectively (Zacherl et al., 2003). Further fossil *Kelletia* species are recorded from both regions as well as Ecuador (e.g. Anderson, 1910; Arnold, 1910; Addicott, 1970, Ozaki, 1954; Olsson, 1964). One species of *Antarctoneptunea* is found in New Zealand waters (Dell, 1995; Vaux et al., 2017a) and another occurs off Antarctica (Dell, 1972, Fig. 1). The high taxonomic diversity of *Penion* in New Zealand corresponds with a high level of endemism for other marine snails (Powell, 1979, Spencer et al., 2009, 2017), which is likely driven partially by the geographical remoteness of the region (Vaux et al., 2017a).

Ecological and behavioural data that might aid the distinction of *Penion, Antarctoneptunea* and *Kelletia* species are scarce. All species are predator-scavengers (Rosenthal, 1971; Harasewych, 1998), and most occur on soft sediment basins in mid-shelf to bathyal depths (50–2000 m, Dell, 1956; Powell, 1979), although *Kelletia* and some *Penion* species occur on rocky coastal substrates in shallow water (0–50 m, Powell, 1979; Vendetti, 2009; Willan et al., 2010; Hwang et al., 2013). All three genera have dioecious sexes (Rosenthal, 1970; Ponder, 1973). There is a size difference in mating pairs of *K. kelletii* (Forbes, 1850) (Rosenthal, 1970), but there is no evidence for secondary sexual dimorphism in the shell shape and size of *P. chathamensis* (Powell, 1938) (Vaux et al., 2017b). Despite apparent variation, there is currently insufficient evidence for developmental traits to be used for the distinction of genera or species (Vaux et al., 2017a). Captive rearing has demonstrated that the larvae of *K. kelletii* undergo indirect development with facultative planktotrophy, potentially permitting long-distance dispersal (Vendetti, 2009). However, estimates regarding the development of all other taxa are based on protoconch morphology, which is highly variable among both extant and fossil species (Ponder, 1973; Powell, 1979; Nielsen, 2003). Some lineages also occupy

geographical ranges at odds with predictions based on protoconch morphology, for instance *Antarctoneptunea* 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 *Penion, Antarctoneptunea* and *Kelletia* and analyse shell shape and size variation using a geometric morphometric method. We compare results of this morphological analysis to a complete molecular phylogeny of the three genera, and determine whether shell morphology can be used to reconstruct evolutionary relationships and incorporate fossil specimens. The landmarks used in this study capture some key shell measurements traditionally used in gastropod taxonomy such as shell and aperture height, along with shape information. Many molluscan studies have compared molecular data with discrete morphological measurements or character states (e.g. Reid et al., 1996; Iguchi et al., 2005; Grahame et al., 2006; Kantor, 2013; Sigwart and Lindberg, 2015; Moussalli and Herbert, 2016; Bapst et al., 2017), but research comparing genetic and geometric morphometric variation for extant (e.g. Pfenninger et al., 2006; Cunha et al., 2014; Dowle et al., 2015; Becker et al., 2016; Gemmell, 2017; Verhaegen et al., 2018) or fossil taxa (e.g. Hills et al., 2012; Smith and Hendricks, 2013) is still developing.

2. Materials and methods

2.1. Taxonomy and sampling

Individual snails were initially assigned taxon names using the traditional examination of shell morphology, which focusses on such features as shell size and shape, sculpture, protoconch morphology, number of teleoconch whorls, and colour and pattern (e.g. Ponder, 1973; Powell, 1979). All specimens were classified by experienced molluscan taxonomists (Bruce A. Marshall and Alan G. Beu).

All extant species of *Penion* from New Zealand and Australia were sampled, including all subspecies recognised by Ponder (1973) and Powell (1979), as well as all extant species of *Antarctoneptunea* and *Kelletia* (Fig. 1 for species distributions). Our sampling of New Zealand *Penion* included two morphological variants, *P.* cf. *cuvierianus cuvierianus* (Powell, 1927) from northern Northland, and *P.* cf. *ormesi* (Powell, 1927) from the West Coast, South Island. The majority of specimens examined came from the Museum of New Zealand Te Papa Tongarewa, and other museum and university collections (acknowledged below), supplemented with snails newly collected in the field (Tables 1 and 2). Specimens were collected by trawling (20–500 m depth for most sampling), snorkelling (2–5 m), and by hand within the intertidal zone (1–3 m). Some specimens were derived from commercial trawling fishery bycatch. Animals used for DNA sequencing were frozen swiftly following capture, and subsequently thawed and removed from their shells, with tissue clips preserved in ample 98% ethanol.

For DNA sequencing at least one individual from each species was sampled, although multiple individuals were sequenced from some taxa to investigate intraspecific variation across geographical ranges (Table 1). For geometric morphometric analysis, a substantial number of shells were sampled for each putative species (see Table 3), with at least 46 shells per species where possible, as this number exceeds the final number of landmarks used (45). For downstream analyses, adequate sampling ensures that the degrees of freedom exceed the shape dimensionality of the data.

Molecular and geometric morphometric sampling was limited (only one individual for DNA sequencing and fewer than 46 shells) for six taxa: *P. lineatus* Marshall, Hills & Vaux, 2018, *P.* cf. *ormesi* West Coast, *P.* cf. *c. cuvierianus* Northland, *A. aurora* (Hedley, 1916), *K. kelletii* (Forbes, 1850) and *K. lischkei* Kuroda, 1938 (Tables 1–3). The first four taxa are known only from remote regions or have restricted geographical ranges (the Three Kings Islands, West Coast, northern Northland, and the Southern Ocean respectively – see Fig. 1a), which makes sampling challenging. Both *Kelletia* species were sampled at low frequency because they occur outside Australasia and could not be resampled as part of this study.

For outgroup taxa, we sampled mtDNA and rDNA sequence data from one individual each of the buccinoid species *Cominella adspersa* (Bruguière, 1789), *Aeneator elegans* (Suter, 1917), *A. recens* (Dell, 1951), *Buccinulum fuscozonatum* (Suter, 1908), and *B. pertinax finlayi* Powell, 1929. The mtDNA *cox1* gene was also PCR amplified and sequenced from additional *Antarctoneptunea, Kelletia* and *Penion* individuals to assess phylogenetic trees with more specimens (Table 2). Sequences for *Kelletia lischkei* from a previous study were also used (Table 2, Kim et al., 2012).

Only complete or near-complete adult specimens, with intact shell margins and points encompassed by landmarks, and reliable provenance data were used for geometric morphometric analysis (n = 1037). Shell maturity was determined from the presence of at least six teleoconch whorls, thickening of the outer shell lip and the ascent of the end of the last whorl (Vaux et al., 2017b). Sampling was restricted to adult shells to avoid the potential allometric effects of development (see Outomuro and Johansson, 2017). Among our shell sampling of *Antarctoneptunea, Kelletia* and *Penion*, 20 specimens also provided genetic data from their soft tissue.

Since many more shells of *Penion* species were sampled than *Antarctoneptunea* or *Kelletia* species (Table 3), it is possible that variation among specimens could be dominated by variation within *Penion*. To address this, we analysed shell shape and size variation using the 'full dataset' (1037 shells), but also reanalysed variation using a 'subsampled dataset' of 155 shells (Table 3). In the subsampled dataset, we randomly selected proportionate numbers of specimens from each *Penion* taxon to yield 61 specimens (equal to the sampling of *Antarctoneptunea*).

2.2. DNA sequencing and molecular phylogenetics

Total genomic DNA was obtained using a standardised extraction protocol (Vaux et al., 2017a). DNA was quantified using the Qubit Fluorometric Quantitation kit (Life Technologies, Thermo Fisher Scientific Inc.).

Whole DNA extracts from 26 individuals of 21 taxa were processed for high-throughput sequencing using the ThruPLEX DNA-seq Kit (Rubicon Genomics). Fragmented genomic DNA was paired-end sequenced on an Illumina HiSeq 2500 (Table 1). Reads for each of the 26 individuals were de-multiplexed using standard indexes incorporated in the library preparation kit. Resulting short-sequence reads that passed standard quality filters had adapter sequences removed using cutadapt 1.11 (Martin, 2011). Following a previous bioinformatics method (Vaux et al., 2017a), we paired sequence reads, and assembled and aligned sequences using Geneious 9.1.3 (Kearse et al., 2012).

Alignments for phylogenetic analyses were concatenated and had

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

Taxon	GenBank accession				Voucher ID	Location
	rDNA 18S rRNA	rDNA 5.8S rRNA	rDNA 28S rRNA	mtDNA genome		
Aeneator elegans	MH277509	MH277578	MH277534	MH198157	SFKH-TMP015	Chatham Rise, NZ
Aeneator recens	MH277510	MH277579	MH277535	MH198159	M.190119	Cape Turnagain, NZ
Antarctoneptunea aurora	MH277511	MH277580	MH277536	MH140430	MNA0094	Hallet Peninsula, Antarctica
Antarctoneptunea benthicola	MH277512	MH277581	MH277537	MH198156	M.183832	Chatham Rise, NZ
Antarctoneptunea benthicola	MH277514	MH277583	MH277539	MH198173	M.274268	Cape Kidnappers, NZ
Antarctoneptunea benthicola	MH277513	MH277582	MH277538	MH198172	M.306257/2	Cape Runaway, NZ
Buccinulum fuscozonatum	MH277515	MH277584	MH277540	MH198158	M.302907/2	Ariel Bank, Gisborne, NZ
Buccinulum p. finlayi	MH277516	MH277585	MH277541	MH198162	M.302870/2	Oneroa Bay, Bay of Islands, NZ
Cominella adpsersa	MH277517	MH277586	MH277542	MH198163	SFKH-TMP009	Urupukapuka Bay, Bay of Islands, NZ
Kelletia kelletii	MH277518	MH277587	MH277543	MH198161	KK12	Santa Barbara, California, USA
Kelletia lischkei	MH277519	MH277588	MH277544	MH198160	KL2	Kansai, Mie Prefecture, Japan
Penion chathamensis	MH277520	MH277589	MH277545	MH140429	M.190082/2	Chatham Rise, NZ
Penion chathamensis	MH277521	MH277590	MH277546	MH140428	M.190085	Chatham Rise, NZ
Penion c. cuvierianus	MH277523	MH277592	MH277548	MH140431	M.183792/1	Red Mercury Island, NZ
Penion c. cuvierianus	MH277524	MH277593	MH277549	MH140432	M.183927	Coromandel, NZ
Penion cf. *c. cuvierianus* Northland	MH277525	MH277594	MH277550	MH198171	M.318615/1	Columbia Bank, Northland, NZ
Penion c. jeakingsi	MH277526	MH277595	MH277551	MH198170	M.279432/6	Tasman Bay, NZ
Penion fairfieldae	MH277522	MH277591	MH277547	MH198165	Phoenix1	Otago Peninsula, NZ
Penion lineatus	MH277527	MH277596	MH277552	MH198166	M.302876	Three Kings Islands, NZ
Penion mandarinus	MH277528	MH277597	MH277553	MG211145	C.456980	Gabo Island, Victoria, Australia
Penion maximus	MH277529	MH277598	MH277554	MG211144	C.487648	Terrigal, New South Wales, Australia
Penion ormesi	MH277530	MH277599	MH277555	MH198168	M.299869/1	Cloudy Bay, NZ
Penion ormesi	MH277531	MH277600	MH277556	MH198167	M.318565/2	Pelorus Sound, NZ
Penion cf. *ormesi* West Coast	MH277532	MH277601	MH277557	MH198169	M.316215/1	Kahurangi Point, NZ
Penion sulcatus	MH277533	MH277602	MH277558	MH198164	Phoenix1	Tauranga, NZ
Penion sulcatus	MG194428	MG194427	MG194426	MG098232	Phoenix9	Auckland, NZ

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).

2.3. Geometric morphometric analysis of shells

Variation in shell morphology was analysed using the same two-dimensional landmark-based geometric morphometric method used to investigate sexual dimorphism in *P. chathamensis* (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,

Table 2
Further snails for *cox1* sequencing. Additional *Penion, Kelletia* and *Antarctoneptunea* specimens used for *cox1* phylogenetic reconstruction.

Taxon	Voucher ID	Location	GenBank accession	Source
Antarctoneptunea aurora	MNA0095	Adare Peninsula, Ross Sea	MH281631	This paper
Antarctoneptunea aurora	MNA0096	Hallet Peninsula, Ross Sea	MH281632	This paper
Kelletia lischkei	KL1	Kansai, Mie Prefecture, Japan	MH281633	This paper
Kelletia lischkei	KL2	Kansai, Mie Prefecture, Japan	MH281634	This paper
Kelletia lischkei		Yeosu, South Korea	HM180632	Kim et al. (2012)
Kelletia lischkei		Yeosu, South Korea	HM180633	Kim et al. (2012)
Kelletia lischkei		Yeosu, South Korea	HM180634	Kim et al. (2012)
Kelletia lischkei		Yeosu, South Korea	HM180635	Kim et al. (2012)
Kelletia lischkei		Yeosu, South Korea	HM180636	Kim et al. (2012)
Penion c. jeakingsi	M.279432/1	Tasman Bay, NZ	MH281635	This paper
Penion c. jeakingsi	M.279432/3	Tasman Bay, NZ	MH281636	This paper
Penion c. jeakingsi	M.279432/5	Tasman Bay, NZ	MH281637	This paper
Penion c. jeakingsi	M.279432/7	Tasman Bay, NZ	MH281638	This paper
Penion c. jeakingsi	Phoenix2	Golden Bay, NZ	MH281639	This paper
Penion c. jeakingsi	Phoenix3	Golden Bay, NZ	MH281640	This paper
Penion ormesi	M.318599/2	Pelorus Sound, NZ	MH281641	This paper

Table 3
Geometric morphometric sampling of snail shells. Sampling of extant, adult shells of *Penion, Kelletia* and *Antarctoneptunea* species used for geometric morphometric analysis.

Genus	Species	Geographical region	Number of shells	Subsampling
Antarctoneptunea	*aurora*	Antarctica	1	1
Antarctoneptunea	*benthicola*	New Zealand	60	60
Kelletia	*kelletii*	USA and Mexico	24	24
Kelletia	*lischkei*	Japan and South Korea	8	8
Penion	*chathamensis*	New Zealand	125	8
Penion	*c. cuvierianus*	New Zealand	200	12
Penion	cf. *c. cuvierianus* Northland	New Zealand	21	2
Penion	*c. jeakingsi*	New Zealand	78	5
Penion	*fairfieldae*	New Zealand	48	3
Penion	*lineatus*	New Zealand	25	2
Penion	*mandarinus*	Australia	89	6
Penion	*maximus*	Australia	114	8
Penion	*ormesi*	New Zealand	50	3
Penion	cf. *ormesi* West Coast	New Zealand	4	1
Penion	*sulcatus*	New Zealand	190	11
TOTAL			1037	155

2010). Experimental error during photography and digitisation is unlikely to be a confounding source of variation, based on a previous error study for *P. chathamensis* (photographic and digitisation error estimated to contribute 1.2% and 0.08% of intraspecific shape variation respectively; Vaux et al., 2017b). We used a total of 45 landmarks; of these, six represent biologically homologous landmarks in the strict sense, and 39 are semi-landmarks (see Webster and Sheets, 2010) that are used to capture the shape of outline segments (Vaux et al., 2017b). The programs tpsUtil, tpsDig (Rohlf, 2013), and CoordGen8 (Sheets, 2014) were used to digitise landmarks and slide semi-landmarks. Semi-landmarks are slid to minimise variation associated with arbitrary placement along a contour, which is this case was achieved by minimising the Procrustes distance between individuals (Bookstein, 1996; Vaux et al., 2017b). “Combs” were added to photographs in Adobe Photoshop CS6 prior to digitisation to ensure approximately consistent placement of semi-landmarks (Vaux et al., 2017b).

Partial Procrustes superimposition, principal components analysis (PCA), and canonical variates analysis (CVA) were conducted using MorphoJ 1.06c (Klingenberg, 2011). The principal components generated by PCA reflect (mathematically independent) variation in the shape of objects, and centroid size acts as a proxy for size variation (independent of shape). For graphical interpretation, the number of ‘meaningful’ principal components (PCs) was determined using the broken-stick test on eigenvalues to identify PCs that explain more variance in the data than expected by chance alone, as implemented in the R (R Core Team, 2017) package vegan 2.2-1 (Jackson, 1993; Zelditch et al., 2004; Oksanen et al., 2015). We used PCA ordinations with 90% mean confidence ellipses plotted in order to estimate the separation and discrimination of *a priori* groups (e.g. monophyletic clades, taxonomic species).

Variation in shell shape and size was examined together by scaling centroid size with the statistically significant PCs, and producing three-dimensional plots using the R package car (Fox and Weisberg, 2011). Scaling was conducted using the base scale function in R (Becker et al., 1988; R Core Team, 2017). This function centres a data column by subtracting the mean from each observation and dividing values by the estimated standard deviation (Becker et al., 1988). For the three-dimensional plots used, all PCs and centroid size scaled in the same manner and weighted equally. The ability to differentiate these *a priori* groups using only shell shape was estimated via cross-validation scores from CVA implemented using the R package MASS 7.3-26 (Venables and Ripley, 2002; R Core Team, 2017) using PCs that accounted for 95% of variation among specimens. CVA employed additional PCs, over and above those identified as meaningful using the broken-stick test (above), to allow for the possibility that useful variance is in fact captured by these PCs; if these additional PCs describe little more than noise, then they are not expected to compromise the CVA. We also used F-tests (2500 bootstraps using Procrustes distances) implemented in TwoGroup8 (Sheets, 2014) to statistically test shell shape differences between genera.

Groupings that could be identified naïvely using only shell shape (significant PCs) and size variation (centroid size) were investigated using model-based cluster analysis implemented in the R package mclust 5.2 (Fraley and Raftery, 2002). Mclust attempts to identify the multivariate clustering model that most efficiently explains variation in a dataset without prior classification of specimens (method in Fraley and Raftery, 2012; Vaux et al., 2017b). Models used by mclust are named based on their parametisation, for example the VVE model uses variable volume, variable shape, and equal orientation for the clustering of data (full list of model explanations in Fraley and Raftery, 2012). Two models with different parameters can support the same number of clusters, although these clusters are unlikely to be identical due to the different settings used. Bayesian information criterion (BIC) scores were used to determine the relative support for competing clustering models. In mclust, BIC scores are multiplied by −1 and therefore higher BIC values indicate higher support. Where centroid size was included with PCs for mclust analyses, variables were scaled using the same method as above and weighted equally, because centroid size is expressed on a much larger numerical scale than the PCs (Vaux et al., 2017b).

3. Results

3.1. mtDNA and rDNA phylogenetics

We assembled new mitochondrial genomes and nuclear 45S rDNA sequences (18S, 5.8S, 28S rRNA genes) from 11 individuals representing 6 putative taxa (Table 1). All sequenced mtDNA genomes contained the standard gene complement and order described for previously sequenced neogastropod species (Simison et al., 2006; Cunha et al., 2009; Hills et al., 2011; Vaux et al., 2017a). A few individuals had short read coverage dropouts for mitochondrial genome sequences, and for two individuals, *P. c. cuvierianus* M.183927 and *P. ormesi* M.318565/2, mtDNA sequencing was only a partial success as there were gaps of up to 500 bp in their cicular genome (Supplementary Table 1 available in Appendix 1). Whole mtDNA genomes varied between 15,227 and 15,251 bp in length, and the concatenated 18S, 5.8S and 28S rRNA genes for the nuclear 45S rDNA cassette varied between 5337 and 5447 bp (Supplementary Tables 1 and 2). There was little variation in nucleotide ratios among sequences (Supplementary Tables 1 and 2).

For mtDNA sequences, gblocks retained 98% of the original mtDNA protein encoding nucleotide positions, and 77% and 90% of the mitochondrial tRNA and rRNA positions respectively. This resulted in sequence lengths of 9349 bp, 995 bp and 984 bp respectively for mtDNA protein-encoding, tRNA and rRNA sequence regions. Most (99%) of the nuclear rDNA (excluding ITS1 and 2) nucleotide positions were also retained, leaving an alignment sequence length of 4576 bp available for phylogenetic reconstruction.

Phylogenetic relationships inferred separately from mtDNA and nuclear rDNA data were broadly similar (Fig. 2), and Bayesian and maximum-likelihood trees were mostly consistent (Fig. 2, Supplementary Figs. 1 and 2 in Appendix 1). Concordant phylogenetic relationships were also inferred from the short-length *cox1* data (Supplementary Fig. 3). However, the phylogenetic placement of *P. lineatus, P. c. cuvierianus, P. cuvierianus jeakingsi* (Powell, 1947), *P. ormesi* (and respective morphological variants) differed between the

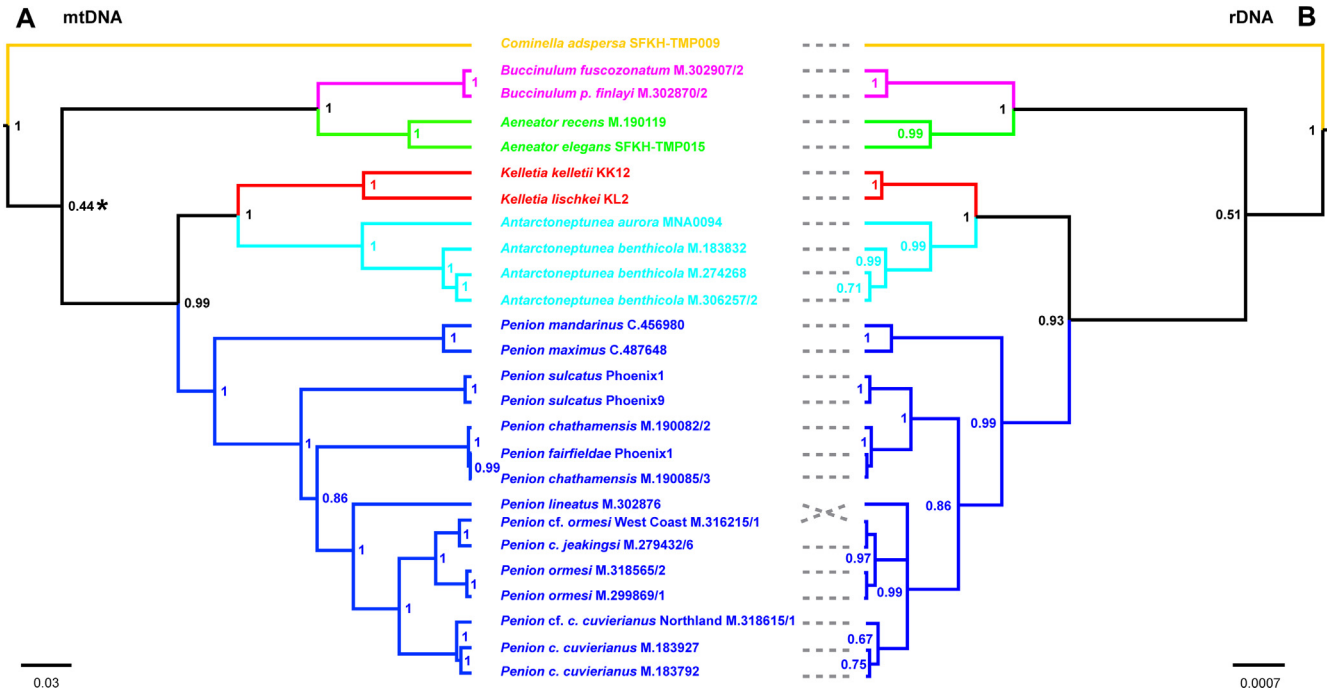

Fig. 2. Concordant evolutionary relationships among *Penion, Antarctoneptunea* and *Kelletia* species inferred using mitochondrial (A) and nuclear (B) DNA sequences. Individuals are coloured according to generic classification. Scale bars denote the estimated substitution rate. Bayesian phylogenies were estimated using BEAST, operated using an MCMC length of 100 million, 1000 sample frequency and a 10% burn-in. No outgroup or monophyly was enforced for either tree. The full list of parameters used to produce trees is provided in the text. Splits with < 0.5 posterior support are not shown, with the exception of a split involving *Aeneator* and *Buccinulum* in the mtDNA tree (A), as this relationship has been supported in wider sampled mtDNA-based trees of buccinoid whelks (Hayashi, 2005; Vaux et al., 2017a). (A) A phylogeny based on an alignment of 26 mitochondrial genome sequences (incorporating protein-encoding, tRNA and rRNA genes). Two sequence partitions were used: (1) protein-encoding genes (9349 bp), and (2) tRNA and rRNA genes (1979 bp) using the GTR + I + G and HKY + I + G substitution models respectively. (B) A phylogeny based on a 4576 bp alignment of 26 nuclear rDNA sequences (18S, 5.8S, 28S rRNA genes). Sequence data were not partitioned and the GTR + I + G substitution model was used.

mtDNA and nuclear rDNA trees (Fig. 2). Since this difference 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).

3.2. Geometric morphometric analysis of shells

Two statistically significant PCs of shell shape variation were identified (broken-stick test) for the full dataset sampling all shells of *Penion, Antarctoneptunea* and *Kelletia* 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).

For the full dataset, naïve cluster analysis using mclust found highest support for a model of three clusters where only shell shape variation (PCs 1 and 2) was analysed (VVE3, based on BIC score using mclust; Fig. 4b, Supplementary Fig. 6). Under this model, shells of *Kelletia* were distinguished from *Penion* and *Antarctoneptunea* (Fig. 4b), with cluster 1 containing 90.6% of sampling for the genus (29 out of 32 specimens), and the genus representing 74.4% of that cluster (29 out of 39 shells). With the inclusion of shell size in the cluster analysis of the full dataset, a model with four clusters received highest support (EVE4; Fig. 4b, Supplementary Fig. 6). However, models using shell shape and size with five clusters received almost exactly equal BIC scores (Supplementary Fig. 6), and we present a PCA plot of the EVE5 model as it exhibits groupings that closely align with generic classification (Fig. 4a). Under this model *Antarctoneptunea* was separated with high accuracy, with cluster 5 containing 93.4% of sampling for the genus (57 out of 61 specimens), and the genus representing 68.7% of the cluster (57 out of 83 shells).

The clusters identified in the subsampled dataset were similar to those in the full dataset (Fig. 4c, Supplementary Figs. 9 and 10). For the subsampled dataset, a model with three clusters received the highest BIC support when only the significant shape PCs were analysed, and models with three or four clusters were best supported where shell size was also included (Supplementary Fig. 9).

Clusters identified in the full dataset appeared to be nest hierarchically after three or four clusters, and groupings were fairly consistent across models (Fig. 4b). Models with > 4 clusters frequently also had similar BIC scores (Supplementary Fig. 6). The hierarchical nature of the data could be best observed by comparing the assignment probability of individuals across models and varying number of clusters (Fig. 4b), and the assignment of specimens across models was compared directly (Supplementary Fig. 7).

In the full dataset, these hierarchically nested clusters sometimes corresponded to particular species or phylogenetic subclades within *Penion*, but usually with low accuracy (Fig. 4b). For example, for the EVE4 model using shell shape and size (Fig. 4b), cluster 2 contains almost all specimens of *P. sulcatus* (Lamarck, 1816) (95.0% of species, 53.6% of cluster 2) and many *P. fairfieldae* (Powell, 1947) (79.2% of species, 11.9% of cluster 2), which are phylogenetically sister according to mtDNA and rDNA data (Fig. 2). In contrast, *P. mandarinus* and *P.*

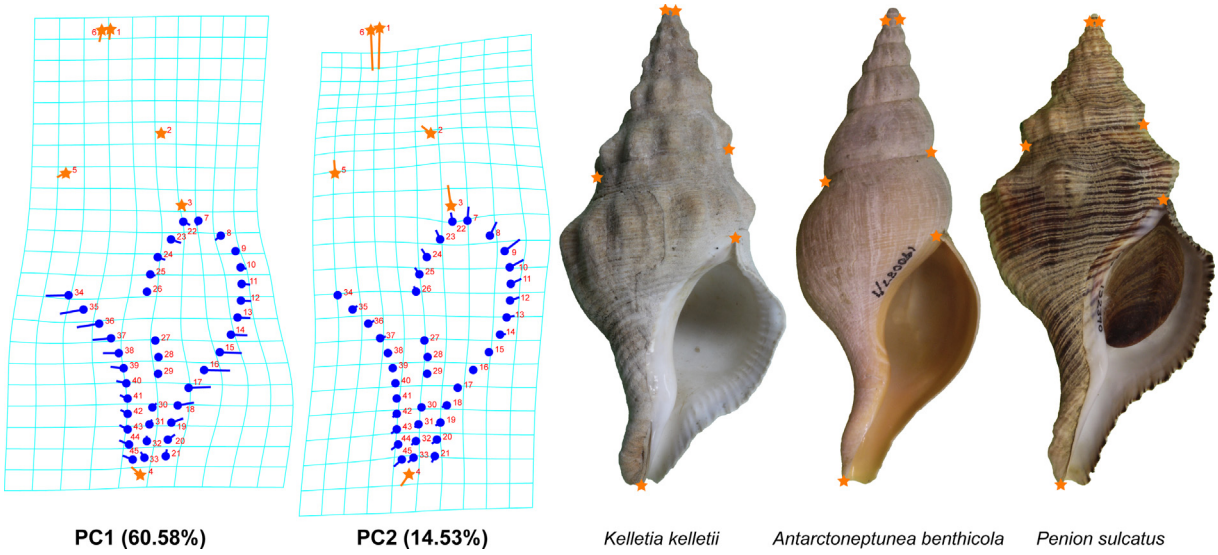

Fig. 3. Shape comparisons of *Penion, Antarctoneptunea* and *Kelletia* shells. Thin plate spline diagrams with a transformation grid produced using MorphoJ, showing the shape differences represented by PC1 (60.6% of variation) and PC2 (14.4%) in the full dataset. See Supplementary Fig. 8 for shape differences in the subsampled dataset. Landmarks and semi-landmarks are illustrated using orange stars and blue circles, respectively. The length of lollipop lines from landmarks demonstrates warping in shape space for each PC. Shells of *K. kelletii, A. benthicola* and *P. sulcatus* (not to scale) with landmarks superimposed are provided for comparison to the TPS thin plate spline diagrams.

maximus from Australia are phylogenetically sister taxa according to mtDNA and rDNA sequence data, but they are not assigned to the same morphological cluster within *Penion.* In contrast, naïve clusters that corresponded to species classification were not resolved in the subsampled dataset (Supplementary Fig. 10).

Similarity between the clusters identified by mclust and generic and species-level classification can be further explored by comparing PCA plots (compare Figs. 3a and 4a, Supplementary Fig. 10a). Where specimens were classified according to *a priori* taxonomy, it was easy to see the separation of *Penion, Antarctoneptunea* and *Kelletia* using shell shape and shell size variation (Fig. 5). Results were very similar between the full and subsampled datasets (Fig. 5). In the full dataset, although *Antarctoneptunea* shells exhibited low variation for PC1 and specimens were very similar in shape to *Penion* species (Fig. 5a), the genus was distinguished by PC2, which appeared to reflect the relatively short length of the spire (Figs. 3 and 5a).

The three genera could also be readily distinguished using CVA ordination of shell shape applied to the full and subsampled datasets (Fig. 6), and were successfully separated based on cross-validation scores (Table 4). Pairwise F-tests using TwoGroup8 found that all genera in the full and subsampled datasets had statistically significant shell shape differences (Supplementary Table 3). Using the full dataset, CVA cross-validation scores, F-test scores and the distance between Procrustes shape means (Table 4, Supplementary Table 3), indicate that *Antarctoneptunea* and *Penion* were similar in shell shape. Although differences were highly significant, this relatively weaker result from the full dataset is likely due to the much higher sampling of *Penion*, and the similarity of these genera on PC1 (60.6% of shape variation).

4. Discussion

4.1. Molecular phylogenetics

Phylogenetic analysis of entire mtDNA and nuclear 45S rDNA sequence data indicate that *Penion, Antarctoneptunea* and *Kelletia* are each monophyletic (Fig. 2, Supplementary Figs. 1–3). The previously predicted sister relationship between *A. aurora* and *A. benthicola* (Vaux et al., 2017a), was confirmed with the both mitochondrial and nuclear data, supporting their generic classification. The evolutionary relationships of the three genera, with *Antarctoneptunea* and *Kelletia* forming a clade sister to *Penion* (Fig. 2), is consistent with previous phylogenetic reconstructions with incomplete sampling of *Penion* species (Vaux et al., 2017a), and morphological comparisons (Ponder, 1973; Beu, 2009; Crame et al., 2014).

New Zealand and Australian *Penion* species are related but reciprocally monophyletic (Fig. 2, Supplementary Figs. 1–3). All phylogenies indicate that two species with adjacent geographical ranges, *P. chathamensis* and *P. fairfieldae* represent the same genetic lineage (Fig. 2, Supplementary Figs. 1–3). This disagrees with previous taxonomic hypotheses based on shell and soft-body morphology (Powell, 1947; Dell, 1956; Powell, 1979). Nuclear rDNA sequence data supported a clade consisting of *P. sulcatus, P. chathamensis* and *P. fairfieldae* (Fig. 2), and the mtDNA splits network (Supplementary Fig. 4) shows that signal for this relationship is likely the source of relatively low posterior support for a split separating these taxa in the mtDNA phylogeny (Fig. 2).

Mitochondrial DNA sequencing results indicate that the morphological variant *P.* cf. *c. cuvierianus* Northland is closely related to other individuals of *P. c. cuvierianus* sampled across a contiguous geographical range (Fig. 2, Supplementary Fig. 1). Similarly, mtDNA data indicate little genetic difference between *P. ormesi* and the variant *P.* cf. *ormesi* West Coast (Fig. 2 and Supplementary Fig. 1). Mitochondrial trees suggest that *P. c. jeakingsi* is more closely related to *P. ormesi* than to *P. c. cuvierianus* (Fig. 2), and a distinct mtDNA lineage supports the existence of the recently described species *P. lineatus* from the Three Kings Islands (Marshall et al., 2018). However, the nuclear rDNA sequence data provides conflicting relationships among these five taxa (Fig. 2, Supplementary Fig. 2). Notably, *P. lineatus* is nested within the nuclear diversity of *P. ormesi* (Fig. 2). Short-length sequence data from the *cox1* mtDNA gene allow us to include five more *Penion* specimens, but the estimated phylogeny did not resolve separate clades for *P. ormesi, P.* cf. *ormesi* West Coast and *P. c. jeakingsi* (Supplementary Fig. 3). Splits network analysis clearly reveals a lower amount of phylogenetic resolution provided by rDNA than mtDNA for derived splits (Supplementary Figs. 4 and 5), and therefore it seems reasonable to treat mitochondrial relationships as being more likely. It is possible though, that mitochondrial introgression has caused the differences in data for these taxa, and a disagreement between mtDNA and rDNA could indicate a hybrid origin for individuals (e.g. M.318565/2*P. ormesi*). A future analysis sampling a wider range of fast-evolving and

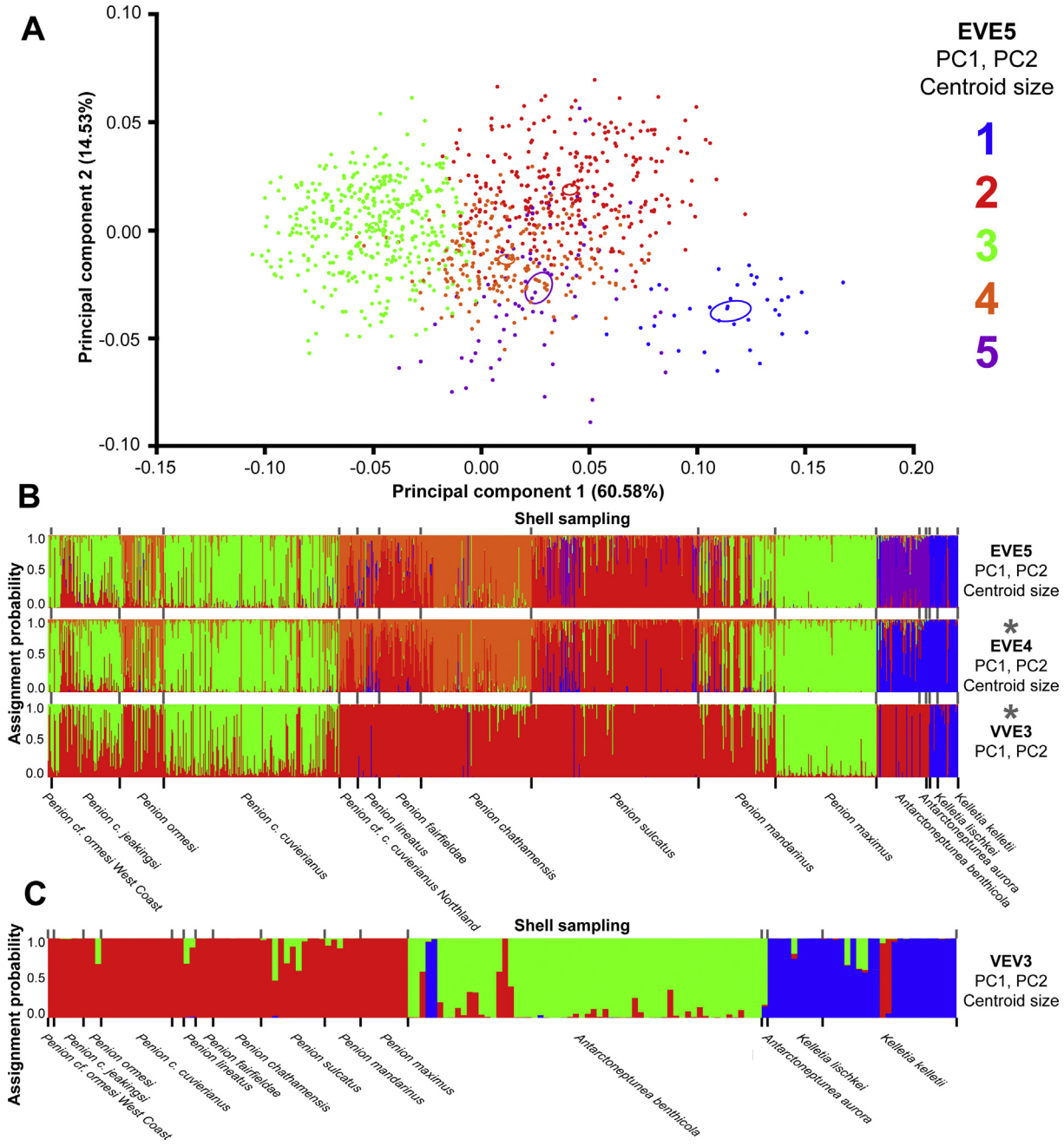

Fig. 4. Naïvely estimated clusters of shell shape and size variation among *Penion, Antarctoneptunea* and *Kelletia* estimated by Bayesian assignment using clustering package mclust. (A) Shell shape variation among specimens in the full dataset, using PCs 1 (60.6% of variation) and 2 (14.5%). The colouration of specimens represents the identification of shells under the five cluster EVE5 model estimated by mclust, using the PCs 1 and 2, as well as centroid size variation. Mean confidence ellipses (90%; same colouration) indicate that the group means are not likely to overlap. (B and C). Bayesian assignment probabilities estimated by mclust for specimens. Specimens (each individual is one vertical line) are coloured by assignment probability to clusters, organised following the mtDNA phylogeny (labelled by species) and by geographical distribution within species (not shown due to space constraints). See Supplementary Fig. 7 for a comparison for BIC values among clustering models. Colours used for each cluster are identified within a key. (B) For the full dataset we present two models that received the highest BIC support (marked with an asterisk) when only the statistically significant shell shape variation (PCs 1 and 2) was analysed (VVE3, bottom plot), and when shell size (centroid size) was also included (EVE4, middle). The top plot presents clusters under the EVE5 model (top) using both PCs 1 and 2 and shell size (centroid). The EVE5 model received almost the same level of support as the EVE4 model, and distinguishes *Kelletia* and *Antarctoneptunea*. (C) For the subsampled dataset we present here only the VEV3 model, which used statistically significant shape variation (PCs 1 and 2) and shell size. The VEV3 model received almost the same level of support as the optimal VII3 and EVI4 models (see Supplementary Fig. 10).

conserved DNA markers would be advantageous to investigate such issues. It remains most likely, however, that the differences observed between mtDNA and rDNA phylogenies are derived from different rates of molecular evolution between the markers, which affects the phylogenetic information provided for earlier and more recent splits (e.g. ND2 and ND5 in mtDNA versus 28S RNA, see Vaux et al., 2017a).

4.2. Morphological variation of shells

Shell shape differences identified by the significant PCs in the full and subsampled datasets (PCs 1 and 2 for both) were almost identical and appeared to be biologically relevant (Fig. 3, Supplementary Fig. 8), reflecting variation in features such as the height of the teleoconch spire

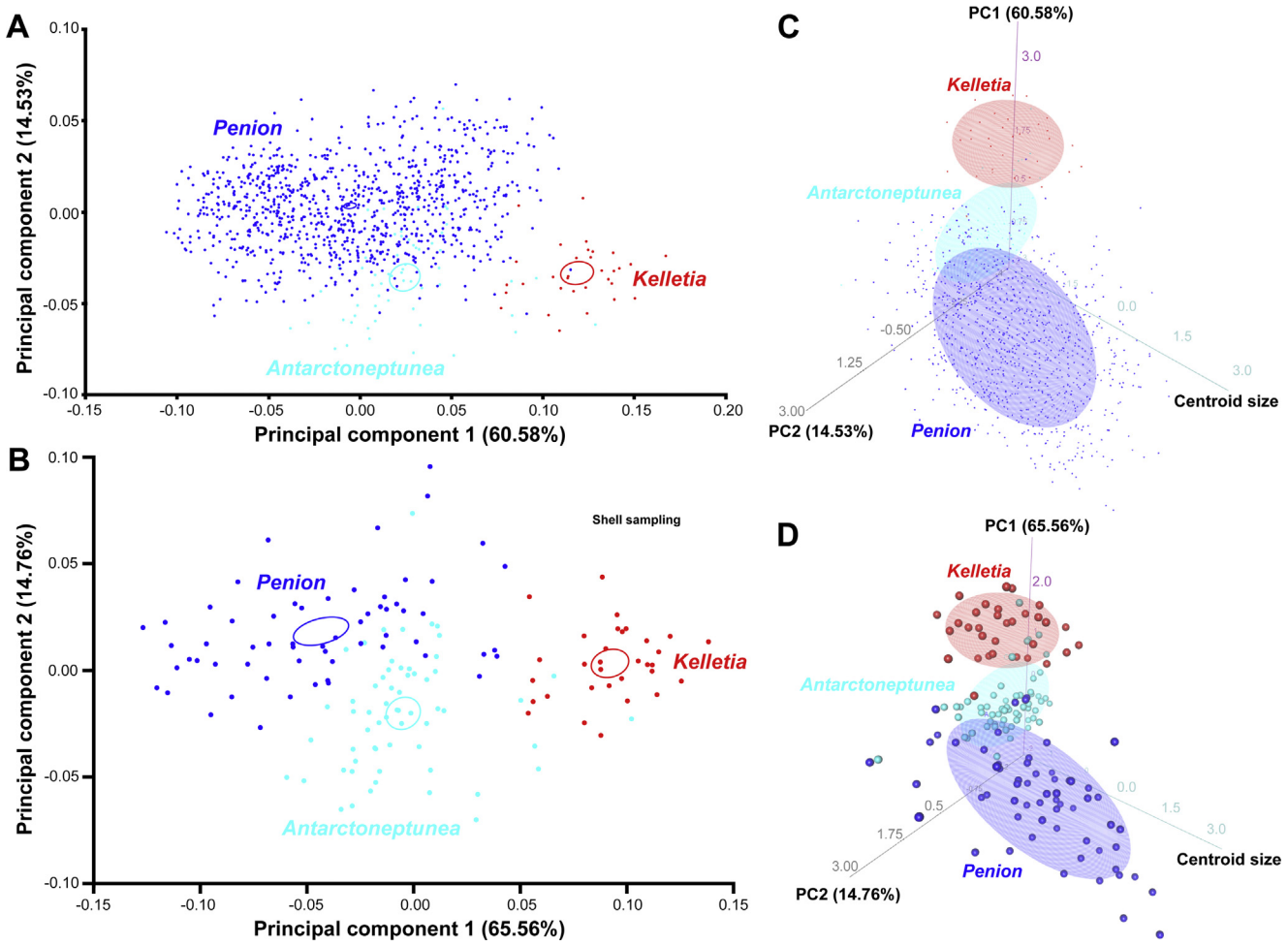

Fig. 5. Morphological variation among our sampled shells of *Penion, Antarctoneptunea* and *Kelletia.* (A and B). Principal component analysis of scatterplots shell shape variation: (A) PCs 1 (60.6% of variation) and 2 (14.5%) for the full dataset, (B) PCs 1 (65.6%) and 2 (14.8%) for the subsampled dataset. The colouration of specimens corresponds to the generic-level classification of shells. Mean confidence ellipses (90%, same colouration) indicate that the group means are not likely to overlap. (C and D). Three-dimensional scatterplots presenting shell shape variation using PCs and shell size variation using centroid size: (C) for the full dataset, (D) for the subsampled dataset. Ellipsoids shown contain 50% of individuals within a genus. In both plots PCs and centroid size were scaled for this comparison using the default function in R. A rotating GIF image of plot (C) is presented in the online supplementary data.

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 sufficient ecological or behavioural data, is unclear how differences 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 *Antarctoneptunea* and *Kelletia* into unique clusters (Fig. 4). Specimens of *Penion* 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 *Penion, Antarctoneptunea* and *Kelletia,* 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). In the full dataset, the shell shape of *Antarctoneptunea* overlapped considerably with *Penion* on PC1 (60.6% variation; Fig. 5a), but shell size improved the distinction of *Antarctoneptunea* (Fig. 5c). Results from the subsampled dataset using shell shape and size were similar (Fig. 5b and d). Based on CVA of shell shape, the three genera were readily distinguished (Fig. 6, Table 4), and F-test results indicated that the genera had statistically significant shell shape differences (Supplementary Table 3). In the full dataset, CVA cross-validation scores were slightly lower for the distinction of *Antarctoneptunea* and *Penion* (Table 4), and F-test scores indicated similarity in shell shape (Supplementary Table 3). Since the distinction of these genera was easier in the subsampled dataset, it is likely that the full dataset was influenced by the higher sampling of *Penion*, and overlap between *Antarctoneptunea* and *Penion* for PC1. This shape overlap stresses the importance of shell size for the distinction of these lineages (Figs. 4 and 5).

Morphometric variation did not distinguish subclades or species within genera (Fig. 4, Supplementary Fig. 10). Using mclust, shells of *Penion* in the full dataset were typically assigned into two or more clusters, but mostly remained separate from *Antarctoneptunea* and *Kelletia* (Fig. 4). These additional clusters in the full dataset appeared to be

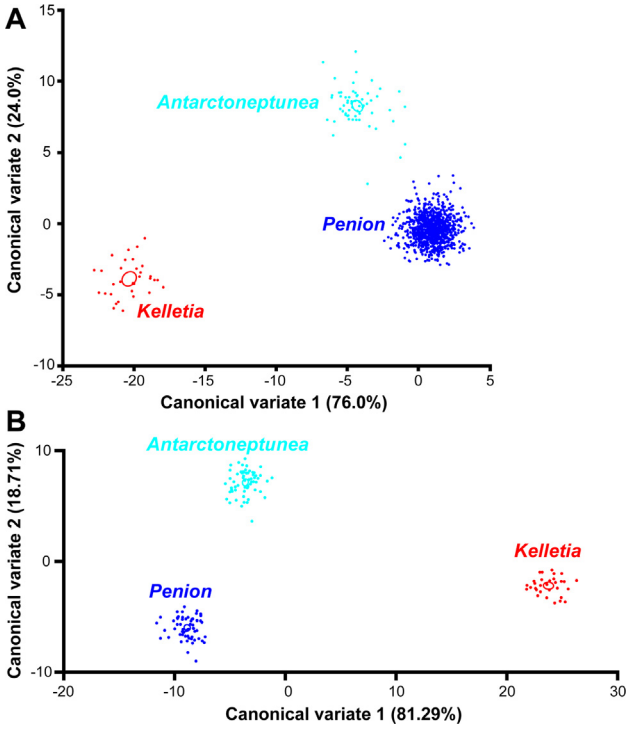

Fig. 6. Two canonical variates analysis plots of shell shape variation among *Penion, Antarctoneptunea* and *Kelletia*: (A) variation in the full dataset, (B) variation in the subsampled dataset. The colouration of specimens corresponds to the generic-level classification of shells. Mean confidence ellipses (90%, same colouration) indicate whether groups overlap.

Table 4
Cross-validation scores estimated for the discrimination of *Penion, Antarctoneptunea* and *Kelletia* using shell shape variation in the full (top) and subsampled dataset (bottom). The table is read horizontally row-wise, for example in the full dataset: of the 61 sampled *Antarctoneptunea* shells, 18 were assigned to the same genus, 4 to *Kelletia* and 39 to *Penion.* Cross-validation scores were estimated via CVA implemented in MASS, using the PCs that accounted for 95% of variation among samples (PCs 1–83 for the full dataset, and 1–9 for the subsampled dataset).

	Antarctoneptunea	*Kelletia*	*Penion*	Total	Percentage correctly assigned (jackknifed cross-validation)
Full dataset					
Antarctoneptunea	56	0	5	61	91.8%
Kelletia	0	32	0	32	100.0%
Penion	1	0	943	944	98.9%
Subsampled dataset					
Antarctoneptunea	58	0	3	61	95.1%
Kelletia	0	32	0	32	100.0%
Penion	1	0	60	61	98.4%

hierarchically nested across models (Fig. 4, Supplementary Fig. 7), but these groupings corresponded with low accuracy to particular subclades or species within *Penion* (compare Figs. 2 and 4). For example, based on shell shape, the sister species from Australia (*P. mandarinus* and *P. maximus*) each cluster with a different New Zealand *Penion* species (*P. sulcatus* and *P. c. cuvierianus* respectively; Fig. 4). Such groupings may highlight ecological similarity and potential evolutionary convergence, as *P. mandarinus* and *P. sulcatus* are both smaller species that can be found on rocky substrates in shallow water (1–50 m), whereas *P. maximus* and *P. c. cuvierianus* are larger and are mostly restricted to soft sediments at greater depths (Ponder, 1973; Powell, 1979). Given the sister relationship of *P. mandarinus* and *P. maximus* 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 *Kelletia* and *Antarctoneptunea* may be due to the limited sampling of these taxa. That said, the consistent placement of *A. benthicola* in a cluster with *A. aurora* (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 *A. benthicola* as a species of *Penion* (Powell, 1979). A future investigation focussed on shell shape and size variation in *Penion* 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 would require additional genetic information.

Although shell size was useful for distinguishing *Antarctoneptunea* from *Penion* (Fig. 4c and d), there was considerable overlap in size of shells among the three genera (Powell, 1979). Likewise, even though mean confidence ellipses indicated that *Penion, Antarctoneptunea* and *Kelletia* specimens could be distinguished using shell shape, large numbers of shells overlapped in morphospace (Fig. 5). Overlap was expected, though, as putative inter- and intraspecific morphological variation is a long-standing challenge within buccinoid whelk taxonomy (e.g. Ponder, 1973; Powell, 1979). This means that the level of congruence between molecular and morphometric evidence detected here, reflecting generic classification, is quite remarkable.

Admittedly, there is a risk of circularity in our investigation, as taxa were primarily identified based on the examination of shell traits. Nevertheless, our Bayesian assignment of specimens to clusters did not use taxon labels, and genera represent evolutionary lineages as demonstrated by our extensive DNA sequence dataset. Despite the seemingly high level of intraspecific morphological variation, we were able to identify three or more large clusters of specimens using just three independent traits, suggesting that reliable evolutionary inferences can be drawn from shell characters in this group of gastropods. Congruence between shell morphology and molecular phylogeny has also been reported in limpets, despite similar, extensive morphological variation within taxa (Reisser et al., 2012). For the PCA and CVA results presented (Figs. 5 and 6), the traditional taxonomic classification of genera considers traits not captured by our two-dimensional landmarks, such as protoconch morphology, the presence, size and persistence of axial ribs on the teleoconch, shell thickness, and shell colouration (Ponder, 1973; Powell, 1979; Beu and Maxwell, 1990). Most of these traits are preserved in the fossil record, and their incorporation into an analysis possibly would aid species discrimination.

5. Conclusions

There is close concordance between variation in shell morphology and molecular phylogeny for the distinction of the marine snail genera *Penion, Antarctoneptunea* and *Kelletia*. The morphometric analysis supports the treatment of *A. benthicola* (formerly *Penion benthicolus*) as a member of the genus *Antarctoneptunea,* in agreement with genetic similarity. Although phylogenetically sister to *Kelletia,* shells of *Antarctoneptunea* are more similar in shape to some *Penion* species. Morphological clusters identified naïvely from shell shape and size variation matched deep phylogenetic splits that correspond to generic classification, although phylogenetic subclades within genera and species could not be identified with high accuracy. This finding complements conclusions drawn in palaeontology, where morphologically defined species taxonomy is often less reliable than genus-level classification for biodiversity analyses (Foote et al., 2007; Eronen et al.,

2011). It would be useful to focus a combined molecular and morphometric analysis on *Penion*, as this genus has the most thorough morphometric sampling for living and extinct populations, and further genetic sampling with additional markers could clarify the evolutionary relationships of some taxa.

The overall concordance between shell morphology and molecular phylogeny is a surprise, given the seeming abundance of morphological variation within and between the species studied. The typical assumption that convergence in shell shape is commonplace and that shell morphology is problematic for the taxonomy of shelled Mollusca may be too pessimistic. If geometric morphometric analysis of shells can identify living taxa, at least to the genus-level, then shell shape and size could be relied upon to identify evolutionary lineages in situations where molecular data are limited, or in the rich molluscan fossil record (Steiner et al., 2007; Parkhaev and Demidenko, 2010), where the distinction of invasion and lineage-splitting is crucial to estimates of diversity and evolutionary rates. If we are to progress in our understanding of biological diversity and its change over time, more systematic investigations of the relationship between morphological and molecular variation are required.

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Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.ympev.2018.06.009.

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