A phylogeny of Southern Hemisphere whelks (Gastropoda: Buccinulidae) and concordance with the fossil record

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ABSTRACT

Under current marine snail taxonomy, the majority of whelks from the Southern Hemisphere (Buccinulidae) are hypothesised to represent a monophyletic clade that has evolved independently from Northern Hemisphere taxa (Buccinidae). Phylogenetic analysis of mitochondrial genomic and nuclear ribosomal DNA sequence data indicates that Southern Hemisphere taxa are not monophyletic, and results suggest that dispersal across the equator has occurred in both directions. New Zealand buccinulid whelks, noted for their high endemic diversity, are also found to not be monophyletic. Using independent fossil calibrations, estimated genetic divergence dates show remarkable concordance with the fossil record of the *Penion* and *Kelletia*. The divergence dates and the geographic distribution of the genera through time implies that some benthic marine snails are capable of dispersal over long distances, despite varied developmental strategies. Phylogenetic results also indicate that one species, *P. benthicolus* belongs in *Antarctoneptunea*.

1. Introduction

Geographic distributions of extant populations often form the basis of taxonomic hypotheses, although the relevance of biogeographic patterns to reconstruct evolutionary history varies. A well-known example is the Old World and New World divide, which accurately predicts shared ancestry and separate evolutionary radiations of monkeys (Catarrhini and Platyrrhini; Perelman et al., 2011), but conversely does not reflect the phylogenetic relationships and convergence exhibited among all vultures (Gypaetinae, Aegypiinae and Cathartidae; Wink, 1995; Gibb et al., 2007).

In this study we investigate a similar biogeographic hypothesis in the taxonomy of whelks. We use 'whelks' here to refer to marine snails classified within the families Buccinidae or Buccinulidae, although the same term is often used to loosely refer to any members of Buccinoidea. Currently, the majority of whelks in the Southern Hemisphere – Buccinulidae, are hypothesised to be the product of an evolutionary radiation in geographic isolation from Northern Hemisphere whelks – Buccinidae (Finlay, 1928; Powell, 1929, 1951; Harasewych and Kantor, 1999; Hayashi, 2005). Only soft-body anatomy provides potential biological traits to separate Southern Hemisphere buccinulid and Northern Hemisphere

buccinid whelks (Powell, 1951; Harasewych and Kantor, 1999; Hayashi, 2005). Buccinidae overall is not a monophyletic group (Couto et al., 2016; Galindo et al., 2016), but the evolutionary relationships of many potential subclades and Buccinulidae have not previously been focussed upon. We investigate whether buccinulid whelks are monophyletic using molecular phylogenetics. Using a dated phylogeny, we also investigate when possible dispersal events may have occurred, and compare estimated divergence dates to fossil record evidence. We focus especially on New Zealand buccinulid whelks as the initial Southern Hemisphere hypothesis was based on endemic taxa (Finlay, 1928; Powell, 1929), and because the region exhibits high species diversity (Powell, 1979; Spencer et al., 2009, 2017).

The whelk lineages recognised within Buccinidae or Buccinulidae are a diverse group of neogastropod marine snails that are typically carnivores or detritivores (Strong et al., 2008; Spencer et al., 2009). Neogastropods are frequently sampled for phylogenetic and biogeographic studies as taxa are diverse, widely distributed, and frequently occur within easily accessible shallow water habitats (Harasewych et al., 1997; Colgan et al., 2007; Cunha et al., 2009). New Zealand waters host a high diversity of endemic neogastropods (Powell, 1979; Spencer et al., 2009, 2017), of which buccinulid whelks represent a significant proportion (Powell, 1951; Powell, 1979), with a rich fossil record (Beu and Maxwell, 1990). New Zealand taxa occupy an unusual variety of niches compared

to other regions (Powell, 1929; Dell, 1956; Willan, 1978; Powell, 1979; Spencer et al., 2009), and they exhibit significant morphological variation (Powell, 1927; Powell, 1947; Dell, 1956; Ponder, 1973; Powell, 1979).

We compare divergence dates estimated from our molecular data using independent fossil calibrations, to the fossil record and geographic distributions of *Penion* P. Fischer, 1884 and *Kelletia* Bayle, 1884 through time. Both genera represent large whelks. Six extant species and one subspecies of *Penion* are currently recognised from New Zealand (Powell, 1979; Spencer et al., 2017), with another two endemic species from Australian waters (Ponder, 1973). A species of *Kelletia* is recognised from the coast of southern California, USA and Baja California, Mexico (Zacherl et al., 2003a; Vendetti, 2009), and another occurs off Japan (Zacherl et al., 2003b; Hayashi, 2005; Kim et al., 2012; Hwang et al., 2014). *Penion* and *Kelletia* are hypothesised to be closely related based on shell morphology (Ponder, 1973), and short-length sequence data (Hayashi, 2005), which we test using mitochondrial genomic and nuclear DNA sequence data. The fossil record for both genera is rich: 17 extinct fossil *Penion* species are recognised from New Zealand (Beu and Maxwell, 1990), along with four from Australia (Ponder, 1973), 11 from Argentina and Chile (Frassinetti, 2000; Nielsen, 2003; Parras and Griffin, 2009; Reichler, 2010), and one species from Antarctica (Beu, 2009; Crame et al., 2014). Similarly, 5 extinct species of *Kelletia* are recorded from North America (Arnold, 1910; Anderson and Martin, 1914; Kanakoff, 1954; Addicott, 1970; Hertlein, 1970), a further two from Ecuador (Olsson, 1964), and one from Japan (Ozaki, 1954).

Only a few previous phylogenetic studies have sequenced buccinulid whelks from the Southern Hemisphere (Hayashi, 2005; Oliverio and Modica, 2010; Donald et al., 2015). Analysis of mitochondrial 16S rRNA gene sequences from a subset of worldwide buccinid and buccinulid whelks, including four species in three genera from New Zealand found mixed support for the monophyly of Buccinulidae distinct from Buccinidae (Hayashi, 2005). Donald et al. (2015) produced a phylogeny of *Cominella* Gray, 1850 in New Zealand and Australia, but no Northern Hemisphere lineages were sequenced and the monophyly of Buccinulidae was not addressed.

Buccinulidae was introduced as a classification to cover New Zealand taxa (Finlay, 1928; Powell, 1929), and the hypothesis of isolation for Southern Hemisphere Buccinulidae taxa was probably influenced by traditional interpretations of New Zealand biodiversity. Perhaps because of its very late colonisation by humans (McGlone and Wilmshurst, 1999; Wilmshurst et al., 2008), and remote geographic location, New Zealand was considered to be almost completely biogeographically isolated by a few early authors (notably Finlay, 1926). This view has led to the perennial popularity of vicariance-based hypotheses for the evolution of New Zealand taxa (especially terrestrial), typically involving former Gondwanan landmasses (Craw et al., 1999; Cooper and Millener, 1993; Trewick et al., 2007). However, many studies of extant populations have demonstrated that migration to and from New Zealand is common (e.g. Fleming, 1975; Battley, 1997; Hernández et al., 2015). Phylogenetic evidence indicates that dispersal events are frequent for some taxa (e.g. Trewick, 2000; Winkworth et al., 2002; Knapp et al., 2005; Goldberg et al., 2008), and not all groups with endemic radiations are monophyletic (e.g. Phillips et al., 2010). The present geographic remoteness of New Zealand has existed for less than 85 Ma (final split of Zealandia from Gondwana; Tulloch et al., 2009), and the accuracy of geological reconstructions affects the likelihood for particular vicariant mechanisms and routes of dispersal (e.g. Turner, 1991; Knapp et al., 2005; Goldberg et al., 2008; Winkworth et al., 2015).

Despite New Zealand being an oceanic archipelago, the phylogeny and dispersal ability of native marine invertebrates has been investigated in only a small number of species (e.g. Sponer and Roy, 2002; Donald et al., 2005; Hills et al., 2011; Cumming et al., 2014; Donald et al., 2015). Like terrestrial species, aquatic organisms can be subject to vicariance and dispersal. Ocean currents provide a means of dispersal across large distances (e.g. Turner, 1991; Dutton et al., 2014), but they change through time (Rahmstorf, 2002), and species vary in their ability to transverse the widest regions of deep water (e.g. Lessios et al., 1998; Parsons, 1998; Baums et al., 2012; Dutton et al., 2014; Hernández et al., 2015). Land formations can represent long-lasting barriers to dispersal (Bacon et al., 2015), but they form gradually in a complex manner (Bacon et al., 2015; Ingley et al., 2015), and can be circumvented (e.g. Miura et al., 2012). Given the high degree of endemism among New Zealand marine snails (Powell, 1979; Spencer et al., 2009, 2017), the group represents a suitable system to investigate marine biogeographic hypotheses.

2. Materials and methods

2.1. Taxonomy

Whelks worldwide are usually classified in the family Buccinidae (Neogastropoda: Buccinoidea) (e.g. Thiele, 1912; Thiele, 1929; Wenz, 1941; Powell, 1951; Harasewych and Kantor, 1999; Donald et al., 2015), but some authors have alternatively treated the majority of taxa from the Southern Hemisphere as a sister family, Buccinulidae (e.g. Finlay, 1928; Powell, 1929, 1951; Harasewych and Kantor, 2004; Bouchet et al., 2005; Pastorino, 2016; Fig. 1). The basis of this distinction is that Buccinulidae represents a Southern Hemisphere radiation, independent from the Northern Hemisphere Buccinidae (Powell, 1951, 1965). New Zealand taxa dominate the Buccinulidae group (Finlay, 1928; Powell, 1929, 1951), due to the high level of regional endemism (Spencer et al., 2009; Spencer et al., 2017). A small number of genera from the Northern Hemisphere are classified within Buccinulidae (and vice versa), but these clades are hypothesised to represent dispersal events following the independent phylogenetic radiations of the two groups (Powell, 1951; Ponder, 1973).

The classification of whelks and distinction of the two families depends upon morphological differences in opercula and radulae (Powell, 1951; Harasewych and Kantor, 1999, 2004). However, it is common for radula morphology to be useless for discriminating species and it is possible that trait variation reflects environmental plasticity (e.g. Dell, 1956, 1972; Willan, 1978). Furthermore, stomach anatomy struggles to distinguish Buccinidae and Buccinulidae, despite separating other neogastropod families (Kantor, 2003).

Buccinulidae have otherwise been treated as subfamily Buccinulinae or tribe Buccinulini (Bouchet et al., 2005; Hayashi, 2005; Fig. 1). *Cominella* and *Pareuthria* Strebel, 1905 are sometimes classified into a separate family, Cominellidae (or Cominellinae or Cominellini), but this group is currently placed within Buccinulidae (Powell, 1951; Hayashi, 2005; Donald et al., 2015). Species classification is based on traditional morphological examination of shells and body parts such as the radula, operculum, stomach, and gonads (Powell, 1951; Dell, 1956, 1972; Ponder, 1973; Powell, 1979; Harasewych and Kantor, 1999; Kantor, 2003; Spencer et al., 2009, 2017).

2.2. Sampling

The majority of specimens were borrowed from museum and university collections (acknowledged below), although some individuals were collected in the field for this study (Tables 1 and 2). Most material examined is stored at Museum of New Zealand Te Papa Tongarewa (NMNZ), with six digit registration numbers
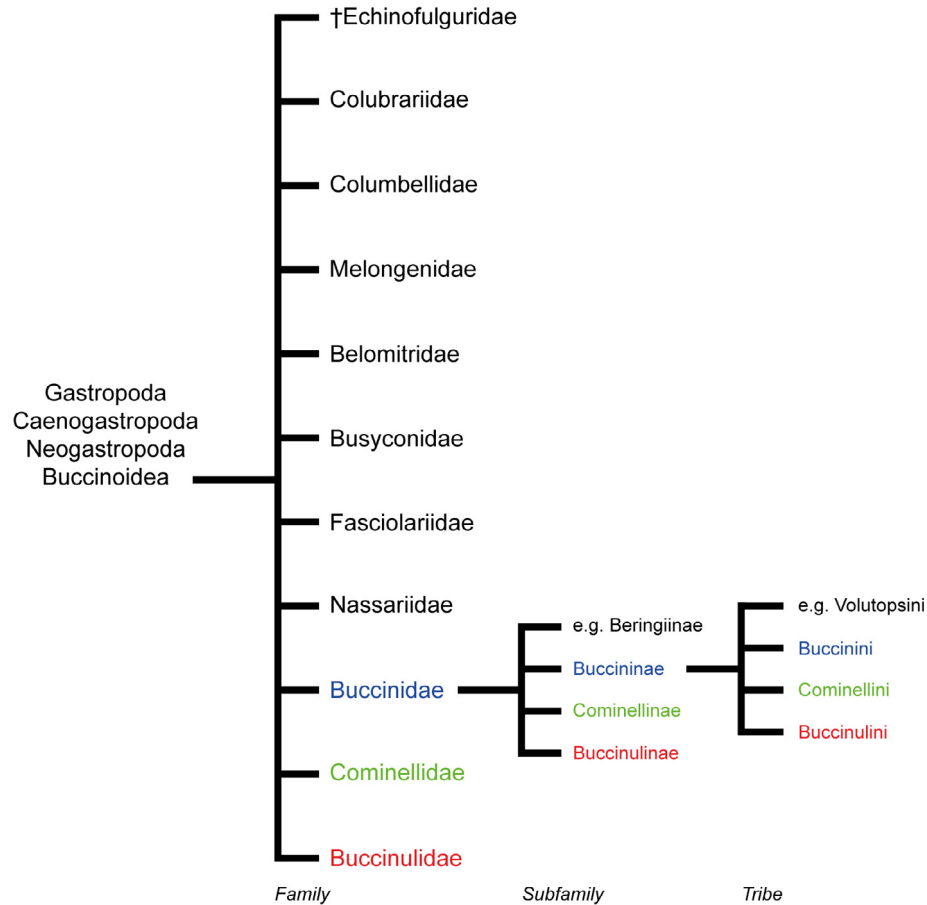
Fig. 1. *Summary of taxonomic problems related to Southern Hemisphere whelks.*
Alternative taxonomic arrangements for buccinulid and buccinid whelks. The tree is not a phylogenetic reconstruction and branch lengths are not meaningful; not all taxa are shown. Evolutionary relationships among groups are uncertain (shown as polytomy). The majority of whelk species from the Southern Hemisphere are often recognised within the family Buccinulidae (red), whereas most Northern Hemisphere taxa are classified within Buccinidae (blue). *Cominella* and *Pareuthria* are sometimes recognised within their own family Cominellidae (green), but are otherwise classified within Buccinulidae. Buccinulidae and *Cominella* can also be nested within Buccinidae at the subfamily or tribe level. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

preceded by "M." Specimens were collected either via trawling (20–500 m depth for most sampling) or by hand from the intertidal zone. Some specimens were caught as trawling fishery bycatch. Captured individuals were swiftly frozen to prevent tissue and DNA degradation. Snails were subsequently thawed and bodies were removed from shells for preservation in 95% ethanol. All sampled specimens were identified by experienced molluscan taxonomists: Bruce A. Marshall (Collection Manager Sciences, Museum of New Zealand Te Papa Tongarewa) and Alan G. Beu (Palaeontologist, GNS). We used a public database (GenBank) to retrieve sequence data from other Northern Hemisphere taxa (from Claremont et al., 2008; Vendetti, 2009; Barco et al., 2010; Oliverio and Modica, 2010; Zou et al., 2011a,b; Kim et al., 2012; see Tables 1 and 2). We were unable to sample the putative buccinulid genera *Antarctodomus* Dell, 1972 and *Euthrenopsis* Powell, 1929 from southern New Zealand and the subantarctic.

We sampled all species of the buccinulid genera *Antarctoneptunea* Dell, 1972 and *Kelletia*, and selected representatives of *Aeneator* Finlay, 1926, *Austrofusus* Kobelt, 1879, *Buccinulum* Deshayes, 1830, *Cominella*, *Pareuthria*, and *Penion* (Tables 1 and 2). These genera are dominated by New Zealand taxa, with the exception of *Antarctoneptunea* and *Kelletia*. *Kelletia* is restricted to the Sea of Japan and the Pacific coast of Honshu (Zacherl et al., 2003b; Hayashi, 2005; Kim et al., 2012; Hwang et al., 2014), and southern California, USA and Baja California, Mexico (Zacherl et al., 2003a; Vendetti, 2009). Species of *Pareuthria* and

Antarctoneptunea aurora (Hedley, 1916), the type species of the genus, are restricted to the polar circle of Antarctica (Dell, 1972; Oliverio and Modica, 2010; Pastorino, 2016). Most species of *Penion* occur off New Zealand and two species are distributed off Australia (Ponder, 1973). A number of extant marine snails from the coast of Chile have been classified in *Aeneator* as well (McLean and Andrade, 1982; Araya, 2013). We sampled representatives of four buccinid genera restricted to the Northern Hemisphere; *Buccinum* Linnaeus, 1758, *Colus* Röding, 1798, *Neptunea* Röding, 1798, and *Volutopsius* Mörch, 1857 (Tables 1 and 2).

We use representatives of Fasciolariidae, which are morphologically similar to buccinid whelks (Kosyan et al., 2009), as the primary outgroup for our analysis. Based on fossil record evidence Fasciolariidae is hypothesised to include some of the earliest evolutionary splits within Neogastropoda (Tracey et al., 1993; Hayashi, 2005; Couto et al., 2016). We also use representatives of Nassariidae, which is considered to be closely related to Buccinidae/Buccinulidae based on molecular (Harasewych et al., 1997; Hayashi, 2005; Cunha et al., 2009), and morphological evidence (Haasl, 2000). However, Nassariidae can only be resolved as monophyletic if some clades recognised as Buccinidae are transferred to the former family (Galindo et al., 2016). We sampled the fasciolariid species *Glaphyrina caudata* (Quoy & Gaimard, 1833), *Pararetifusus carinatus* (Ponder, 1970) (here newly referred to *Pararetifusus* Kosuge, 1967), and *Taron dubius* (Hutton, 1878), which are all endemic to New Zealand waters. From Nassariidae,

Table 1

Individual marine snails that were Illumina sequenced to yield whole mitochondrial genome and nuclear ribosomal sequence data. Specimen with origins marked with an asterisk (*) were obtained from aquaria or fish markets (Simison et al., 2006; Vendetti, 2009), and precise localities are unknown. Highlighted taxa are genera assigned to Buccinulidae, grey coloured taxa are Buccinidae, or Fasciolaridae and Nassariidae species used as outgroups. Colours used for each "buccinulid" genus correspond to the highlighting of lineages in phylogenetic trees (Figs. 2 and 3, Supplementary Figs. 1–3 in Data in Brief article). 'P' indicates partial success of sequencing and 'C' a complete sequence.

Taxon	rDNA cassette	mtDNA genome	Voucher ID	Location	GenBank Accession	Source
Putative 'Southern' whelks (Buccinulidae)						
Aeneator benthicolus	C	C	M.274111	Cape Palliser, NZ		This paper
Aeneator elegans	C	C	SFKH-TMP015	Chatham Rise, NZ		This paper
Aeneator otagoensis	C	C	M.279437	Tasman Bay, NZ		This paper
Aeneator recens	C	C	M.190119	Cape Turnagain, Manawatu-Wanganui, NZ		This paper
Aeneator valedictus	P	C	SFKH-TMP013	TAN 616/83		This paper
Austrofusus glans	C	C	SFKH-TMP014	Island Bay, Wellington, NZ		This paper
Buccinulum linea	C		SFKH-TMP016	Nelson, NZ		This paper
Buccinulum fuscozonatum	C	C	M.302907/2	Ariel Bank, Gisborne, NZ		This paper
Buccinulum pallidum	C	C	M.258277/6	Stewart Island, NZ		This paper
Buccinulum pertinax finlayi	C	C	M.302870/2	Point Gibson, NZ		This paper
Buccinulum robustum	C	C	M.314755/1	Oneroa Bay, Bay of Islands, NZ		This paper
Buccinulum vittatum littorinoides	C		SFKH-TMP011	Mahia Peninsula, NZ		This paper
Buccinulum vittatum vittatum	C	C	SFKH-TMP012	Hicks Bay, Gisborne, NZ		This paper
Cominella adspersa	C	C	SFKH-TMP009	Urupukapuka Bay, Bay of Islands, NZ		This paper
Cominella virgata brookesi	C	C	SFKH-TMP010	Spirits Bay, Northland, NZ		This paper
Kelletia kelletii	C	C	KK12	Santa Barbara, California, USA*		This paper
Kelletia lischkei	C	C	KL2	Kansai, Mie Prefecture, Japan		This paper
Penion benthicolus	C	C	M.183832	Chatham Rise, NZ		This paper
Penion chathamensis	C	C	M.190082/2	Chatham Rise, NZ		This paper
Penion chathamensis	C	C	M.190085	Chatham Rise, NZ		This paper
Penion cuvierianus cuvierianus	C	P	M.183792/1	Red Mercury Island, NZ		This paper
Penion cuvierianus cuvierianus	C	C	M.183927	Coromandel, NZ		This paper
Penion mandarinus	C	C	C.456980	Gabo Island, Victoria, Australia		This paper
Penion maximus	C	C	C.487648	Terrigal, New South Wales, Australia		This paper
Penion sulcatus	C	C	Phoenix1	Tauranga, NZ		This paper
Penion sulcatus	C	C	Phoenix9	Auckland, NZ		This paper
Putative 'Northern' whelks (Buccinidae)						
Buccinum undatum	C	C	20140783	Reykjanesskagi, Iceland		This paper
Colus islandicus	C	C	20140782	Moray Firth, Scotland, UK		This paper
Volutopsius norwegicus	C	C	20140781	Hornsund Fjord, Svalbard, Norway		This paper
New Zealand tulip and spindle snails (Fasciolariidae)						
Glaphyrina caudata	C	C	SFKH-TMP004	Off Farewell Spit, Golden Bay, NZ		This paper
Pararetifusus carinatus	C	C	SFKH-TMP005	Chatham Rise, NZ		This paper
Taron dubius	C	C	SFKH-TMP006	Hot Water Beach, Coromandel, NZ		This paper
Californian dog whelks (Nassariidae)						
Tritia obsoleta		C		California, USA*	NC_007781	Simison et al. 2006
Tritia reticulata		C		California, USA*	NC_013248	Cunha et al. 2009

we sampled whole mitochondrial genome sequences for *Tritia obsoleta* (Say, 1822) and *T. reticulata* (Linnaeus, 1758) generated by previous studies (Simison et al., 2006; Cunha et al., 2009). Both of these species are restricted to the Northern Hemisphere.

2.3. DNA extraction and sequencing

50 mg sections of foot or columella muscle tissue were cut from preserved specimens using a sterile scalpel blade. These sections were pressed and dried to remove ethanol and were diced into a dozen pieces, and sometimes also crushed using a sterile pestle. Tissue was transferred to a clean 2 mL Eppendorf microtube and placed in 300 μl CTAB buffer (2% hexadecyl-trimethyl-ammonium bromide, 100 mM Tris-HCl pH 8.0, 1.4 M NaCl, 20 mM EDTA). Tissue was digested using 15 μl of 1/10 Proteinase K and incubated overnight (15–20 h) at 57 °C. To reduce RNA contamination, 4 μl of 1/10 RNase A was added to each sample following digestion and then incubated for a further 15 min. DNA was isolated using high-salt precipitation, following a purification using chloroform (24:1 chloroform-isoamyl alcohol), sodium acetate (3 M NaOAc), and −20 °C chilled 70% ethanol, which is a modification of previous molluscan DNA extraction methods (Thomaz et al., 1996; Trewick et al., 2009). This extraction method has been found to be the most successful for attaining high molecular weight DNA while avoiding the potential problem, common when

studying neogastropod tissue, of mucopolysaccharide contamination interfering with enzymatic reactions (Winnepenninckx et al., 1993). Samples were re-suspended in 50 μl of TE buffer (10 mM Tris, 0.1 mM EDTA), or 100 μl for larger yields of DNA. DNA was quantified using the Qubit Fluorometric Quantitation kit (Life Technologies, Thermo Fisher Scientific Inc.).

Total DNA extracts from 32 individuals of 29 putative 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 32 individuals were de-multiplexed using standard indexes incorporated in the library-preparation kit. Resulting Illumina short-sequence reads that passed standard quality filters had adapter sequences removed using cutadapt 1.11 (Martin, 2011). Geneious 9.1.3 (Kearse et al., 2012), was used to pair sequence reads and to edit, assemble and align sequences. The whole mitochondrial genome and 45S nuclear ribosomal cassette (18S, ITS1, 5.8S, ITS2, 28S) were both constructed by mapping paired reads to reference annotated molluscan mitochondrial genomes/gene regions. A new target sequence, using only reads from the sequenced individual was generated, and an iterative re-mapping of reads to the target reference sequence was used to extend coverage of each genomic region.

To investigate relationships among species with greater sampling and to include specimens with low DNA quality, the

Table 2
Individual marine snails that were PCR amplified and sequenced for the mitochondrial *cox1*, 16S or nuclear ribosomal 28S genes. Specimen with origins marked with an asterisk (*) were obtained from aquaria or fish markets (Simison et al., 2006; Vendetti, 2009; Barco et al., 2010), and precise localities are unknown. Colours used for each "buccinulid" genus correspond to the highlighting of lineages in phylogenetic trees (Supplementary Figs. 4–6 in Data in Brief article). 'Y' indicates that whether genes were sequenced for each individual.

Taxon	mtDNA cox1	mtDNA 16S	rDNA 28S	Voucher ID	Location	GenBank Accession	Source
Antarctoneptunea aurora	Y			MNA0095	Adare Peninsula, Ross Sea		This paper
Antarctoneptunea aurora	Y			MNA0096	Hallet Peninsula, Ross Sea		This paper
Buccinulum linea		Y		NUGB-G2011	Leigh Harbour, Auckland, NZ	AB044256	Hayashi 2005
Cominella adspersa		Y		NUGB-G2012	Orewa, Auckland, NZ	AB044265	Hayashi 2005
Kelletia kelletii			Y	UCMP-557057	Monterey Bay, California, USA	FJ710099	Vendetti 2009
Kelletia kelletii		Y		NUGB-G2051	Santa Barbara Island, California, USA	AB121037	Hayashi 2005
Kelletia lischkei	Y			KL1	Kansai, Mie Prefecture, Japan		This paper
Kelletia lischkei	Y			KL3	Kansai, Mie Prefecture, Japan		This paper
Kelletia lischkei		Y		NUGB-G2031	Wakasa Bay, Fukui Prefecture, Japan	AB044263	Hayashi 2005
Kelletia lischkei	Y				Yeosu, South Jeolla, South Korea	HM180632	Kim et al. 2012
Kelletia lischkei	Y				Yeosu, South Jeolla, South Korea	HM180633	Kim et al. 2012
Kelletia lischkei	Y				Yeosu, South Jeolla, South Korea	HM180634	Kim et al. 2012
Kelletia lischkei	Y				Yeosu, South Jeolla, South Korea	HM180635	Kim et al. 2012
Kelletia lischkei	Y				Yeosu, South Jeolla, South Korea	HM180636	Kim et al. 2012
Pareuthria fuscata	Y			M.317715	Campbell Island, NZ	KP694137	Donald et al. 2015
Pareuthria fuscata	Y			M.317715	Campbell Island, NZ	KP694138	Donald et al. 2015
Pareuthria fuscata	Y			M.317716	Campbell Island, NZ	KP694139	Donald et al. 2015
Pareuthria fuscata	Y	Y		IM-2009-4613	Ushuaia, Tierra del Fuego, Argentina	FM999174 FM999126	Oliverio and Modica 2010
Penion benthicolus	Y			M.274268	Cape Kidnappers, Hawkes Bay, NZ		This paper
Penion chathamensis		Y		NUGB-G2009	Chatham Rise, NZ	AB044266	Hayashi 2005
Penion sulcatus		Y		NUGB-G2016	New Zealand	AB044267	Hayashi 2005
Buccinum bayani	Y			UCMP-556091	Tokyo, Kantō, Japan*	FJ710068	Vendetti 2009
Buccinum bayani	Y				Tokyo, Kantō, Japan*	FJ710069	Vendetti 2009
Buccinum middendorfi	Y			UCMP-556105	Nagoya, Japan*	FJ710071	Vendetti 2009
Buccinum middendorfi	Y				Nagoya, Japan*	FJ710072	Vendetti 2009
Buccinum opisoplectum		Y		NUGB-G2029	Japan	AB044257	Hayashi 2005
Buccinum pemphigus	Y			LSGB2320301	Bohai Strait, China	HQ834057	Zou et al. 2011a
Buccinum pemphigus	Y			LSGB2320303	Shaungtaizi River Estuary, China	HQ834059	Zou et al. 2011a
Buccinum shenshumaruae			Y	UCMP-556095	Jōetsu, Niigata Prefecture, Japan*	FJ710095	Vendetti 2009
Buccinum tenuissimum			Y	UCMP-556096	Joetsu, Japan*	FJ710096	Vendetti 2009
Buccinum tsubai			Y	UCMP-556097	Jōetsu, Niigata Prefecture, Japan*	FJ712705	Vendetti 2009
Buccinum undatum			Y	BAU-2008004	London, UK*	FN677456	Barco et al. 2010
Buccinum undatum			Y	BMNH-20070640	Rekjanes, Iceland	EU391567	Claremont et al. 2008
Buccinum yokomaruae	Y				Yellow Sea, China	JN052995	Zou et al. 2011b
Buccinum yokomaruae	Y				Yellow Sea, China	JN052996	Zou et al. 2011b
Buccinum yokomaruae	Y				Yellow Sea, China	JN052997	Zou et al. 2011b
Neobuccinum eatoni			Y	IM-2009-4614	Terra Nova Bay, Antarctica	FM999149	Oliverio and Modica 2010
Neptunea arthitica			Y	UCMP-556104	Nagoya, Aichi Prefecture, Japan*	FJ710101	Vendetti 2009
Neptunea constricta			Y	UCMP-556094	Joetsu, Japan*	FJ710102	Vendetti 2009
Neptunea eulimata			Y	UCMP-556098	Wakkanai, Hokkaido, Japan	FJ710103	Vendetti 2009
Neptunea frater			Y	UCMP-556110	Sōma, Fukushima Prefecture, Japan	FJ710104	Vendetti 2009
Neptunea intersculpta		Y		NUGB-G2032	Hokkaido, Japan	AB044265	Hayashi 2005
Neptunea kuroshio			Y	UCMP-556093	Awa-gun, Japan	FJ710101	Vendetti 2009
Neptunea polycostata			Y	UCMP-556108	Sendai, Miyagi Prefecture, Japan*	FJ710107	Vendetti 2009

mitochondrial genes cytochrome oxidase I (*cox1*) and 16S rRNA, as well as the nuclear ribosomal RNA 28S gene from additional individuals were amplified using PCR and Sanger sequencing (Table 2). Alignments used for regions of these genes were assembled with reference to the whole genome and nuclear riboosomal cassette sequences produced from the high-throughput sequencing above.

2.4. Molecular phylogenetic analysis and divergence date estimation

All sequence alignments used for phylogenetic analyses were concatenated to remove missing regions and sequence ambiguities. Gblocks 0.91b (Castresana, 2000), operating under standard settings was used to eliminate poorly aligned positions and regions with low homology from DNA alignments used for phylogenetic reconstruction. SplitsTree 4 (Huson and Bryant, 2006), was used to investigate the unrooted phylogenetic network derived from the DNA sequence alignments used to produce phylogenies, in order to examine the structure of the phylogenetic signal. Partitions in sequence data were investigated for protein-encoding, tRNA and rRNA genes. jModelTest 2.1.6 (Guindon and Gascuel, 2003; Darriba et al., 2012), was used to statistically identify the best fitting nucleotide substitution model for each gene partition. The generalised time-reversible substitution model (GTR + I + G) (Tavaré, 1986), was found to be most appropriate for substitution

model for the mtDNA protein-encoding, rRNA and nuclear rDNA sequences, whereas the HKY + I + G model (Hasegawa et al., 1985), was most suitable for the mtDNA tRNA regions. Alignments of 31 sequences were used for non-calibrated phylogenetic reconstructions. When sequence data were partitioned, the respective substitution models were unlinked. Molecular phylogenies were estimated using Bayesian MCMC inference in MrBayes 3.2 (Ronquist et al., 2012), and BEAST 1.8.3 (Drummond et al., 2012). Tracer 1.6 (Rambaut et al., 2014) was used to evaluate posterior statistics for Bayesian MCMC parameters. Maximum-likelihood phylogenetic trees were also estimated using RAxML 8.2.8 (Stamatakis, 2014). Figtree 1.4.2 (Figtree, 2015), was used to graphically view and edit tree outputs, and support for phylogenetic nodes was inferred using posterior probability. All phylogenetic reconstruction was processed using CIPRES Science Gateway (Miller et al., 2010).

The timing of genetic divergences among the putative buccinulid taxa, in particular among lineages of *Penion*, were investigated using BEAST 1.8.3. A sequence alignment of mtDNA from 25 individuals, and another of mtDNA and nuclear rDNA from 27 individuals, were both fossil calibrated and used to phylogenetically estimate divergence dates among taxa. 25 and 27 sequences were used respectively, rather than the maximum of 31, because divergence dating methods require only one sequence per taxon. For the

combined mtDNA and rDNA calibrated phylogeny using 27 sequences, two partitions were used based on jModelTest results: (1) mtDNA protein-encoding and rRNA genes and nuclear rDNA genes (15,891 bp), and (2) tRNA genes (1065 bp) using the GTR + I + G and HKY + I + G substitution models respectively. The calibrated phylogeny of 25 mtDNA sequences also used two partitions: (1) protein-encoding and rRNA genes (10,635 bp), and (2) tRNA genes (1065 bp) using the GTR + I + G and HKY + I + G substitution models respectively.

An MCMC analysis of 100 million generations, with a sample frequency of 1000, and a burn-in of 10% was used to generate phylogenies. Time calibrated phylogenetic analysis was carried out using the lognormal-relaxed clock model (Drummond et al., 2006), and the speciation birth-death process tree prior (Gernhard, 2008). Priors for calibrations based on fossil data outside of New Zealand were fitted with a normal distribution. Using the earliest known occurrence of buccinoid fossils, which are classified as *Khetella* (Kaim and Beisel, 2005), we estimated the mean tree root height to be 165 Ma (SD = 4.0 Ma). Likewise, based on earliest known fossil occurrences of Fasciolariidae (Allison, 1955; Tracey et al., 1993), we estimated the earliest mean convergence date with Nassariidae and Buccinidae/Buccinulidae to be 139.8 Ma (SD = 3.0 Ma).

A recent divergence was calibrated for our phylogeny, incorporating the earliest known fossil occurrence of the extant species *Buccinulum v. vittatum* (Quoy & Gaimard, 1833) (3.0 Ma; Beu and Maxwell, 1990). This calibration set a minimum divergence time between the sampled living species *B. v. vittatum* and *B. robustum* Powell, 1929 in the resulting phylogeny. Following the method of Hills (2010), the prior for this calibration was fitted with a lognormal distribution modelled on estimates of sampling biases in the New Zealand geological record (Crampton et al., 2003). This method means that our date estimates for these lineages incorporates measured uncertainty in the fossil record (i.e. whether fossils of a species may occur earlier in time than known under current sampling). Crucially, to avoid circularity, no fossil calibrations were used from *Penion* or its immediate sister clades (*Kelletia, Antarctoneptunea*, see results). Divergence dates estimated from our phylogenetic trees (using Fasciolariidae and *B. v. vittatum*) were therefore independent of the fossil record of *Penion* (and allies) during subsequent comparisons. The maximum clade credibility tree was generated from BEAST MCMC sampling using TreeAnnotator 1.7.5, and visualised in FigTree 1.4.2.

3. Results

3.1. Sequence data

We assembled new mitochondrial genome sequences from 30 individuals belonging to 27 putative taxa (Table 1). We also assembled new nuclear rDNA sequences (18S, 5.8S, 28S rRNA genes) for 32 individuals belonging to 28 taxa (Table 1). In addition, sequences from 15 further individuals for the mtDNA 16S rRNA and *cox1* genes were amplified and Sanger sequenced or downloaded from GenBank (Table 2). 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). Mitochondrial genome sequences varied between 15,104 and 15,264 bp in length, and nuclear rDNA sequences varied between 5334 and 5340 bp in length. Statistics concerning sequence length and nucleotide ratios are summarised in Supplementary Tables 1 and 2 in the Data in Brief article, along with all other Supplementary Figures mentioned below.

Most of the original 32 individuals yielded complete mtDNA and rDNA sequence data, but three specimens had low sequence coverage for regions of mtDNA or rDNA, and so the set of taxa and number of individuals varies slightly for trees based on marker (see Table 1). One specimen of *P. c. cuvierianus* (Powell, 1927), *B. linea* (Martyn, 1758) and *B. vittatum littorinoides* (Reeve, 1846) had low sequencing read coverage for the mitochondrial genome, but all three specimens provided nuclear ribosomal cassette sequences (Table 1). The 3′ end of the 28S rRNA gene was poorly covered for *Aeneator valedictus* (Watson, 1886) (Table 1). Although our estimated sequence scaffolds for the nuclear ribosomal data include internal spacer region 1 (ITS1) and ITS2, these regions were excluded from phylogenetic analysis as individuals contain multiple ITS sequence variants. A third of the nuclear rDNA 18S rRNA gene was removed from the 5′ end for phylogenetic analyses, as all high-throughput sequenced specimens had reduced read coverage at this region.

Mean pair wise mtDNA variability across all whelks (Buccinidae and Buccinulidae) was 22.5%, whereas values within putative Buccinidae and Buccinulidae were 16.6% and 22.6% respectively (see Table 1 for putative assignment of genera to families). This suggests that the sampled, putative buccinulids have (on average) more divergent mtDNA genomes than buccinid taxa sampled in this study. The three sampled fasciolariid species had a mean pair wise mtDNA variability of 17.5%. At the generic-level, mtDNA mean pair wise variability was 7.8%, 29.6% and 21.2% among species of *Aeneator*, *Buccinulum* and *Penion* respectively. Pair wise mtDNA variability for *Cominella* and *Kelletia* (both n = 2), was 19% and 10.7% respectively. Based on the proportion of variable sites per gene, some genes such as ND2 and ND5 convey more phylogenetic information than others such as 16S rRNA at different levels of phylogenetic investigation (see Supplementary Fig. 7), which agrees with previous results from other local buccinulid taxa (e.g. *Cominella* species, Donald et al., 2015). Compared to the mtDNA, variation among rDNA sequences was very low (Supplementary Fig. 7).

3.2. Phylogenetic reconstruction

Sequence alignments used for phylogenetic reconstruction had gaps and ambiguous nucleotides manually removed for the regions and specimens mentioned above. For mtDNA sequences, gblocks retained 97% of the original mtDNA protein-encoding nucleotide positions, and 61% and 76% of the mtDNA tRNA and rRNA positions respectively. This analysis resulted in sequence lengths of 9251, 983 and 894 bp respectively for mtDNA protein-encoding, tRNA and rRNA sequence regions. 99% of the nuclear rDNA nucleotide positions were also retained, leaving an alignment sequence length of 4667 bp available for phylogenetic reconstruction.

The phylogenetic relationships inferred from mitochondrial and nuclear ribosomal markers were broadly similar, and both indicate that Southern Hemisphere whelks (Buccinulidae) and Northern Hemisphere taxa (Buccinidae) are not reciprocally monophyletic (Fig. 2). Results also indicate that New Zealand buccinulid whelks are not monophyletic (Fig. 2). Bayesian and maximum-likelihood derived phylogenies were similar (Fig. 2; Supplementary Figs. 1 and 2). Phylogenies using the two markers inferred contrasting evolutionary relationship for *Aeneator* and *Buccinulum* (Fig. 2); the mitochondrial data suggested a sister relationship with *Penion*, whereas nuclear markers suggested a sister relationship with a clade of southern and northern buccinid genera. Relationships among some closely related taxa also differed between phylogenies (e.g. *P. c. cuvierianus* and *P. chathamensis*; Fig. 2). When a phylogeny was produced using both mtDNA and nuclear rDNA
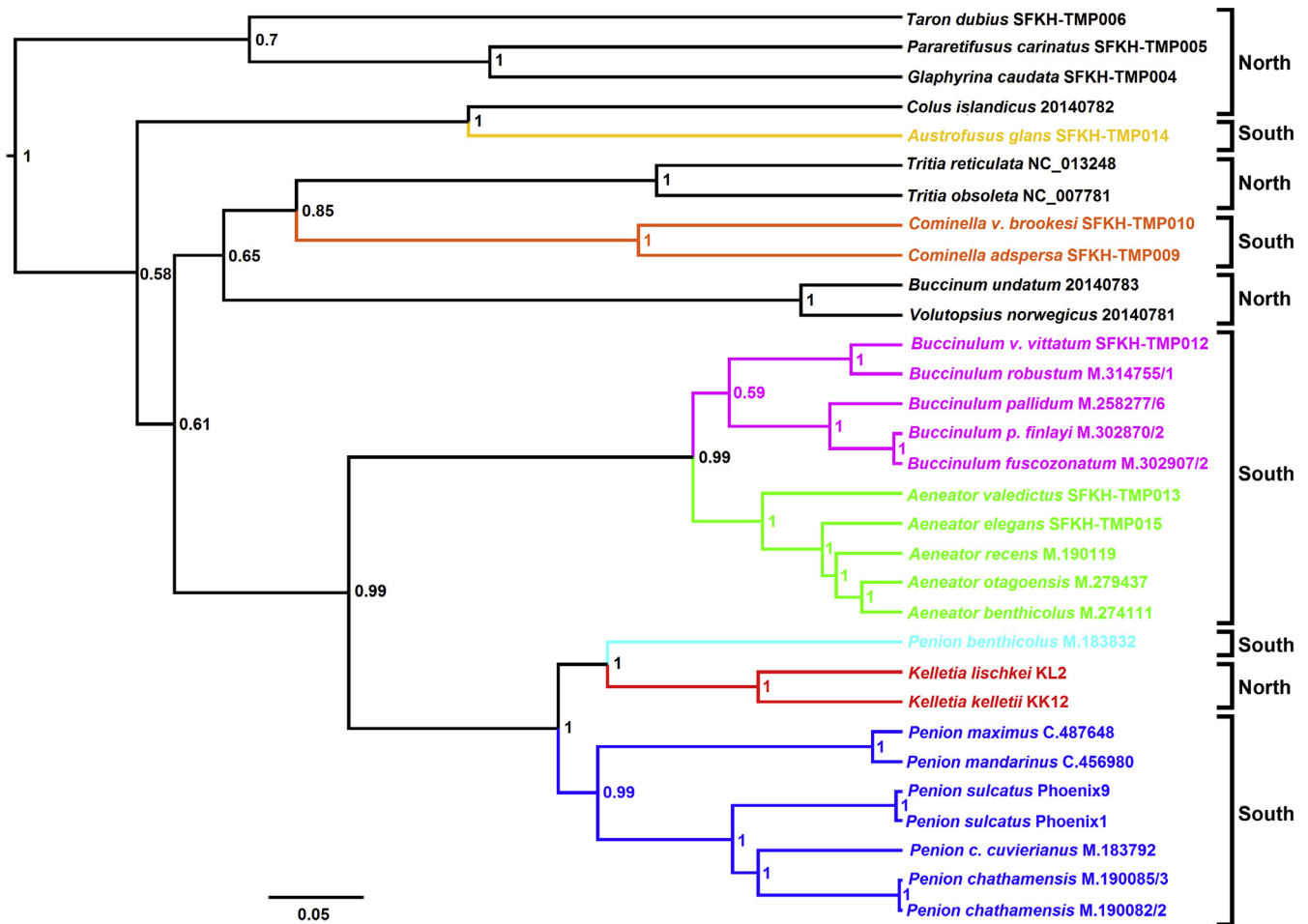
Fig. 2. *Southern Hemisphere whelks are not monophyletic.*
(A) Bayesian mtDNA phylogeny for buccinid and buccinulid whelks. An mtDNA phylogeny demonstrating the relationship of Northern and Southern Hemisphere whelks (Buccinidae and Buccinulidae). The phylogeny is based on an alignment of 31 concatenated mitochondrial genome sequences (incorporating protein-encoding, tRNA and rRNA genes). (B) A nuclear 45S rDNA phylogeny demonstrating the relationship of Northern and Southern Hemisphere whelks (Buccinidae and Buccinulidae). The phylogeny is based on a 4667 bp alignment of concatenated nuclear rDNA gene sequences (18S, 5.8S, 28S rRNA) from 31 specimens. For both trees, node posterior support values are shown where >0.5. Genera putatively belonging to Buccinulidae are shown in different colours, and the geographic origin of specimens (Northern and Southern Hemisphere) is listed on the right. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

sequence data (Fig. 3), the inferences were dominated by the phylogenetic signal present in the mitochondrial genomic data.

In order to increase the number of taxa studied, three additional phylogenetic trees were inferred from short-length sequence data from the rDNA 28S (Supplementary Fig. 5), mtDNA *cox1* (Supplementary Fig. 4), and 16S rRNA (Supplementary Fig. 6) fragments. Aligned sequences were too short for robust phylogenetic analyses (1486, 502 and 261 bp for 28S, *cox1* and the 16S respectively), but all markers consistently placed *P. benthicolus* Dell, 1956 as sister to *A. aurora* (Supplementary Fig. 4).

3.3. Divergence date estimation

We estimated divergence dates among extant taxa by fossil calibrating a combined mtDNA and rDNA sequence phylogeny (Fig. 3), as well as a phylogeny based only on mtDNA sequence data (Supplementary Fig. 3). Based on posterior outputs, we were able to successfully calibrate these trees using earliest known fossil occurrences for *Buccinulum v. vittatum*, Fasciolariidae and the earliest known buccinoidean fossils. Highest 95% posterior density ranges for estimated divergence dates do not differ substantially between the two phylogenetic trees (Fig. 3; Supplementary Fig. 3), likely due to the dominance of phylogenetic signal from mtDNA sequence data.

Posterior results also indicated that the inclusion of a calibration for the earliest occurrence of Nassariidae (estimated at 66.0 Ma, SD = 5.0 Ma; Palmer and Bran, 1965; Haasl, 2000; Sessa and Patzkowsky, 2009), did not have a significant impact upon our results. This calibration may not have had a significant impact because only two mtDNA sequences from *Tritia reticulata* and *T. obsoleta* were sampled. Alternatively, this calibration may have little impact because our phylogenies find Nassariidae to be closely related to Buccinidae/Buccinulidae (Fig. 2; Supplementary Fig. 3). This finding corroborates previous molecular (Hayashi, 2005; Galindo et al., 2016), and morphological findings (Haasl, 2000).

4. Discusssion

4.1. Evolution of Southern Hemisphere and New Zealand whelks

All phylogenies in this study fail to support monophyly of the Buccinulidae Southern Hemisphere whelks, including taxa from New Zealand (Figs. 2 and 3; Supplementary Figs. 1–6). Although not all New Zealand buccinulid whelks are sampled, and only a tiny proportion of all putative buccinulid and buccinid whelks, it is apparent that the two families are not reciprocally monophyletic as phylogenies support clades comprising of Southern and Northern Hemisphere species (Figs. 2 and 3). For long-length sequence
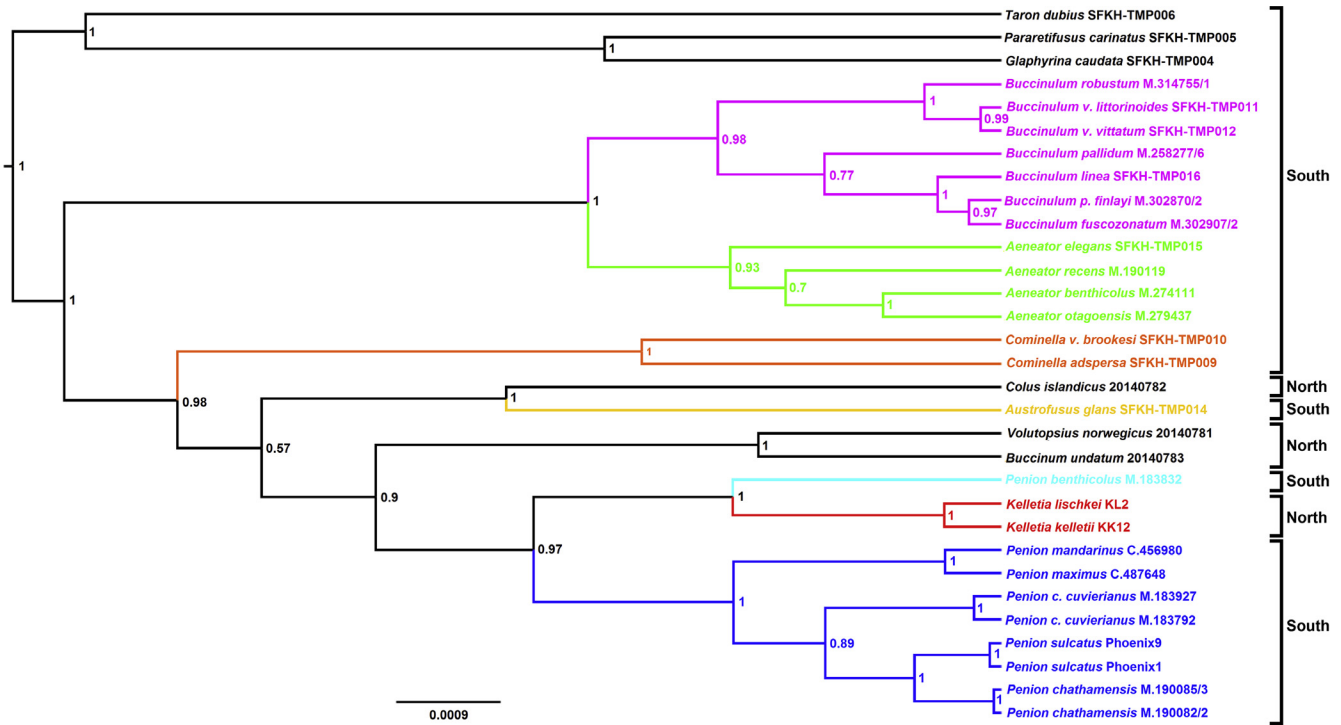
Fig. 2 (*continued*)

data, the closest sampled relatives of *Cominella* appear to be the Northern Hemisphere taxa *Buccinum undatum* Linnaeus, 1785 and *Volutopsius norwegicus* (Gmelin, 1791), and likewise *Austrofusus glans* (Röding, 1798) is more closely related to the *Colus islandicus* (Mohr, 1786) specimen sampled from the North Sea than to any of the sampled Southern Hemisphere taxa (Figs. 2 and 3). Short-length sequence data implies that *Cominella* and *Pareuthria* are sister (Supplementary Figs. 4 and 6), however most phylogenetic results indicate that this clade is more closely related to Northern Hemisphere taxa than putative Buccinulidae (Figs. 2 and 3, Supplementary Fig. 6).

Placement of the buccinulid genera *Aeneator* and *Buccinulum* was sensitive to the marker used, and probably reflected the weaker phylogenetic signal in the nuclear rDNA data compared to mtDNA. Based on the proportion of variable sites per gene, sequence variation exhibited for the nuclear rDNA 18S, 5.8S and 28S rRNA genes was small (see Supplementary Fig. 7). Investigating the phylogenetic signal in the two datasets confirmed that the more conserved and shorter rDNA sequences result in greater phylogenetic conflict (splits networks; Supplementary Figs. 7–9). Specifically, the shorter branch lengths in the rDNA data (Supplementary Fig. 9) indicate smaller genetic distances among specimens, and the box structures shown between many taxa (especially *Aeneator* and *Buccinulum*; Supplementary Fig. 9) for rDNA indicate a strong signal for alternative relationships. In contrast for the mtDNA sequence data, most relationships are resolved with few significant incompatible splits, and the branch lengths between individuals are long (Supplementary Fig. 8). The area with the most possible splits for the mtDNA sequence alignment focussed on our sampling of Nassariidae and Fasciolariidae (Supplementary Fig. 8), where there is low Bayesian posterior probability support on our phylogenetic tree (Fig. 2).

Based on mtDNA data, some of the sampled, putative buccinulid whelks form a monophyletic group, with *Aeneator, Antarctoneptunea, Buccinulum, Kelletia* and *Penion* appearing to be closely related (Fig.2A). This relationship was affected by the uncertain

placement of *Aeneator* and *Buccinulum* in the rDNA data however (Fig.2B). It is also entirely possible that an unsampled Northern Hemisphere snail lineage could nest within this clade if included (in addition to *Kelletia* north of the equator). If the short DNA fragments provide correct phylogenetic relationships (Supplementary Fig. 7), the Northern Hemisphere genus *Neptunea* is not closely related to *Antarctoneptunea* and *Penion*, despite previous studies noting their morphological and ecological similarities (Ponder, 1973; Dell, 1972).

If we accept the mtDNA and combined mtDNA and rDNA phylogenetic reconstruction (Figs. 2 and 3), Buccinulidae could be retained as a valid taxonomic clade by being restricted to only include *Aeneator, Antarctoneptunea, Buccinulum, Kelletia* and *Penion*. Its ranking as a family or subfamily is dependent upon necessary developments with the phylogeny of Buccinoidea overall and Buccinidae (which is currently not monophyletic; Couto et al., 2016; Galindo et al., 2016). However, biogeographic hypotheses relating this clade to the potential biogeographic isolation of New Zealand should be avoided. As noted above, *Kelletia* is distributed in the Northern Hemisphere and might represent a dispersal event from this otherwise Southern Hemisphere restricted group (Powell, 1929, 1951; Ponder, 1973). This group therefore inherently challenges an assumption of isolation in the Southern Hemisphere. Likewise, the extant distribution of these taxa does not support the assumption of biogeographic isolation for New Zealand buccinulid whelks, as *Antarctoneptunea* is restricted to the polar circle of Antarctica, New Zealand and the Tasman Sea, and extant species of both *Penion* (Ponder, 1973), and *Aeneator* (McLean and Andrade, 1982; Araya, 2013), are currently recognised outside of New Zealand. Fossils of *Penion* also are documented from Australia (Ponder, 1973), Chile and Argentina (Ponder, 1973; Frassinettii, 2000; Nielsen, 2003; Parras and Griffin, 2009; Reichler, 2010), and Antarctica (Beu, 2009). Similarly, fossil species of *Kelletia* are known from Ecuador (Olsson, 1964), the USA (Arnold, 1910; Anderson and Martin, 1914; Kanakoff, 1954; Addicott, 1970; Hertlein, 1970), and Japan (Ozaki, 1954).
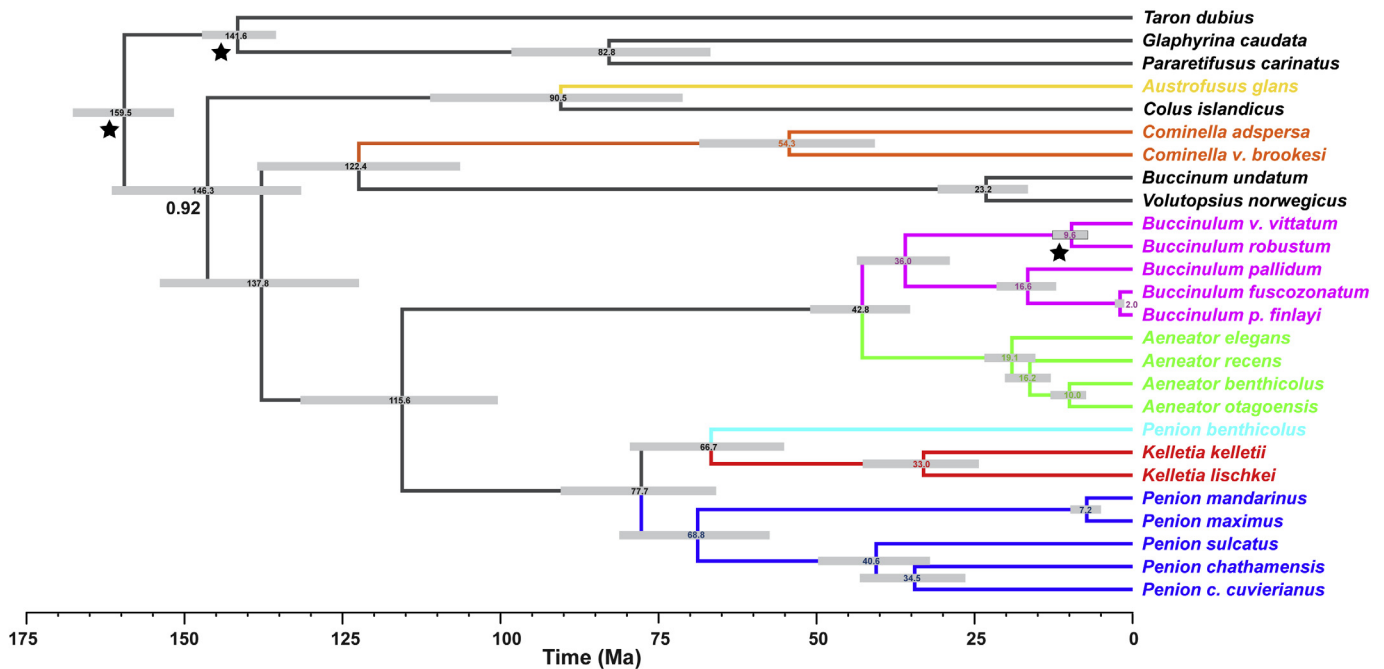
Fig. 3. *The timing of buccinulid whelk evolution.*
(A) Bayesian calibrated mtDNA and 45S rDNA phylogeny of buccinid and buccinulid whelks. A Bayesian phylogeny based on an alignment of 27 concatenated mitochondrial genome (incorporating protein-encoding, tRNA and rRNA genes) and nuclear ribosomal rRNA 18S, 5.8S and 28S sequences, which has been fossil calibrated to estimate divergence dates among the buccinid and buccinulid whelk lineages. The entire phylogeny is shown in A), whereas B) focusses on the divergence dates estimated for *Penion* and *Kelletia,* with comparison to the partial fossil record of the clade (shading shows estimated time range for referenced fossil taxa), with photos of extant shells and fossils for illustration. Black stars indicate fossil calibrated splits. Node labels are estimated median divergence dates with the 95% highest posterior density (HPD) range shown as a horizontal bar (grey in A), yellow in B)). Node posterior values are shown if support was < 1. Genera of putative Buccinulidae are shown in different colours (B). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The key implication of this phylogenetic analysis is therefore that assumptions of geographic isolation and separate evolutionary radiation in the Southern Hemisphere are not valid for all lineages of buccinulid whelks. The occurrence of multiple, separate lineages in New Zealand implies that whelks have transversed long distances over evolutionary time. As in other marine molluscs, these findings indicate that dispersal can be common on an evolutionary timescale, even in lineages that undergo direct development (e.g. Donald et al., 2005; Huelsken et al., 2013; Cumming et al., 2014; Donald et al., 2015). New Zealand may be sufficiently remote to allow an increased frequency of endemism in benthic marine snail species, but over millions of years the islands are clearly not so biologically isolated. This finding corresponds with many studies of terrestrial fauna (e.g. Battley, 1997; Trewick, 2000; Goldberg et al., 2008). Studies of other marine molluscs have demonstrated that a high rate of endemism, as observed in *Aeneator, Cominella* and *Penion*, is not mutually exclusive with dispersal ability (e.g. Huelsken et al., 2013).

4.2. Comments on Fasciolariidae and Buccinidae

The sampled Fasciolariidae in our phylogenies (*Glaphyrina caudata, Pararetifusus carinatus, Taron dubius*), are monophyletic and sister to all other taxa included (Figs. 2 and 3; Supplementary Figs. 1–3). Recent taxonomic summaries of Buccinidae (e.g. Bouchet et al., 2005), have suggested that *Buccinum* and *Volutopsius* reside within tribes Buccinini and Volutopsini respectively. However, the relatively small genetic distance (0.44% and 2.30% pair wise variability for rDNA and mtDNA respectively) estimated by our phylogenetic analysis suggests a close relationship between these taxa (Figs. 2 and 3; Supplementary Figs. 1–3). A previous assessment of soft-body and radula morphology hypothesised that *Penion* represent an early split among Buccinidae (Harasewych,

1990), but this might instead be an example of morphological convergence.

4.3. Penion benthicolus and Antarctoneptunea

Our molecular phylogenies indicate that *Penion* and *Kelletia* are closely related (Figs. 2 and 3; Supplementary Figs. 1–6). This result agrees with the previous analysis of mitochondrial 16S rRNA gene data (Hayashi, 2005), and hypotheses based on shell morphology and soft-body anatomy (Powell, 1929; Wenz, 1941; Ponder, 1973; Stilwell and Zinsmeister, 1992), and it justifies previous taxonomic confusion of the genera (Palmer and Bran, 1965). In addition, our phylogenetic evidence also indicates that *P. benthicolus* is not closely related to other *Penion*, forming instead a clade with *Antarctoneptunea aurora* (Supplementary Fig. 4). Since its discovery, the evolution and classification of *A. aurora* has puzzled malacologists (Powell, 1958; Dell, 1972). Morphological comparison has been made to *Penion* (Dell, 1972), but radula morphology (Dell, 1956), and the small size of *P. benthicolus* shells has been noted to be unusual within *Penion* (Powell, 1979). Comparison of the shells of *P. benthicolus* and *A. aurora* reveals their similarity (Fig. 4). Both taxa share a relatively large, beehive-shaped protoconch (Fig. 4), and occur at water depths beyond most other species of *Penion* (Dell, 1956, 1972; Fig. 5). Although genetic evidence is limited (477 bp of *cox1* from two individuals of *A. aurora*), we recommend that the species are treated as sister and that *P. benthicolus* is referred to *Antactoneptunea*.

4.4. Molecular divergence dates and fossil record of Antarctoneptunea, Kelletia and Penion

It seems likely that the common ancestor of the monophyletic *Antarctoneptunea, Kelletia* and *Penion* clade evolved in the Southern
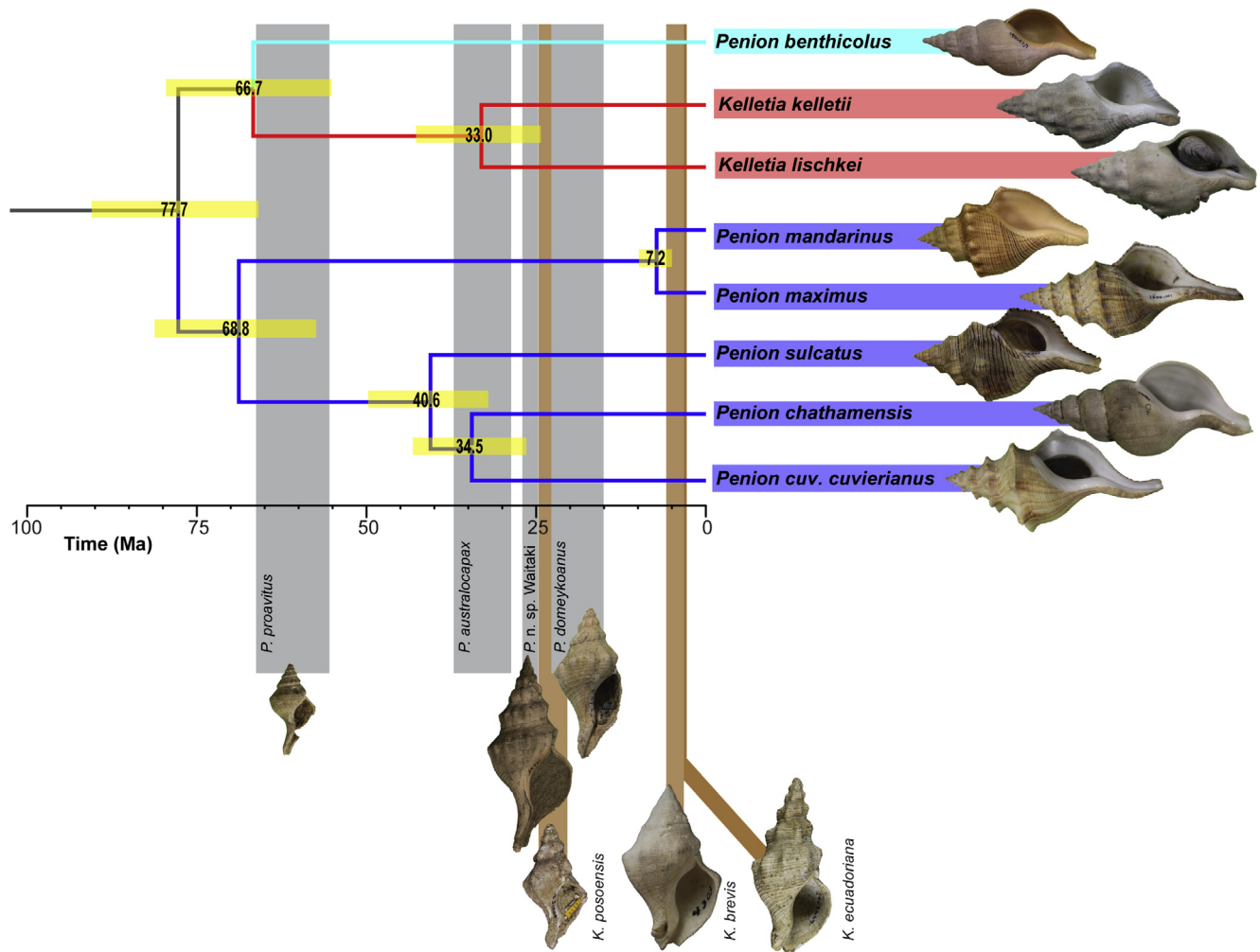
Fig. 3 (*continued*)

Hemisphere, most likely on the Zealandian continental shelf or in the Southern Ocean around 76 million years ago (based on fossil occurrences discussed below and the occurrence of the related taxa *Aeneator* and *Buccinulum* in New Zealand). The divergence dates estimated from molecular phylogenies using fossil calibrations from independent lineages (*Buccinulum*, Fasciolariidae; Fig. 3; Supplementary Fig. 3) show close concordance with the documented fossil record of *Penion* and *Kelletia*. The earliest occurrences within regions also hint at the possible route of dispersal for the clade.

The earliest known fossil belonging to the clade is *P. proavitus* Finlay and Marwick, 1937 from 66.0 to 55.80 Ma in New Zealand (Fig. 5 label 1; Beu and Maxwell, 1990; Beu et al., 1997). Based on our molecular phylogenetic estimates of divergence dates (Fig. 3), we suggest that this fossil species may represent a crown lineage of either the entire clade (median divergence date 77.77 Ma; Fig. 3) or monophyletic *Penion* (median 68.84 Ma; Fig. 3). It is therefore unfortunate that the type specimens of *P. proavitus* are juveniles and the only known adult specimen is poorly preserved (Finlay and Marwick, 1937). The next-earliest known Australasian fossils are *P.* n. sp. Waitaki and *P.* n. sp. Waimumu from 27.3 to 25.2 Ma, again from New Zealand (Fig. 5 label 2; pers. comm. Alan G. Beu, GNS Science 2016). These fossils occur later than the estimated period of divergence for New Zealand and Australian *Penion*, and occur within the range estimated for the split of *P. chathamensis* and *P. c. cuvierianus* (median 34.51 Ma; 95% HPD 430 – 26.78 Ma; Fig. 3). Although undescribed, these

fossil specimens are adult and well preserved – allowing future further study. The Antarctic fossil species *P. australocapax* Stilwell and Zinsmeister, 1992 is a little earlier (approximately 37.0– 28.1 Ma), but the chronostratigraphy for the region is also less certain (Fig. 5 label 3; Stilwell and Zinsmeister, 1992; Beu, 2009). This fossil range does however overlap with the estimated period of divergence for speciation among the genetically sampled New Zealand *Penion* (Fig. 3). Afterwards, numerous fossils classified as *Penion* are documented from Argentina and Chile (Frassinettii, 2000; Nielsen, 2003), the earliest of which are dated approximately to 23.03–15.90 Ma (stratigraphy uncertain; Fig. 5 label 4), or potentially 20.43–15.97 Ma (more reliable stratigraphy for a subset of fossils; Reichler, 2010). These fossils occur after divergence estimated for New Zealand *Penion* (median 40.62 Ma; 95% HPD 50.04– 32.53 Ma; Fig. 3). The earliest, reliable fossils of Australian *Penion* (4.3–4.0 Ma; Fig. 5 label 5; Ponder, 1973) occur close to (but not technically within) the date range predicted from the phylogeny (median 7.25 Ma; 95% HPD 9.86–5.05 Ma; Fig. 3). Other Australian taxa that are currently classified as *Penion* do occur much earlier, but these fossils have quite different shells, and likely represent unrelated Buccinidae or Fasciolariidae (Ponder, 1973).

The earliest known fossils of *Kelletia* are *K. posoensis* Anderson and Martin, 1914, dated to 25.2–21.7 Ma from California. This is within the geographic range of extant *K. kelletii* (Fig. 5 label 8; Anderson and Martin, 1914; Addicott, 1970), and *K. posoensis* occurs within the estimated period of divergence for the split
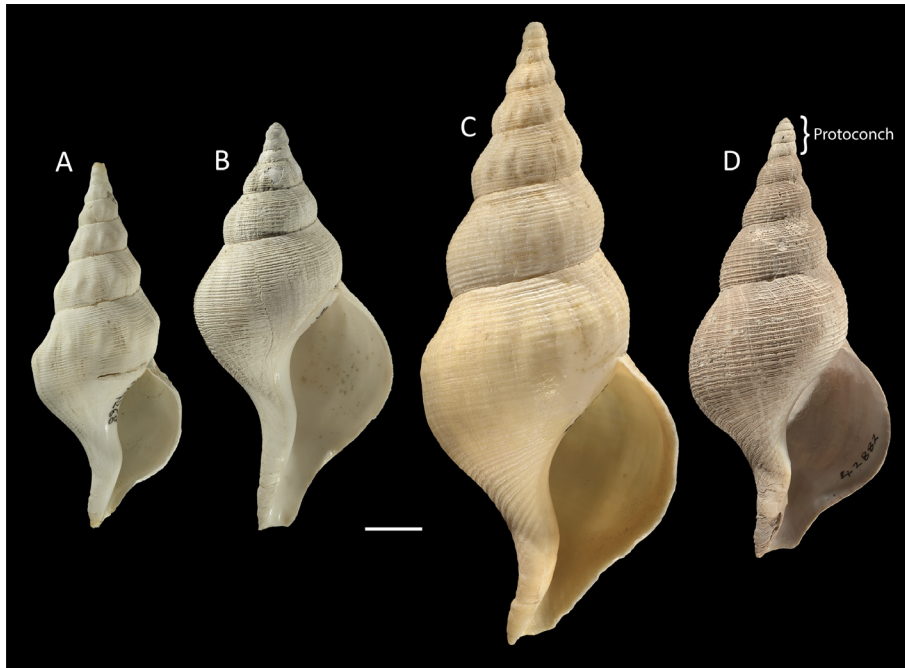
Fig. 4. *Shells of Penion benthicolus and Antarctonetpunea aurora.*
A comparison of *Penion benthicolus* and *Antarctoneptunea aurora* shells. (A) *P. benthicolus* M.274268 from 815 m deep off Cape Kidnappers, note that the last teleoconch whorl is broken; (B) *P. benthicolus* M.059741 from 1549 to 1723 m deep in Hikurangi Trench; (C) *P. benthicolus* M.118756 from 390 to 400 m deep east of Auckland Islands; (D) *A. aurora* M.242882 from 494 to 498 m deep in the Ross Sea. The scale bar is 1 cm and the larval shell (protoconch) is labelled for D.

between *K. lischkei* and *K. kelletii* (median 33.0 Ma; Fig. 3). In addition, later fossil species of *Kelletia* are also known from Ecuador, dated to approximately 5.33–7.00 Ma (stratigraphy uncertain; Fig. 5 label 7; Olsson, 1964). Previously these fossils were hypothesised to represent a southward dispersal of *Kelletia* from California (Lindberg, 1991), but instead it now seems plausible that these species descended from lineages that moved northward from the Southern Hemisphere. A similar dispersal route is hypothesised for *Haliotis* Linnaeus, 1758 abalone (Bester-van der Merwe et al., 2012). The earliest known fossils of *Kelletia* in Japan are *K. brevis* (Ozaki, 1954) from 5.6 to 8.0 Ma (Fig. 5 label 9; Ogasawara, 2002; Wade et al., 2011; Shiba et al., 2012), which is compatible with the estimated period of divergence between the two extant *Kelletia* lineages (Fig. 3). The fossil record for *K. lischkei* and presumed close, extinct relatives may be incomplete due to poor preservation. Modern populations of *K. lischkei* occur on rocky substrates within coastal waters (Hwang et al., 2014), an environment that is inconsistently represented in the marine fossil record (Crampton et al., 2003), with preservation rates affected by lithology (Foote et al., 2015). This is born out by the fact that the earliest fossil occurrence of *K. lischkei* itself is from only 0.13 Ma (Ogasawara, 2002).

Antarctoneptunea aurora is not represented by fossils, although it has been suggested that *P. australocapax* from the Antarctic Peninsula (within the range of extant *A. aurora*) may be a misclassified species of *Antarctoneptunea* (Beu, 2009). *Antarctoneptunea benthicola* is recorded from New Zealand at 2.4 Ma (Fig. 5 label 6; Beu and Maxwell, 1990). This is unlikely to represent the origin of *Antarctoneptunea* though as deep-water localities are sparsely represented in the New Zealand fossil record (Crampton et al., 2003; e.g. Beu, 1979).

Given the wide distribution of extant species and fossils (Fig. 5), the evolution of *Antarctoneptunea, Kelletia* and *Penion* indicates that the potential for long-distance dispersal in benthic, marine

gastropods should not be overlooked. The Buccinulidae hypothesis of geographic isolation is clearly incorrect for this clade. Similarly, the prediction of southward migration of *Kelletia* from the Northern Hemisphere now seems unlikely (Lindberg, 1991). Considering the rich fossil record for this clade across the Pacific (e.g. Ozaki, 1954; Olsson, 1964; Addicott, 1970; Ponder, 1973; Beu and Maxwell, 1990; Nielsen, 2003; Beu, 2009), *Antarctoneptunea, Kelletia* and *Penion* represent a valuable system for future investigations of speciation and long-distance dispersal in marine invertebrates.

Research is needed to determine whether *Penion, Kelletia* and *Antarctoneptunea* exhibit different developmental strategies, which may have affected the potential for dispersal and phylogeny of the clade. Captive rearing has shown that *Kelletia kelletii* Forbes, 1850 undergo indirect development (facultative planktotrophy) with larvae well suited to dispersal (Vendetti, 2009). However, hypotheses for the development of *Penion* and *Antarctoneptunea* species are based only on protoconch and egg morphology, which is highly variable between and within taxa (Ponder, 1973; Powell, 1979; Beu and Maxwell, 1990). It is likely that extant New Zealand *Penion* exhibit direct development from eggs that hatch into miniature adult snails (Ponder, 1973; Powell, 1979). On the other hand, some fossil *Penion* from New Zealand and Chile (Beu and Maxwell, 1990; Nielsen, 2003), and extant species from Australia (Ponder, 1973), have small protoconchs suggestive of indirect development. *Antarctoneptunea* have very large protoconchs indicative of direct development (Fig. 4), but the vast geographic range occupied by both species (Fig. 5) and bathyal depth distributions imply that adaptations for long-distance dispersal are necessary.

Although phenotypic convergence is the most probable explanation for similarities in the shell morphology of snails divided by large distances, shared ancestry remains a possibility. Perhaps the classification of some fossils in the North Atlantic as *Penion* or *Kelletia* (sometimes *Boreokelletia* Anderson, 1964; e.g. Palmer
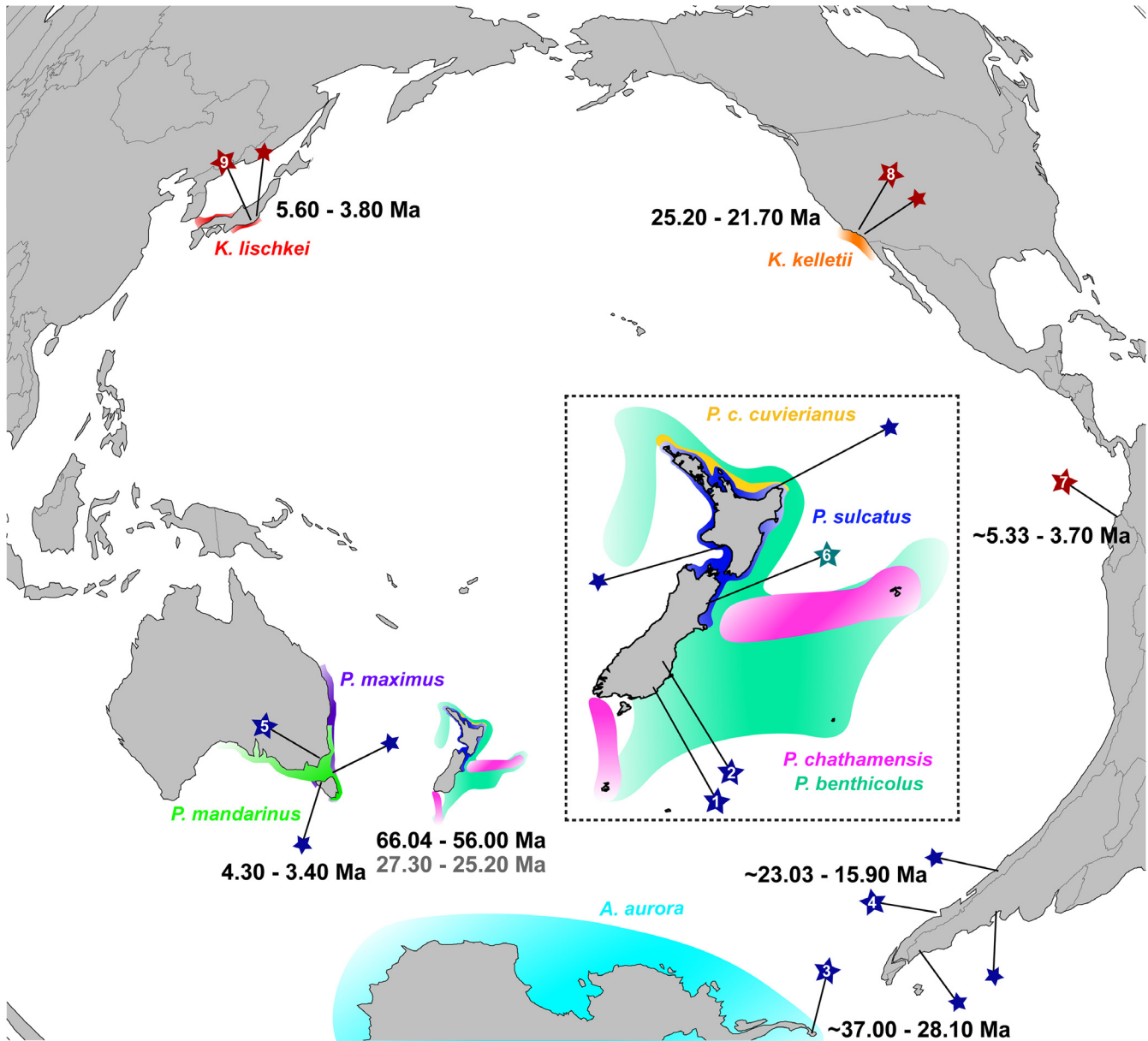
Fig. 5. *Divergence times and fossil record.*

Extant distributions of *Antarctoneptunea* (*A. aurora* in cyan, *P. benthicolus* in mint green), *Kelletia* (*K. lischkei* in red, *K. kelletii* in orange), and *Penion* (*P. chathamensis* in pink, *P. c. cuvierianus* in yellow, *P. mandarinus* in green, *P. maximus* in purple, *P. sulcatus* in blue). Stars mark the location of key fossils: (1) *P. proavitus* from Wangaloa, Otago (66.04–56.00 Ma); (2) *P.* n. sp. Waitaki from Lake Waitaki, Canterbury (27.3–25.2 Ma); (3) *P. australocapax* from Seymour Island, Antarctic Peninsula (approximately 37.0–28.1 Ma); (4) *Penion* spp. from numerous locations in Chile and Argentina (approximately 23.03–15.90 Ma); (5) *P. mandarinus* from Kalimna, Victoria (4.3–4 Ma); (6) *P. benthicolus* from Oaro, Canterbury (2.40–1.63 Ma); (7) *K. ecuadoriana* and *K. rugosa* from Esmeraldas, Ecuador (approximately 5.33–3.70 Ma); (8) *K. posoensis* from San Luis Obispo County, California (25.2–21.7 Ma); (9) *K. brevis* from Cape Inuwaka, Chiba Prefecture (5.6–8 Ma). The colour of fossil markers reflects putative classification (*P. benthicolus* in dark green, *Kelletia* in burgundy, *Penion* in navy blue). Markers without numbers show the location of further fossil sites not discussed within the text. The age estimates shown are the earliest known fossil occurrences of the clade within each region (Antarctica, Argentina and Chile, Australia, Japan, New Zealand, USA). Two date ranges are shown for New Zealand; black for *P. proavitus* and grey for *P.* n. sp. Waitaki. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and Bran, 1965; Anderson, 1973; Gilbert, 1973; Kollmann and Peel, 1983; CoBabe and Allmon, 1994; Moths and Albrecht, 2010), is not as incongruous as it first appeared.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2017.06.018.

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