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ORIGINAL ARTICLE

Multiple fossil calibrations, nuclear loci and mitochondrial genomes provide new insight into biogeography and divergence timing for true seals (Phocidae, Pinnipedia)

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ABSTRACT

Aim To better understand the historical biogeography of the true seals, Phocidae, by combining nuclear DNA (nDNA) and mitochondrial DNA (mtDNA) in a divergence time analysis using multiple fossil calibrations.

Location Arctic, Antarctic, Pacific and Atlantic Oceans, Lake Baikal, Caspian Sea.

Methods Fifteen nuclear genes totalling 8935 bp plus near-complete mitochondrial genome sequences were used in a Bayesian divergence time analysis, incorporating eight soft-bound fossil calibrations across the phylogeny. All species of true seals were included, plus the walrus, three otariids and seven carnivore outgroups. The majority of the nuclear sequences and four phocid mitochondrial genomes (plus three non-phocid mitochondrial genomes) were newly generated for this study using DNA extracted from tissue samples; other sequences were obtained from GenBank.

Results Using multiple nuclear genes and multiple fossil calibrations resulted in most divergence time estimations within Phocidae being much more recent than predicted by other molecular studies incorporating only mtDNA and using a single calibration point. A new phylogenetic hypothesis was recovered for the Antarctic seals.

Main conclusions Incorporating multiple nuclear genes and fossil calibrations had a profound effect on the estimated divergence times. Most estimated divergences within Phocinae (Arctic seals) correspond to Arctic oceanic events and all occur within the last 12 Myr, a time when the Arctic and Atlantic oceans were freely exchanging and perennial Arctic sea ice existed, indicating that the Arctic seals may have had a longer association with ice than previously thought. The Monachinae ('southern' seals) split from the Phocinae *c.* 15 Ma on the eastern US coast. Several early trans-Atlantic dispersals possibly occurred, leaving no living descendants, as divergence estimates suggest that the *Monachus* (monk seal) species divergences occurred in the western Atlantic *c.* 6 Ma, with the Mediterranean monk seal ancestor dispersing afterwards. The tribes Lobodontini (Antarctic seals) and Miroungini (elephant seals) are also estimated to have diverged in the eastern Atlantic *c.* 7 Ma and a single Lobodontini dispersal to Antarctica occurred shortly afterwards. Many of the newly estimated dates are used to infer how extinct lineages/taxa are allied with their living relatives.

Keywords

Carnivora, fossil calibration, historical biogeography, molecular clock, Phocidae, phylogeny, phylogeography, Pinnipedia, seals.

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INTRODUCTION

The historical biogeography of the true seals (Phocidae, Pinnipedia) has been debated throughout the last century, with widely varying hypotheses and little consensus. The debate concerning the historical classification of pinnipeds as monophyletic (Illiger, 1811; Flower & Lyddekker, 1891; Gregory & Hellman, 1939; Simpson, 1945) vs. the resurrected theory of diphyly (Mivart, 1885) during the 1960s to 1980s influenced the interpretation of the phylogenetic affinity of early pinnipeds, Enaliarctidae, and the initial origins of Pinnipedia. Based on a diphyletic origin, an Atlantic (or Palaearctic) origin was proposed for the Phocidae and a Pacific origin for the Otariidae (fur seals and sea lions) and Odobenidae (walrus), the latter often including the Enaliarctidae (McLaren, 1960a; Mitchell & Tedford, 1973; Ray, 1976; Repenning *et al.*, 1979). Pinnipedia is now known to be a monophyletic group that is sister to the Musteloidea within the arctoid Carnivora (Flynn *et al.*, 2005; Fulton & Strobeck, 2006; Sato *et al.*, 2006). Most recent hypotheses support a Pacific origin of pinnipeds, continued early otariid and odobenid evolution in the Pacific and movement of the phocid ancestor through the Central American Seaway, leading to an Atlantic origin of the Phocidae (Bininda-Emonds & Russell, 1996; Deméré *et al.*, 2003; Fyler *et al.*, 2005). A southern North American non-marine pinniped origin has also been proposed (Árnason *et al.*, 2006), with otarioids (including enaliarctids) dispersing west into the Pacific and phocids eastward into the Atlantic. However, the recent discovery of the most primitive known pinniped, *Puijila darwini*, 21–24 Ma in the Canadian archipelago (Rybczynski *et al.*, 2009) has dramatically influenced pinniped biogeography, providing fossil evidence for early theories of an Arctic origin of pinnipeds (Matthew, 1915; Davies, 1958b).

Pinnipedia comprises three families: Otariidae (fur seals and sea lions), Odobenidae (walrus) and Phocidae (true seals). The family Phocidae comprises two subfamilies, Monachinae ('southern' seals) and Phocinae (northern seals), and each is divided into three tribes (Table 1, Fig. 1). Prior to King (1966), a third subfamily, Cystophorinae (bladder-nosed seals), was used, grouping *Cystophora cristata* (hooded seal) and the genus *Mirounga* (elephant seals), and further complicating early interpretations of biogeography and fossil descriptions. Although the two-subfamily system placing *Cystophora* in Phocinae is nearly universally accepted, the only recent comprehensive analysis of many phocid fossil taxa was framed within the three-subfamily system (Koretsky, 2001). The present tribal system as proposed by Burns & Fay (1970) is strongly supported by recent molecular work (Davis *et al.*, 2004; Árnason *et al.*, 2006; Higdon *et al.*, 2007). Therefore, although extensive lists of existing fossils have been compiled (Miyazaki *et al.*, 1994; Deméré *et al.*, 2003), rigorous phylogenetic study including fossil taxa remains to be performed within the present, well-defined taxonomic system.

Several recent studies have employed molecular dating techniques to address aspects of phocid biogeography and divergence times using mitochondrial (mt) restriction fragment length polymorphisms (RFLPs; Sasaki *et al.*, 2003), mtDNA (Árnason *et al.*, 2006; Palo & Vainola, 2006), or combined nuclear (n) DNA and mtDNA (Fyler *et al.*, 2005; Higdon *et al.*, 2007). All but one of these studies (Sasaki *et al.*, 2003) employed some method of relaxing the molecular clock with one or more fossil calibration points. Because of the different focus of each study, the calibration times used have been variable, although all of these studies are united by the use, primarily or exclusively, of mtDNA.

Just as a range of methods can be used to relax the molecular clock (Welch & Bromham, 2005), various methods of enforcing fossil calibrations can be employed. Fossils can be used to calibrate the clock across the tree by fixed-point calibrations or through a number of different techniques of creating hard or soft bounds on divergence times (Yang & Rannala, 2006). Point calibrations are difficult to implement properly if uncertainty exists regarding the age of the fossil or its placement on the tree. The assignment of hard minimum bounds allows fossils to represent minimum divergence times and such bounds are often implemented with a very liberal upper bound, or with no upper bound at all. While this allows flexibility in assigning a molecular date to the node in question, as opposed to a point estimate, using only minimum limits can lead to overestimated divergence times (Hugall *et al.*, 2007). Recent Bayesian techniques allow fossil calibrations to be implemented as prior distributions that can more accurately reflect the uncertainty involved (Drummond *et al.*, 2006; Yang & Rannala, 2006).

Here, 16 nuclear markers, in combination with mtDNA, are used to examine the effect of including nDNA and the use of multiple markers on the estimated divergence times. Multiple soft-bound fossil-based calibrations are implemented in a relaxed clock framework. The use of multiple DNA markers spanning a variety of evolutionary rates, combined with multiple calibration points, provides a framework for evaluating previous biogeographical hypotheses and examining the potential associations of some fossils of uncertain phylogenetic placement within the currently estimated topological framework and divergence times.

MATERIALS AND METHODS

Sequence acquisition and alignment

Forty-seven taxa were included in this study. Seven non-pinniped carnivores were included as outgroups: two feliforms, two canids and three mustelids. Appendix S1 in the Supporting Information lists GenBank accession numbers and references for all loci. New sequences and alignments have been deposited in GenBank under the numbers GU167260–GU167670 and GU174609–GU174639.

Total genomic DNA was isolated from either tissue or blood using the QIAgen DNeasy tissue extraction kit (Qiagen, Mississauga, ON, Canada). Sixteen nuclear regions were selected (Appendix S2), representing 15 genes (two fragments

Table 1 Taxonomy of the recent Phocidae (and otarioid outgroups).

Classification	Species name	Common name	Authority
Order Carnivora			Bowdich, 1821
Suborder Caniformia			Kretzoi, 1943
Infraorder Arctoidea			Flower, 1869
Pinnipedia			Illiger, 1811
Superfamily Phocoidea			Smirnov, 1908
Family Phocidae (true, earless seals)			Gray, 1821
Subfamily Phocinae (northern true seals)			Gray, 1821
Tribe Erignathini			Chapskii, 1955
	Erignathus barbatus	Bearded seal	(Erxleben, 1777)
Tribe Cystophorini			Burns & Fay, 1970
	Cystophora cristata	Hooded seal	(Erxleben, 1777)
Tribe Phocini			Gray, 1821
Subtribe Histriophocina			Chapskii 1955
	Histriophoca fasciata	Ribbon seal	(Zimmerman, 1783)
	Pagophilus groenlandicus	Harp seal	(Erxleben, 1777)
Subtribe Phocina			Chapskii 1955
	Phoca largha	Spotted seal	Pallas, 1811
	Phoca vitulina	Harbour seal	Linnaeus, 1758
	Halichoerus grypus	Grey seal	(Fabricius, 1791)
	Pusa caspica	Caspian seal	(Gmelin, 1788)
	Pusa sibirica	Baikal seal	(Gmelin, 1788)
	Pusa hispida	Ringed seal	(Schreber, 1775)
Subfamily Monachinae ('southern' true seals)			Trouessart, 1897
Tribe Monachini (monk seals)			Gray, 1869
	Monachus schauinslandi	Hawaiian monk seal	Matschie, 1905
	Monachus monachus	Mediterranean monk seal	(Hermann, 1779)
	Monachus tropicalis†	Caribbean monk seal	(Gray, 1850)
Tribe Miroungini (elephant seals)			de Muizon, 1982
	Mirounga angustirostris	Northern elephant seal	(Gill, 1866)
	Mirounga leonina	Southern elephant seal	(Linnaeus, 1758)
Tribe Lobodontini (Antarctic seals)			Scheffer, 1958
	Lobodon carcinophagus	Crabeater seal	(Hombron & Jacquinot, 1842)
	Ommatophoca rossii	Ross seal	Gray, 1844
	Leptonychotes weddellii	Weddell seal	(Lesson, 1826)
	Hydrurga leptonyx	Leopard seal	(de Blainville, 1820)
Superfamily Otarioidea			Lucas, 1899
Family Odobenidae (walrus)			Allen, 1880
	Odobenus rosmarus	Walrus	(Linnaeus, 1758)
Family Otariidae (fur seals & sea lions; eared seals)*			Gray, 1825
Subfamily Arctocephalinae			Gray, 1837
	Arctocephalus australis	South American fur seal	(Zimmerman, 1783)
	Arctocephalus forsteri	New Zealand fur seal	(Lesson, 1828)
Subfamily Otariinae			Gray, 1825
	Eumetopias jubatus	Steller's sea lion	(Schreber, 1776)

*Only otariids included in this study are listed.
†Extinct, not included in this study.

from *BRCA1*) and 14 unlinked regions (*RAG1* and *RAG2* are linked in the dog genome). Primer sequences and amplification polymerase chain reaction (PCR) conditions for each locus are listed in Appendix S2. The PCR amplification products were purified using the QIAgen PCR purification kit (Qiagen). *IRBP* amplifications yielded more than one amplified region, as separated in a 1% agarose gel and visualized using ethidium bromide. The PCR product of interest was excised from the agarose gel and isolated using the QIAquick gel extraction kit (Qiagen). Bi-directional direct sequencing was performed using BigDye v.3.1 (Applied Biosystems, Carlsbad, CA, USA). The amplification primers were used for sequencing, with two loci (*IRBP* and *RAG1*) requiring additional internal sequencing primers (Appendix S2). Sequences were resolved

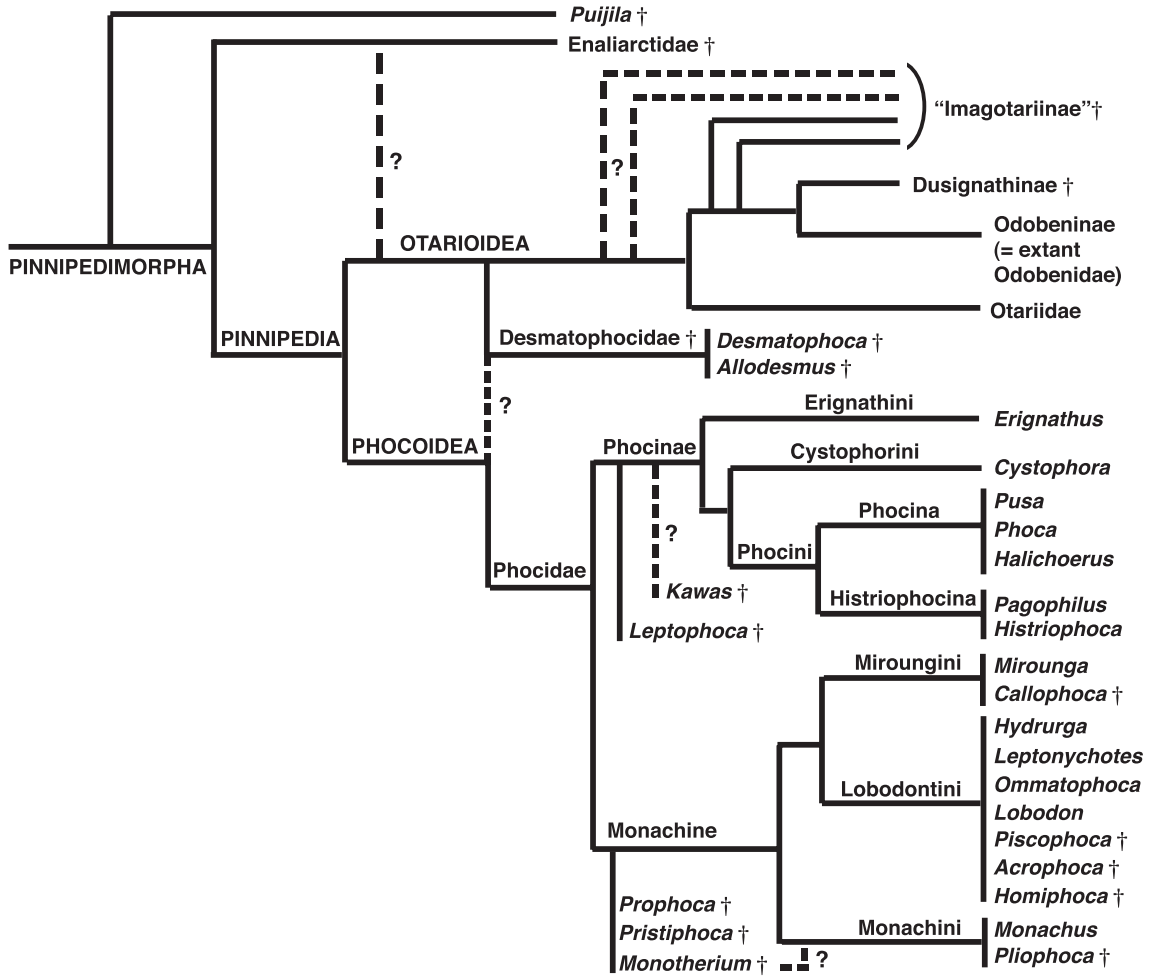

Figure 1 Overview of pinniped relationships of extant and some extinct taxa. Only fossil taxa discussed in the text are included. The relationships between extinct and extant taxa and clades are a composite of the results of the most compatible molecular and morphological studies. Dashed lines represent possible alternative branching structures.

using an Applied Biosystems 3730 capillary sequencer. Sequences were analysed, basecalled and aligned using the 3730 DNA Analyzer Data Collection Software (Foundation Data Collection) v.3.0, Sequence Navigator v.1.0.1, and ABIPrism SeqScape v.2.1 (all from Applied Biosystems). Heterozygous sites (two distinct peaks in the electropherograms observed from both directions of a sequence) were coded as polymorphisms.

Sequences were aligned by eye or using ABIPrism SeqScape and corrected by eye. Mitochondrial 12S rRNA alignment was performed using mafft v.6.240 (Katoh *et al.*, 2002, 2005) under the default settings for FFT-NSi (fast Fourier transform, iterative refinement) and adjusted manually in Se-Al v.2.0a11 (Rambaut, 2002) according to the carnivore 12S rRNA structural model of Ledje & Árnason (1996). *BRCA1* fragments 1 and 2 represent different sections of the same exon and were thus concatenated for all analyses.

Base composition homogeneity across taxa was assessed in paup* v.4.0b10 (Swofford, 2003) for each data set as a whole and partitioned by codon position. The nDNA data set did not show any base composition bias among taxa ($P = 0.9999$). The mtDNA data set did show significant base composition bias across taxa ($P = 0.0000$), but no bias was observed after the third codon position bases were removed ($P = 0.9999$). Third codon position bases of protein-coding mitochondrial genes were excluded from further analysis.

Phylogeny and divergence time estimation

A likelihood ratio test for clock-like evolution (Felsenstein, 1981) was performed using paup* v.4.0b10 (Swofford, 2003) for nDNA, mtDNA and combined (nDNA + mtDNA) data sets. For both tests including all taxa and those tests including only the ingroup taxa (Phocidae) a molecular clock was strongly rejected ($P = 0.000$).

Bayesian estimation of divergence times was performed using beast v.1.4.8 (Drummond & Rambaut, 2007) for the combined nDNA + mtDNA data set. Each gene was allowed its own independent evolutionary model and parameters by manually editing the XML control file produced by beauti

v.1.4.8 (Drummond & Rambaut, 2007). Models were selected using Akaike's information criterion (AIC) selection in MrModeltest (Nylander, 2004), a restricted version of Modeltest (Posada & Crandall, 1998). Selected models are listed in Appendix S2. Base frequencies were set to be estimated for all partitions, except for *CHRNA1* and *RAG1*, where the K80 + Γ (Kimura, 1980) and SYM+I+Γ (Zharkikh, 1994) models were implemented, respectively. A Yule process tree prior was used and rate variation across branches was uncorrelated and lognormally distributed. Tuning parameters for the Markov chain Monte Carlo (MCMC) operators were set to auto-optimize and successive runs were tuned accordingly. Each MCMC chain was started from a random tree and run for 50,000,000 generations. Three independent runs were performed; each run was sampled every 1000 generations and 10% of samples were removed from each run as burn-in. The runs were combined using LogCombiner v.1.4.8 to obtain a number of independent samples from the marginal posterior distribution (ESS, effective sample size) greater than 200, determined using Tracer v.1.4 (Rambaut & Drummond, 2007). The analysis was run without data to determine that fossil calibration priors were being implemented properly (and not interacting unexpectedly) and that the data were informative (i.e. the posterior values with data were different from those without data) to ensure that the final results were not solely the result of the priors (Drummond *et al.*, 2006).

Fossil calibrations were implemented as normal, lognormal or gamma-distributed priors and are listed in Appendix S3. A tree prior was used for all other nodes. All fossil-calibrated priors represent soft-bounded priors to allow for possible uncertainty in fossil dates or node assignment. The age range of each calibration prior is listed in Table 2. Detailed reasoning for each calibration is given in Appendix S3.

Analyses were also run for the nDNA and mtDNA data sets separately, using the same calibration set, with beast v.1.4.7 (Drummond & Rambaut, 2007). For these, two runs of 15,000,000 generations were combined to achieve ESS values >200 for all parameters. Samples were taken every 1000 generations and 10% of these were removed for burn-in.

To ensure that the root age (Caniformia–Feliformia split) and deep node calibrations (canid–arctoid and musteloid–pinniped divergences) were not heavily influencing the other divergence times, a second set of calibrations was used. A normally distributed prior was applied to the caniform–feliform divergence (root node) with a mean age of 65.0 Ma (Table 2). The Caniformia and Mustelida calibrations, to which fossil-based priors were previously applied, were set to have a tree prior. Two runs of 30,000,000 were performed and combined as above.

Table 2 beast calibrations imposed on the divergence estimates of eight nodes during divergence time estimation. Dates are represented in millions of years ago (Ma). Details on the reasoning and implementation of each calibration are included in the Supporting Information.

Calibration	Fig. 1 node no.	Median age (Ma)	95% range (Ma)
1. Carnivora	1	44.48	40.87–63.22
2. Caniformia	2	40.00	35.07–44.93
3. Musteloidea–Pinnipedia	3	29.95	26.57–40.66
4. Otarioidea	22	17.56	14.91–23.69
5. Phocidae	5	16.92	14.73–28.28
6. Phocina	10	4.98	1.37–23.72
7. Lobodontini–Miroungini	17	9.96	6.83–15.75
8. Lobodontini	19	9.39	5.25–18.82
Alternative dates:			
1b. Carnivora	1	65.0	56.78–73.22

RESULTS AND DISCUSSION

Nuclear vs. mitochondrial divergence time estimates and the effect of multiple fossil calibrations

All higher-level relationships, from tribal to superfamily, are strongly supported, in congruence with taxonomy and other phylogenetic works (Fig. 2). Only two regions of the tree were not resolved with 100% support: basal relationships within Lobodontini and species relationships within the subtribe Phocina. Both regions of the phylogeny have been contentious (Davis *et al.*, 2004; Árnason *et al.*, 2006; Higdon *et al.*, 2007) and these species relationships are discussed both here and in greater detail elsewhere (Fulton & Strobeck, 2009).

The examination of nDNA, in addition to mtDNA, and the inclusion of multiple calibration points across the phylogeny had a strong impact on the recovered divergence estimations and subsequent biogeographical hypotheses. A comparison of nDNA and mtDNA analysed separately (Table 3) indicated up to two- to threefold differences, in some cases without overlap in the 95% highest posterior density interval (95% HPD). In general, family-level nodes and higher were estimated to be older using nDNA, while more recent nodes (within families) were estimated to be older using mtDNA. This is unsurprising, as mtDNA mutates more quickly than most of the nuclear genes employed, accumulating more mutations near the tips of the tree but leading to saturation at the deeper nodes and an underestimation of these divergence times (e.g. see Hugall *et al.*, 2007). When the two data sets were combined (mtDNA + nDNA), the recovered dates were quite similar to those estimated by nDNA alone, but the more recent clades (within subfamilies) had slightly older divergence times (Tables 3 and 4). To include both the information about recent divergences (i.e. species divergences) provided by the mtDNA and the information for deeper nodes (i.e. tribes to superfamilies) provided by the more slowly evolving nDNA, the combined data set was used to infer biogeography and phylogeny (Fig. 2).

While the difference between mitochondrial and nuclear estimations was not unexpected, it has important implications for comparisons with previous studies that primarily utilized mtDNA. The dates recovered from the nDNA + mtDNA analysis (Table 4) for the divergences within the two Phocidae subfamilies are slightly more recent, but most similar to the

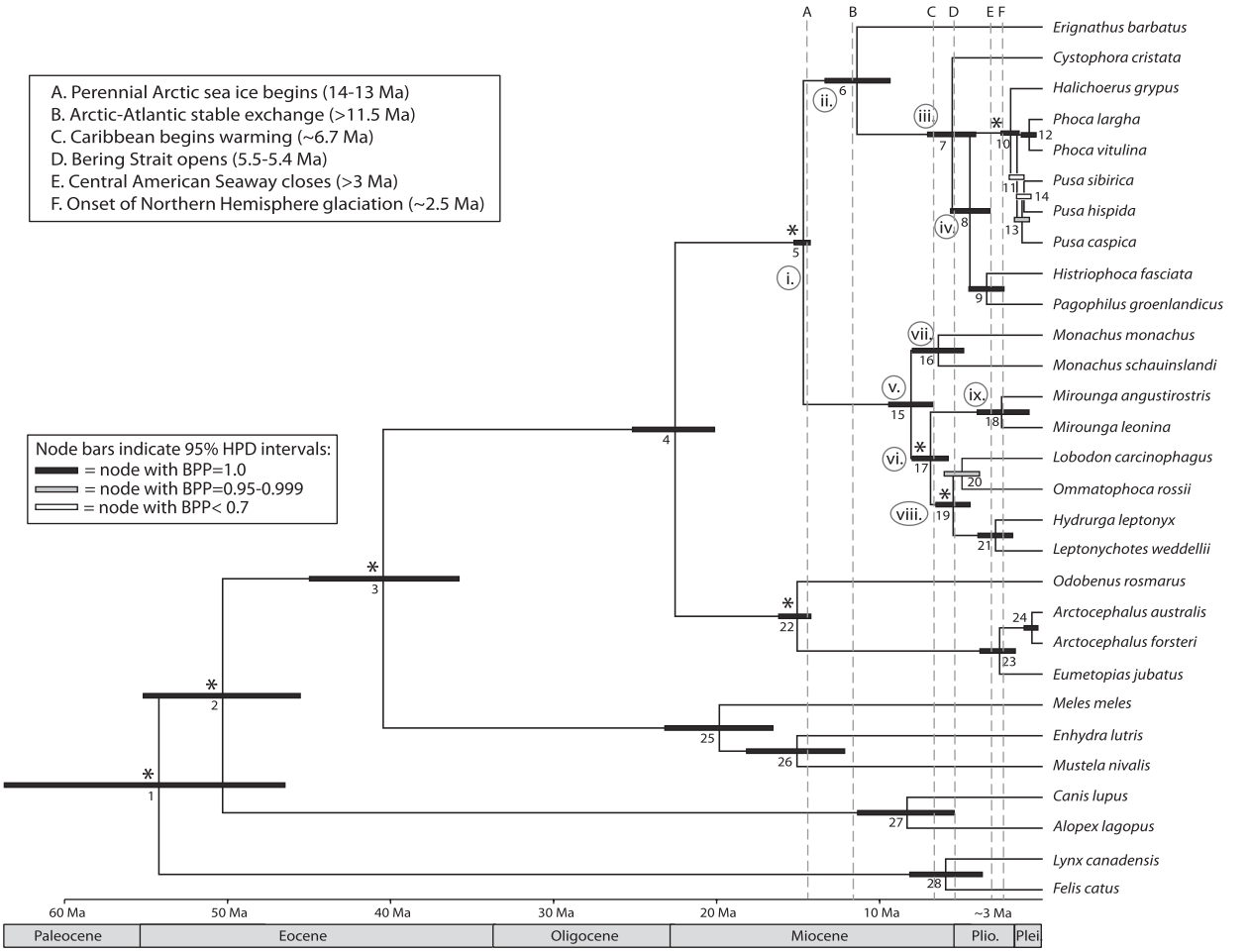

Figure 2 Combined nuclear plus mitochondrial phylogeny of Phocidae. Node numbers correspond to those in Table 4 for divergence time estimates. Stars represent fossil calibrated nodes (Table 2), roman numerals indicate events in Fig. 3, and letters indicate oceanic events relevant to their associated node. Node bars represent the 95% highest posterior density (HPD) interval for nodal age; the bars are coloured corresponding to the Bayesian posterior probability (BPP) of each clade.

other study that included both nuclear and mtDNA using a supertree dating technique based on several fossil calibrations (Higdon *et al.*, 2007), although Higdon *et al.*'s (2007) supermatrix estimated dates were generally about twice as old as those estimated here. Similarly, dates within subfamilies recovered here also tended to be younger than those obtained by other studies that primarily used mtDNA and only calibrated the root node (Fyler *et al.*, 2005; Árnason *et al.*, 2006; Palo & Vainola, 2006). As the dates recovered in this study correspond closely to those used as calibrations in other studies (Fyler *et al.*, 2005; Phocinae–Monachinae, 15 and 17 Ma; Árnason *et al.*, 2006; feliform–caniform, 52 Ma; Palo & Vainola, 2006; feliform–caniform, 52 Ma), the differences between this and previous studies may be attributed to the DNA markers used (nuclear or mitochondrial), the relaxed clock method employed and the program in which it was implemented, or to the number of fossil calibrations enforced. As these studies implemented several different relaxed clock methods, we did not examine the effect of the program and method employed but instead examined the effect of using mitochondrial vs. nuclear data within the same relaxed clock framework. As discussed above, the mtDNA estimated dates for the more recent nodes were considerably older than those estimated using nDNA (Table 3). Our estimated mtDNA divergence times within the Monachinae are comparable to those obtained by Fyler *et al.* (2005), who used one nuclear and three mitochondrial genes. The mitochondrial-only dates are slightly younger across both Monachinae and Phocinae than those obtained by Árnason *et al.* (2006) in their analysis of the complete mitochondrial amino acid sequence. This difference may be attributable to the use of amino acid sequence vs. DNA sequence, the exclusion of mitochondrial third-position bases in this study, or to the use of different relaxed clock methods. However, given that all analyses utilizing primarily mtDNA are more comparable to the mtDNA-only analysis than to the nuclear-only analysis performed here, it is reasonable to assume that most of the discrepancies between the present and previous studies are due to the different evolutionary patterns of mtDNA compared with those estimated by the combination of several independent nuclear loci. As 15 nuclear

Table 3 Separate nuclear (n) and mitochondrial (mt) DNA divergence times for the most recent common ancestor of clades recovered in this study. Node numbers correspond to node labels in Fig. 2.

		nDNA only		mtDNA only	
Node	Group	Mean divergence time (Ma)	95% HPD range	Mean divergence time (Ma)	95% HPD range
1	Carnivora (root)	57.27	48.24–67.27	42.70	40.21–46.49
2	Caniformia	51.21	46.77–55.93	38.77	34.53–42.56
3	Musteloidea + Pinnipedia	39.98	35.54–44.23	33.03	28.57–37.11
4	Pinnipedia	22.05	19.70–24.64	24.65	21.05–28.31
5	Phocidae	14.71	14.26–15.29	16.27	14.38–18.62
6	Phocinae	11.22	8.05–13.95	12.97	10.35–15.63
7	Cystophorini + Phocini	4.03	2.88–5.22	9.087	6.94–11.37
8	Phocini	3.42	2.47–4.42	7.01	5.25–8.96
9	Histriophocina	2.73	1.82–3.64	5.07	3.36–6.83
10	Phocina	1.85	1.16–2.54	2.90	2.11–3.71
–	*Halichoerus* + *Pusa*	1.27	0.74–1.82	–	–
11	*Phoca* + *Pusa*	–		2.33	1.70–2.99
12	*Phoca*	0.67	0.25–1.17	1.34	0.86–1.85
13	*Pusa*	0.88	0.47–1.32	–	–
–	*Phoca* + *Pusa sibirica* + *Pusa hispida*	–	–	2.16	1.57–2.78
14	*Pusa sibirica* + *Pusa hispida*	–	–	1.89	1.28–2.55
–	*Pusa caspica* + *Pusa hispida*	0.66	0.32–1.03	–	–
15	Monachinae	7.093	5.98–8.25	11.92	9.78–14.20
16	Monachini	5.48	3.93–7.13	9.74	7.30–12.21
17	Miroungini + Lobodontini	6.34	5.48–7.35	9.90	8.02–11.93
18	Miroungini	–	–	2.68	1.59–3.84
19	Lobodontini	5.27	4.14–6.40	7.19	5.40–9.00
–	*Ommatophoca rossii* + *Hydrurga leptonyx* + *Leptonychotes weddellii*	–	–	6.51	4.72–8.30
20	*Ommatophoca rossii* + *Lobodon carcinophagus*	4.48	3.27–5.78	–	–
21	*Leptonychotes weddellii* + *Hydrurga leptonyx*	2.40	1.25–3.63	3.82	2.40–5.41
22	Otarioidea	15.03	14.21–16.12	18.07	15.21–20.97
23	Otariidae	1.83	0.93–2.87	7.00	4.67–9.48
24	*Arctocephalus*	0.28	0.04–0.59	1.79	1.01–2.73
25	Mustelidae	18.63	15.49–21.76	18.72	14.27–23.76
26	*Mustela nivalis* + *Enhydra lutris*	13.37	10.62–16.21	15.51	11.01–19.98
27	*Canis lupus* + *Alopex lagopus*	6.63	3.51–7.30	16.51	10.34–23.13
28	*Felis catus* + *Lynx canadensis*	5.33	4.41–8.99	9.88	5.80–14.67

HPD, highest posterior density.

loci plus mtDNA data were utilized in this study, the combination of many genes of differing evolutionary rate may provide a more accurate estimation of both the species tree and divergence times, as opposed to analysis of a single hereditary unit (mtDNA) alone.

Another large contributor to the difference between this and previous studies is the number of fossil calibrations used. The dates estimated by this study are notably different from those obtained by Árnason *et al.* (2006). Although we obtain a very similar divergence time as that which was used to calibrate their tree, calibrations in our study were placed across the tree to allow calibration at multiple levels to 'anchor' the clock locally (Benton & Donoghue, 2007). When an older prior was enforced on the root node to test the effect of the root age calibration (Table 2, calibration 1b), the deeper nodes were estimated to be unreasonably old: Carnivora *c.* 76 Ma, Caniformia *c.* 75 Ma, Arctoidea *c.* 58 Ma. However, estimated dates within Pinnipedia were virtually unchanged. The estimated divergence between Otarioidea and Phocidae was slightly older (*c.* 24.9 Ma) than the estimate using more reasonable root ages (*c.* 22.5 Ma). Estimated divergence times within Phocidae were very similar, and those within Monachinae were slightly older (<1 Myr difference) and well within the 95% HPD of the 'reasonably' aged root node. This illustrates the benefit of using multiple calibrations across the tree. When the root node was set to be older and the calibrations of close nodes (Caniformia and Arctoidea) were removed, all divergences before Pinnipedia (the next calibration point) were pulled back much deeper in time. This is consistent with other findings that the use of a single deep

Table 4 Combined nuclear + mitochondrial DNA divergence times for Phocidae. Nodes correspond to those in Fig. 2.

Node	Group	Mean divergence time (Ma)	95% HPD range
1	Carnivora (root)	54.21	
2	Caniformia	50.30	55.16–45.54
3	Musteloidea + Pinnipedia	40.45	35.81–44.98
4	Pinnipedia	22.55	25.16–20.13
5	Phocidae	14.68	15.24–14.26
6	Phocinae	11.39	13.35–9.37
7	Cystophorini + Phocini	5.55	7.06–4.11
8	Phocini	4.47	5.65–3.23
9	Histriophocina	3.44	4.52–2.38
10	Phocina	1.97	2.56–1.43
11	*Phoca* + *Pusa*	1.58	2.05–1.15
12	*Phoca*	0.83	1.31–0.41
13	*Pusa*	1.27	1.73–0.81
14	*Pusa sibirica* + *Pusa hispida*	1.15	1.60–0.72
15	Monachinae	8.08	9.44–6.75
16	Monachini	6.40	7.99–4.85
17	Miroungini + Lobodontini	6.88	8.00–5.80
18	Miroungini	2.52	4.02–0.84
19	Lobodontini	5.49	6.55–4.45
20	*Ommatophoca rossii* + *Lobodon carcinophagus*	4.95	6.04–3.88
21	*Leptonychotes weddellii* + *Hydrurga leptonyx*	2.89	3.97–1.84
22	Otarioidea	15.06	16.19–14.22
23	Otariidae	2.64	3.85–1.68
24	*Arctocephalus*	0.66	1.14–0.30
25	Mustelidae	19.83	23.17–16.55
26	*Mustela nivalis* + *Enhydra lutris*	15.07	18.16–12.15
27	*Canis lupus* + *Alopex lagopus*	8.32	11.35–5.44
28	*Felis catus* + *Lynx canadensis*	5.95	8.15–3.71

HPD, highest posterior density.

external calibration can yield inflated divergence times (Hugall *et al.*, 2007). Of other studies that enforced only a single root calibration, those that enforced a calibration very close to the group of interest (Fyler *et al.*, 2005) yielded more similar results to ours. Use of a single calibration in most previous studies or using only minimum constraints (i.e. Higdon *et al.*, 2007), combined with the primary use of mtDNA, appears to have led to inflated ingroup divergence times compared with those estimated here.

Origin of Pinnipedia, Otarioidea and Phocidae

With the recent discovery of the early Miocene fossil *Puijila*, a relict stem pinniped representing a transitional form between terrestrial and aquatic carnivores (Rybczynski *et al.*, 2009), the origin of pinnipeds seems most likely to have occurred in what is now the Canadian Arctic. Our molecular estimate places the divergence of pinnipeds from musteloids *c.* 36–45 Ma, nearly 20 Myr prior to the existence of *Puijila*. But although *Puijila* is the most primitive known pinniped, it is not the oldest. A slightly older (28–25 Ma), but more derived, pinnipedimorph fossil genus, *Enaliarctos*, is known from the north-east Pacific Ocean (Berta, 1991), where the family Enaliarctidae persisted until around at least 16 Ma (Miyazaki *et al.*, 1994; Deméré *et al.*, 2003). As enaliarctids appear prior to the estimated Otarioidea–Phocidae split *c.* 20–25 Ma (Fig. 2, Table 4), these molecular results are consistent with their placement as a stem lineage (Fig. 1). Around the estimated time of the otarioid–phocid divergence, *Desmatophoca* (Desmatophocidae) existed in the north-east Pacific *c.* 23–20 Ma (Barnes, 1987). Desmatophocids are commonly grouped with otarioids (Barnes, 2007), although a phocoid link has been proposed (Wyss & Flynn, 1993; Berta & Wyss, 1994). Based on the fossil locations and abundance, it is generally unquestioned that otarioids evolved in the northern Pacific and phocids diversified in the North Atlantic, but the place of divergence of these two lineages remains unresolved. Consensus on the affinity of the extinct Desmatophocidae will help resolve this question, as this lineage appears at the estimated time of divergence between otarioids and phocoids. If desmatophocids are phocoids, then it is likely that the unknown phocid ancestor travelled through the Central American Seaway between North and South America to reach the area where phocid fossils are first found – the eastern US coast (Bininda-Emonds & Russell, 1996; Deméré *et al.*, 2003; Fyler *et al.*, 2005). If desmatophocids are otarioids, a Central American Seaway scenario is not precluded, but it is more parsimonious that the divergence occurred in or near the Arctic, with phocids dispersing directly into the North Pacific, where all early fossil phocids are found.

The *c.* 22.5 Ma divergence between Phocidae and Otarioidea is congruent with Higdon *et al.*'s (2007) supertree estimate of *c.* 23 Ma, but very different from the *c.* 33 Ma age estimated by Árnason *et al.* (2006). On the basis of their estimated date, Árnason *et al.* (2006) postulated a non-marine pinniped origin in southern North America, including the 'Oligocene seal' (Koretsky & Sanders, 2002) in the Phocidae and including Enaliarctidae in Otarioidea. The 'Oligocene seal' from South Carolina *c.* 27–24 Ma was assigned to Phocidae based on two partial femora (Koretsky & Sanders, 2002), but its membership in Phocidae is inconsistent with the molecular findings here (Fig. 2) and is extremely anomalous with the known fossil record, as its occurrence would be contemporaneous with the earliest pinnipeds. However, the age of this fossil has been questioned (Deméré *et al.*, 2003), so it is also possible that the fossil was correctly assigned to the Phocidae but is actually much younger than the Oligocene. While our estimates provide no support for or against a non-marine origin (although a southern North American origin now seems unlikely), the inclusions of the Oligocene seal within Phocidae and of Enaliarctidae within Otarioidea are inconsistent with our molecular estimates, as the fossils pre-date the estimated molecular divergence.

The divergence between the two otarioid families, Otariidae and Odobenidae, was estimated to be at *c.* 15.1 Ma (16.2–14.2). When no calibration was applied to this node, the estimated date was considerably more recent and with a

broader estimated range (*c.* 10.7 Ma, 13.5–7.6). All other nodes were virtually unaffected by the removal of this calibration. The extinct Imagotariinae is generally considered to be a paraphyletic assemblage within the Odobenidae (Deméré, 1994; Kohno, 1994, 2006; Kohno *et al.*, 1994; Deméré & Berta, 2001), but was first described as a subfamily within Otariidae (here referred to as Otarioidea), as were the extant Otariinae and Odobeninae (Mitchell, 1968; Mitchell & Tedford, 1973). However, the oldest known members of the Imagotariinae, *Proneotherium repenningi* and *Prototaria*, pre-date our molecular divergence, having existed *c.* 16–15 Ma in the north-east Pacific (Kohno *et al.*, 1994; Miyazaki *et al.*, 1994; Kohno, 2006). As the molecular dates tend to push the lower (younger) bounds of the calibration and are younger still when uncalibrated, it seems that at least some of the Imagotariinae may be stem otarioids as opposed to odobenids. In his initial description of the Imagotariinae, Mitchell (1968) raised the possibility that the otarioid ancestor may have been more 'walrus-like' than 'sea lion-like' and that otariids arose from a stock 'paralleling' the Imagotariinae. In contrast, the extinct subfamily Dusignathinae is generally unquestioned as the sister group to the extant Odobenidae (Odobeninae) (Deméré, 1994; Kohno, 1994, 2006; Deméré *et al.*, 2003). The earliest known Dusignathinae are only *c.* 7–5 Myr old (Deméré *et al.*, 2003; Kohno, 2006), and the early fossil otariid, *Pithanotaria starrii* occurs *c.* 12–7 Ma (Miyazaki *et al.*, 1994; Deméré *et al.*, 2003) which are both consistent even with the unconstrained estimated divergence time between otariids and odobenids of *c.* 13.5–7.6 Ma. Very recently (Yonezawa *et al.*, 2009), the divergence between otariids and odobenids was estimated to be *c.* 21 Ma based on mitochondrial protein-coding regions. This estimate is somewhat older than ours. Although the dates may be pushed back because of the faster rate of evolution of mtDNA, it is also probable that the inclusion of many more otariids (the focus of the study) provided more information regarding factors such as local mutation rate and improved evolutionary model parameter and phylogenetic estimates. Comprehensive taxon sampling for many nuclear loci, as in Yonezawa *et al.* (2009), will provide the best understanding of the relationships and timing within Otarioidea.

Phocidae

Fossils assigned to the subfamilies Phocinae and Monachinae are first found together in the western north Atlantic at *c.* 15 Ma (Repenning *et al.*, 1979), and were used to set a minimum divergence of these subfamilies (Table 2). Despite more prior weight having been placed on an earlier divergence, the molecular estimate suggests that the two subfamilies split *c.* 15 Ma in the vicinity of what is now Maryland and Virginia, USA, and the known fossils represent the earliest members of each subfamily (Figs 2i and 3i). After the split between Monachinae and Phocinae, the late Middle Miocene (*c.* 14.6–11 Ma) represents a time of high dispersal for phocids, as fossils have been found across the North Atlantic, Mediterranean, Paratethys (Deméré *et al.*, 2003) and as far south as Argentina (Cozzuol, 2001), with many genera persisting until *c.* 5.2–3.4 Ma (Deméré *et al.*, 2003). If Phocidae did diverge from Otarioidea in the Arctic and ancestral phocoids travelled southwards into the North Atlantic along the eastern North American coastline, many of these taxa could belong to extinct lineages bearing no extant relatives. For example, the basal phocine *Kawas benegasorum* of Argentina (dated *c.* 14–12 Ma) may represent an early lineage that dispersed south and became extinct. An early fossil phocid, *Desmatophoca claytoni*, from the central Paratethys *c.* 15 Ma (Koretsky & Holec, 2002) is roughly synchronous with the estimated molecular divergence between the two extant subfamilies, consistent with its basal placement within Phocidae (Koretsky & Holec, 2002). *Desmatophoca claytoni* could represent evidence for phocine entry into the North Atlantic from the Arctic, rather than via the Central American Seaway. Many of the Paratethyan species have been specifically associated with the Phocini (Repenning *et al.*, 1979; de Muizon, 1982; Koretsky, 2001), but this is inconsistent with a Phocini–Cystophorini divergence *c.* 5.5 Ma. The one extensive morphological phylogenetic study (Koretsky, 2001) could not assign many taxa to a tribe and several aspects of the recovered phylogeny are inconsistent with currently accepted relationships among extant taxa. However, many of the early Mediterranean or Paratethyan species, assigned as either monachines or phocines, are regarded as having tendencies to the other subfamily (Repenning *et al.*, 1979; de Muizon, 1982). This may be explained in two ways: early migrations across the Atlantic immediately following the phocine–monachine split and the retention of pleisiomorphies, or that many of these subfamily-ambiguous taxa are indeed stem taxa, belonging to neither Phocine nor Monachinae. Determining how these northern Atlantic taxa are related to the extant phocids will be influential in determining the geographical origin of Phocidae.

Associations with ice

Puijila lived in a cool temperate environment, with seasonally frozen freshwater lakes (Rybczynski *et al.*, 2009). Thus, northern ice may have played a lengthy role in shaping phocid evolution. Episodic northern ice sheets may have existed as early as *c.* 23 Ma (DeConto *et al.*, 2008), coincident with the divergence between Otarioidea and Phocidae, and perhaps driving this divergence in the Arctic. After both the Monachinae and Phocinae spread across the Atlantic, the first phocine tribe (Erignathini) emerged at *c.* 11.4 Ma (Table 4, node 6; Figs 2ii and 3ii) corresponding to a possibly stable exchange between the Arctic and Atlantic oceans beginning <11.5 Ma (Haley *et al.*, 2008) (Fig. 2B). At this time, perennial ice cover probably already existed, having begun 14–13 Ma (Darby, 2008; Krylov *et al.*, 2008). After a long temporal gap, the Cystophorini diverged *c.* 5.5 Ma, coincident with the proposed opening of the Bering Strait *c.* 5.5–5.4 Ma (Gladenkov *et al.*, 2002) (Fig. 2D), suggesting continued influence of shifting Arctic oceanic events on Phocinae evolution. Despite being the oldest extant lineage, the earliest known Erignathini is the

c. 2-Myr-old *Erignathus* fossil from England (Harington, 2008). Early entry or evolution in Arctic sea ice could explain the absence of fossils associated with this 11-Myr-old lineage.

After the Phocini subtribes diverged from one another *c.* 4.5 Ma, the two Histriophocina genera, *Histriophoca* and *Pagophilus*, split *c.* 3.4 Ma, roughly coincident with the closure of the Central American Seaway *c.* 3 Ma (Fig. 2E). However, this may be unrelated, as the rise of the Isthmus of Panama (closing the Central American Seaway) may only be a minor factor in Northern Hemisphere glaciation and not the primary cause of it (Lunt *et al.*, 2008; Molnar, 2008). Glacioeustatic-forced allopatric speciation during the Pleistocene has previously been hypothesized for the split between the Histriophocina species (Davies, 1958a; Deméré *et al.*, 2003), although this speciation event is estimated to have occurred earlier than the onset of major glaciation *c.* 2.5 Ma. However, given their present distributions, the ribbon seal probably evolved in the North Pacific and the harp seal in the North Atlantic, with this separation reinforced by glaciation. By the late Pleistocene, the first fossils of each are found in these respective locations (Deméré *et al.*, 2003).

Thus, it seems that phocids have had a long association with ice, from an Arctic origin, to the onset of episodic Arctic ice as the potential driver of the divergence between otarioids and phocids. All Phocinae, except the harbour seal, exhibit some level of pagophilic (ice-loving) behaviour (Berta *et al.*, 2006) and a long-standing association with cold water was proposed just over 50 years ago (Davies, 1958b). The white lanugo (fur at birth) of the Phocini has been used to indicate that the tribe's ancestor inhabited ice (McLaren, 1960b; Árnason *et al.*, 2006), although life in a frozen polar environment is not likely to be the only factor in the evolution of this white coat, as the Antarctic Lobodontini retain the dark lanugo shared by their temperate-water monachine relatives. This white lanugo was probably fixed in a small population, perhaps due to selective predation pressure, as they also share a reduced chromosome number (Árnason, 1974; Árnason *et al.*, 2006). The non-pagophilic harbour seal, *Phoca vitulina*, now sheds its white lanugo *in utero*, suggesting that this trait is highly selectable, as this change is not shared by its congener, *Phoca largha* (0.8 Myr divergence, Table 4).

Phocini

Although the topologies differ within Phocina, the same recovered age for the clade by analysis of three very different data sets (this study; Palo & Vainola, 2006; Higdon *et al.*, 2007) and divergence estimation methods, lends considerable support for the origination of Phocina *c.* 2 Ma. This divergence follows the rapid cooling of the Arctic Ocean associated with the Northern Hemisphere glaciation *c.* 2.5 Ma (Lyle *et al.*, 2008). Isotope analyses indicate a regional climatic shift in the Canadian Basin 1–2 Ma and that central Arctic glacial/interglacial cyclicity and pronounced Arctic climate change also occurred *c.* 1 Ma (Haley *et al.*, 2008). Within this same short time frame, nearly all Phocina species radiate rapidly (Fig. 2), indicating that these Arctic shifts were influential in Phocina evolution. Between 1.64 and 0.79 Ma, fossils resembling *Phoca vitulina* are found in Oregon, USA, and by the late Pleistocene (0.79–0.01 Ma), *Phoca* species are found in both the north Pacific and Atlantic, including the Champlain Sea (present-day Ontario and Quebec, Canada). All other fossils of extant species are not found until the Late Pleistocene, generally close to the locations where they occur today (Deméré *et al.*, 2003). A Greenland Sea/Barents Sea centre for Phocina divergence has previously been suggested (Deméré *et al.*, 2003), and given the present North Atlantic distribution of the first diverging Phocina lineage, *Halichoerus*, an Arctic Atlantic origin is probable.

Approximately 1.5 Ma, *Pusa* and *Phoca* diverged. *Pusa hispida* retained an Arctic distribution while *Phoca vitulina* was present in the eastern North Pacific *c.* 1.3–1.2 Ma (Miyazaki *et al.*, 1994) and the western North Atlantic *c.* 1.64–0.79 Ma (Deméré *et al.*, 2003). Between 1.3 and 0.4 Ma, the two *Phoca* species split, probably as *Phoca largha* either retained or reattained an affinity for ice, in contrast to the land-breeding *Phoca vitulina*. As the *Phoca vitulina concolor* individual included here is of Atlantic origin, the divergence between *Phoca* species may be an overestimate, as it may actually represent the divergence of the Pacific and Atlantic *Phoca vitulina*, rather than the Pacific *Phoca vitulina* from *Phoca largha*. However, *Phoca largha* is differentiated from *Phoca vitulina* on several morphological and behavioural grounds (Shaughnessy & Fay, 1977); thus, the genetic divergence estimate is not likely to be significantly overestimated unless many morphological/behavioural changes have occurred rapidly. Subspecific relationships are beyond the scope of this paper and timing within *Phoca* cannot be definitive based on the present data.

Relationships within *Pusa* are not confidently resolved; *Pusa hispida* and *Pusa sibirica* were recovered, but not supported, as sister [Bayesian posterior probability (BPP) = 0.61]. Importantly, *Pusa* is supported (BPP = 0.9999) as monophyletic (Fulton & Strobeck, 2009), which has not been previously recovered in comprehensive molecular analyses including all three taxa (Árnason *et al.*, 2006; Fulton & Strobeck, 2006; Palo & Vainola, 2006; Higdon *et al.*, 2007). Divergence between the three *Pusa* species is estimated to have occurred between 1.7 and 0.8 Ma (Table 4). These Phocina divergence times are generally congruent with estimates of Sasaki *et al.* (2003) based on mtRFLPs, once their results are corrected to represent the mammalian molecular divergence (not substitution) rate of 2% Myr^{-1} (see Palo & Vainola, 2006). The sister relationship recovered here (Fig. 2) between *Pusa sibirica* and *Pusa hispida* has been proposed numerous times (for a review, Palo & Vainola, 2006). This is consistent with the existence of an ancestral Arctic *Pusa* population, from which some individuals were isolated first in the Caspian Sea, becoming *Pusa caspica*, and shortly thereafter a separate invasion of Lake Baikal, which became *Pusa sibirica*. Davies (1958a) proposed a virtually simultaneous invasion of both the Caspian Sea and Lake Baikal by a *Pusa* ancestor, via the formation of a large lake due to the

southern advance of Arctic glaciation, with the remaining *Pusa hispida* re-joining those populations that remained in the Arctic following glacial retreat. A Miocene Paratethyan origin of *Pusa* has also been proposed (Chapskii, 1955; McLaren, 1960b), but is not supported here, nor by other recent work (Deméré *et al.*, 2003; Palo & Vainola, 2006).

Early Monachinae and the Monachini

The tribe Monachini (monk seals) diverged from other monachines *c.* 8.1 Ma, and the two extant monk seal species share their common ancestor *c.* 6.4 Ma (Table 4, Figs 2 and 3vii). The extinct *Pliophoca etrusca* has also been included in the Monachini and may represent the ancestor to the extant *Monachus* species (de Muizon, 1982). It is so morphologically similar to the extant *Monachus monachus* that it was originally described as the same species (de Muizon, 1982). *Pliophoca etrusca* has been found in the western north Atlantic *c.* 5.2–3.4 Ma and in the Mediterranean region *c.* 3.4–1.64 Ma (Deméré *et al.*, 2003). Given these ages, several possibilities for the relationship between *Pliophoca* and *Monachus* exist. *Pliophoca etrusca* may be a sister lineage to one or both of the extant *Monachus* species that followed a similar geographical pattern, spreading across the Atlantic. But if it does represent the ancestor to *Monachus*, the divergence between extant *Monachus* species probably occurred in the western Atlantic (Fig. 3vii), given the earlier occurrence there, as opposed to the more common hypothesis of a European divergence between monachine species (Hendey, 1972; Fyler *et al.*, 2005). This could have involved some members of the ancestral *Pliophoca etrusca* stock remaining in the western Atlantic, eventually leading to the extinct Caribbean monk seal (*Monachus tropicalis*) and the Hawaiian monk seal (*Monachus schauinslandi*) and others dispersing to the Mediterranean to become the Mediterranean monk seal (*Monachus monachus*).

The hypothesis for a Mediterranean origin of the extant monk seals is often based on the appearance of *Pristiphoca*, and sometimes on the presence of early monachines in several areas of Europe (Hendey, 1972). However, *Pristiphoca,* first found *c.* 14–11 Ma in the Paratethys region, and several *Monotherium* species and other genera found across the north Atlantic *c.* 16.3–6.7 Ma are more likely to represent stem monachines, as many of their occurrences pre-date the molecular divergence of the Monachinae tribes 6.8–9.4 Ma (Table 4). For a Mediterranean origin of the extant monk seals and inclusion of *Pristiphoca* in the Monachini, the estimated divergence of the Monachini, *c.* 7.8 Ma, would have to be erroneously underestimated by at least 3 Myr and requires an additional east to west dispersal to account for the ancestor of the Caribbean and Hawaiian monk seals (Hendey, 1972; Repenning *et al.*, 1979; de Muizon, 1982). Based on the divergence times estimated here, the occurrence of *Pliophoca* in North America prior to its

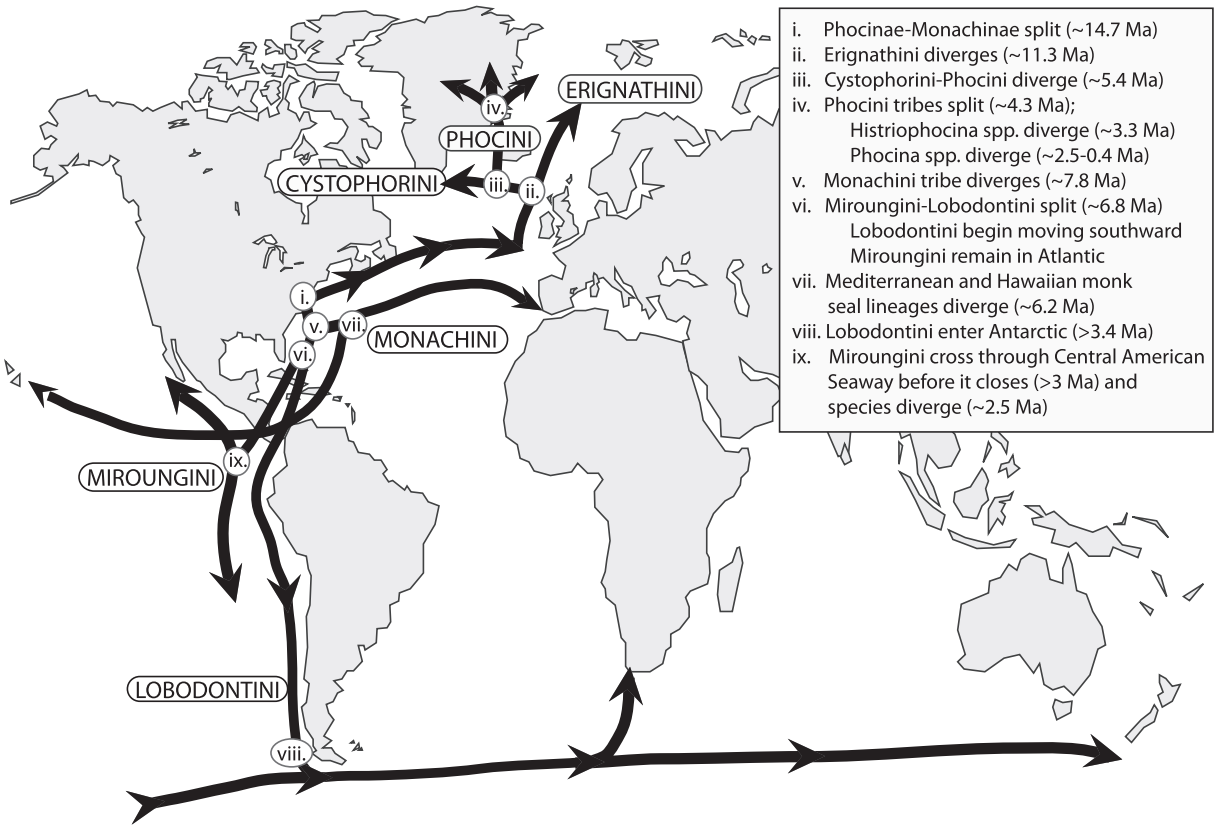

Figure 3 Hypothesized historical movements of phocid lineages. Divergence times with 95% highest posterior density ranges are shown in Table 4 and the complete phylogeny is shown in Fig. 2.

appearance in Europe, and the likely North American origin of the remaining monachine tribes (below), we propose a North American origin for the Monachini (see also Árnason *et al.*, 2006).

Miroungini

The sexually dimorphic *Callophoca*, found across the Atlantic *c.* 5.2–3.4 Ma (Deméré *et al.*, 2003), has been proposed as the ancestor of the extant *Mirounga* (Ray, 1976; de Muizon, 1982), as part of the elephant seal tribe, Miroungini. A North American origin for *Callophoca* has been proposed, with dispersal eastward either across the Gulf Stream in the North Atlantic or through equatorial currents, explaining the European occurrence (de Muizon, 1982). Extant *Mirounga* evolved in the eastern Pacific, thus their ancestor (potentially *Callophoca*) crossed through the Central American Seaway some time before it closed *c.* 3 Ma, although the two species did not diverge until *c.* 2.5 Ma (Fig. 3ix). At this time (*c.* 2.5 Ma), they began spreading northwards and southwards along the eastern Pacific until they reached their present distributions, where *Mirounga angustirostris* resides along the north-eastern Pacific coast and *Mirounga leonina* in the Antarctic to sub-Antarctic. *Mirounga leonina* probably achieved its circumpolar Antarctic distribution during or prior to the late Pleistocene (790–10 ka), as fossils probably belonging to *Mirounga* are found in Australasia in this interval (Deméré *et al.*, 2003). *Callophoca* fossils from Florida from between 3.4 and 1.64 Ma (Deméré *et al.*, 2003) allow for the possibility that the genus may have persisted in the western North Atlantic after the *Mirounga* ancestor crossed through to the Pacific, but any remnant Atlantic populations would have gone extinct (Ray, 1976).

Lobodontini

Though the rise of the Isthmus of Panama began *c.* 13 Ma (Lunt *et al.*, 2008), between 6.7 and 6.4 Ma the Caribbean warmed relative to the Pacific as deep water exchange ceased (McDougall, 1996). The Lobodontini diverged *c.* 6.9 Ma and these changing Central American waters may have influenced the movement of the lobodontine ancestor, heading south along the western South American coastline, possibly to maintain an association with cooler waters. However, the timing and effects of the rise of the Isthmus of Panama are not fully understood or agreed upon (Molnar, 2008). The Lobodontini probably spread eastwards around the Antarctic via the Antarctic Circumpolar Current (West Wind Drift), starting at least 3.4 Ma, based on the occurrence of *Homiphoca capensis* in South Africa *c.* 5.2–3.4 Ma (Hendey & Repenning, 1972).

Lobodontini relationships are not confidently resolved, and it is possible that either *Ommatophoca* or *Lobodon* may be the basal lineage or that the two may be sister taxa, as recovered here (BPP = 0.96) and by another recent study (Dasmahapatra *et al.*, 2009). Molecular evidence yields consistent, strong support for *Hydrurga* and *Leptonychotes* as sister taxa (Davis *et al.*, 2004; Fyler *et al.*, 2005; Árnason *et al.*, 2006; Fulton & Strobeck, 2006; Higdon *et al.*, 2007). However, an early morphological study placed *Hydrurga* and *Lobodon* as sister and *Ommatophoca* and *Leptonychotes* as sister (de Muizon, 1982) and most researchers have since followed this framework. A European or American origin for the Lobodontini was originally discussed, based on a hypothesized close relationship with the eastern and western Atlantic *Monotherium* species *c.* 10.4–6.7 Ma (de Muizon, 1982). The *c.* 6.9 Ma origin of the tribe obtained here suggests that *Monotherium* was not part of the Lobodontini and a European origin is unlikely.

As the specific associations of the fossil lobodontine taxa *Piscophoca*, *Acrophoca* and *Homiphoca* remain uncertain (de Muizon, 1982; Berta & Wyss, 1994), their presence can be used to infer movement patterns, but not divergence times of the Lobodontini species. *Acrophoca* has been allied with the leopard seal (de Muizon, 1982) but the *Acrophoca* species of Peru are *c.* 8–5 Myr old (de Muizon & DeVries, 1985; Deméré *et al.*, 2003), making them most likely to be stem lobodontines, based on the Lobodontini divergence estimates (Table 4, Fig. 3). While early species of *Acrophoca* may be stem lobodontines, later species, such as *Acrophoca longirostris* of Peru (*c.* 5–3.5 Ma) and the *c.* 5 Ma possible Chilean occurrence of *Acrophoca* (Walsh & Naish, 2002), may represent members of the *Hydrurga–Leptonychotes* lineage based on the similarity of *Acrophoca* to *Hydrurga. Homiphoca capensis* has been considered the potential ancestor of *Lobodon* (de Muizon, 1982), but in their original species description, Hendey & Repenning (1972) did not ally *Homiphoca* with a single extant species or lineage, instead suggesting that the reduction of the last upper post-canine tooth in *Homiphoca capensis* would 'preclude the possibility of it being ancestral to *Hydrurga* or *Lobodon*'. However, as *Lobodon* diverged from other lobodontines *c.* 5 Ma, the association between the extant *Lobodon* and the 5.2–3.4 Ma South African *Homiphoca capensis* (Hendey & Repenning, 1972) is consistent with either a sister or ancestor–descendant relationship.

Because the divergence times between *Ommatophoca*, *Lobodon* and *Hydrurga+Leptonychotes* are highly overlapping with each other (Fig. 2), none of the lobodontine fossil lineages can be excluded from any position within the Lobodontini, except excluding specific association with only *Hydrurga* or *Leptonychotes*, as they did not diverge from one another until *c.* 2.9 Ma. Depending on the association of fossil and extant taxa, it is possible that up to three lineages of Lobodontini (*Ommatophoca*, *Lobodon* and *Hydrurga+Leptonychotes* ancestors) entered the Antarctic after a single, initial southward dispersal along the Pacific coast of South America. These lineages most probably obtained most of their specialized differences once they colonized this new environment (Hendey, 1972) and began to adapt to life with ice. The ancestor of all Southern Hemisphere otariids was also recently estimated (Yonezawa *et al.*, 2009) to have diverged from their Northern Hemisphere relatives at nearly the exact same time (*c.* 7.2 Ma) as the Lobodontini diverged from the Miroungini, suggesting

that major oceanic changes affecting pinnipeds may have occurred at that time.

An interesting twist to Lobodontini evolution is the possible existence of *Homiphoca* along the central to south-eastern US coast *c.* 5.2–3.4 Ma (Ray, 1976). 'A few bones' were first mentioned by Ray (1976) as resembling the South African *Homiphoca capensis* (then referred to as *Prionodelphis*). Though the genus appears well described from South Africa, only recently have the North American samples been extensively studied. The North American material remained assigned to *Homiphoca*, though the assignment was stated to be quite tentative (Koretsky & Ray, 2008). Complex biogeographical hypotheses have been put forward to accommodate this unusual finding of a possible lobodontine (Deméré *et al.*, 2003). The referred species is found at the same time in South Africa, indicating that long transoceanic dispersals would be required within a short time frame. It seems most parsimonious at present to accept the hypothesis of a single southward Lobodontini dispersal, with a wayward lineage retreating back northwards and across the Central American Seaway to the Atlantic. Provided the North American material does not represent *Homiphoca capensis*, and represents other lobodontines or even other monachines, the simple single-dispersal theory holds. Further evaluation of fossil genera with extant genera, particularly with consideration for the molecular topology, will be required to be more confident in understanding lobodontine evolution.

CONCLUSIONS

The use of nuclear and mitochondrial markers has presented both a new topology for the Phocidae and, in combination with multiple soft-bound fossil calibrations, new divergence time estimates. With the recent discovery of a primitive Arctic pinniped and suggestions of occurrence of Arctic ice at the same time as the phocid–otarioid divergence, an Arctic origin of Pinnipedia *c.* 40 Ma and an Arctic divergence between otarioids (fur seals and sea lions) and phocids (true seals) *c.* 23 Ma are proposed.

The primarily Arctic subfamily Phocinae may have maintained an association with icy conditions, or at least entered into icy conditions earlier than previously thought. The bearded seal diverged from the other phocines *c.* 11.4 Ma, as the Arctic and Atlantic Oceans became freely exchanging and perennial Arctic sea ice existed. The ancestor to the remaining Phocinae probably remained in the seas between the Arctic and Atlantic Oceans, with genera diverging between *c.* 2 and 5 Ma in association with changing Arctic conditions. Speciations within *Phoca* and *Pusa* are not estimated to have occurred until *c.* 1 Ma, when Arctic ringed seals invaded the Caspian Sea and Lake Baikal from the north.

The two phocid subfamilies, Monachinae and Phocinae, split from one another *c.* 15 Ma, probably on the eastern US coast. If *Pliophoca etrusca* is assumed to be the ancestor of the extant *Monachus*, ancestral populations of *Pliophoca etrusca* may have split in the western North Atlantic *c.* 6 Ma. A subset of individuals would have dispersed across the Atlantic, eventually becoming the Mediterranean monk seal, while the rest of the ancestral population remained, leading to the now-extinct Caribbean monk seal and the extant Hawaiian monk seal. The two *Mirounga* species diverged from the Lobodontini *c.* 6.9 Ma, crossing through the Central American Seaway and diverging from one another in the Pacific *c.* 2.5 Ma. A single dispersal to the Antarctic along the western South American coast is hypothesized for the Lobodontini beginning *c.* 6.8 Ma and with up to three lineages entering and eventually spreading around Antarctica.

Most uncertainty in these hypotheses stems from questions regarding the association of fossil taxa with extant taxa. A comprehensive morphological examination of all fossil and extant genera now holds the greatest promise for understanding phocid biogeography.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Individual GenBank accession numbers for sequences analysed in this study.

Appendix S2 Loci included in this study and associated information.

Appendix S3 Fossil calibrations and priors.

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BIOSKETCHES

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