Journal of Biogeography (J. Biogeogr.) (2005) 32, 1267–1279

ORIGINAL ARTICLE

Historical biogeography and phylogeny of monachine seals (Pinnipedia: Phocidae) based on mitochondrial and nuclear DNA data

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ABSTRACT

Aim To determine the origin and diversification of monachine seals using a phylogenetic framework.

Methods Molecular sequence data from three mitochondrial genes (cyt *b*, ND1 and 12S), and one nuclear marker (an intron from the α-lactalbumin gene) were examined from all extant species of monachine seals. Maximum likelihood and partitioned Bayesian inference were used to analyse separate and combined (mitochondrial + nuclear) data sets. Divergence times were estimated from the resultant phylogeny using nonparametric rate smoothing as implemented by the program r8s.

Results *Mirounga*, *Monachus* and the Lobodontini form three well-supported clades within a monophyletic Monachinae. Lobodontini + *Mirounga* form a clade sister to *Monachus*. Molecular divergence dates indicate that the first split within the Monachinae (Lobodontini + *Mirounga* clade and *Monachus*) occurred between 11.8 and 13.8 Ma and *Mirounga*, *Monachus* and the Lobodontini originated 2.7–3.4, 9.1–10.8 and 10.0–11.6 Ma, respectively.

Main conclusions Two main clades exist within Monachinae, *Monachus* and Lobodontini + *Mirounga*. *Monachus*, a warm water clade, originated in the North Atlantic and maintained the temperate water affinities of their ancestors as they diversified in the subtropic regions of the Northern Hemisphere. The cold-water clade, Lobodontini + *Mirounga*, dispersed southward to the cooler climates of the Southern Hemisphere. The Lobodontini continued south until reaching the Antarctic region where they diversified into the present-day fauna. *Mirounga* shows an anti-tropical distribution either reflective of a once cosmopolitan range that was separated by warming waters in the tropics or of transequatorial dispersal.

Keywords
Historical biogeography, mixed model, molecular divergence estimates, Monachinae, nonparametric rate smoothing, partitioned Bayesian inference, phylogeny, Pinnipedia, seal biogeography.

INTRODUCTION

The Pinnipedia includes marine carnivores that share a single common evolutionary origin within arctoid carnivores 25–27 Ma in the North Pacific (Berta & Adam, 2001). Three major groups of pinnipeds are recognized: the Phocidae (true seals), the Otariidae (fur seals and sea lions) and the Odobenidae (walruses). Phocid seals, the focus of this study, are typically divided into two subfamilies that reflect previously hypothesized phylogenetic groupings and present distribution. The Phocinae (northern seals) includes 10 species that inhabit the Arctic and sub-Arctic, whereas the Monachinae (southern seals) consists of three geographically widespread groups (Lobodontini, *Mirounga* and *Monachus*). The Lobodontini (Antarctic seals) are restricted to the Antarctic region, primarily in a circumpolar distribution and includes four species:

 doi:10.1111/j.1365-2699.2005.01281.x

Ommatophoca rossii (Ross seal), *Lobodon carcinophagus* (crabeater seal), *Leptonychotes weddelli* (Weddell seal), and *Hydruga leptonyx* (leopard seal). *Mirounga* (elephant seals) comprises two species. *Mirounga angustirostris* (northern elephant seal) is distributed in the eastern North Pacific from northern California to the Baja California peninsula in Mexico, and *Mirounga leonina* (southern elephant seal) occupies islands scattered around the sub-Antarctic. *Monachus* (monk seals) includes two of the world's most elusive and endangered pinnipeds and one recently extinct species. *Monachus schauinslandi* (Hawaiian monk seal), an endemic to the Hawaiian Islands, includes approximately 1300 individuals with the majority of the population occurring at six locations in the north-western Hawaiian Islands. Hawaiian monk seal populations have declined 60% since the late 1950s and future demographic trends do not favour recovery of the species (Forney *et al.*, 2000). *Monachus monachus* (Mediterranean monk seal) once inhabited the entire Mediterranean Basin and the eastern North Atlantic and is now classified as the world's most endangered pinniped with an estimated 400–600 individuals split among a number of severely contracted breeding populations. The largest aggregations occur in the eastern Mediterranean (Greece and Turkey) and off the coast of the western Sahara (Cap Blanc peninsula) in the Atlantic Ocean (Aguilar, 1999; Androukaki *et al.*, 1999; Forcada *et al.*, 1999). *Monachus tropicalis* (Caribbean monk seal) was once widely distributed throughout the West Indies. Their exploitation as a source of meat and oil began in the late 1400s and eventually led to the species extinction in the 1950s (Timm *et al.*, 1996; Adam & Garcia, 2003).

At present, the historical biogeography and present-day distribution of monachine seals is not well understood. Furthermore, few studies have examined the evolutionary relationships within this group even though one species is recently extinct (*Monachus tropicalis*), and two other species are endangered (*M. schauinslandi* and *M. monachus*). Phylogenetic relationships among monachines are especially intriguing because of their widespread distribution and diverse ecology. For instance, monk seals are unique among all modern phocid seals in retaining an ancestral exclusivity to temperate subtropical waters and yet they are thought to be closely related to the Lobodontini which live on islands scattered around the Antarctic. Resolving monachine seal phylogeny is essential in determining when the ecological shifts occurred which led to present-day monachine seal distributions.

Historical biogeography

Phocid seals have a widespread geographical range that spans both hemispheres (Fig. 1). Previously unresolved relationships among phocids have led to primarily narrative accounts of historical biogeography based solely on the analysis of historical, geological and climatic factors. In one notable exception, Deméré *et al.* (2003) proposed a hypothesis for the evolutionary biogeography of pinnipedimorphs (pinnipeds and their fossil relatives) using a comparative approach and multiple lines of evidence including physical and ecological factors controlling modern pinniped distributions, past geological events, the fossil record and phylogenies of the various groups.

A North Atlantic origin of monachine seals is widely accepted (Repenning *et al.*, 1979; de Muizon, 1982; Deméré *et al.*, 2003). However, there has been controversy concerning the origin of *Monachus*. de Muizon (1982) suggested a European origin of *Monachus*, which later crossed the Atlantic from east to west following the warm equatorial currents in the southern North Atlantic. Subsequent isolation and allopatric speciation in the Caribbean basin resulted in the evolution of *M. tropicalis*. Dispersal of *Monachus* into the Pacific via the Central American Seaway led to the divergence of *M. schauinslandi* by 4 Ma. Conversely, Repenning *et al.* (1979) supported a Caribbean origin for *Monachus* with subsequent radiation to the eastern Atlantic and Pacific Oceans as early as 15 Ma.

Past biogeographical hypotheses for the Lobodontini suggest southern migration of the group in the late Miocene to early Pliocene and diversification in the colder waters of Antarctica. Basal Lobodontine fossils from the Pisco Formation in Peru suggest migration south along the Pacific coast of South America (de Muizon, 1982; de Muizon & De Vries, 1985).

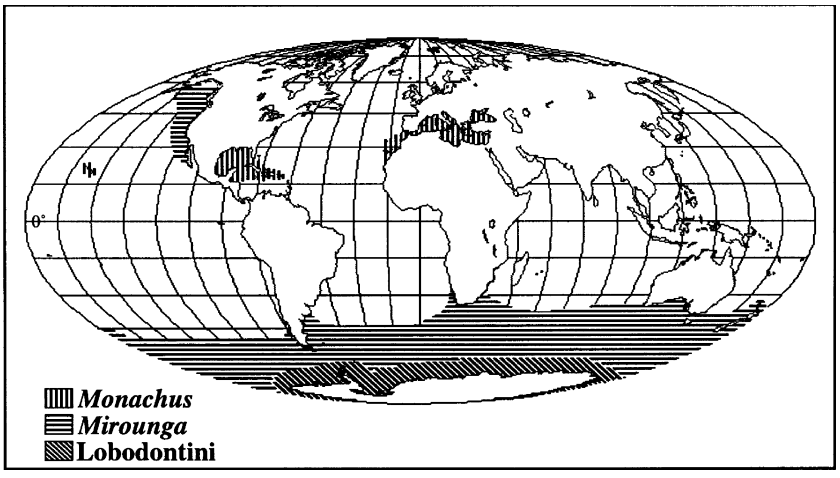

Figure 1 Historical distribution of monachine seals. The current distributions of *Monachus* species are severely reduced, with the Caribbean species being extinct and the Hawaiian and Mediterranean populations reduced to small fragmented populations.

A hypothesis for the historical biogeography of *Mirounga* suffers from a limited and poorly documented fossil record. *Callophoca*, the purported sister taxon to *Mirounga*, occurs in the early Pliocene of the western North Atlantic and Europe. Deméré *et al.* (2003) suggested that some members of this lineage could have dispersed through the then open Central American Seaway to establish the group in the eastern South Pacific. Subsequent speciation resulted in the evolution of *M. leonina*. The antitropical occurrence of the presumed sister species *M. angustirostris* in the eastern North Pacific suggests a transequatorial event and allopatric speciation.

Previous phylogenetic hypotheses

Studies examining phylogenetic relationships of monachines using morphological and molecular data are incongruent, providing conflicting hypotheses for both the monophyly and paraphyly of Monachinae, the Lobodontini, and *Monachus*. For example, in a study based primarily on the ear region Repenning & Ray (1977) positioned *Monachus* as sister to all other phocid seals. In another morphological study, de Muizon (1982) suggested monophyly of the Monachinae, the Lobodontini, and *Monachus*, as well as the sister relationship between *Mirounga* and the Lobodontini. More recent parsimony-based analyses support incongruent relationships among monachines. A study based on 39 osteological and soft anatomical characters suggested the paraphyly of both the Monachinae and *Monachus* (Wyss, 1988; Fig. 2a) whereas a second study of 168 primarily osteological characters supported the monophyly of the Monachinae, the monophyly of *Monachus*, and the paraphyly of lobodontine seals (Bininda-Emonds & Russell, 1996; Fig. 2b). Bininda-Emonds *et al.* (1999) used supertree construction techniques to infer the phylogeny of carnivores suggesting the monophyly of the Monachinae, *Monachus*, *Mirounga*, and the Lobodontini (Fig. 2c).

Molecular studies elucidating monachine seal relationships are limited to three prior studies. Arnason *et al.* (1995) analysed mitochondrial cytochrome *b* (cyt *b*) gene sequences from four of the nine monachine species to position the Hawaiian monk seal as the sister taxon to all other phocids (Fig. 2d). A second molecular study based on mitochondrial 12S rDNA and cyt *b* included three monachine species and supported the monophyly of the Monachinae (Ledje & Arnason, 1996; Fig. 2e). Several shortcomings reduce the influence and importance of these two previous molecular studies. First, taxon sampling was limited to half of the extant monachines. This makes it impossible to assess subgroup monophyly and resolve species level relationships within the Monachinae. Second, phylogenetic analyses were conducted using only uniformly weighted parsimony which does not adequately model the heterogeneous nature of DNA evolution

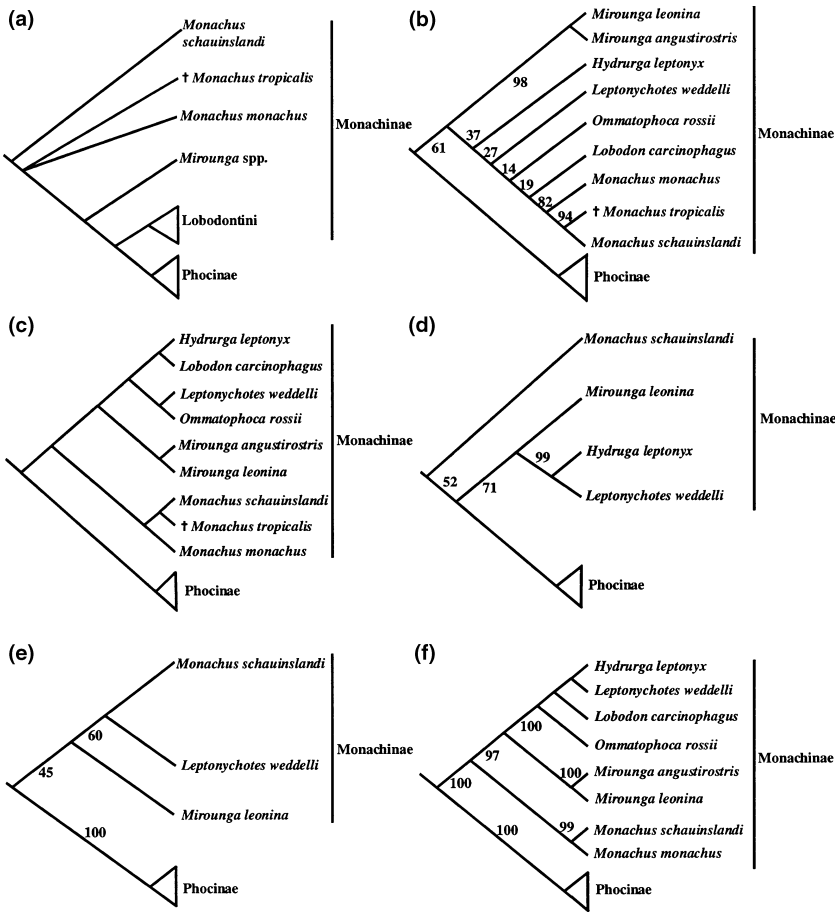

Figure 2 Previous hypotheses regarding Monachinae relationships. (a) Wyss (1988). Parsimony analysis based on 39 morphological characters. (b) Bininda-Emonds & Russell (1996). Parsimony analysis based on 168 morphological characters. (c) Bininda-Emonds *et al.* (1999). Parsimony analysis based on supertree construction using 21 source trees. (d) Arnason *et al.* (1995). Parsimony analysis based on the mitochondrial cyt *b* gene sequence. (e) Ledje & Arnason (1996). Parsimony analysis based on the combined mitochondrial cyt *b* and 12S gene sequences. (f) Davis *et al.* (2004). Maximum likelihood tree based on the complete mitochondrial DNA coding regions. Bootstrap values are indicated on the nodes. †Extinct taxon.

(Huelsenbeck, 1995; Swofford *et al.*, 1996; Yang, 1996a). In a recent more inclusive study of molecular sequence data (entire mitochondrial genome) and taxa (all phocids except for land locked species of Phoca) Davis *et al.* (2004) found strong support for the Monachinae, *Monachus*, *Mirounga* and the Lobodontini (Fig. 2f). All previous molecular studies used only mitochondrial data, which essentially represent only one genealogical history.

MATERIALS AND METHODS

Taxon sampling

All species of monachine seals were sampled, with the exception of the recently extinct *Monachus tropicalis*. The monophyly of the Monachinae has been questioned in previous phylogenetic studies (Wyss, 1988; Arnason *et al.*, 1995); therefore, three phocine seals were included: *Phoca vitulina* (harbour seal), *Cystophora cristata* (hooded seal) and *Erignathus barbatus* (bearded seal). Debate exists concerning the sister taxon to the Phocidae. While some phylogenetic studies based on morphological data support a sister relationship between the Phocidae and the Odobenidae (Wyss, 1987; Wyss & Flynn, 1993; Berta & Wyss, 1994), other morphological data (Bininda-Emonds & Russell, 1996; Bininda-Emonds *et al.*, 1999) suggest an odobenid + otariid clade. Molecular data have consistently supported an odobenid + otariid clade (Arnason *et al.*, 1995; Ledje & Arnason, 1996; Davis *et al.*, 2004). Given the morphological and molecular support for an odobenid + otariid clade, the tree was rooted with *Odobenus rosmarus* (walrus; an odobenid) and *Zalophus californianus* (California sea lion; an otariid). One representative from each species was sequenced and used in the phylogenetic analyses (see Table 1 for complete taxon list and sources).

Molecular techniques

Gene regions were selected based on their established utility in resolving interspecific phylogenetic relationships in other carnivores (Arnason *et al.*, 1995; Ledje & Arnason, 1996; Carr & Perry, 1997). Two protein coding genes (cyt *b* and ND1) and one rRNA gene (12S) were selected from the mitochondrial genome. Additionally, sequence data were obtained from an intron of the nuclear gene α-lactalbumin.

Total genomic DNA was extracted from tissue using the standard phenol-chloroform protocol (Hillis *et al.*, 1996), or came from previously extracted DNA. PCR primers used in this study were either designed from conserved gene regions of pinniped sequences available in GenBank or obtained from previously published studies (Table 2). PCR was performed in 25 μL reactions using 50–200 ng of template DNA, 20 mM

Table 1 Taxon list. Sources for tissue, extracted DNA or sequence data (GenBank accession numbers) noted in parentheses

Ingroup

Phocidae

Monachus monachus (Hermann, 1779) – Mediterranean monk seal – Greece (MOm*)

Monachus monachus – Mediterranean monk seal – western Sahara (BMTB†)

Monachus schauinslandi (Matchie, 1905) – Hawaiian monk seal (NMFS/PIR‡) (Y08524) (X72209)

Ommatophoca rossii (Gray, 1844) – Ross seal (UofA§)

Lobodon carcinophagus (Hombron & Jacquinot, 1842) – Crabeater seal (LU¶)

Leptonychotes weddellii (Lesson, 1826) – Weddell seal (LU) (Y08522) (X72005)

Hydruga leptonyx (Blainville, 1820) - Leopard seal (LU) (X82297)

Mirounga angustirostris (Gill, 1866) – Northern elephant seal (NMFS/SWMF**)

Mirounga leonina (Linnaeus, 1758) – Southern elephant seal (SAM††) (Y08523) (X82298)

Outgroup

Phocidae

Phoca vitulina (Linnaeus, 1758) – Harbour seal (NC_001325)

Cystophora cristata (Erxleben, 1777) – Hooded seal (DFO‡‡) (X82294)

Erignathus barbatus (Erxleben, 1777) – Bearded seal (DFO) (X82295)

Otariidae

Zalophus californianus californianus (Lesson, 1828) – California sea lion (LU) (Y08525) (X82310)

Odobenidae

Odobenus rosmarus (Linnaeus, 1758) – Walrus (SWMF/SD) (NC_004029)

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¶Lund University – Lund, Sweden.

**National Marine Fisheries Service Southwest Marine Fisheries – La Jolla, California.

††South Australian Museum – Adelaide, Australia.

‡‡Department of Fisheries and Oceans – Newfoundland, Canada.

Table 2 Primers used in PCR and sequencing of pinnipeds

Gene fragment	Primer	Sequence (5′–3′)	Source
Cyt *b*			
1	Cyt *b* EF	aggcgtcgaagcttgacatgaaaagccatcgttg	Arnason *et al.* (1995)
	Cyt *b* IR	tartabgggtgraatgggattttgtctgagt	This study
2	Cyt *b* ER	cgaattccatttttggtttacaagac	Arnason *et al.* (1995)
	Cyt *b* IF	caaccytaacacgattyttygcyytcca	This study
12S			
1	12S EF	gagcgtcgaagcttgcaaggcactgaaaatgcc	Ledje & Arnason (1996)
	12S IR	ttcctttttaagggtttgctgyygatggcggtatatagac	This study
2	12S ER	gtggtcgaattctgtgaaatcttctgggtgta	Ledje & Arnason (1996)
	12S IF	aactgggattagataccccactatgcttagccctaaa	This study
ND1			
1	tMET	tcggggtatgggcccraragctt	Leaché & Reeder (2002)
	ND1 IR	attgtytgrgctacggctcg	This study
2	16dR	ctacgtgatctgagttcagaccggag	Leaché & Reeder (2002)
	ND1 IF	taggagtrytattyatrytagcaatatcaag	This study
α–Lactalbumin			
1	Lac IR	ctcactgtcacaggagatgt	Milinkovitch *et al.* (1998)
	Lac IIF	ccaaaatgatgtcctttgtc	Milinkovitch *et al.* (1998)

Tris–HCl (pH 8.4), 50 mM KCl, 1 mM $MgCl_2$, 0.12 mM each dNTP, 0.25 μM each primer, and 1.25 units of *Taq* DNA polymerase using annealing temperatures between 50 and 60 °C. The mitochondrial PCR templates were purified using polyethylene glycol (PEG) precipitation (20% PEG 8000, 2.5 M NaCl), cycle sequenced using Big Dye™ dideoxy terminators (Applied Biosystems, Inc., Foster City, CA, USA) and sequenced samples ran on an ABI 377 automated sequencer. The nuclear gene (α-lactalbumin) fragment was purified using polyacrylamide gel purification (Sambrook *et al.*, 1989) because of the presence of multiple non-specific PCR products. Final DNA sequence data were compared to previously sequenced α-lactalbumin data in GenBank to verify that the correct gene was amplified and sequenced.

Due to conserved amino acid codon positions, the alignment of the protein coding sequences (cyt *b* and ND1) was obvious and performed by eye. The nuclear intron (α-lactalbumin) and the 12S rRNA gene were aligned using CLUSTAL X (Thompson *et al.*, 1997). Alignment of the 12S data was also performed manually using a secondary structure model proposed by Ledje & Arnason (1996). Ambiguously aligned regions were identified by constraining gaps to loops and aligning under differing gap costs (6, 9, 12). Regions that varied across different gap costs were considered ambiguously aligned and excluded from subsequent phylogenetic analyses (Gatesy *et al.*, 1993). Heterozygous sites found in the nuclear sequences (three sites in *Monachus schauinslandi* and two sites in *Lobodon carcinophagus*) were coded with the appropriate IUPAC ambiguity codes.

Phylogenetic analysis

Maximum likelihood (ML) and partitioned Bayesian phylogenetic analyses were performed on three data sets: the nuclear data set, the mitochondrial data set, and the combined (mitochondrial + nuclear) data set. The mitochondrial and combined data sets were partitioned to investigate existing heterogeneity across and within the gene regions. Data partitions were chosen based on the independent evolution of nuclear vs. mtDNA, as well as the different functional constraints on protein coding vs. structural genes. Based on the previous criteria, a total of eight data partitions were distinguished: separate codon positions for each of the protein coding cyt *b* and ND1 genes (= 6 partitions), and a single partition each for the structural 12S gene and the nuclear intron of α-lactalbumin. Appropriate models of sequence evolution for each data partition were determined using the hierarchical likelihood ratio test as implemented by MrModeltest (a variant of Modeltest; Posada & Crandall, 1998; Nylander, 2002).

Maximum likelihood heuristic searches using PAUP* 4.0b10 (Swofford, 2003) were performed under the appropriate models of sequence evolution determined for each of the three data sets (i.e. nuclear, mitochondrial and combined). At present, truly partitioned (i.e. 'mixed model') ML analyses are not possible in PAUP*, so one single best model (and estimated parameters) was determined using MrModeltest for the nuclear, mitochondrial and combined data sets. Each heuristic ML analysis consisted of 1000 random addition sequence replicates, with TBR branch swapping. Support for the inferred clades was estimated by bootstrap analysis (100 pseudoreplicates with 5 random sequence additions per pseudoreplicate; TBR branch swapping). Clades with bootstrap values of ≥ 70% were considered strongly supported (Hillis & Bull, 1993).

A combined phylogenetic analysis potentially includes genes and gene regions (e.g. codon positions) with different models of evolution; thus, a single model may provide a poor explanation and perhaps introduce significant systematic error (Yang, 1996b; Wilgenbusch & de Queiroz, 2000; Reeder, 2003; Nylander *et al.*, 2004; Brandley *et al.*, 2005). Given this, a partitioned or mixed model approach in molecular phylogenetic analyses provides a better explanation for the evolution of data (Nylander *et al.*, 2004; Brandley *et al.*, 2005). While not

possible in PAUP*, applying multiple partition-specific models in a single phylogenetic analysis is now possible in the most recent version of MrBayes (MrBayes 3.0b4; Huelsenbeck & Ronquist, 2001). The Bayesian analyses were conducted for the same three data sets as in the ML analyses, but with the previously identified seven data partitions in the mitochondrial data set and eight data partitions in the combined data set.

The best tree topology, indicated by the highest log-likelihood (ln *L*) scores, estimated in the partitioned and non-partitioned Bayesian analyses were identical for each mixed model data set (i.e. mitochondrial and combined). Therefore, the effectiveness of data partitioning could be investigated by comparing ln *L* scores. Likelihood scores were calculated in PAUP* for the partitioned and non-partitioned tree and a likelihood ratio test was conducted to determine if the likelihood scores were significantly improved by partitioning the data. The number of parameters included in each independently estimated partition was calculated by adding branch lengths, relative rates, base frequencies, and among site rate variation (Table 3). The degrees of freedom were calculated as the difference in parameterization between the partitioned and non-partitioned models being compared. Additionally, Bayes factors (Newton & Raftery, 1994; Kass & Raftery, 1995) were calculated to compare the evidence against the non-partitioned data sets (for other phylogenetic examples, see Nylander *et al.*, 2004; Brandley *et al.*, 2005). Log-transformed harmonic means were calculated using the *sump* command in MrBayes. Bayes factors were calculated as the ratio of harmonic means of the likelihoods sampled from the posterior of the two analyses and Bayes factors > 10 were considered very strongly supported based on hypothesized cut off values by Kass & Raftery (1995).

Bayesian phylogenetic analyses included partition-specific models and were conducted with four independent Markov chains (one cold and three incrementally heated chains) run for 2 million metropolis-coupled MCMC generations (trees sampled at intervals of 100 generations). Stationarity was determined by plotting ln *L* scores against number of generations and was assumed at the point where likelihood scores levelled off after an initial burn in. For each data set, three separate Bayesian analyses were run to ensure the analyses for a given data set had converged on the same posterior distribution (as evident from similar mean ln *L* scores). The frequency that a particular clade is present in the estimated posterior is represented by that clade's posterior probability (PP). Posterior probabilities were mapped onto the ML phylogram and nodes with PP ≥ 0.95 were considered strongly supported (Wilcox *et al.*, 2002).

Assessing biogeographical hypotheses

Biogeographical hypotheses were assessed using the combined ML phylogeny, the fossil record, and molecular rates of evolution. The mtDNA data were used in analyses investigating molecular rates of evolution as it represented the largest and most complete data set. When estimating divergence times within a phylogeny, molecular clock assumptions are sometimes invoked. A molecular clock assumes that on average the rate of molecular evolution is invariable throughout long periods of evolutionary time across multiple lineages (Zuckerkandl & Pauling, 1965; Kimura, 1968; Kimura & Ohta, 1974). However, comparisons of relative rates between lineages have provided abundant evidence for departures from constant rate of substitution (e.g. Wu & Li, 1985; Britten, 1986; Li, 1993).

Likelihood ratio tests were performed on a series of combined and partitioned data sets and rejected the assumption of a uniform molecular clock (Felsenstein, 1981), suggesting rate heterogeneity among lineages in the combined ML phylogeny (results not shown). Therefore, divergence times were calculated using nonparametric rate smoothing (NPRS), as implemented by the program r8s (Sanderson, 2002). Instead of assuming global rate consistency (i.e. a molecular clock), r8s places a constraint on the way rates can vary across a clade. NPRS relies on the minimization of ancestor-descendant local rate changes and is motivated by the likelihood that evolutionary rates are limited in the speed with which their rates can change from an ancestral to a descendant lineage (Sanderson,

Table 3 Models used in partitioned and non-partitioned Bayesian analyses with the number of parameters for each corresponding model listed

Data set	Model	Branch length parameters	Relative rate parameters	Base frequency parameters	ASRV shape parameters
Non-partitioned mitochondrial	GTR + Γ	25	5	3	1
Non-partitioned complete	GTR + Γ	25	5	3	1
α-Lactalbumin	HKY	25	1	3	0
12S	GTR + I + Γ	25	5	3	2
Cyt *b*					
First codon	SYM + Γ	25	5	0	1
Second codon	HKY + I + Γ	25	1	3	2
Third codon	GTR + Γ	25	5	3	1
ND1					
First codon	SYM + Γ	25	5	0	1
Second codon	HKY + I + Γ	25	1	3	2
Third codon	HKY + Γ	25	1	3	1

1997). NPRS can enforce constraints on node ages by incorporating fossil evidence, which exists for the phocid clade. Basal fossil phocids (*Leptophoca lenis* and *Monotherium? wymani*) support the phocine–monachine split occurring during the mid-Miocene (15–17 Ma) in the North Atlantic (Repenning *et al.*, 1979; de Muizon, 1982; Berta & Adam, 2001; Deméré *et al.*, 2003). The phylogeny was calibrated twice; once with the phocid clade fixed at 15 Ma and again at 17 Ma and 95% confidence intervals were estimated by the program. Divergence time estimates were compared with the fossil record to infer monachine seal origin and diversification events.

RESULTS

Data partitioning

The complete combined data set contained a total of 3502 unambiguously aligned nucleotide positions, of which 940 sites were variable and 619 were parsimony informative (Table 4). The nuclear sequence, α-lactalbumin, was not included for one ingroup taxon (*Ommatophoca rossii*) and two outgroup taxa (*Odobenus rosmarus* and *Phoca vitulina*). Models used in partitioned Bayesian analyses can be found in Table 3. Models and their estimated parameters used in the ML analyses are listed in Table 5. Likelihood ratio tests demonstrated that the partitioned Bayesian analyses are significantly better explanations of the evolution of the data than the non-partitioned analyses. Additionally, the Bayes factors demonstrate very strong positive evidence for the partitioned data set (Table 6). Therefore, the posterior probabilities reported hereafter are those from the partitioned analyses.

Phylogeny inferred from mitochondrial and nuclear DNA using maximum likelihood and mixed-model Bayesian analyses

The ML heuristic searches always resulted in a single optimal tree. In each replicate Bayesian analysis stationarity was achieved by at least the 2000th generation, and 15% of the trees were excluded as 'burn in' to assure the retention of only the trees at stationarity. Separate Bayesian analyses for a given data set converged on the same posterior distribution. Therefore, post burn-in trees for a given data set were combined and posterior probabilities are based on the pooled set of data points.

Table 5 Models and their estimated parameters used in ML analyses

Data set	Base frequency	Model	Substitution rates	α
Mitochondrial (ND1, 12S and cyt *b*)	A = 0.329 C = 0.314 G = 0.138 T = 0.218	GTR + Γ	A ↔ C 9.653 A ↔ G 119.853 A ↔ T 18.991 C ↔ G 0.000 C ↔ T 373.513	0.157
Combined (ND1, 12S, cyt *b* and α-lactalbumin)	A = 0.312 C = 0.305 G = 0.145 T = 0.238	GTR + Γ	A ↔ C 9.143 A ↔ G 92.080 A ↔ T 14.093 C ↔ G 1.206 C ↔ T 269.485	0.144
α-lactalbumin	A = 0.221 C = 0.239 G = 0.172 T = 0.369	HKY	Ti/Tv = 2.906	

Nuclear, mitochondrial and combined data sets all resulted in congruent Monachinae phylogenies. Phylogenetic analyses with more sequence data provided increased nodal support. As there were no conflicting results between the data sets the complete data set will be presented here.

The ML and Bayesian analyses of the complete DNA data (Fig. 3) Davis *et al.*, (2004) confirm and support the monophyly of monachine seals, the monophyly of the three groups within the Monachinae (*Monachus*, *Mirounga* and Lobodontini), and the sister relationship between *Mirounga* and the Lobodontini. In the ML analyses all clades were strongly supported with bootstrap values ≥ 70%, with the exception of the *Mirounga* + Lobodontini clade (BS = 67%) and the *Ommatophoca* + *Leptonychotes* + *Hydruga* clade (BS = 68%) which were 'marginally' strongly supported. Similarly, the Bayesian analyses resulted in well-supported nodes with posterior probabilities of 1.0 for all ingroup nodes with one exception (*Ommatophoca* + *Leptonychotes* + *Hydruga* clade, PP = 0.98). These results strongly support the monophyly of the Monachinae, the Lobodontini, *Mirounga* and *Monachus*, as well as the sister relationship between the Lobodontini and *Mirounga*.

Table 4 Data partitions implemented in the Bayesian analyses

Data set	Data partitions	Total number of sites	Variable sites	Parsimony informative sites
Nuclear (1 partition)	α-Lactalbumin	526	23	3
Mitochondrial (7 partitions)	12S cyt *b* – codon positions 1, 2 and 3 ND1 – codon positions 1, 2 and 3	2976	917	616
Combined (8 partitions)	12S cyt *b* – codon positions 1, 2 and 3 ND1 – codon positions 1, 2 and 3 α-Lactalbumin	3502	940	619

Table 6 ln *L* scores and harmonic means for partitioned and non-partitioned data sets with results of the likelihood ratio test (*P*-value), and Bayes factor

Data set	Non-partitioned ln *L*	Partitioned ln *L*	*P*-value	Non-partitioned harmonic mean of ln *L*	Partitioned harmonic mean of ln *L*	Bayes factor in favour of the partitioned model
Combined	−13,083.27	−12,726.97	< 0.001	−13,116.12	−12,819.07	> 10
Mitochondrial	−12,050.90	−11,805.62	< 0.001	−12,072.89	−11,366.20	> 10

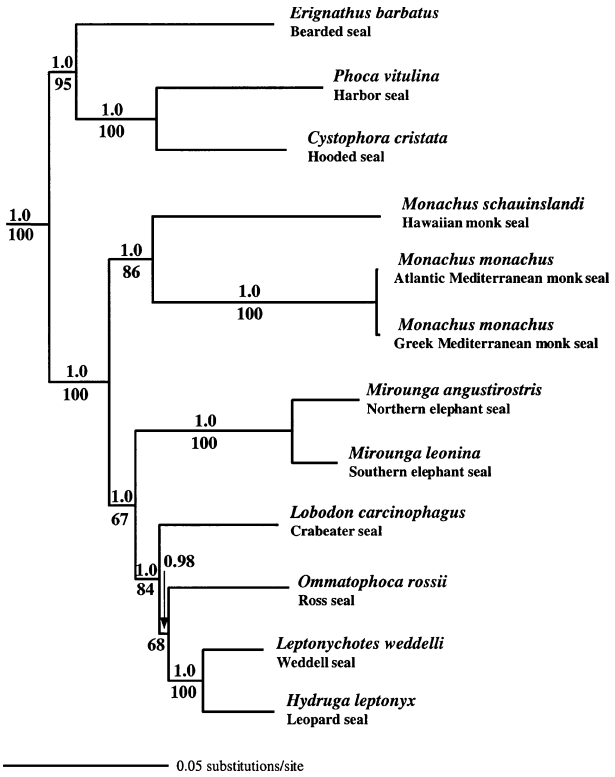

Figure 3 Maximum likelihood phylogeny of combined mitochondrial and nuclear data sets analysed under the GTR + Γ model. ln $L = -12{,}785.83$. Posterior probabilities shown above nodes. Maximum likelihood bootstraps shown below nodes. Walrus and sea lion outgroups not shown.

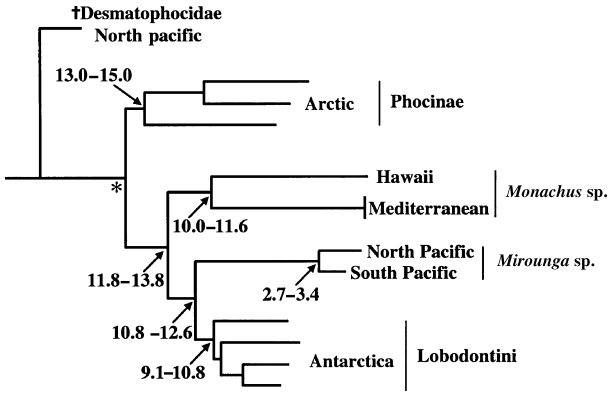

Figure 4 Area cladogram with divergence times (Ma) indicated for each node. *Phocinae–Monachinae divergence occurring in the North Atlantic 15–17 Ma.

Biogeographical patterns

The biogeographical results are displayed as an area cladogram with current species distributions mapped onto the combined ML phylogeny obtained in this study (Fig. 4). The Desmatophocidae, the extinct sister group to phocid seals with a fossil record in the North Pacific was added to the phylogeny (Deméré & Berta, 2002). Monachine seal divergence times estimated with the program r8s range from the lower 95% confidence interval of the 17 Ma calibration point to the upper 95% confidence interval of the 15 Ma calibration point and are indicated in Fig. 4.

DISCUSSION

Monachinae phylogeny

This study has resolved phylogenetic relationships among extant monachine seals using mitochondrial and nuclear DNA sequence data. These analyses provided strong support for the monophyly of the Monachinae, *Monachus*, *Mirounga* and the Lobodontini and the sister relationship between the Lobodontini and *Mirounga*. The mitochondrial data were better at resolving monachine seal relationships compared with the nuclear data due to higher rates of nucleotide substitution in mitochondrial DNA as well as the inclusion of six times as many characters in the mitochondrial data set compared with the nuclear data set. The nuclear data (which represents a data set evolving independently of the mitochondrial DNA) supported the same phylogenetic hypothesis as the mitochondrial data set therefore increasing support for the combined data analysis and the resultant phylogeny as an accurate portrayal of the evolutionary history of this group.

The use of mixed-model Bayesian analyses led to a dramatic improvement in mean ln *L* scores and slight increases in nodal posterior probabilities when compared with the non-partitioned analyses. The increase in mean ln *L* and posterior probabilities can be attributed to more of the data being modelled more appropriately in the partitioned phylogenetic analyses.

Historical biogeography

To investigate historical biogeography of any group it is best to use as many lines of evidence as possible (i.e. phylogenetic relationships, fossil record, molecular rates of evolution, past geological events). One reality of the fossil record is that fossils impose minimum ages and can therefore only underestimate actual divergence dates (i.e. the oldest fossil will almost always be younger than the origin of its group). Conversely, using

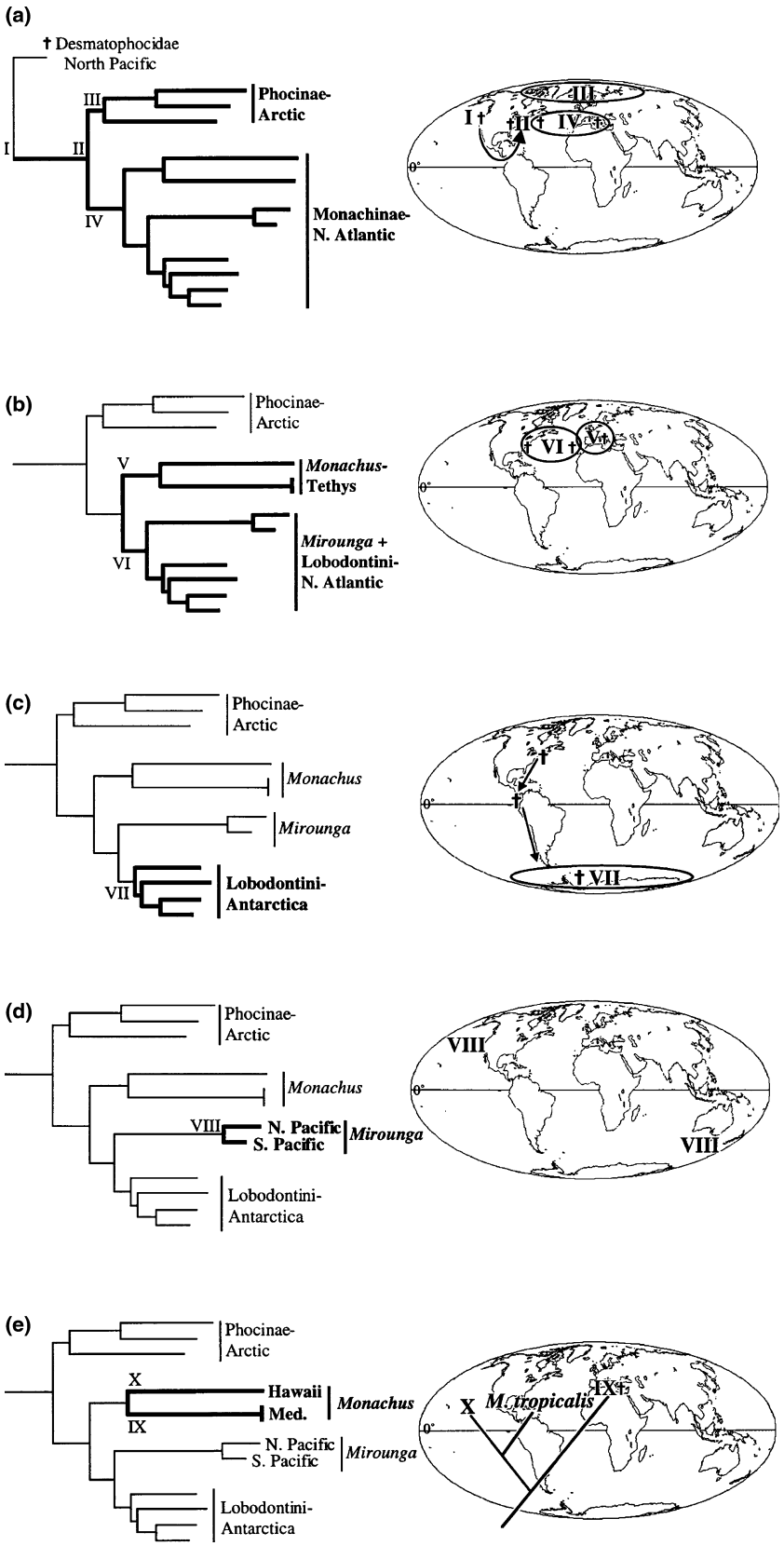

Figure 5 (a) Phocid seals and their close relatives (Desmatophocidae) and the hypothesized events leading to current distributions. I – Desmatophocidae in the North Pacific 20–23 Ma. II– Ancestral fossil phocids (*Leptophoca lenis* and *Monotherium? wymani*) in the North Atlantic 15–17 Ma. Multiple lines of evidence suggest a southern route for ancestral phocid seals from the North Pacific to the North Atlantic. III – Phocine seal dispersal to the cooler waters of the Arctic and sub-Arctic regions. IV – Monachine seal diversification in the eastern and western North Atlantic. †Fossil evidence. (b) Monachinae clade and the hypothesized events leading to current distributions. Diversification of the two main clades within Monachinae occurred between 11.8 and 13.8 Ma based on molecular estimates. V – Ancestral fossil *Monachus* representatives in the Mediterranean support the origin of the *Monachus* clade in the Tethys region. VI – Fossil evidence supports diversification of the *Mirounga* + Lobodontini clade in the North Atlantic. (c) Lobodontini clade and the hypothesized events leading to current distributions. VII – Molecular evidence supports lobodontine seal diversification between 9.1 and 10.8 Ma. Fossil evidence of lobodontines from the Pisco Formation in Peru suggests dispersal to Antarctica through the Central American Seaway followed by southward migration to Antarctica by at least 4 Ma. † Fossil evidence. (d) *Mirounga* clade and the hypothesized events leading to current distributions. *Callophoca*, the purported sister taxon of *Mirounga* is known from the early Pliocene. VIII – Two *Mirounga* species occur in an anti-tropical distribution. Molecular evidence estimates the divergence of the northern and southern species between 2.7 and 3.4 Ma. (e) *Monachus* clade and the hypothesized events leading to current distributions. According to molecular evidence, the Hawaiian and Mediterranean lineages split between 10 and 11.6 Ma. IX – Fossil evidence supporting ancestral monk seals in the Tethys region. X – Current distribution of Hawaiian monk seals. Placement of the recently extinct Caribbean monk seal as sister taxon to the Hawaiian monk seal supports the hypothesis of dispersal of an ancestral Mediterranean monk seal to the Caribbean and then subsequent dispersal to the South Pacific. †Fossil evidence.

molecular rates of evolution will impose maximum ages because nucleotide changes will start accumulating independently for each lineage at the first occurrence of restricted gene flow. Because of these factors it is common for divergence times resolved by molecular evidence to estimate maximum divergence times and those resolved by the fossil record to estimate

minimum divergence times. With this in mind, both lines of evidence can be valuable in elucidating origin and diversification events.

By integrating our well-supported phylogeny with fossil evidence and divergence time estimates, we propose the following biogeographical scenario to infer the origin and diversification of monachine seals. Fossils of the extinct phocid sister taxon (Desmatophocidae) are found in the North Pacific 20–23 Ma (Deméré & Berta, 2002). Basal phocine (*Leptophoca lenis*) and monachine (*Monotherium? wymani*) representatives appeared for the first time in the fossil record in the western north Atlantic between 15 and 17 Ma (Deméré *et al.*, 2003). Basal phocid seals could have made the transition from the North Pacific to the North Atlantic following two possible routes: southward through the Central American Seaway or north through the Arctic. Multiple lines of evidence point to a southern route (Fig. 5a). First, the Central American Seaway was open during the early Miocene coupled with an absence of a marine corridor through Beringia. Second, a southern route would allow the desmatophocids to maintain temperate water affinities. Finally, desmatophocid fossils can be found as far south as southern California.

Once in the Atlantic, basal phocid seals diversified. One group dispersed north to the cooler climates of the Arctic and sub-Arctic regions (Phocinae), while the other group retained their warm water affinities and diversified in the eastern and western North Atlantic (Monachinae). Basal monachine fossil taxa include *Monotherium aberrratum* (Ray, 1976) of the eastern and western North Atlantic, along with *Pristiphoca vetusa* (de Muizon, 1982) and *Pontophoca sarmatica* (Grigorescu, 1976) from the Paratethys region (a northern arm of the Tethys Sea which historically stretched across what is now the Black, Caspian and Aral Seas of Asia). The circum-Atlantic distribution of basal monachine seals suggest a trans-Atlantic dispersal event from east to west sometime in the mid-Miocene.

According to estimated molecular divergence dates, the divergence of the Lobodontini and *Mirounga* from the subtropically distributed *Monachus* occurred between 11.8 and 13.8 Ma (Fig. 5b). There is limited fossil evidence by which to date the split of the two main clades within Monachinae. However, Pliocene fossils (*Pristiphoca* and *Pliophoca*) in southern Europe thought to be basal *Monachus* representatives would support the evolution of *Monachus* in the warm waters of the Tethys Sea (de Muizon, 1982). Fossil evidence (*Monotherium aberratum*, *M. affine* and *M. delognei*) suggests the origin of the *Mirounga* + Lobodontini clade in the North Atlantic 7–9 Ma (Deméré *et al.*, 2003). Molecular evidence suggests that the *Mirounga* + Lobodontini clade appeared between 10.8 and 12.6 Ma.

The well-studied fauna from the Pisco Formation in Peru contains fossils which support basal lobodontine seal dispersal from the Atlantic to the Pacific through the Central American Seaway followed by a period of southward migration along the Pacific Coast of South America (Fig. 5c). Divergence of *Mirounga* and the Lobodontini occurred between 9.1 and 10.8 Ma based on molecular data. The age of the Pisco Formation suggests origin of the Lobodontini clade by 7 Ma (*Acrophoca longirostris*, *Piscophoca pacifica*; de Muizon, 1982; de Muizon & De Vries, 1985).

Callophoca, the extinct sister taxon of *Mirounga*, is represented by early Pliocene fossils of the western North Atlantic and Europe (Deméré *et al.*, 2003). Currently, there are two extant lineages of elephant seals, one in the North Pacific and one in the South Pacific (Fig. 5d). According to molecular evidence, divergence of the northern and southern species occurred between 2.7 and 3.4 Ma. A limited fossil record for this group makes it hard to determine whether it was dispersal or vicariance that led to their present distribution. If a dispersalist hypothesis is evoked, dispersal of the *Callophoca* + *Mirounga* basal taxon would have occurred from east to west through the Central American Seaway followed by south-west migration from the equatorial Pacific to the eastern South Pacific. South Pacific to North Pacific dispersal of *Mirounga* in the Pleistocene would account for the current anti-tropical distribution seen today. A vicariance hypothesis would explain the anti-tropical distribution as a relict of a once cosmopolitan range that was separated due to increased water temperature in the tropics. Antitropical distributions in closely related marine mammals were first described by Davies (1963) and have since been documented using phylogenetic relationships and area cladograms in other marine mammals (Rosel *et al.*, 1995; Cipriano, 1997).

The occurrence of fossil *Monachus* representatives in southern Europe (*Pristiphoca* and *Pliophoca*), and their exclusion in all other formations with abundant phocid remains (e.g. Yorktown Formation, Pisco Formation) supports the evolution of this clade in the Tethys Sea (de Muizon, 1982). According to molecular divergence date estimates, the Mediterranean and Hawaiian Monk seal lineages split between 10 and 11.6 Ma (Fig. 5e). Movement from the Mediterranean to the Pacific most likely took place during two dispersal events; one from the Mediterranean to the Caribbean via the North Equatorial Current followed by a second dispersal event from the Caribbean to the Hawaiian Islands through the Central American Seaway. Dispersal from Atlantic to Pacific through the seaway could have occurred until at least its closure 3.5 Ma and perhaps more recently due to the seal's ability to move successfully across land. The position of the recently extinct Caribbean Monk seal as the sister species to the Hawaiian monk seal based on morphological data (Bininda-Emonds & Russell, 1996; Bininda-Emonds *et al.*, 1999) also supports this hypothesis.

CONCLUSIONS

We presented ML and partitioned Bayesian analyses of four molecular markers to infer the phylogenetic relationships of monachine seals. The phylogeny played a crucial role in providing a framework for constructing biogeographical hypotheses based on dispersal and vicariant events that led to the present-day distribution of monachine seals. Results from this study support two main clades within a monophy-

letic Monachinae, *Monachus*, and Lobodontini + *Mirounga*. *Monachus*, a warm water clade, maintained the temperate water affinities of their ancestors and diversified in the subtropic regions of the Northern Hemisphere. Several lines of evidence including phylogenetic relationships based on morphology, the fossil record, and past geological events support the origin of *Monachus* in the Tethys Sea region, with dispersal from east to west, first in the Caribbean and then in the Pacific Ocean.

The cold water clade, Lobodontini + *Mirounga*, dispersed southward occupying new niches in higher latitudes characterized by high oceanic primary productivity that was initially driven by glaciation in the Southern Hemisphere. The migration and diversification of lobodontine seals continued in the cold nutrient-rich waters of Antarctica. The *Mirounga* clade likely had a widespread distribution in the temperate waters of the North Atlantic. Increasing temperatures in the tropics would have displaced the wide-ranging temperate-adapted taxa into higher latitudes, leading to the modern antitropical distribution of *Mirounga*.

ACKNOWLEDGEMENTS

This project was only possible with the cooperation of scientists world-wide who donated tissues and provided advice, among those we would like to thank are Ulfur Arnason, Jason Baker, Steven Carr, Cory Davis, Steve Donnellan, John Henderson and Kelly Robertson. Samples from the Mediterranean monk seals were supplied by the BMA bank with the support of the Pew Fellows Program in Marine Conservation and Earthtrust. Funding for this project came from an SDSU RSCA grant to A. Berta and a Lerner-Grey grant to C.A. Fyler from the American Museum of Natural History. Molecular work for this project was conducted in the laboratories of Tod Reeder and Andy Bohonak at SDSU. We would like to thank Matthew Brandley for his help with partitioned Bayesian analyses and comments on multiple drafts of this manuscript, the Systematics Seminar Group of the Department of Ecology and Evolutionary Biology at UCONN, and Paul Lewis who provided helpful insight on mixed model analysis. This research was in partial fulfilment of an MS degree to C. A. Fyler in the Department of Biology at San Diego State University.

REFERENCES

Adam, P.J. & Garcia, G.G. (2003) New information on the natural history, distribution, and skull size of the extinct (?) West Indian monk seal, *Monachus tropicalis*. *Marine Mammal Science*, **19,** 297–317.

Aguilar, A. (1999) *Status of Mediterranean monk seal populations*. RAC-SPA. United Nations Environment Program (UNEP), Aloès Editions, Túnez, 60 pp.

Androukaki, E., Adamantopoulou, S., Dendrinos, P., Tounta, E. & Kotomatas, S. (1999) Causes of mortality in the Mediterranean monk seal (*Monachus monachus*) in Greece. *Contributions to the Zoology and Ecology of the Eastern Mediterranean Region*, **1,** 405–411.

Arnason, U., Bodin, K., Gullberg, A., Ledje, C. & Mouchaty, S. (1995) A molecular view of pinniped relationships with particular emphasis on the true seals. *Journal of Molecular Evolution*, **40,** 78–85.

Berta, A. & Adam, P. (2001) The evolutionary biology of pinnipeds. *Secondary adaptation of tetrapods to life in the water* (ed. by V. de Buffrenil and J.-M. Mazin), pp. 235–260. Verlag Dr Frederich Pfeil, München Germany.

Berta, A. & Wyss, A.R. (1994) Pinniped phylogeny. *Proceedings of the San Diego Society of Natural History*, **29,** 33–56.

Bininda-Emonds, O.R.P. & Russell, A.P. (1996) A morphological perspective on the phylogenetic relationships of the extant phocid seals (Mammalia: Phocidae). *Bonner Zoologische Monographien*, **41,** 1–256.

Bininda-Emonds, O.R.P., Gittleman, J.L. & Purvis, A. (1999) Building large trees by combining phylogenetic information: a complete phylogeny of the extant Carnivora (Mammalia). *Biological Reviews*, **74,** 143–175.

Brandley, M.B., Schmitz, A. & Reeder, T.W. (2005) Partitioned Bayesian analyses, partition choice, and the phylogenetic relationships of scincid lizards. *Systematic Biology*, in press.

Britten R.J. (1986) Rates of DNA sequence evolution differ between taxonomic groups. *Science*, **231,** 1393–1398.

Carr, S.M. & Perry, E.A. (1997) Intra- and Interfamilial systematic relationships of phocid seals as indicated by mitochondrial DNA sequences. *Molecular genetics of marine mammals* (ed. by A.E. Dizon, S.J. Chivers and W.E. Perrin), pp. 277–290. Society for Marine Mammalogy Special Publication No. 3, Allen Press, Kansas.

Cipriano, F. (1997) Antitropical distributions and speciation in dolphins of the genus *Lagenorhynchus*. *Molecular genetics of marine mammals* (ed. by A.E. Dizon, S.J. Chivers and W.E. Perrin), pp. 305–316. Society for Marine Mammalogy Special Publication No. 3, Allen Press, Kansas.

Davies, J.L. (1963) The antitropical factor in cetacean speciation. *Evolution*, **17,** 107–116.

Davis, C.S., Delisle, I., Stirling, I., Siniff, D.B. & Strobeck, C. (2004) A phylogeny of the extant Phocidae inferred from complete mitochondrial DNA coding regions. *Molecular Phylogenetics and Evolution*, **33,** 363–377.

Deméré, T.A. & Berta, A. (2002) The Miocene pinniped *Desmatophoca oregonensis* Condon, 1906 (Mammalia: Carnivora) from the Astoria Formation, Oregon. *Smithsonian Contributions to Paleobiology*, **93,** 113–148.

Deméré, T.A., Berta, A. & Adam, P.J. (2003) Pinnipedimorph evolutionary biogeography. *Bulletin of the American Museum of Natural History*, **279,** 32–76.

Felsenstein, J. (1981) Evolutionary trees from DNA sequences: a maximum likelihood approach. *Journal of Molecular Evolution*, **17,** 368–376.

Forcada, J., Hammond, P.S. & Aguilar, A. (1999) Status of the Mediterranean monk seal *Monachus monachus* in the wes-

tern Sahara and the implications of a mass mortality event. *Marine Ecology Progress Series*, **188,** 249–261.

Forney, K.A., Barlow, J., Muto, M.M., Lowry, M., Baker, J., Cameron, G., Mobley, J., Stinchcomb, C. & Carretta, J.V. (2000) *US Pacific marine mammal stock assessments: 2000*. US Department of Commerce, NOAA Technical Memorandum. NOAA-TM-NMFS-SWFSC-300, 276 pp.

Gatesy, J., DeSalle, R. & Wheeler, W. (1993) Alignment-ambiguous nucleotide sites and the exclusion of systematic data. *Molecular Phylogenetics and Evolution*, **2,** 152–157.

Grigorescu, D. (1976) Paratethyan seals. *Systematic Zoology*, **25,** 407–419.

Hillis, D.M. & Bull, J.J. (1993) An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Systematic Biology*, **42,** 182–192.

Hillis, D.M., Moritz, C. & Mable, B.K. (1996) *Molecular Systematics*, 2nd edn. Sinauer, Sunderland, MA.

Huelsenbeck, J.P. (1995) Performance of phylogenetic methods in simulation. *Systematic Biology*, **44,** 17–48.

Huelsenbeck, J.P. & Ronquist, F. (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics*, **17,** 754–755.

Kass, R. & Raftery, A. (1995) Bayes factors. *Journal of the American Statistical Association*, **90,** 773–795.

Kimura, M. (1968) Evolutionary rate at the molecular level. *Nature*, **217,** 624–626.

Kimura, M. & Ohta, T. (1974) On some principles governing molecular evolution. *Proceedings of the National Academy of Sciences of the United States of America*, **71,** 2848–2852.

Leaché, A.D. & Reeder, T.W. (2002) Molecular systematics of the Eastern Fence Lizard (*Sceloporus undulatus*): a comparison of parsimony, likelihood, and Bayesian approaches. *Systematic Biology*, **51,** 44–68.

Ledje, C. & Arnason, U. (1996) Phylogenetic relationships within caniform carnivores based on analyses of the mitochondrial 12S rRNA gene. *Journal of Molecular Evolution*, **43,** 641–649.

Li, W-H. (1993) So, what about the molecular clock hypothesis? *Current Opinion in Genetics and Development*, **3,** 896–901.

Milinkovitch, M.C., Bérubé, M. & Palsbøll, P.J. (1998) Cetaceans are highly specialized Artiodactyls. *The emergence of whales: evolutionary patterns in the origin of Cetacea* (ed. by N.G.M. Thewissen), pp. 113–131. Plenum, New York.

de Muizon, C. (1982) Phocid phylogeny and dispersal. *Annals of the South African Museum*, **89,** 175–213.

de Muizon, C. & De Vries, T.J. (1985) Geology and paleontology of late Cenozoic marine deposits in the Sacaco area (Peru). *Sonderdruck aus Geologische Rundschau*, **74,** 547–563.

Newton, M.A. & Raftery, A.E. (1994) Approximate Bayesian inference with the weighted likelihood bootstrap (with discussion). *Journal of the Royal Statistical Society*, **56,** 3–48.

Nylander, J.A.A. (2002) *MrModeltest v1.0b. Program distributed by the author*. Department of Systematic Zoology, Uppsala University (http://www.ebc.uu.se/systzoo/staff/nylander.html).

Nylander, J.A. A., Ronquist, F., Huelsenbeck, J.P. & Nieves-Aldrey, J.L. (2004) Bayesian phylogenetic analysis of combined data. *Systematic Biology*, **53,** 47–67.

Posada, D. & Crandall, K.A. (1998) MODELTEST: testing the model of DNA substitution. *Bioinformatics*, **14,** 817–818.

Ray, C.E. (1976) Geography of phocid evolution. *Systematic Zoology*, **25,** 391–406.

Reeder, T.W. (2003) A phylogeny of the Australian *Sphenomorphus* group (Scincidae: Squamata) and the phylogenetic placement of the crocodile skinks (*Tribolonotus*): Bayesian approaches to assessing congruence and obtaining confidence in maximum likelihood inferred relationships. *Molecular Phylogenetics and Evolution*, **27,** 384–397.

Repenning, C.A. & Ray, C.E. (1977) The origin of the Hawaiian monk seal. *Proceedings of the Biological Society of Washington*, **89,** 667–688.

Repenning, C.A., Ray, C.E. & Grigorescu, D. (1979) Pinniped biogeography. *Historical biogeography, plate tectonics, and the changing environment* (ed. by J. Gray and A.J. Boucot), pp. 357–369. Oregon State University Press, Corvalis, OR.

Rosel, P.E., Haygood, M.G. & Perrin, W.F. (1995) Phylogenetic relationships among the true porpoises (Cetacea: Phocoenidae). *Molecular Phylogenetics and Evolution*, **4,** 463–474.

Sambrook, J., Fritsch, E. & Maniatis, T. (1989) *Molecular cloning: a laboratory manual*, 2nd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Sanderson, M.J. (1997) A nonparametric approach to estimating divergence times in the absence of rate consistency. *Molecular Biology and Evolution*, **14,** 1218–1232.

Sanderson, M.J. (2002) *r8s, version 1.5 (beta), user's manual*. Distributed by M.J. Sanderson. University of California Davis, Davis, CA.

Swofford, D.L. (2003) PAUP*. *Phylogenetic analysis using parsimony (*and other methods), Version 4*. Sinauer Associates, Sunderland, MA.

Swofford, D.L., Olsen, G.J., Waddell, P.J. & Hillis, D.M. (1996) Phylogenetic inference. *Molecular systematics*, 2nd edn (ed. by D.M. Hillis, C. Moritz and B.K. Mable), pp. 407–514. Sinauer Associates, Sunderland, MA.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. & Higgins, D.G. (1997) The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research*, **24,** 4876–4882.

Timm, R.M., Salazar, R.M. & Peterson, A.T. (1996) Historical distribution of the extinct tropical seal, *Monachus tropicalis* (Carnivora: Phocidae). *Conservation Biology*, **11,** 549–551.

Wilcox, T.P., Zwickl, D.J., Heath, T. & Hillis, D.M. (2002) Phylogenetic relationship of the dwarf boas and a comparison of Bayesian and bootstrap measures of phylogenetic support. *Molecular Phylogenetics and Evolution*, **25,** 361–371.

Wilgenbusch, J. & de Queiroz, K. (2000) Phylogenetic relationships among the phrynosomatid sand lizards inferred from mitochondrial DNA sequences generated by heterogeneous evolutionary processes. *Systematic Biology*, **49,** 592–612.

Wu, C-I. & Li, W-H. (1985) Evidence for higher rates of nucleotide substitution in rodents than in man. *Proceedings of the National Academy of Sciences of the United States of America*, **82,** 1741–1745.

Wyss, A.R. (1987) The walrus auditory region and monophyly of pinnipeds. *American Museum Novitates*, **2871,** 1–31.

Wyss, A.R. (1988) On 'retrogression' in the evolution of the Phocinae and phylogenetic affinities of the monk seals. *American Museum Novitates*, **2954,** 1–38.

Wyss, A.R. & Flynn, J. (1993) A phylogenetic analysis and definition of the Carnivora. *Mammal phylogeny: placentals* (ed. by F.S. Szalay, M.J. Novacek and M.C. McKenna), pp. 32–52. Springer-Verlag, New York.

Yang, Z. (1996a) Phylogenetic analysis using parsimony and likelihood methods. *Journal of Molecular Evolution*, **42,** 294–307.

Yang, Z. (1996b) Maximum-likelihood models for combined analyses of multiple sequence data. *Journal of Molecular Evolution*, **42,** 587–596.

Zuckerkandl, E. & Pauling, L. (1965) Molecules as determinants of evolutionary history. *Journal of Theoretical Biology*, **8,** 357–366.

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Editor: Philip Stott