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Resurrection of *Mesoplodon hotaula* Deraniyagala 1963: A new species of beaked whale in the tropical Indo-Pacific

Merel L. Dalebout,[1] School of Biological, Earth, and Environmental Sciences, University of New South Wales, Sydney, New South Wales 2052, Australia; **C. Scott Baker** and **Debbie Steel**, School of Biological Sciences, University of Auckland, Private Bag 92019, Auckland 1000, New Zealand and Marine Mammal Institute, Hatfield Marine Science Center, Oregon State University, 2030 Marine Science Drive, Newport, Oregon 97365, U.S.A.; **Kirsten Thompson**, School of Biological Sciences, University of Auckland, Private Bag 92019, Auckland 1000, New Zealand; **Kelly M. Robertson, Susan J. Chivers** and **William F. Perrin**, Southwest Fisheries Science Center, NMFS, NOAA, 8901 La Jolla Shores Drive, La Jolla, California 92037, U.S.A.; **Manori Goonatilake**, Department of National Museums, PO Box 854, Colombo 7, Sri Lanka; **R. Charles Anderson**, Manta Marine Pvt. Ltd., PO Box 2074, Malé, Republic of Maldives; **James G. Mead** and **Charles W. Potter**, National Museum of Natural History, Mailstop NHB 108, Smithsonian Institution, Washington, DC 20560, U.S.A.; **Lisa Thompson** and **Danielle Jupiter**, Island Conservation Society Fondation pour la Conservation des Iles Pointe Larue, PO Box 775, Victoria, Seychelles; **Tadasu K. Yamada**, National Science Museum, Tokyo, 3-23-1 Hyakunin-cho, Shinjuku-ku, Tokyo 169-0073, Japan.

Abstract

We present genetic and morphological evidence supporting the recognition of a previously synonymized species of *Mesoplodon* beaked whale in the tropical Indo-Pacific, *Mesoplodon hotaula*. Although the new species is closely-related to the rare ginkgo-toothed beaked whale *M. ginkgodens,* we show that these two lineages can be differentiated by maternally (mitochondrial DNA), biparentally (autosomal), and paternally (Y chromosome) inherited DNA sequences, as well as by morphological features. The reciprocal monophyly of the mtDNA genealogies and the largely parapatric distribution of these lineages is consistent with reproductive isolation. The new lineage is currently known from at least seven specimens: Sri Lanka (1), Gilbert Islands, Republic of Kiribati (1+), Palmyra Atoll, Northern Line Islands, U.S.A. (3), Maldives (1), and Seychelles (1). The type specimen (Sri Lanka) was described as a new species, *M. hotaula*, in 1963, but later synonymized with *M. ginkgodens.* This discovery brings the total number of *Mesoplodon* species to 15, making it, by far, the most speciose yet least known genus of cetaceans.

Key words: speciation, taxonomy, species delimitation, mtDNA, nuclear introns, Y-chromosome, morphology, Mesoplodon, beaked whale.

[1]Corresponding author: (e-mail: mereldalebout@gmail.com).

On 26 January 1963, a female beaked whale washed ashore at Ratmalana, Sri Lanka. The stranding was reported by the director of the National Museums of Ceylon, P. E. P. Deraniyagala (1963*a*, *b*), who described the whale as a new species, *Mesoplodon hotaula*, deriving the species name from the local Sinhala words for "pointed beak." Deraniyagala provided no diagnosis by which *M. hotaula* could be differentiated from the other *Mesoplodon* beaked whales known at that time, except to note that the position of the teeth differed from that of *M. bidens* and *M. hectori.* Two years after it was described, *M. hotaula*, still known only from the holotype, was synonymized with the ginkgo-toothed beaked whale, *M. ginkgodens* Nishiwaki and Kamiya 1958, by Moore and Gilmore (1965). Although Deraniyagala was apparently unaware of the existence of *M. ginkgodens* when he described *M. hotaula,* now it seems he was correct regarding its uniqueness.

Here we present genetic and morphological evidence for the distinctiveness of *M. hotaula*, now known from at least seven specimens. We consider the taxonomic ranking of the new taxon using the Genealogical Concordance Species Concept (GCC; Avise and Ball 1990), and discuss its sister-species relationship with *M. ginkgodens*. In choosing the GCC, we are aware that there are many definitions of what constitutes a species (De Queiroz 2007). The Biological Species Concept (BSC) defines a species as a group of actually or potentially interbreeding natural populations that are reproductively isolated from other such groups (Mayr 1963); a criterion that is generally difficult if not impossible to assess in many wild populations. The Phylogenetic Species Concept (PSC), a character-based approach originating from cladistic principles, defines a species as the smallest cluster of organisms that can be diagnosed as distinct from other clusters based on fixed character differences, showing a parental pattern of ancestry and descent (Cracraft 1989, Davis and Nixon 1992). The GCC attempts to reconcile these two approaches by requiring multiple lines of evidence, including phylogenetic analysis of DNA sequences and morphology, to establish the distinctiveness of the evolutionary lineages in question. This addresses the problem that application of a strict PSC, especially using molecular data, could lead paradoxically to a vast increase in the number of recognized species at a time when most biologists agree that global biodiversity is decreasing (Avise 2000, Zachosa *et al.* 2013).

The GCC also accounts for the fact that gene phylogenies can differ greatly from locus to locus due to recombination in meiosis, mating patterns, and varying reproductive success of the individuals through which alleles are transmitted. The GCC therefore stresses that phylogenetic diagnoses should be based on broad agreement at multiple loci. A group of organisms is considered to constitute a distinct species under the GCC if the following criteria are met (Avise and Ball 1990, Avise 2000): (1) concordance across sequence characters within a genetic locus leading to conclusive exclusion; (2) concordance in these genealogical patterns across multiple loci, both mitochondrial and nuclear; (3) concordance with biogeographical patterns; and (4) concordance with morphological characters. These criteria were reviewed and supported by a specialist workshop on shortcomings in cetacean taxonomy (Reeves *et al.* 2004) and the GCC has subsequently been used by several authors to describe new species of cetaceans (*e.g.*, Dalebout *et al.* 2004, Caballero *et al.* 2007).

The GCC is especially useful for rare or poorly described taxa. Beaked whales (family Ziphiidae) are deep-diving odontocetes that live in the offshore waters of all the world's oceans except the highest latitudes of the Arctic. They are rarely seen at sea due to their elusive habits, long dive capacity, and, for some species, probable low abundance (Reeves *et al.* 2002). Most information has come from stranded animals, and several species are known from only a handful of specimens. To assist with beaked

whale identification and discovery, a comprehensive, validated DNA taxonomy for all known species in this group was established using sequences from mitochondrial DNA (mtDNA) control region (CR) and cytochrome *b* (CYB) genes (Dalebout *et al.* 2004). This database was subsequently expanded for the most speciose genus, *Mesoplodon*, using up to six specimens per species from throughout the range where possible, to assess the robustness of genetic patterns observed at these loci (Dalebout *et al.* 2007). The patterns originally observed were again evident in this expanded sample: intraspecific variation within species was generally low (mean; CR $0.6\% \pm 0.06\%$, CYB $0.8\% \pm 0.09\%$), while interspecific divergence was generally high (mean; CR $7.4\% \pm 0.04\%$, CYB $11.8\% \pm 0.04\%$), with little overlap. In phylogenetic analyses, these mtDNA sequences formed strongly supported, species-specific clades that were reciprocally monophyletic with respect to all other such clades. In short, sequences from each of the known *Mesoplodon* species clustered together to the exclusion of sequences from other known *Mesoplodon* species. Each *Mesoplodon* species also possessed multiple diagnostic nucleotide substitutions at these genes distinguishing each species from all other species in the group, *sensu* Davis and Nixon (1990). While intraspecific variation may be underestimated because of the small sample sizes available, Dalebout *et al.* (2007) sampled as many specimens as possible, which in some cases included all currently known specimens for that species. Overall these mitochondrial markers were found to be well suited for DNA taxonomy in this genus, with the results from phylogenetic analyses concordant with morphological diagnoses and other requirements for species distinctiveness under the GCC. The application of this DNA taxonomy to beaked whales has already led to some significant discoveries: the description of a new species from the North Pacific (Perrin's beaked whale *M. perrini*; Dalebout *et al.* 2002); the resurrection of a long-forgotten species in the Southern Hemisphere (the spade-toothed whale *M. traversii*; van Helden *et al.* 2002, Thompson *et al.* 2012); and, confirmation of the identity of the enigmatic "tropical bottlenose whale" (*Indopacetus pacificus*; Dalebout *et al.* 2003).

Within the robust framework offered by this comprehensive DNA taxonomy and the guidelines provided by the GCC, the discovery of a divergent lineage could therefore indicate the existence of an unrecognized species or subspecies. Just such a lineage was reported by Dalebout *et al.* (2007), based on analyses of several specimens which appeared to be related to *M. ginkgodens* yet were genetically distinct from this species. Further specimens representing this divergent mtDNA lineage have since been discovered. One of these was the Sri Lankan specimen described as a new species, *M. hotaula*, by Deraniyagala (1963*a*, *b*) and subsequently synonymized with *M. ginkgodens* (Moore and Gilmore 1965). *M. ginkgodens* is one of the least-known of beaked whale species. It is known from less than 30 strandings and there has yet to be a confirmed sighting of a living whale at sea.

To assess the taxonomic status of "*M. hotaula*," we analyzed three mtDNA genes, seven nuclear autosomal introns, and one Y-chromosome intron, as well as morphological characters. Detection of genetic differences among recently diverged taxa can be difficult with slowly evolving, single-copy nuclear autosomal loci (Hare 2001). Our inclusion of data from a Y-chromosome intron therefore has several advantages. Firstly, under random mating, the effective population size of this nonrecombining chromosome is ¼ that of single-copy autosomal markers. Therefore the accumulation of mutations through genetic drift occurs far more rapidly. Secondly, the Y-chromosome is subject to mutations that have arisen only in the male germline, giving us a male-specific marker to compare to the female-specific mtDNA. Based on diagnostic genetic characters and morphological features consistent with the GCC and the

criterion of "irreversible divergence," as recommended by a workshop on cetacean taxonomy (Reeves *et al.* 2004), we present a formal proposal for the recognition of *M. hotaula* as a valid species.

MATERIALS AND METHODS

Material Examined

Seven specimens of *M. hotaula* were examined (Table 1, Fig. 1)[2] and compared to all other known *Mesoplodon* species *via* phylogenetic analyses of mtDNA and nuclear gene sequences. Museums and institutions holding specimens of *M. hotaula* are as follows: the National Museum, Colombo, Sri Lanka (1), Smithsonian National Museum of Natural History, Washington, DC, U.S.A. (USNM, 3), a private collection in the Republic of Maldives (1), and the Island Conservation Society, Seychelles (1). Specimen 2 from the Gilbert Islands, Republic of Kiribati, is known only from a soft-tissue sample held in the University of Auckland DNA and Tissue Archive, Auckland, New Zealand. Information on additional specimens identified by DNA analysis from fragmentary osteological material from Kiribati can be found in Baker *et al.* (2013).

Genetic and morphological comparisons were made to six specimens of *M. ginkgodens* (Table 1, Fig. 1), including the holotype (Nishiwaki and Kamiya 1958). For genetic comparisons to other *Mesoplodon* species, up to six specimens per species were sampled (see Dalebout *et al.* 2007 for details).

DNA Extraction, PCR, and Sequencing

Six of the seven specimens of *M. hotaula* (Nos. 1, 3–7) were represented only by osteological material requiring the use of "ancient DNA" methods. A hand-held electric drill with a 2 mm diameter drill bit was used to obtain 0.01–0.02 g of bone or tooth powder from each specimen as described by Pichler *et al.* (2001*a*). DNA was extracted using the silica-guanidinium thiocyanate method (Boom *et al.* 1990, Höss and Pääbo 1993, Matisoo-Smith *et al.* 1997) as modified by Rohland and Hofreiter (2007). These methods were also used to extract DNA from the holotype and California specimens of *M. ginkgodens* (Dalebout *et al.* 2004). Only the Kiribati specimen of *M. hotaula* (No. 2) was represented by soft tissue that was several months old and dried for preservation.[3] DNA was extracted from this sample using standard phenol:chloroform methods (Sambrook *et al.* 1989), as modified for small samples by Baker *et al.* (1994).

Specimens 1 and 6 were analyzed at the University of New South Wales, Sydney, Australia (by MLD). Specimens 2 and 7 were analyzed at the University of Auckland, New Zealand (by DS, KT, and MLD). Specimens 3 through 5 were analyzed at the NOAA/NMFS Southwest Fisheries Science Center, La Jolla, California (by KMR).

The polymerase chain reaction (PCR) was used to amplify fragments from three mitochondrial genes (control region–CR, cytochrome *b*–CYB, cytochrome *c* oxidase I–COXI), seven nuclear autosomal introns (biglycan–BGN, catalase–CAT, rhodopsin–RHO, cytotoxic T-lymphocyte-associated serine esterase 3–CTLA3, cholinergic

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[2]The initial conclusions of Dalebout *et al*. (2007) were based on specimen Nos. 2–4.

[3]Personal communication from R. Grace, 56 Bertram Street, Warkworth, New Zealand, 19 August 2003.

Table 1. Specimens of *Mesoplodon hotaula* and *M. ginkgodens* examined for this study. See text for details of museum holdings. H, holotype.

Specimen code	Other codes	Date found	Location	Coordinates	Total length (cm)	Sex
Mesoplodon hotaula						
1 3WZS (H)		26 January 1963	Ratmalana, Sri Lanka	6°49'N, 79°52'E	445	F
2 UKIRI		11 July 2003	Tabiteuea Atoll, Republic of Kiribati	1°07'S, 174°40'E[a]	?	M[b]
3 USNM593418	SW53473, PANWR 12533-06001[c]	9 November 2005	Palmyra Atoll, northern Line Islands, USA	5°52'N, 162°06'W	480	F[b]
4 USNM593414	SW53474, PANWR 12533-06002[c]	9 November 2005	Palmyra Atoll, northern Line Islands, USA	5°52'N, 162°06'W	470	F?
5 USNM593426	SW70984, PANWR 12533-06003[c]	8 July 2006	Palmyra Atoll, northern Line Islands, USA	5°52'N, 162°06'W	386	M
6 MDV-X		January 2007	Hulhudhuffaru, Raa Atoll, Republic of Maldives	5°45'N, 73°00'E	?	M
7 MM-0001		20 June 2009	Desroches Island, Seychelles	5°67'S, 53°65'E	432	M
Mesoplodon ginkgodens						
1 MginUSNM298237		10 June 1954	Del Mar, California	32°57'N, 117°15'W	?	F
2 MginTSM8744 (H)	NSMT M8744	22 September 1957	Oiso, Tokyo, Japan	35°18'N, 139°18'E	472	M
3 MginMV29623		26 June 1983	Cape Reamur, Victoria, Australia	38°23'S, 142°08'E	?	?
4 MginTW01	NMNS-SU-94-29	1994	northeast coast Taiwan	23°46'N, 121°00'E	?	F
5 MginNZ03	NMNZ 2901	11 April 2003	Taranaki, New Zealand	39°18'S, 174°08'E	480	M
6 MginNZ04	NMNZ2618	1 November 2004	Pakawau, Nelson, New Zealand	40°48'S, 172°48'E	496	M

[a]Note that coordinates given in Dalebout *et al.* (2007) were incorrect.
[b]Determined or confirmed by molecular sexing.
[c]US Fish and Wildlife Service "Palmyra Atoll National Wildlife Refuge" Accession number.

Note: other specimens which may represent *M. hotaula*: adult male (BMNH1957.4.5.1) from Malaysia, November 1954, material held at the Natural History Museum, London, U.K.; identification by MLD based on skull morphology from photographs courtesy of R. Sabin; neonate female (End002) from Phuket, Thailand, 7 June 1988 or 1989 (Andersen and Kinze 1999, Chantrapornsyl *et al.* 1999); based on stranding location only.
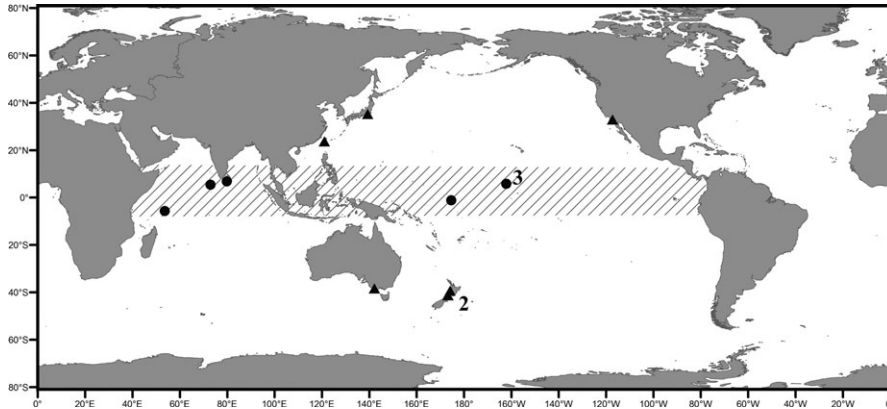
Figure 1. Map showing stranding locations of specimens sampled for this study: *M. hotaula* (black circles), *M. ginkgodens* (black triangles). Area with diagonal lines indicates suggested distribution of *M. hotaula.* Each symbol represents one specimen per location, except where noted (see Table 1).

receptor-nicotinic alpha polypeptide 1–CHRNA1, muscle actin–ACT, major histocompatibility complex class II–DQA) and one nuclear Y-chromosome intron (DBY7). For COXI amplification, we used the primers, BatL5310 (5′-CCT ACTCRGCCATTTTACCTATG-3′) and Bat6871tSer (5′-GTTCGATTC CTTCCTTTCTT-3′), courtesy of the Alan Wilson Centre, Massey University, New Zealand (T. McLenachan). For DBY7 amplification, we used the primers, DBY7-F (5′-GGTCCAGGAGARGCTTTGAA-3′) and DBY7-R (5′-CAGCCAATTCTC TTGTTGGG-3′), from Hellborg and Ellegren (2003). PCR information for COXI and DBY7 can be found in the online supplementary material. Information for other loci is in Dalebout *et al.* (2004, 2008*b*). PCR products were prepared for sequencing by enzymatic purification, using shrimp alkaline phosphatase and exonuclease I (Werle *et al.* 1994). Products were sequenced on an ABI 377, modified ABI 373, or ABI 3700 automated sequencer (Applied Biosystems, Inc.) using BigDye Dye Terminator Chemistry Vs. 3.1. Fragments were sequenced at least twice in both directions for confirmation in the majority of cases. Sequences were edited manually and aligned using the program SEQUENCER Vs. 4.0 (Gene Codes Corporation, Inc.).

Genetic Analyses

Mitochondrial DNA—Phylogenetic reconstructions of individual CR and CYB data sets were presented by Dalebout *et al.* (2007). Here, we concatenated these two data sets (819 base pairs, bp) in an attempt to obtain a stronger phylogenetic signal. Cuvier's beaked whale *Ziphius cavirostris* was used as an outgroup. COXI data were not included in these analyses as only a single representative of *M. hotaula*, and only a subset of other ziphiid species have been sequenced for this locus to date. Maximum likelihood (ML) analyses were performed using PAUP* 4.0b10 (Swofford 2003), with parameters estimated by Modeltest (Posada and Crandall 1998) and starting trees for heuristic searches obtained *via* neighbor joining. Full model details for ML analyses can be found in the online supplementary material. The robustness of the nodes was assessed using 1,000 full heuristic, nonparametric ML bootstrap replicates.

Bayesian analyses were performed using MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001) using an ML model with six substitution types and empirical base frequencies. Rate variation across sites was modelled using a gamma distribution, with a proportion of sites estimated as being invariant. The Markov chain Monte Carlo search was run with four chains for 1 million generations, with trees being sampled every 100 generations (first 1,000 trees were discarded as burn-in). Details on methods used to assess convergence can be found in the online supplementary material.

Building on the results of Dalebout *et al.* (2007), we made pairwise comparisons of the CR, CYB, and COXI gene fragments to determine the number of diagnostic nucleotide substitutions (putative fixed differences) distinguishing *M. hotaula* from *M. ginkgodens.* The program MEGA3 (Kumar *et al.* 2004) was used to calculate net divergence (dA $\pm$ SE; Nei 1987) between taxa using the Kimura 2-parameter model, and determine the proportion of synonymous and nonsynonymous substitutions for the two protein-coding genes.

Nuclear introns—Phylogenetic reconstruction of the relationships among *Mesoplodon* beaked whales based on autosomal nuclear introns was presented by Dalebout *et al.* (2008*b*) but did not include *M. hotaula.* Initial screening of these slowly evolving loci for the present study revealed only limited differentiation between *M. hotaula* and *M. ginkgodens,* such that phylogenetic assessment would not be able to differentiate between them. Therefore, we focused on a character-based diagnostic approach (Davis and Nixon 1992) for these autosomal loci, as well as for the Y-chromosome intron, DBY7. Under a character-based approach, a clade is characterized by one or more synapomorphies, defined as shared derived character states inferred to have been present in the first member of the taxon (most recent common ancestor), inherited by its descendants (unless secondarily lost), and not inherited by any other taxa.

Morphological Data

Cranial and mandibular measurements were obtained for five specimens, following the methods of Moore (1963), as adapted by JGM for the Smithsonian US National Museum of Natural History collections. Specimens USNM593418, USNM593414, and USNM593426 were measured by JGM and CWP. Measurements for the holotype (3SWZ) were taken from Deraniyagala (1963*a*), and checked by RCA and MG in consultation with JGM. Specimen MDV-X was also measured by RCA. Comparative measurements for the *M. ginkgodens* holotype were obtained from Nishiwaki and Kamiya (1958). Measurements from additional *M. ginkgodens* were provided by TKY. Measurements were obtained using calipers and rounded to the nearest whole mm.

Results

Genetics—Mitochondrial DNA

CR fragments (658 bp) were successfully sequenced from all seven specimens of *M. hotaula.* CYB fragments (384–706 bp) were successfully sequenced from only four specimens due to the degraded nature of the material available. A COXI fragment (987 bp) was successfully sequenced only from the dried meat from Kiribati (Table 2). These CR, CYB, and COXI fragments were also successfully sequenced from up to six specimens of *M. ginkgodens*.

In phylogenetic analyses of the combined CR and CYB (819 bp) including all known *Mesoplodon* species, the *M. hotaula* and *M. ginkgodens* specimens clustered together in two strongly supported clades (bootstrap scores, BS 100%, posterior probabilities, BPP 1.00) that were reciprocally monophyletic to one another, consistent with the proposed species differences (Fig. 2). The sister-species relationship of these taxa was also strongly supported (bootstrap 91%, posterior probability 1.00). All other recognized *Mesoplodon* species formed similar strongly supported, species-specific clades, with branch lengths reflecting the relatively low genetic diversity observed *within* species and the comparatively large genetic divergence observed *between* species (see also Dalebout *et al*. 2002, 2004, 2007). Individual analyses of the CR and CYB data sets revealed the same pattern (Dalebout *et al*. 2007).

It is worth noting the deep mtDNA divergence observed among True's beaked whales, *M. mirus* (Fig. 2). This is the only *Mesoplodon* species with a disjunct, allopatric distribution, with populations found in the North Atlantic and in the Southern Hemisphere (South Africa and Australia). These northern and southern populations also have different color patterns (Ross 1969) and likely represent unique subspecies or species in their own right. This divergence, however, is far less than that observed between the *M. ginkgodens* and *M. hotaula* lineages.

For the CR, pairwise comparisons between *M. hotaula* and *M. ginkgodens* over 658 bp revealed 35 variable sites, of which 18 appear to represent diagnostic characters distinguishing these taxa from one another (Table S1A). In pairwise comparisons between all *Mesoplodon* species (435 bp), net divergence (dA) ranged from 3.1 % to 8.3% (mean, 5.4% ± 0.99%). Net divergence between *M. hotaula* and *M. ginkgodens* was 3.6% ± 0.91% over this fragment. Similar levels of genetic divergence were observed between other sister-species pairs (as identified by Dalebout *et al*. (2008*b*) based on nuclear introns, the results of which were concordant with cranial morphology): *M. perrini* and *M. peruvianus* (3.2% ± 0.83%); *M. densirostris* and *M. stejnegeri* (3.6% ± 0.90%); *M. mirus* and *M. europeaus* (3.7% ± 0.87%); and *M. bowdoini* and *M. carlhubbsi* (5.2% ± 1.10%; Table S2A).

For CYB (384 bp), comparisons between *M. hotaula* and *M. ginkgodens* revealed 31 variable sites, of which 26 appear to represent diagnostic characters distinguishing these taxa from one another, including four nonsynonymous substitutions (Table S1B). In pairwise comparisons between all *Mesoplodon* species (384 bp), net divergence ranged from 5.5% to 16.6% (mean, 11.4% ± 1.97%). Net divergence between *M. hotaula* and *M. ginkgodens* was 8.2% ± 1.79%. This is slightly lower than that observed between other recognized sister-species pairs: *M. mirus* and *M. europeaus* (8.7% ± 1.85%), *M. densirostris* and *M. stejnegeri* (10.4% ± 2.01%), *M. perrini* and *M. peruvianus* (11.7% ± 2.24%), and *M. bowdoini* and *M. carlhubbsi* (11.8% ± 2.24%; Table S2B).

For COXI (987 bp), comparisons between *M. hotaula* and *M. ginkgodens* revealed 64 variable sites, of which 49 appear to represent diagnostic characters distinguishing these taxa from one another (all synonymous substitutions, Table S1C). Although COXI is not considered a good "DNA barcode" for cetaceans due to significant overlap between intra- and interspecific variation in some groups, Viricel and Rosel (2012) similarly observed species-specific sequences for *Mesoplodon* beaked whales. In pairwise comparisons between a subset of species (*M. hotaula, M. ginkgodens, M. europaeus, M. mirus,* and *M. densirostris*), net divergence ranged from 5.5% to 10.0% (mean, 8.5% ± 1.24%) over 958 bp. Net divergence between *M. hotaula* and *M. ginkgodens* was 5.5% ± 0.76%. The only other sister-species pair sampled in our

Table 2. Summary of genetic data used in the comparison of *Mesoplodon botaula* and *M. ginkgodens*. Auto, autosomal; CR, control region; COXI, cytochrome oxidase I; CYB, cytochrome *b*; DBY7,[a] sex intron; MtDNA, mitochondrial DNA.

Specimen code	Source of DNA	MtDNA			Nuclear introns		GenBank accession Numbers
		CR	CYB	COXI	Auto	DBY7	
Mesoplodon botaula							
1 3WZS	osteological material	Y					KF027298
2 UKIRI	dried soft tissue	Y	Y	Y	Y	Y	JX470545, JX470546, KF027311, KF027315–KF027321, KF027328
3 USNM593418	osteological material	Y	Y				JX470543, JX470547
4 USNM593414	osteological material	Y	Y				JX470544
5 USNM593426	osteological material	Y	Y				KF027299, KF027306
6 MDV-X	osteological material	Y					KF027300
7 MM-0001	osteological material	Y	Y				KF027301, KF027307
Mesoplodon ginkgodens							
1 MginUSNM298237	osteological material	Y					KF027302
2 MginTSM8744	osteological material	Y					AY579518
3 MginMV29623	osteological material	Y	Y				KF027303, KF027310
4 MginTW01	soft tissue	Y	Y	Y	Y		AY579517, AY579544, KF027312, EU447764, EU476111, EU476141
5 MginNZ03	soft tissue	Y	Y	Y	Y	Y	KF027304, KF027308, KF027313, EU447749, EU476126, EU476156, EU476171, KF027329
6 MginNZ04	soft tissue	Y	Y	Y			KF027305, KF027309, KF027314

[a]DBY7 GenBank Accession Numbers for other *Mesoplodon* species: KF027328–KF027337.

study, *M. mirus* and *M. europaeus*, differed by 7.8% ± 1.05% at this locus (Table S2C).

Note that for the CR and CYB data sets, the lowest divergences were observed between taxa that are not recognized sister species (CR, *M. hectori* and *M. hotaula*, 3.11% ± 0.83%; CYB, *M. layardii* and *M. mirus*, 5.53% ± 1.42%). This is the due to the rapid rate of accumulation of mutations at these highly variable loci, resulting in multiple substitutions/site (saturation). Nucleotide substitutions in ancestral lineages are undetectable as subsequent substitutions erase the evidence. This issue is particularly apparent when genetic differences are reduced to pairwise distances, as phylogenetic divergence between taxa will be underestimated even further (Avise 1994). For this reason, while well suited to addressing questions of species identity, the CR and CYB are generally not well suited for resolving higher-level relationships in the genus *Mesoplodon*, including some of the deeper, older divergences between sister species (Fig. 2, gray-shaded area, bootstrap scores <50%; see also Dalebout *et al.* 2004, 2007). A robust, higher-level phylogeny for this group has been provided by phylogenetic analyses of more slowly evolving nuclear markers (Dalebout *et al.* 2008*b*).

Intraspecific diversity for *M. hotaula* at the CR and CYB was low, in line with trends observed for these loci in other *Mesoplodon* species (Dalebout *et al.* 2007). For CR, the Kiribati and Palmyra specimens shared the same haplotype, the Maldives specimen differed from this by 4 bp (0.61%), and the holotype of *M. hotaula* differed from this by 7 bp (1.06%). For CYB, two Palmyra specimens shared the same haplotype, and the Kiribati specimen differed from this by 1 bp.

Genetics-Nuclear Introns

Due to the degraded nature of much of the material available, nuclear intron sequences were obtained only from the Kiribati specimen of *M. hotaula.* For this same reason, other *Mesoplodon* species were also generally represented by only a single specimen for these analyses. Partial introns were successfully amplified from seven nuclear genes: BGN, 706 bp; CAT, 559 bp; RHO, 166 bp; CTLA3, 305 bp; CHRNA1, 366 bp; ACT, 925 bp; and, DQA, 456 bp (Dalebout *et al.* 2008*b*). Over all these introns combined (3,348 bp), each previously recognized *Mesoplodon* species possessed between one (*M. grayi, M. ginkgodens* proper) and 13 (*M. bidens*) diagnostic nucleotide substitutions, *sensu* Davis and Nixon (1990), distinguishing them from all other species in the group (Table 4). One nucleotide substitution distinguished *M. ginkgodens* proper from all other species including *M. hotaula*, and four nucleotide substitutions distinguished the *M. ginkgodens–M. hotaula* complex from all other species. In sister-species comparisons, one nucleotide substitution (position 2199, ACT) distinguished *M. ginkgodens* from *M. hotaula*, while 10 substitutions distinguished *M. perrini* from *M. peruvianus,* 12 substitutions distinguished *M. bowdoini* from *M. carlhubbsi,* 14 substitutions distinguished *M. densirostris* from *M. stejnegeri,* and 17 substitutions distinguished *M. europeaus* from *M. mirus.* The divergence date estimates for these latter species pairs range from 5.3 to 10.4 Mya (Dalebout *et al.* 2008*b*), while the split of the *M. ginkgodens* and *M. hotaula* lineages appears to be a more recent occurrence.

DBY7 fragments (241 bp) were successfully amplified from 12 *Mesoplodon* species. Due to the poor quality of much of the material available and the male-only nature of this marker, each species was represented by only a single specimen for these analyses. For this marker, most though not all, *Mesoplodon* species sampled possessed at least
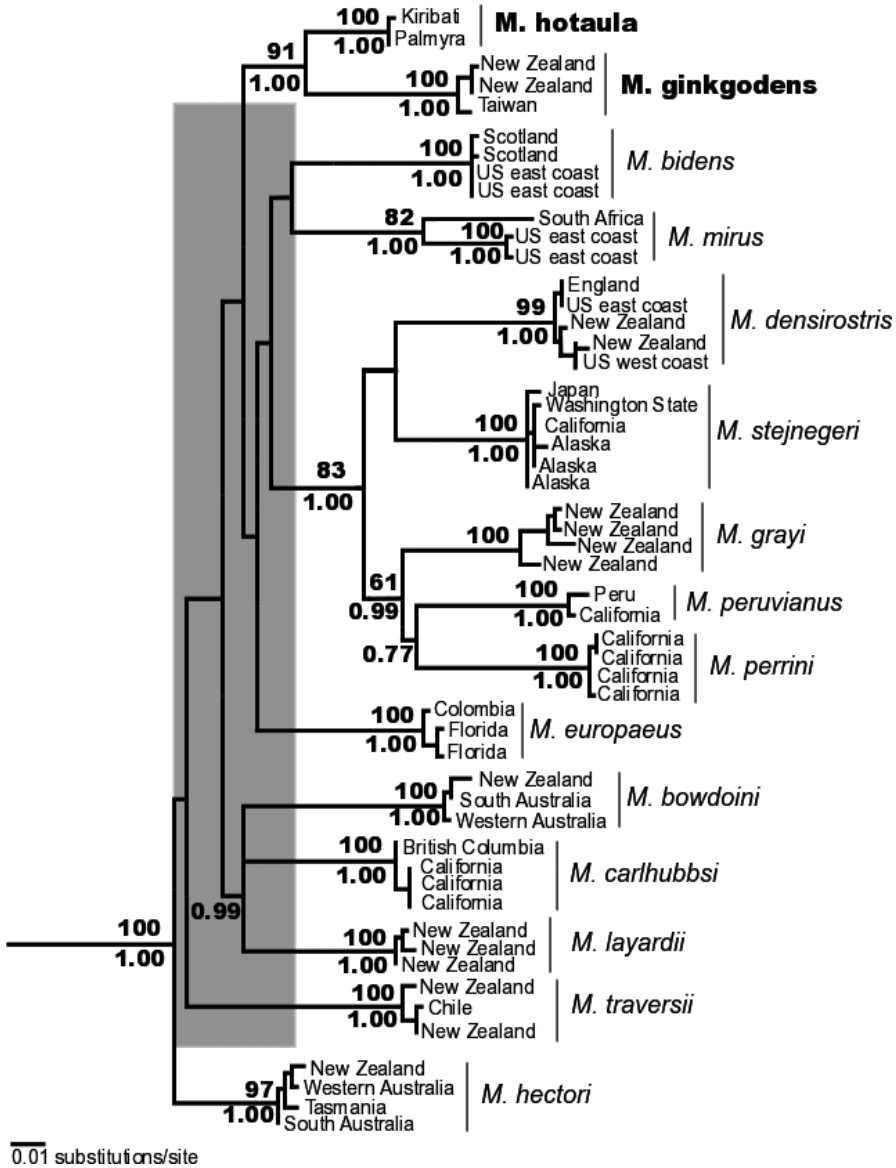
Figure 2. Maximum likelihood (ML) reconstruction of phylogenetic relationships among *Mesoplodon* beaked whales based on combined CR and CYB mtDNA sequences. Clade robustness is shown by bootstrap scores, BS (≥60%, above branches) and Bayesian posterior probabilities, BPP (≥0.90, below branches). Note strong support of all species-specific groupings (majority of bootstrap scores >80%, posterior probabilities >0.95) and consistent patterns of low intraspecific genetic variation and high interspecific genetic divergence in this group. Higher-level relationships between species were generally not well resolved by these markers (gray-shaded regions, most bootstrap scores <50%). A single tree was retained from the search (score –ln 4363.84942). Cuvier's beaked whale *Ziphius cavirostris* was used as an outgroup.

one diagnostic nucleotide substitution that distinguished them from the other species in the group (Table S3). Although three taxa did not yield species-specific sequences for this locus, both *M. ginkgodens* and *M. hotaula* represented unique lineages. *M. ginkgodens* possessed one nucleotide substitution (position 156) that distinguished it from all other *Mesoplodon* species, including *M. hotaula,* and *M. hotaula* possessed one nucleotide substitution (position 191) that reciprocally distinguished it from all other *Mesoplodon* species, including *M. ginkgodens.* For the other sister-species pairs sampled, two substitutions distinguished *M. europeaus* from *M. mirus*, and no substitutions distinguished *M. bowdoini* from *M. carlhubbsi.*

Description

Order Cetartiodactyla Montgelard, Catzefils and Douzery 1997
Cetacea (Brisson 1762)
Family Ziphiidae Gray 1865
Genus *Mesoplodon* Gervais 1850
Mesoplodon hotaula Deraniyagala 1963*a, b*

Holotype

Adult female (3WZ5): skull, mandible, tooth, and some postcranial elements held at the National Museum, Colombo, Sri Lanka. Specimen washed ashore "in a dying condition" on 26 January 1963. Collected, described, and named by P. E. P. Deraniyagala (1963*a, b*).[4] See also Deraniyagala (1965).

Type Locality

Ratmalana (6º49′N, 79º52′E), approximately 8 km south of Colombo, on the west coast of Sri Lanka.

Paratypes

Adult female (USNM593418): skull, jaw, teeth, and postcranial elements held at the Smithsonian National Museum of Natural History, Washington, DC. Tissue sample (bone powder, SW53473) held at US National Marine Fisheries Southwest Fisheries Science Center (SWFSC). Collected from Cooper Island, Palmyra Atoll, one of the Northern Line Islands, *ca.* 1,770 km SW of Honolulu, Hawaii, by staff of The Nature Conservancy.[5]

Subadult, possible female (USNM593414): skull only held at the Smithsonian National Museum of Natural History, Washington, DC. Tissue sample (bone powder, SW53474) held at SWFSC. Collected from Eastern Island, Palmyra Atoll, by staff of The Nature Conservancy.

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[4]Note typographical error in Deraniyagala (1963*a*); figure is labeled incorrectly as *Mesoplodon ulhota.*

[5]Palmyra Atoll was purchased by The Nature Conservancy in 2000. In 2001, the US Fish and Wildlife Service designated the coral reef habitat surrounding this atoll as a National Wildlife Refuge. Baumann-Pickering *et al.* (2010) recorded echolocation signals from an unknown species of beaked whale at Palmyra Atoll, which they suggest could be attributed to *M. hotaula.*

Adult male (USNM593426): skull and mandible held at the Smithsonian National Museum of Natural History, Washington, DC. Tissue sample (bone powder, SW70984) held at SWFSC. Collected from eastern lagoon, Palmyra Atoll by staff of The Nature Conservancy. Teeth present at time of collection but were lost in transit to the Smithsonian.

Adult male (MDV-X): skull, mandible, teeth, and postcranial elements held in a private collection in the Republic of Maldives.

Adult male (MM-0001): skull, mandible, teeth, and postcranial elements held in the collection of the Island Conservation Society (ICS) Fondation pour la Conservation des Iles in Victoria, Seychelles. Collected from Desroches Beach, Seychelles on 20 June 2009 by L. and W. Thompson with assistance from ICS members.

Male (UKIRI): tissue sample held in the University of Auckland DNA and Tissue Archive, New Zealand, collected by Roger V. Grace from Tabiteuea Atoll in the Gilbert Islands, Republic of Kiribati in 2003. The dried meat was a gift from the islanders, a leftover from a recent festival feast. It was reportedly obtained from one of seven whales driven onto the beach and killed in October 2002 when the whales came into the shallow water of the lagoon (Baker *et al.* 2013). The description of this hunt is extremely unusual. Beaked whales generally only come into shallow water prior to stranding, and such behavior usually involves only single individuals or cow-calf pairs. With the exception of *M. grayi* (von Haast 1876, Reeves *et al.* 2002), beaked whales do not generally mass strand. The islanders reported that such events occurred several times a year and provided a common source of food, not just for ceremonial occasions. The whales were described as "long ones," *ca.* 15–20 ft (457–609 cm) in length. An expedition to Kiribati in June–July 2009 did not find any further remains on Tabiteuea Atoll, but recovered osteological material from one or more whales identified as *M. hotaula* through DNA analysis from nearby Onotoa Island (Baker *et al.* 2013).

Etymology

The specific name, *hotaula*, is derived from the Sinhala words, *hota* = beak, and *ula* = pointed (Deraniyagala 1963*a*). Recommended pronunciation is as follows: *ho* as in hot, *ta* as in tuppence, *ul* as in school, and *a* as in uh. We propose that this species be known by the common name, "Deraniyagala's beaked whale."

DIAGNOSIS

Molecular Characters

M. hotaula can be differentiated from *M. ginkgodens* and all other species of *Mesoplodon* beaked whales based on molecular genetic characters (Fig. 2, Tables S1–S3).

Mitochondrial DNA—In phylogenetic analyses of combined CR and CYB sequences (Fig. 2), *M. hotaula* specimens cluster together in a strongly supported clade (BS 100%, BPP 1.00) that is reciprocally monophyletic to the clade formed by the *M. ginkgodens* specimens. The number of apparently fixed nucleotide substitutions (diagnostic characters) that distinguish *M. hotaula* from *M. ginkgodens* (Table S1), together with the overall degree of genetic differentiation (% net divergence, Table S2), is similar to what is observed between other recognized *Mesoplodon* species: CR, $n = 18$ (dA, 3.6% $\pm$ 0.91%), CYB, $n = 26$ (dA, 8.2% $\pm$ 1.79%), COXI, $n = 49$

(dA, 5.5% ± 0.76%). Branch lengths in phylogenetic reconstructions using these sequences reflect this trend.

Nuclear DNA—Autosomal introns: a nucleotide substitution at position 2199 (ACT) distinguishes *M. ginkgodens* from *M. hotaula* and all other *Mesoplodon* species sampled. Y-chromosome DBY7: nucleotide substitutions at positions 156 and 191 distinguish *M. ginkgodens* and *M. hotaula* from one another, and from all other *Mesoplodon* species sampled (Table S3).

Morphological Characters

The following characters of the teeth and skull are, when combined, diagnostic for *M. hotaula* (Fig. 3–6).

(1) Single pair of very large, triangular, laterally compressed mandibular teeth.
(2) Alveoli of the teeth fully posterior to the mandibular symphysis.
(3) Teeth with vertical growth form, taller than they are wide, asymmetric; posterior margin convex, anterior margin almost planar.
(4) Short mandibular symphysis (distal portions of mandibles appear "stubby").
(5) Greatest transverse span of combined premaxillary bones in adults ≥60 mm (Table S4, measurement 32) and "flattened" in cross section.

Character 1 is shared with *M. ginkgodens, M. bowdoini, M. carlhubbsi, M. densirostris,* and *M. stejnegeri*. Character 2 is shared with *M. ginkgodens, M. densirostris, M. peruvianus,* and *M. stejnegeri*. Characters 3, 4, and 5 distinguish *M. hotaula* from *M. ginkgodens*. Tooth form in adult males is particularly distinct. In contrast, the teeth of *M. ginkgodens* are generally wider than they are tall, both the posterior and anterior margins are convex, and they are nearly symmetrical (Fig. 5). In *M. ginkgodens*, the distal portion of the mandibles appears long and gracile (Fig. 4), and the greatest transverse span of the combined premaxillary bones at the midpoint of the length of the beak is greater than 40 mm but less than 60 mm (diagnostic feature 5, Moore and Gilmore 1965; also TKY, unpublished data). Further, the premaxillary bones in *M. ginkgodens* are angled upwards (*ca.* 30°–45°) rather than flattened (*ca.* 10°–15°) as in *M. hotaula* (Fig. 6). Adult male *M. ginkgodens* also appear to be larger in size (total length, 472–496 cm) than adult male *M. hotaula* (total length, 386–432 cm; Table 1). Additional images of *M. hotaula* and *M. ginkgodens,* together with details of skull and mandibular measurements, can be found in the online supplemental material (Fig. S1–S6).

External Appearance

To date, we only have information on external appearance for two specimens; the holotype (3WZS), an adult female from Sri Lanka, and an adult male (MM-0001) from the Seychelles. The holotype, which was freshly dead when examined, was described as having a relatively compressed body, a strong lateral ridge, a slender head with an elongate beak, and eyes located about half a beak length behind the angle of the gape. It was blue gray ventrally and the tail had a median lobe with a small caudal notch. There was a single, unerupted pair of teeth in the mandible, located slightly behind the symphysis (Deraniyagala 1963*a, b,* 1965).

The Seychelles specimen (Fig. 7), also freshly dead, was similar in overall appearance, though the tail lacked a median notch. It was examined in the early morning, shortly after its discovery, by W. and L. Thompson. The specimen was blue-black dor-

Figure 3. Holotype of *M. hotaula,* 3WZS, adult female, Sri Lanka: (A) Dorsal view, (B) lateral view, (C) ventral view, (D) mandibles with left tooth *in situ*, right tooth missing, (E) right tooth, labial, lingual, and posterior views. Scale for (A)–(D), 300 mm metal ruler. Scale for (E), 5 mm card tag. Photo credits: (A–D), R. C. Anderson, courtesy of National Museum of Colombo; (E) reproduced from Deraniyagala 1965, Plate III.

sally, grading to a slightly lighter shade ventrally. There were a small number of white cookie-cutter shark (*Isistius* spp.) scars on its ventral surface, predominantly at the posterior end. There were also two large, fresh shark bites; one out of the ventral peduncle and one out of the head-neck area, behind the blowhole. The blue-black color of the body continued on the head, forming a dark cap that extended along the anterior surface of the rostrum and to the posterior end of the mouth line. Coloration around the eye was a lighter mottled gray, becoming lighter ventrally. The tip of the lower jaw was gray but the lower jaw itself was predominantly white. This white color pattern extended on the lower jaw to behind the tooth and continued above the mouthline to the rostrum. The upper lips were whitish, grading to gray and blue-black on the rostrum. The gray mottling of the cheek and eye area formed a distinct wedge of color

against the white of the ventral chin and throat region, cutting across the posterior ends of the throat grooves (see also Fig. S5). Note that the tips of the teeth of both this and the Palmyra adult male (USNM593426) were broken (see also Fig. S3). This suggests that male:male combat using the teeth as weapons does occur in this species, although the Seychelles specimen did not have any of the white linear tooth rake scars that appear to result from such behavior (Mead *et al*. 1982, Heyning 1984).

In contrast, the two New Zealand adult male *M. ginkgodens* were brownish-gray dorsally (though blue-black coloration has been described from Japanese animals; Nishiwaki and Kamiya 1958, Nishiwaki *et al*. 1972), grading to lighter tones ventrally (Fig. 7, see also Fig. S6). There was a darker patch around the eye that extended further in front and a bit below the eye. The beak was white-tipped, both upper and lower jaws. This white coloration reappeared on the upper lip behind the tooth. Where *M. hotaula* appears to have a gray tip to the lower jaw and a white chin and throat region (Fig. 7, arrows), *M. ginkgodens* appears to have a white tip to the lower jaw and a gray-brown chin and throat region (Fig. 8, arrows). However it is difficult to tell from such a small number of animals whether these are fixed color pattern differences or individual variation. These suggested differences need to be confirmed from additional fresh strandings or sightings of living whales at sea.

Distribution

M. hotaula has an equatorial distribution in the Indo-Pacific (Fig. 1), which broadly overlaps, or is mostly parapatric, with the more temperate distribution of *M. ginkgodens*. The majority of confirmed records of *M. ginkgodens* are from temperate and cold-temperate waters such as those around Japan, Taiwan, and New Zealand. Based on tooth form, the record from the Galapagos (Palacios 1996) also appears to represent *M. ginkgodens* proper, which is not surprising given the cold Humboldt Current that flows around these islands. There are three further tropical records for *M. ginkgodens*; one from the Federated States of Micronesia and one from the Marshall Islands (both based on DNA analysis; Dalebout *et al.* 2008*a*; KMR, unpublished data), and one from the Republic of Maldives, based on tooth form (Anderson *et al.* 1999). The latter specimen consists of a single tooth held in the Maldives National Museum, with no information on its provenance (Anderson *et al.* 1999). The species identity of this specimen should be re-examined, but could suggest a zone of overlap in distribution of the two species around the Maldives.

DISCUSSION

The genetic and morphological evidence presented here supports recognition of *M. hotaula* and *M. ginkgodens* as full species based on the four criteria of the GCC (Avise and Ball 1990, Avise and Wollenberg 1997, Avise 2000):

(1) Concordance across sequence characters within genetic locus leading to conclusive exclusion—Over the three mtDNA loci, 93 nucleotide substitutions (CR 18, CYB 26, COX1 49) were observed between *M. hotaula* and *M. ginkgodens*, which appear to represent fixed differences based on a limited number of specimens. For the CYB, this included four nonsynonymous substitutions, which would translate to amino-acid level differences in this key metabolic protein. Two nucleotide substitutions further distinguished these species at the Y-chromosome and
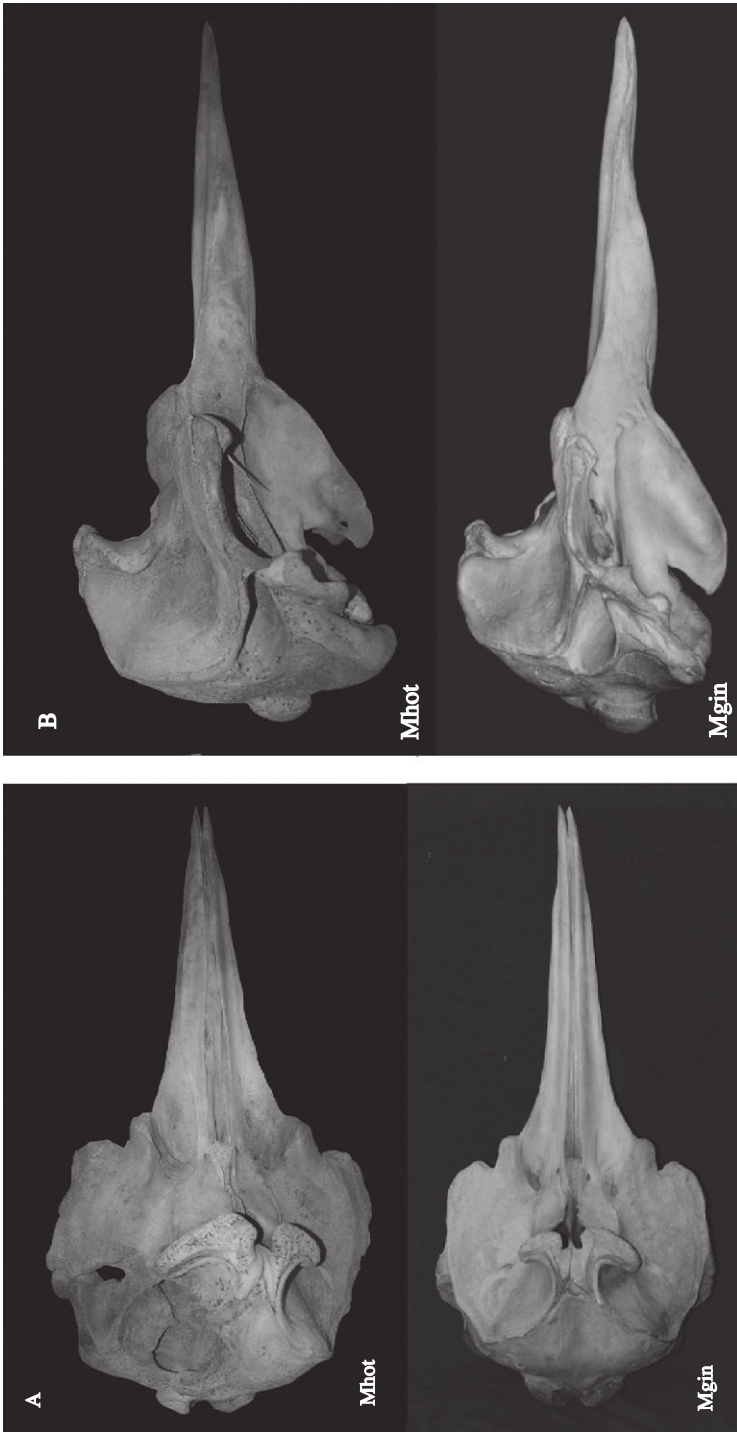
Figure 4. Comparison of adult males; crania and mandibles: *M. botaula* (MDV-X, Maldives) and *M. ginkgodens* (MginTSM8744, holotype). (A) Dorsal view, (B) lateral view, (C) ventral view, (D) mandibles. Photo credits: *M. botaula*, R. C. Anderson; *M. ginkgodens*, T. K. Yamada.
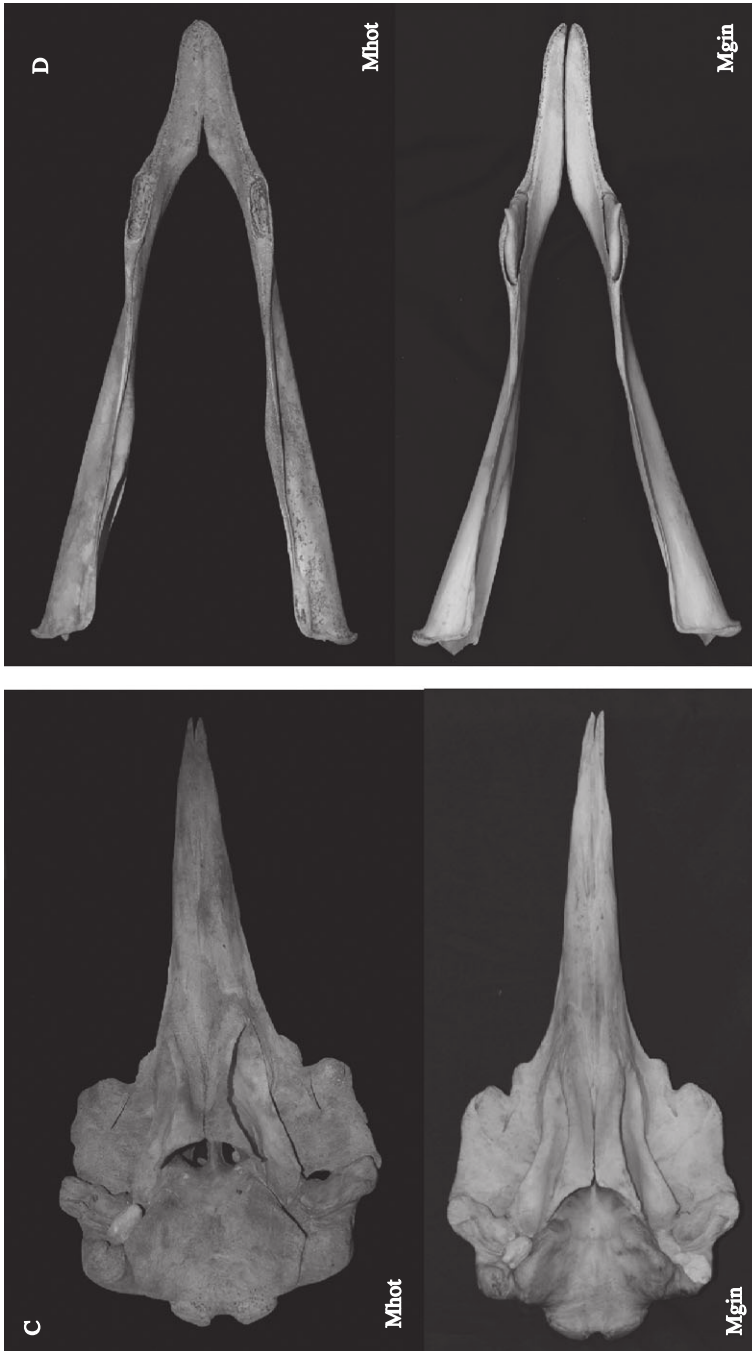
Figure 4. (Continued).

Figure 5. Comparison of adult male teeth: *M. botaula* (MDV-X, Maldives) and *M. ginkgodens* (MginTSM8744, holotype). (A) L tooth, medial face; R tooth, lateral face. Leading edges facing right. (B) *M. botaula* adult males, distal end of skull and mandibles with teeth *in situ.* (i) MDV-X, Maldives; (ii) MM-0001, Seychelles; (iii) teeth of MM-0001. Photo credits: MDV-X, R. C. Anderson; MM-0001, L. Thompson; MginTSM8744, T. K. Yamada.

Figure 6. Comparison of premaxilla morphology; distal and oblique lateral views of rostrum: (A) *M. botaula* USNM593418, (B) *M. botaula* USNM593414, (C) *M. ginkgodens* USNM298237. Photo credit: M. L. Dalebout.
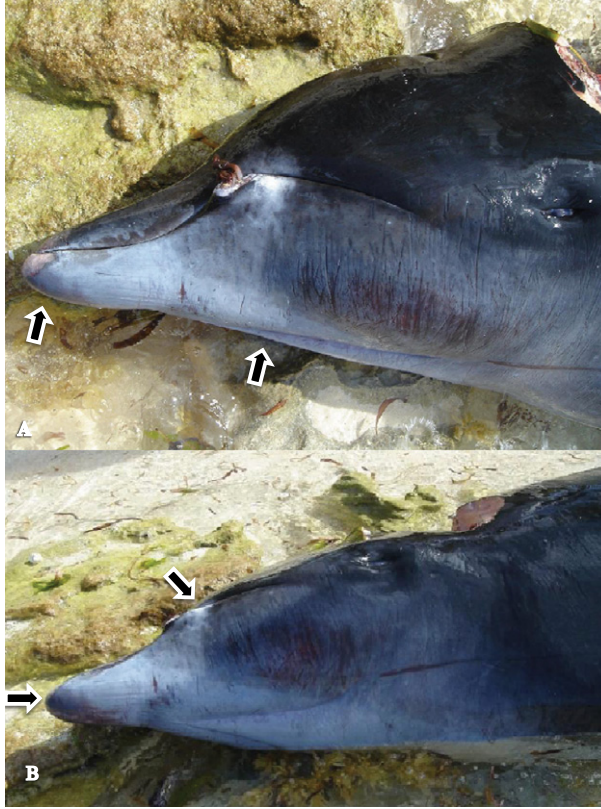
Figure 7. External appearance of *M. hotaula*: MM-0001, adult male, Seychelles. (A) Lateral view of head, (B) antero-ventral view of head. Photo credit: L. Thompson.

autosomal introns surveyed. Lower levels of divergence are expected at single copy nuclear loci due to the slower rate of evolution of these markers (Hare 2001). Overall, the levels of divergence observed were similar to but somewhat lower than those between other known sister-species pairs in this genus. Divergence date estimates for these other species range from 5.3 (*M. perrini* and *M. peruvianus*) to 10.4 Mya (*M. bowdoini* and *M. carlhubssi*; Dalebout *et al.* 2008*b*), while the split of the *M. ginkgodens* and *M. hotaula* lineages appears to be a more recent occurrence.

(2) Concordance in genealogical patterns across multiple loci, both mitochondrial and nuclear—Phylogenies constructed from mtDNA lineages (haplotypes) showed a strongly supported pattern of reciprocal monophyly. Divergence at the nuclear loci was too low to be detected by such analyses but the character-based analyses were concordant with patterns observed in the mtDNA. Based on these results, there was no evidence of mitochondrial (maternal), Y-chromosome (paternal), or autosomal (bi-parental) gene flow between these taxa.

(3) Concordance with biogeographical patterns—The distributions of *M. hotaula* and *M. ginkgodens* appear to be largely parapatric, with *M. hotaula* found in more

Figure 8. External appearance of *M. ginkgodens*: MginNZ04, adult male, New Zealand. (A) Lateral view of head, (B) antero-ventral view of body. Photo credit: H. Stoffregen, courtesy of New Zealand Department of Conservation.

    tropical waters and *M. ginkgodens* found predominantly in more temperate waters, but with some zones of potential overlap (*e.g.,* in the Republic of Maldives).

(4) Concordance with morphological characters—*M. hotaula* and *M. ginkgodens* differ in features of the teeth, mandibles, and cranium, as well as potentially in color pattern. Together, these differences, while perhaps comparatively subtle, indicate that there is indeed morphological divergence between the species.

    It is recognized that small sample sizes and limited geographic sampling can lead to underestimates of intra-specific genetic variability (Meyer and Paulay 2005). When evaluating the utility of the COXI gene for cetacean species identification, Viricel and Rosel (2012) also faced this issue. Although unable to consistently differentiate between closely related taxa in the *Stenella-Delphinus-Tursiops* complex, that study also found that diagnostic characters (species-specific sequences) distinguished all *Mesoplodon* species sampled. Using regression analyses, they also found that there was no significant relationship between the number of individuals analyzed per species and mean intraspecific diversity in their data sets (Viricel and Rosel 2012).

Further, they noted that cetaceans generally show relatively low levels of genetic variability (Shimura and Numachi 1987; Schlotterer *et al.* 1991; Dalebout *et al.* 2004, 2007; Kingston and Rosel 2004). In mysticetes, low rates of molecular evolution have been attributed to a combination of low metabolic rate, large body size, and long generation times (Jackson *et al.* 2009). Slower rates of molecular evolution will limit the amount of genetic diversity that can accumulate within a species and the amount of divergence that can accumulate between species over a given time. Previous studies of mtDNA CR and CYB in beaked whales have shown that intraspecific diversity is generally low, while inter-specific divergence is generally considerably higher, with little overlap (Dalebout *et al.* 2002, 2004, 2006, 2007). So while examination of additional specimens of *M. hotaula* and *M. ginkgodens* would be very useful, the low intraspecific diversity of beaked whales, and the low level of genetic variability in cetaceans overall, will together have reduced the potential biases of limited sampling in this study.

At a comprehensive, specialist workshop on cetacean taxonomy, evidence of "irreversible divergence" was considered of primary importance in the recognition and delimitation of species (Reeves *et al.* 2004). Irreversible divergence was considered to require at least two independent lines of evidence. Genetic characters from unlinked loci were considered to represent multiple lines of evidence. Confirming the independence of multiple morphological characters is difficult, and morphology was therefore considered to represent only a single line of evidence. Based on these guidelines, the proposed species-level ranking of both *M. hotaula* and *M. ginkgodens* is supported by multiple lines of evidence: mtDNA, nuclear autosomal DNA, Y-chromosome DNA, and morphology.

No guidelines were offered by the workshop on the degree of genetic divergence required to warrant species status. Here we have used a comparative approach based on the patterns of divergence observed between other recognized *Mesoplodon* species in phylogenetic reconstructions and pairwise distances to evaluate the proposed species status of *M. hotaula.* For the mtDNA, the divergence of *M. hotaula* and *M. ginkgodens* (CR 3.6%, CYB 8.2%, COXI 5.5%) was within the range observed for other *Mesoplodon* species, though generally on the lower end of the scale. MtDNA divergence between species in this group is, however, considerably higher on average than that observed between many other recognized cetacean species. For example, the net CR divergence between Chilean and Commerson's dolphins (*Cephalorynchus commersoni* and *C. eutropia*) was 2.5% over 442 bp, with three fixed differences (Pichler *et al.* 2001*b*), while the net CYB divergence between dusky and Pacific white-sided dolphins (*Lagenorhynchus obscurus* and *L. obliquidens*) was 1.2% over 496 bp, with five fixed differences (Hare *et al.* 2002). The lower levels of mtDNA divergence among these delphinids are consistent with speciation events initiated in the Pleistocene, approximately 1–1.5 Mya (Avise *et al.* 1998, Caballero *et al.* 2007). While not as deep as some of the other sister species in the genus *Mesoplodon*, it is clear that the divergence of the lineages representing *M. hotaula* and *M. ginkgodens* began well before the Pleistocene, and constitutes an ongoing and "irreversible" trend.

In conclusion, we present genetic and morphological evidence demonstrating that *M. hotaula* is a valid species, closely related to, but distinct from, *M. ginkgodens*. It is known to occur from at least the Seychelles to Palmyra Atoll, and likely ranges right across the tropical Indo-Pacific. This discovery brings the total number of *Mesoplodon* species to 15, making this by far the most speciose cetacean genus, although it remains among the least known.

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Supporting Information

The following supporting information is available for this article online at http://onlinelibrary.wiley.com/doi/10.1111/mms.12113/suppinfo.

Analytical Methods

Figure S1. External appearance of *M. hotaula* holotype adult female (3WZS). Clockwise from top; right ventro-lateral view of anterior half (length of section, 205.7 cm); dorsal view of flukes with caudal notch; dorsal view of head; left side of head. Reproduced from Deraniyagala (1965), Plate I.

Figure S2. External appearance, artist's impression of *M. hotaula* holotype (3WZS). Reproduced from Deraniyagala (1965), fig. 2.

Figure S3. Skulls of *M. hotaula* paratypes. (A) USNM593418, adult female, Palmyra Atoll: (i) dorsal view, (ii) lateral view, (iii) ventral view, (iv) mandibles with teeth *in situ*. Scale, pen 138 mm (i–iii), US nickel, 21 mm (iv). Photo credit: M. L. Dalebout. (B) USNM593414, subadult female?, Palmyra Atoll: (i) dorsal view (scale, US nickel, 21 mm), (ii) lateral view (scale, 138 mm pen). Scale, pen 138 mm (i), US nickel, 21 mm (ii). Photo credit: M. L. Dalebout. (C) USNM593426, adult male, Palmyra Atoll: (i) dorsal view of skull and mandibles, teeth removed, (ii) lateral view of skull and mandibles, teeth removed, (iii) mandibles with teeth *in situ*. Photo credit: A. Hoke. (D) MM-0001, adult male, Seychelles: (i) dorsal view of skull and mandibles with teeth *in situ,* (ii) lateral view of skull and mandibles with teeth *in situ,* (iii) ventral view of skull, (iv) lateral view of mandibles with teeth *in situ.* Photo credit: L. Thompson.

Figure S4. Other *M. ginkgodens* skulls. (A) MginNZ04, adult male, New Zealand: (i) dorsal view, (ii) lateral view, (iii) mandibles with teeth *in situ* lateral view, (iv) mandibles with teeth *in situ* ventral view. Courtesy of A. van Helden and the Museum of New Zealand Te Papa Tongarewa. (B) MginUSNM298237, adult female, California: (i) dorsal view of skull (scale, 300 mm ruler), (ii) dorsal view of mandibles with right tooth *in situ* (scale, US nickel, 21 mm). Photocredit: M. L. Dalebout.

Figure S5. Additional images, external appearance of *M. hotaula,* MM-0001, adult male, Seychelles. (A) Ventral view of stranded animal, (B) postero-lateral view, (C) antero-dorsal view of head. Photo credit: L. Thompson.

Figure S6. Additional images, external appearance of *M. ginkgodens,* MginNZ03, adult male, New Zealand. (A) Lateral view of head, (B) anterior view of head. Photo-credit: B. Williams, courtesy of New Zealand Department of Conservation.

Table S1. MtDNA data. (A) Control region (CR), variable sites over 658 bp. (B) Cytochrome *b* (CYB), variable sites over 384 bp. (C) Cytochrome oxidase I (COXI), variable sites over 987 bp. Gray shading highlights the nucleotide substitutions differentiating *M. hotaula* from *M. ginkgodens*.

Table S2. Pairwise net divergence between species, Kimura 2-parameter distances, as percentages, below diagonal. SE, above diagonal. Values for sister-species pairs highlighted in gray with bold type. (A) Control region, CR (435 bp); (B) cytochrome *b*, CYB (384 bp); (C) cytochrome oxidase I, COXI (958 bp). See footnotes of Table S2A for translation of species codes.

Table S3. Y-chromosome intron, DBY7. Variable sites over 241 bp. The diagnostic nucleotide substitutions that distinguish *M. ginkgodens* and *M. hotaula* from each another, and from the other *Mesoplodon* species sampled are highlighted in gray.

Table S4A. Cranial measurements for *Mesoplodon hotaula*. Measurements (in mm) are taken on the right hand side (R) where possible, following Moore (1963). Where two measurements are given, R, then L. E, estimated length. See Table S3B for definitions of measurements.

Table S4B. Definitions of cranial measurements. Numbers in parentheses refer to Moore (1963).

Table S5A. Mandibular measurements for *Mesoplodon hotaula*. Measurements (in mm) are taken on the right hand side (R) where possible, following Moore (1963). Where two measurements are given, R, then L. E, estimated length. See Table S4B for definitions of measurements.

Table S5B. Definitions of mandibular measurements. Numbers in parentheses refer to Moore (1963).