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True´s beaked whale (*Mesoplodon mirus*) in Macaronesia

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The True´s beaked whale (*Mesoplodon mirus*, True 1913) is a poorly studied member of the Ziphiidae family. Its distribution in the northern hemisphere is thought to be restricted to the temperate or warm temperate waters of the North Atlantic, while a small number of stranding records from the southern hemisphere suggest a wider and antitropical distribution, extending to waters from the Atlantic coast of Brazil to South Africa, Australia and the Tasman Sea coast of New Zealand. This paper i) reports the first molecular confirmation of the occurrence of True´s beaked whales at the southern limit of its distribution recorded in the northeast Atlantic: the Azores and Canary Islands (macaronesian ecoregion); ii) describes a new colouration for this species using evidence from a whale with molecular species confirmation; and iii) contributes to the sparse worldwide database of live sightings, including the first underwater video recording of this species and close images of a calf. In November 2012, a subadult male True's beaked whale stranded in El Hierro, Canary Islands. In July 2004, a subadult male was found floating dead near Faial, the Azores. Species identification was confirmed in both cases

using mitochondrial DNA control region and cytochrome *b* gene markers. The whale that stranded in the Canary Islands had a clearly delimited white area on its head, extending posteriorly from the tip of the beak to cover the blowhole dorsally and the gular grooves ventrally. This colouration contrasts with previous descriptions for the species and it may be rare, but it exemplifies the variability of the colouration of True´s beaked whales in the North Atlantic, further confirmed here by live sightings data. Given the presence of this species around the Azores and the Canary Islands, it would be expected that True´s beaked whales also occur in the area between these archipelagos, including the islands of Madeira. The recording of several observations of this species in deep but relatively coastal waters off the Azores and the Canary Islands suggests that these archipelagos may be unique locations to study the behaviour of the enigmatic True´s beaked whale.

True´s beaked whale (*Mesoplodon mirus*) in Macaronesia

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ABSTRACT

The True´s beaked whale (*Mesoplodon mirus*, True 1913) is a poorly studied member of the Ziphiidae family. Its distribution in the northern hemisphere is thought to be restricted to the temperate or warm temperate waters of the North Atlantic, while a few stranding records from the southern hemisphere suggest a wider and antitropical distribution, extending to waters from the Atlantic coast of Brazil to South Africa, Australia and the Tasman Sea coast of New Zealand. This paper i) reports the first molecular confirmation of the occurrence of the True´s beaked whale at the southern limit of its distribution recorded in the northeast Atlantic: the Azores and Canary Islands (macaronesian ecoregion); ii) describes a new colouration for this species using evidence from a whale with molecular species confirmation; and iii) contributes to the sparse worldwide database of live sightings, including the first underwater video recording of this species and close images of a calf. Species identification was confirmed in two cases using mitochondrial DNA control region and cytochrome *b* gene markers: a subadult male True's beaked that whale stranded in El Hierro, Canary Islands, in November 2012, and a subadult male found floating dead near Faial, the Azores, in July 2004. The whale that stranded in the Canary Islands had a clearly delimited white area on its head, extending posteriorly from the tip of the beak to cover the blowhole dorsally and the gular grooves ventrally. This colouration contrasts with previous descriptions for the species and it may be rare, but it exemplifies the variability of the colouration of True´s beaked whales in the North Atlantic, further confirmed here by live sightings data. The recording of several observations of this species in deep but relatively coastal waters off the Azores and the Canary Islands suggests that these archipelagos may be unique locations to study the behaviour of the enigmatic True´s beaked whale.

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50

51 **INTRODUCTION**

52 Studies on animal distribution rely on the correct identification of the focal species during surveys.
53 This can be challenging for marine mammals that are present at the sea surface for short time
54 periods, particularly when they share colouration patterns and morphology with closely related
55 species. These challenges are exemplified by the family *Ziphiidae,* which contains 22 species of
56 beaked whales. Within ziphiids, species of the genus *Mesoplodon* have been proposed as some of
57 the most poorly known of all the genera of large mammals (Jefferson *et al*., 2015). Ziphiids dive
58 to deep waters for long periods of time, with only short breathing intervals at the sea surface (Tyack
59 *et al*., 2006; Aguilar de Soto *et al*., 2012). Furthermore, they show large intraspecific variability in
60 colouration and interspecific similarities in general morphology, including colouration patterns
61 (Mead, 2009; Jefferson *et al*., 2015). Due to the inherent difficulties in identifying beaked whales
62 to species level at sea, sightings of different ziphiid species are often pooled for analyses of survey
63 data (e.g. Moore & Barlow, 2013). This results in a loss of precision in our knowledge about the
64 distribution of individual species.
65 Beaked whales are broadly distributed in all oceans of the world. Six species of three genera can
66 be found regularly in the North Atlantic: Cuvier´s beaked whales (~ 5-7 m) and northern bottlenose
67 whales (~ 9-10 m) (*Ziphius cavirostris* and *Hyperoodon ampullatus*, respectively), and four
68 species of the genus *Mesoplodon* (~ 4.5-5.5 m): Blainville´s, Sowerby´s, Gervais' and True´s
69 beaked whales (*M. densirostris*, *M. bidens*, *M. europaeus* and *M. mirus*, respectively) (MacLeod
70 *et al*., 2006). The large size and distinctive head morphology of Cuvier´s beaked whales and
71 bottlenose whales facilitate their differentiation at sea. In addition, adult Cuvier´s beaked whales

72 often show clear colour patches in the head, dorsum and other parts of the body, and this provides
73 a further identification cue (examples at www.cetabase.info). Mesoplodonts are similar in size and
74 often difficult to identify at sea to species level. The position of the teeth along the lower jaw is
75 the most reliable cue to distinguish adult males at sea. Females and subadult males do not have
76 erupted teeth but the location of alveoli in the lower jaw, diagnostic of species identity, can be
77 uncovered during necropsy of stranded whales (Jefferson *et al*., 2015). Beak and melon size and
78 shape can be used as defining characteristics of the *Mesoplodont* species. For example, both
79 Blainville´s and Sowerby´s beaked whales have relatively long beaks. However, these species can
80 be distinguished in the field, as an arched lower jaw with protruding teeth often covered in
81 barnacles is typical of the male Blainville's beaked whales, while a bulky melon and thin long beak
82 are characteristic of Sowerby's beaked whales. In addition, the distribution of these two species
83 seems to only partially overlap, with the former preferring warmer waters than the latter (MacLeod
84 *et al*., 2006). True´s and Gervais' beaked whales can share a general grey colouration including a
85 dark eye patch and a pale ventral area in some cases; both have shorter, mostly straight beaks.
86 These two species are very difficult to distinguish at sea. The position of the teeth in the jaw of
87 males provide the most definitive cue but teeth, even when present, are not always easy to observe
88 at sea. A species-defining characteristic is the melon, which is bulbous and well defined in
89 Sowerby´s beaked whales and also in True´s beaked whales, albeit less pronounced in the later. In
90 contrast, the melon of Gervais´ and Blainville´s beaked whales slopes gently towards the beak
91 (Weir *et al*., 2004).
92 Live sightings of many beaked whale species are rare events and just a few have been made for
93 True´s beaked whales. Only three live sightings have been reported in the peer-reviewed literature
94 for the North Atlantic (Weir *et al*., 2004) and some (Tove, 1995) may be misidentified Gervais´

beaked whales. The distribution of True´s beaked whales was once thought to be restricted to temperate or warm-temperate waters of the North Atlantic. However, strandings of animals in South Africa (McCann and Talbot, 1964; Ross, 1969, 1984), Australia (Dixon & Frigo, 1994), and Brazil (Mead, 1989; de Souza *et al*., 2004), revised in MacLeod *et al*., (2006), as well as in New Zealand (Constantine *et al*., 2014), extended its known range to temperate waters of the Southern Indian and South Atlantic Oceans and the Tasman Sea. Thus, True's beaked whales have an antitropical distribution that is unique among ziphiids (Mead, 1989; MacLeod *et al*., 2006). In the North Atlantic, the southernmost limit of the distribution of True´s beaked whales is Macaronesia. The macaronesian ecoregion contains the Azores, Madeira and Canary Islands archipelagos (Spalding *et al*., 2007). For highly migratory species, it has been proposed that the southern Cape Verde archipelago should also be included in the ecoregion (Brito Hernández, 2010). In the Azores, a 3.7 m long subadult male True´s beaked whale with unerupted teeth was found dead in 2004, drifting south of the Faial-Pico channel (Silva *et al*., 2014). In the Canary Islands, a 5 m long adult male True´s beaked whale stranded in 1984 on the island of Lanzarote (Vonk & Martin, 1988). Both animals were identified as True´s beaked whales by their general morphology. The species has neither been recorded in Madeira (Freitas *et al*., 2012) nor Cape Verde archipelagos (Hazevoet *et al*., 2010).

Molecular markers are highly useful to diagnose species among cetaceans (Ross *et al*., 2003) and often more definitive than morphological characteristics that can be difficult to distinguish at sea or when animals strand in a decomposed state (e.g. Dalebout *et al*., 2002; Constantine *et al*. 2014). This is particularly applicable to beaked whales (Dalebout *et al*., 2004; Thompson *et al*., 2013). This paper reports the first occurrence of True´s beaked whales in the Canary Islands and the Azores confirmed with molecular markers. Furthermore, the whale that stranded in the Canary

Islands showed a colouration pattern that has not been previously described for this species. These findings are augmented with new live sighting data of True´s beaked whales off the Azores and the Canary Islands, suggesting that these archipelagos are potentially good areas to study the natural behaviour of this species. Sightings are supported with video and photographic material including the first underwater recording of True´s beaked whales in the wild and close images of a calf.

METHODS

Strandings and genetic analysis.

A 3.9 m long beaked whale stranded at Timijiraque, El Hierro, the Canary Islands, on 30 November 2012 (Figure 1). Observers at the beach reported that the animal might have live-stranded. The whale was identified as an immature male True´s beaked whale by its external morphology. No teeth had erupted nor were any present in the lower jaw. A 3.7 m long subadult male was found drifting south of the Faial-Pico channel, the Azores, on 11 July 2004 (Silva *et al*., 2014) (Figure 2). It was identified to species-level by its general morphology and two small non-erupted teeth in the tip of the lower jaw.

Skin samples were taken from both carcasses and preserved in 95% ethanol. Total genomic DNA was isolated using standard proteinase K digestion and phenol/chloroform methods (Sambrook *et al*., 1989) or a DNeasy kit (Qiagen). Sex was confirmed by amplification of the male-specific *SRY* gene, multiplexed with an amplification of the *ZFY/ZFX* region as a positive control (Aasen & Medrano, 1990; Gilson *et al*., 1998). In order to confirm species identification, we amplified regions of both the mitochondrial DNA (mtDNA) control region and cytochrome *b* gene using

141 polymerase chain reaction (PCR). Approximately 300 bp of the mtDNA control region were
142 amplified using primers M13dlp1.5 (Baker *et al*., 1998) and Dlp4-H (Dalebout *et al*., 2005) and
143 approximately 200 bp of the cytochrome *b* gene using CYBMF-L and CYBMR-H primers using
144 standard protocols (following Dalebout, 2002). These short fragments were targeted because the
145 tissue, and hence DNA, was degraded as samples were collected sometime after death. PCR
146 products were purified for sequencing with AMPURE^{XP} (Agilent) and sequenced with BigDye^{TM}
147 v3.1 Terminator Chemistry (Applied Biosystems) on an ABI 3130 XL. Resulting sequences were
148 aligned against other beaked whale mtDNA sequences and edited by eye in Geneious v7
149 (http://www.geneious.com, Kearse *et al*., 2012) for sequence quality. Species identification was
150 made using the DNA surveillance website, constructing a neighbour joining tree with the support
151 of 1000 bootstraps (Ross *et al*., 2003), and by comparing the target sequences with other beaked
152 whale sequences available from GenBank using blast (http://www.ncbi.nlm.nih.gov/genbank/)
153 (Dalebout et al., 2004). A 3.5 immature female True´s beaked whale was found stranded in
154 Fuerteventura, Canary Islands, in 2004 (Figure 3) and identified initially as Gervais's beaked
155 whale, but then re-classified as True´s due to the typical mouthline of the species and general
156 morphology. This female stranded with fishing gear (long-line) entangled in the caudal peduncle.
157 No tissue was preserved for molecular analysis.
158


159

160 **Figure 1**: True´s beaked whale stranded at El Hierro (Canary Islands) in 2010 showing a head
161 colouration not described previously for this species (report 6 Table 1). Photos: Baudilio Quintero.
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168 **Figure 2**:True´s beaked whale found drifting south of Pico-Faial channel (the Azores) in 2004
169 (report 4 Table 1). Photo: Mónica Silva (MARES-IMAR. UA)
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176 **Figure 3**: True´s beaked whale stranded at Fuerteventura (Canary Islands) in 2004 (report 3 Table
177 1). Photo: Vidal Martín (SECAC).
178
179 Live sightings
180 Here we report data on eight live sightings of True's beaked whales in Macaronesia (Table 1).
181 Sightings were categorised as sure (n=5) or possible (n=3) True´s beaked whales by close
182 inspection of the colouration and morphology of the whales in the photographs taken during

surveys. Teeth were observed in only one individual. This, and the poor quality of the photographs in some of the sightings, made identification challenging. Photos of live sightings were sharpened and their contrast augmented with software packages Photoshop and GIMP.

Five live sightings (four classified as sure) were recorded in the Azores. One of these sightings (report 8 in Table 1) occurred during a field cruise of the educational program Master Mint (www.master-mint.de). A group of 3 beaked whales surfaced and milled near a drifting small inflatable boat for about 10 min, breathing every 9.7 s on average. This allowed the observers to film the animals underwater (Figure 4 and Supporting Information – SI Video 1) providing high-quality images for species identification. Shorter sightings were recorded in the Azores by whale watching companies (Table 1, Figures 5 to 8).

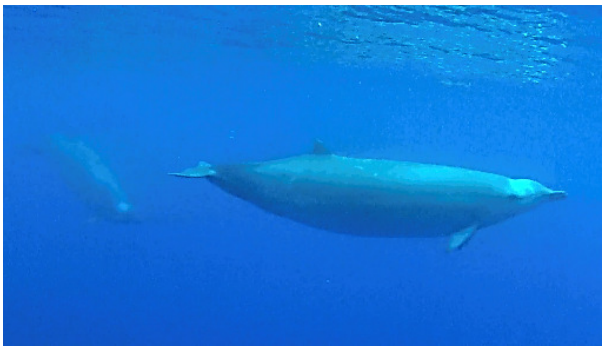

Figure 4: True´s beaked whale observed off Pico showing a pale blaze on the melon (report 8 Table 1; SI video 1). Photo: Roland Edler (Duisburg Zoo).

202 **Figure 5**: Possible True´s beaked whale observed off Pico (report 2 Table 1). Photo: Lisa Steiner
203 (Whale Watch Azores).
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210 **Figure 6**: True´s beaked whale observed off Pico showing a pale blaze on the melon (report 7
211 Table 1). Photo: Petra Szlama (CW Azores).
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214 **Figure 7**: True´s whale with calf observed south of San Miguel, Azores (report 10 Table 1). Photo:
215 Ida Eriksson (Futurismo).
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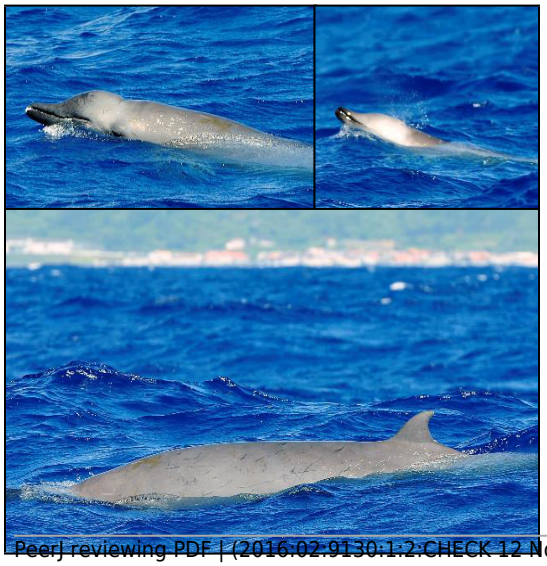
221 

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224 **Figure 8**: True´s beaked whale observed off Pico (report 12 Table 1). Photo João Quaresma
225 (Espaço Talassa).

226

227 Three live sightings were also recorded in the Canary Islands. Only one was classified as sure: a
228 group of four True´s beaked whales observed in 2009 for 8 min during a cetacean research cruise
229 performed by the Society for the Study of Cetaceans in the Canary Islands Archipelago (SECAC,
230 Figure 9, report 5 in Table 1). The whales were estimated to measure 3.5 to 4.5 m in length; the
231 largest whale did not present erupted teeth and was tentatively classified as an adult female. Shorter
232 sightings classified as possible True´s beaked whale were recorded by the Cetacean and Seabird
233 Sighting Network of the Canary Islands "CETAVIST" (www.aviste.me) (Figures 10 and 11;
234 reports 9 and 11 in Table 1). CETAVIST undertook 1300 surveys onboard passenger ferries from
235 December 2012 to October 2016; these were performed by volunteer observers resulting in a
236 heterogeneous observation effort in the different inter-island channels of the archipelago.

237

238

239 **Figure 9:** True´s beaked whale observed off Lanzarote (report 5 Table 1). Photo: Vidal Martín
240 (SECAC).

241

242

243 **Figure 10:** Possible True´s beaked observed in the Canary Islands (report 9 Table 1). Photos:

244 Cristel Reyes (ULL).

245

246

247 **Figure 11**: Possible True´s beaked whales observed in the Canary Islands (report 11 Table 1).

248 Photos: Antonio Portales (Cetavist, ULL).

249

250 Ethics

251 Samples of two dead whales in the Canary Islands and the Azores were gathered for genetic

252 analysis with authorization obtained from the Cabildo Insular of El Hierro (permit number

253 7021/12_dic_2012) and from the Government of the Azores (permit number 4/CN/2004, issued

by the Environment Directorate of the Azores). The sightings data from SECAC were gathered under research permit number 659 125 MAOT/15202 from the Spanish Ministry of Agriculture and Environment (MAGRAMA). Sightings gathered opportunistically from regular ferry and permitted whale watching/educational boat operations did not require specific research ethics authorisations or government permits.

RESULTS

Strandings and genetic analysis.

Genetic sex identification confirmed that both the whale that stranded on El Hierro (Canary Islands) and the whale found drifting in the Faial-Pico channel (Azores) were males. Robust support placed both the El Hierro query sequence (mtDNA control region: 98% bootstrap support; cytochrome *b* gene: 94% bootstrap support) and the Azores' query sequence (mtDNA control region: 97% bootstrap support; cytochrome *b* gene: 94% bootstrap support) in a species-specific clade with True's beaked whale sequences using DNA surveillance.

Furthermore, both the mtDNA control region and cytochrome *b* sequences from both the El Hierro and Azores' males closely matched GenBank sequences identified as True's beaked whales (BLAST accessed May and November 2015, respectively). True's beaked whale sequences from whales that had stranded on the Atlantic coast of the U.S.A. (accession numbers U70465.2 and AY579525.1) were the closest matches to both the El Hierro sample (U70465.2: 99% sequence identity, E-value 9e-153 and AY579525.1: 98% sequence identity, E-value 4e-151) and the Azores' sample mtDNA control region sequences (U70465.2: 98% sequence identity, e-value 7e-152 and AY579525.1: 100% sequence identity, E-value 7e-147). The two top matches against the cytochrome *b* sequence for both samples were: i) a True´s beaked whale that stranded on the

277 Atlantic coast of the U.S.A., accession number AY579551.1 (El Hierro sample: 95% sequence
278 identity, E-value 3e-70; Azores' sample: 99% sequence identity, E-value 3e-112); and ii) a
279 sequence from a True´s beaked whale that stranded in New Zealand, accession number
280 KF435028.1 (El Hierro sample: 94% sequence identity, E-value 3e-61, Azores' sample: 95%
281 sequence identity, E-value 4e-81). The sequences generated in this study have been archived on
282 GenBank (accession numbers Azores CytB: KX375801, KX375802; Azores Dlp: KX150446; El
283 Hierro CytB: KX375803 and El Hierro Dlp KX150445).
284
285 Live sightings
286 Photographs and a video recording from a live sighting of True´s beaked whales from across
287 Macaronesia revealed individuals with a diagonal pale blaze on the head. Data from report 8 (Table
288 1, Figure 4 and SI video 1) revealed that this blaze extends dorsally from behind the blowhole to
289 the top of the melon and reaches ventrally to the eye and the start of the mouthline. No obvious
290 size differences were observed among the individuals in the group and none of the three whales
291 had erupted teeth. The same pale blaze colouration pattern on the head was present on other whales
292 observed in different encounters off the Azores, including a female-calf pair where both whales
293 showed the pale blaze on their heads (Figure 7; report 10 in Table 1) and a breaching female or
294 subadult male (Figure 6; report 7 in Table 1). In contrast, this pale-coloured blaze was not evident
295 on other individuals identified as possible True´s beaked whales in the same archipelago (Figure
296 5; report 2 in Table 1).

297 Table 1: Reports of strandings (Strand.) and live sightings (Sight.) of True´s beaked whales in the
298 Azores and the Canary Islands (CI) with information on location, date, number of whales and
299 certainty on the identification ("sure", achieved using molecular genetic markers (G) or
300 morphology (M), or "possible"). Sightings classified as "possible" may be of True´s or Gervais'
301 beaked whales. The entities that gathered the reports were: SECAC: Society for the Study of
302 Cetaceans in the Canary Islands Archipelago; WW Azores: Whale watch Azores; UA: University

303 of the Azores; ULL: University of La Laguna; CW Azores whale watch; Master Mind educational
304 program; Futurismo whale watch; Espaço Talassa whale watch. # Sighting with underwater video;
305 * sighting with small calf.
306

Report	Location	Date (entity)	Lat, N	Lon, W	Depth	nº	behaviour	certainty
1. Strand.	Lanzarote, CI	23/03/1984 (SECAC)	28.984	13.5	-	1	stranded	sure (M)
2. Sight.	Pico, Azores	07/09/1994 (WW Azores)	38.36	28.3767	1200	3	travelling	possible (M)
3. Strand.	Fuerteventura, CI	06/06/2004 (SECAC)	28.1336	14.24	-	1	stranded	sure (M)
4. Strand.	Faial, Azores	11/07/2004 (UA)	38.47	28.64	-	1	drifting dead	sure (G)
5. Sight.	Lanzarote, CI	29/09/2009 (SECAC)	28.8346	13.5915	1100	4	travelling	sure (M)
6. Strand	El Hierro, CI	30/11/2010 (ULL)	27.268	17.914	-	1	stranded	sure (G)
7. Sight.	Pico, Azores	31/07/2010 (CW Azores)	38.3396	28.3573	1300	3	breaching	sure (M)
8. Sight.	Pico, Azores	05/05/2013 (MasterMind)	38.28	28.341	1600	3#	milling	sure (M)
9. Sight.	Offshore. CI	27/09/2013 (ULL)	28.31	14.99	2500	2	breaching	possible (M)
10. Sight.	S. Miguel, Azores	07/07/2015 (Futurismo)	37.6386	25.5101	600	2*	travelling	sure (M)
11. Sight.	Offshore. CI	11/07/2015 (ULL)	28.4503	14.7048	2500	2	breaching	possible (M)
12. Sight.	Pico, Azores	10/08/2016 (EspaçoTalassa)	38.3242	28.3517	1250	2	travelling	sure (M)

307

308 A ‘white mask’ was observed only in the male subadult whale that stranded on El Hierro, the
309 Canary Islands (Figure 1 and report 6, Table 1). This white colouration covering the whole anterior
310 part of the head, including the melon, beak and lower jaw, has not been previously described for
311 this species. The live sightings in the Canary Islands (Figures 9, 10, 11; reports 5, 6, 9 in Table 1)
312 showed a grey colouration pattern in the melon and pale lower jaw consistent with previous
313 descriptions of the species. The two sightings classified as “possible” in this archipelago witnessed
314 whales breaching in repetition (Figures 10, 11). This behaviour has been observed previously on
315 True´s beaked whales (Figure 12) but was observed also on Gervais’ beaked whales in the Canary

316 Islands (Figure 13, Supplementary video 2). Given the morphological and behavioural similarities
317 between these two species (Figure 14), we cannot exclude that whales classified as possible True´s
318 beaked whales in this paper were, in fact, Gervais' beaked whales.
319

320

321 **Figure 12**. True´s beaked whale breaching at the Bay of Biscay. Photos. Dylan Walker (WCA).
322

323

324 **Figure 13**: Gervais´ beaked whales observed south of La Gomera in the Canary Islands (SI Video
325 2). Photos: Michael Scheer (M.E.E.R)
326

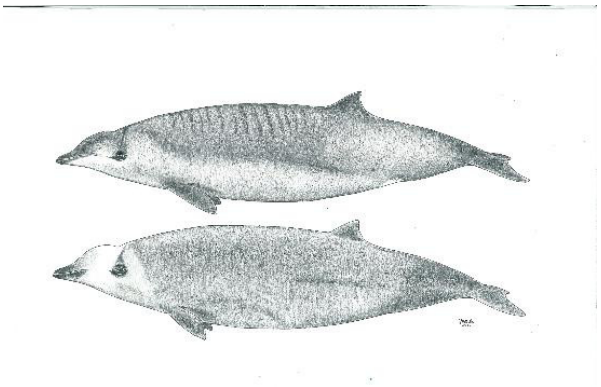

Figure 14. Schematic drawing showing differences between Gervais' (above) and True´s (below) beaked whales. Note the more pronounced melon of True´s beaked whales and the lines in the dorsum of Gervais´ beaked whales. These lines are not present in all individuals of Gervais´ beaked whales, but have never been observed in True´s beaked whales. The genital white patch shown here in Gervais' may appear in True´s beaked whales also. Drawing by Vidal Martín (SECAC).

DISCUSSION

New records of data-scarce species, such as True´s beaked whales, are highly valuable in increasing our knowledge about the morphology, behaviour and distribution of these species. True´s beaked whales in the North Atlantic are described as greyish in colouration. Some individuals show a dark eye mark and a dark blaze in the upper part of the body from behind the blowhole to past the dorsal fin; some animals may show a pale ventral colouration, sometimes extending to the lower jaw, while other animals may have a pale blaze on their melon (Weir *et al*., 2004; Jefferson *et al*., 2015). Contrasting colour patterns have been found for individuals from the southern hemisphere. For example, a female stranded in South Africa showed a whitish dorsal colouration including the dorsal fin and extending to the tail peduncle (Ross, 1984).

346 The True´s beaked whale stranded in El Hierro (Canary Islands) had a clearly delimited white
347 mask covering the anterior part of the head from the blowhole and the gular grooves to the rostrum
348 (Figure 1). This pigmentation pattern does not seem to be the result of a post mortem
349 discolouration. In fact, beaked whale colouration tends to darken after stranding because of
350 decomposition, a phenomenon that does not explain the striking white colouration pattern on the
351 head of the whale stranded on El Hierro (A. van Helden, T. Pusser, pers. comm.), but might
352 increase the contrast between the white head and the grey body after stranding. However, the
353 animal might have stranded alive and was in a fresh state when the photographs were taken. This
354 would suggest that the contrasting white cephalic patch observed in the specimen stranded in El
355 Hierro was present in the living animal. It cannot be dismissed that this unique colour pattern might
356 be an ontogenetic trait. However, this pattern was not observed on the calf nor on any of the other
357 individuals reported in this paper, some of which are likely to be subadults as the whale stranded
358 at El Hierro.
359 The white head colouration described for True´s beaked whales increases the probability of
360 confusing this species with Cuvier´s beaked whales in sightings when the beak is not observed.
361 Cuvier´s beaked whales often have a white colouration on their rostrum and frontal part of the
362 head. Moreover, the female True´s beaked whale observed with a calf off the Azores (Figure 7)
363 had a neck and melon colouration very similar to one of the typical head and neck colour
364 patterns shown by Cuvier´s beaked whales (e.g. www.cetabase.info whale code ZCH15
365 http://bit.ly/1sbuzVA), underlining the possibility of misidentifying these two species when the
366 beak is not observed.
367 In contrast with its mother, the calf in Figure 7 shows a very distinctive head colouration with a
368 white diagonal band observed in other live sightings of True´s beaked whales. The light colour

369 blaze on the melon may be a common feature for True's beaked whales inhabiting the North
370 Atlantic: it has been observed off the Azores in whales of different age classes, from a calf to
371 adult or subadult animals, and previously in the eastern North Atlantic (Weir *et al*., 2004). A
372 similar light coloured head blaze has been observed in True´s beaked whales of both sexes and
373 with different sizes in the western North Atlantic, albeit the pale colour disappears rapidly in
374 stranded whales (T. Pusser, pers. comm.). This suggests that the pale blaze in the melon may
375 pass unnoticed in strandings unless stranded animals are very fresh.
376 In contrast with the colouration patterns described above, some True´s beaked whales in the North
377 Atlantic tend to be more uniformly grey, although they may have a small pale mark in the genital-
378 anus area (http://vertebrates.si.edu/mammals/beaked_whales/pages/mmi/mmi_sp_pg7.htm) (Weir
379 *et al*., 2004). A whale with this mark was photographed at the Bay of Biscay (Figure 12) and
380 identified as a True´s beaked whale thanks to the consistent location of white points at the tip of
381 the lower jaw in several successive photographs, strongly suggesting that these points are the teeth
382 of the whale. A white genital patch, similar to that described in Trues's beaked whales, were
383 observed in a live sighting and strandings of Gervais´ beaked whales in the Canary Islands (Figure
384 13; unpublished data from the Canary Islands Stranding Network by V. Martín and M. Carrillo).
385 True´s and Gervais' beaked whales in the North Atlantic have several similar morphological
386 features that challenge their differentiation at sea: the relatively short beak, mostly straight
387 mouthline, overall colouration and dark eye patches. Figure 14 shows a schematic view of the
388 morphological differences between True´s and Gervais' beaked whales, including a straighter
389 mouthline in True´s than in Gervais´, slightly upwards in True´s; a pale band on the melon of
390 True´s (not always); and a distinctive pattern of pale/dark stripes (or a pale patch) frequent in the
391 dorsum of Gervais' beaked whales. Also, a cue to differentiate these species is the pronounced and

392 rounded melon of True´s beaked whales, contrasting with the relatively more flat-topped melon of
393 Gervais' beaked whales sloping gently towards the beak (Figures 13, 15). However, this may be
394 difficult to judge from photos taken from different perspectives. When present and visible, scarring
395 patterns can also be used to distinguish between species due to the different position of the erupted
396 teeth in males. While True's beaked whales show parallel and linear scars with small gaps between
397 adjacent scars, Gervais' whales are expected to have single linear scars (Weir *et al*., 2004). Parallel
398 scars are visible in Figure 5 (report 2 in Table 1), suggesting that these animals were indeed True´s
399 beaked whales.
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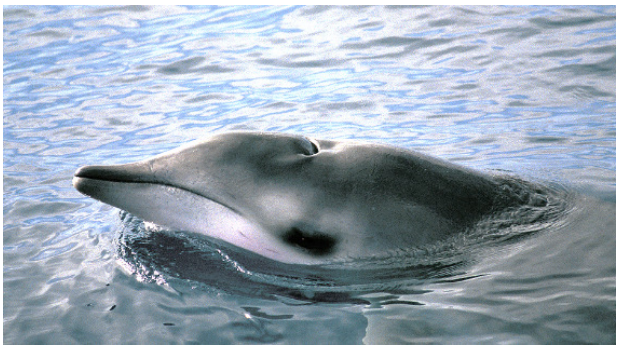

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402 **Figure 15**. Gervais´s beaked whale observed off Tenerife (Canary Islands). Note the head
403 morphology of this whale in comparison with True´s beaked whales. Photo: Sergio Hanquet.
404
405 Variability in colour patterns is not surprising for ziphiids. These animals often undergo
406 ontogenetic changes in colouration, and this trait can vary even among individuals of the same size
407 and sex class (Mead, 2009). For this reason, molecular markers providing definite identification
408 among ziphiid species (Dalebout *et al*., 2004; Thompson *et al*., 2013) should be used, where
409 possible, to confirm identification obtained using morphological cues (Dalebout *et al*., 2002;
410 Constantine *et al*., 2014). Intraspecific variability in colouration for many Ziphiids, as well as

411 similarities in general colouration patterns, size/morphology and behaviour among most
412 *Mesoplodon* species, challenge taxonomic identification at sea. This may cause a bias when
413 assessing the relative abundance of *Mesoplodon* species in the North Atlantic. Animals for which
414 recognition is challenging will be often classified during surveys as unidentified beaked whales,
415 while recognizable animals will be classified to species level. This will result in an apparent lower
416 relative abundance of species difficult to recognize at sea.
417 Given the presence of True´s beaked whales in the Azores and the Canary Islands, it would be
418 expected that this species also occurs in the area between both archipelagos, including Madeira.
419 However, knowledge about the distribution of beaked whales in the eastern North Atlantic Ocean
420 is limited by the relative scarcity of offshore cetacean surveys in Macaronesia. Strandings and
421 sightings of True´s and possible True´s beaked whales in the Azores and Canary Islands occurred
422 from March to November (Table 1). However, sample size is too low to infer any conclusion about
423 seasonality in occurrence. The scarcity of sightings of True´s beaked whales may reflect a low
424 abundance and/or a general preference for deep waters far from the slope where limited survey
425 effort has been made. The latter is supported by the near lack of sightings of True´s beaked whales
426 in relatively nearshore deep waters along the slope of the Canary Islands where other beaked whale
427 species are found routinely. Seasonal surveys in coastal deep waters off the Canary Islands up to
428 1800 m depth in the last decade have recorded only one sighting of True´s beaked whales, while
429 Cuvier´s, Blainville´s and Gervais´ beaked whales are observed or strand year round (Aguilar de
430 Soto, 2006; Martín, 2011; Arranz *et al*., 2014). Most sightings of True´s beaked whales reported
431 in this paper occurred at deeper depths (Table 1) although not far from the slope (Figure 16),
432 showing that True´s beaked whales visit deep waters near the coast in some areas. The closest
433 sighting to the coast included a small calf (report 10 Table 1). Preference of mother-calf pairs for

434 coastal deep waters in oceanic archipelagos has been suggested for other species such as
435 Blainville´s beaked whales (Claridge 2013).
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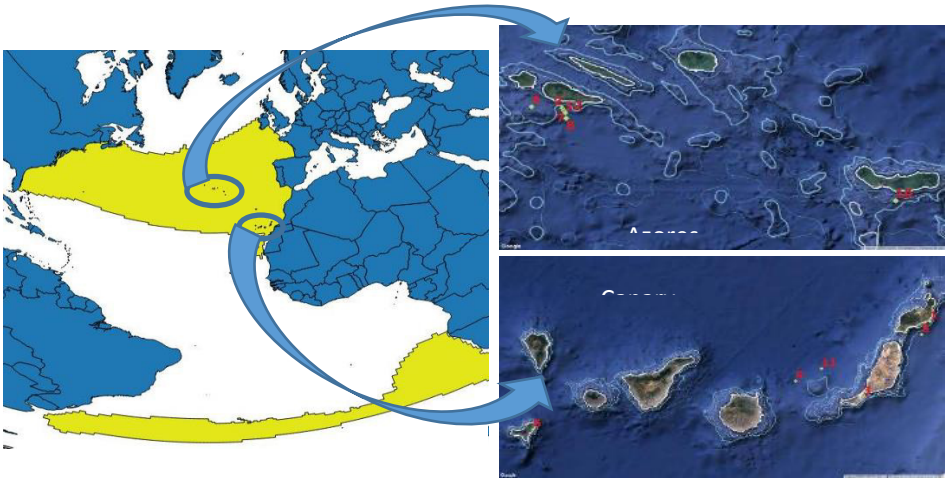

444 **Figure 16**: Right: locations of the reports of True´s beaked whale included in this paper. The
445 1000m depth contour is marked as a thicker light blue line. Left: distribution of the species in the
446 Atlantic, courtesy of the Digital Beaked Whale Atlas of GIS in Ecology, based on data from
447 MacLeod et al. (2006)
448 (http://www.gisinecology.com/Digital_Beaked_Whale_Atlas/Accessing_Data_From_The_DBWA.htm)
449
450

451 The relative abundance of live sightings of True´s beaked whales in deep coastal waters off the
452 Azores, and to some extent off the Canary Islands, suggests that these archipelagos could be ideal
453 areas to research True´s beaked whales in the wild. This is relevant because the identification of
454 hot-spots where some species of beaked whales are found with reliability has provided most of our
455 current knowledge about the natural behaviour of ziphiids (Hooker *et al*. 1999; Tyack *et al*., 2006;
456 Baird *et al*., 2006; Minamikawa *et al*., 2007; Aguilar de Soto *et al*., 2012; Claridge 2013).

The disjointed global distribution of True´s beaked whales has led some authors to suggest that there may be some degree of genetic isolation between the populations of the southern and northern hemispheres (MacLeod *et al*., 2006; Dalebout *et al*., 2007). These authors propose that more research is required in order to assess if the northern and southern hemisphere populations might represent different species. The precedents for this include cetacean species that were thought to have an anti-tropical distribution and were finally separated as different species: Hector´s and Andrews´ beaked whales. *M. hectori* and *M. bowdoini*, were separated from *M. perrini* and *M. carlhubbsi*, respectively. Furthermore, right whales (*Eubalaena spp.*) were separated into three species (Rosenbaum *et al*., 2000): the southern right whale (*E. australis*), with circumpolar distribution in the southern hemisphere, and the North Atlantic (*E. glacialis*) and North Pacific (*E. japonica*) right whales. The results of the genetic analysis shown here suggest a potential genetic structure with a phylogeographic pattern for True´s beaked whales, as the sequences from the Canary Islands and Azores' matched most closely those True's sequences on GenBank from the North Atlantic. However, more data are required to test this hypothesis. Given adequate sampling, a global analysis of connectivity would provide useful in understanding gene flow among the seemingly disparate areas of distribution of True´s beaked whales worldwide.

ACKNOWLEDGEMENTS

Thanks to the Insular Government (Cabildo) of El Hierro for providing access to the stranded True´s beaked whale, and to the Government of the Canary Islands and MAGRAMA for their support to the Canary Islands cetacean stranding network. Thanks also to the ferry companies Armas, Fred Olsen and Trasmediterránea for embarking observers of the CETAVIST sighting net (a project supported by Fundación Biodiversidad-MAGRAMA from 2014 to 2016), and thanks to

the enthusiastic work of these volunteering observers. Thanks to Sergio Hanquet and to Antonio Portales for their photographs of Gervais´ and probable True´s beaked whales, respectively, observed off the Canary Islands. Also, thanks to T. Sneddon and D. Steel for help in the laboratory and to A. van Helden, T. Pusser, M. Arbelo, B. Brederlau, E. Pérez-Gil, J. Mead and R. Pitman for helpful comments on the reports of this paper. We are grateful to researchers in Madeira (Filipe Alves, Luis Freitas) and Cape Verde (Vanda Marques Monteiro, Evandro Lopes and Cornelis Hazevoet) for their information on True´s beaked whales from these archipelagos. Thanks also to the academic editor J. Reimer and to K. Thompson and an anonymous reviewer for their positive comments to the paper.

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609
610
611 **Supporting information: video captions**
612 S1. Underwater video of True´s beaked whales recorded off the Azores by R. Edler within the
613 Master Mint program.
614
615 S2. Gervais´ beaked whales in a group of four whales breaching repetitively in the Canary Islands,
616 recorded by Roland Gocker (M.E.E.R.).

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