Zootaxa 3956 (1): 029–055
www.mapress.com/zootaxa/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

http://dx.doi.org/10.11646/zootaxa.3956.1.2
http://zoobank.org/urn:lsid:zoobank.org:pub:A803164C-9B49-4744-BE99-286E75648002

Merluccius tasmanicus Matallanas & Lloris 2006 is a junior synonym of *M. australis* (Hutton 1872) (Gadiformes: Merluciidae) based on morphological and molecular data

MARIANA Y. DELI ANTONI[1,3], SERGIO M. DELPIANI[1], ANDREW L. STEWART[2], MARIANO GONZÁLEZ-CASTRO[1] & JUAN M. DÍAZ DE ASTARLOA[1]

[1]*Grupo de Biotaxonomía Morfológica y Molecular de Peces (BIMOPE), Instituto de Investigaciones Marinas y Costeras (IIMyC, CONICET-UNMdP), Funes 3350, Mar del Plata 7600, Argentina. Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Av. Rivadavia 1917, CABA C1033AAJ, Buenos Aires, Argentina.*
E-mail: deliantoni@mdp.edu.ar; sdelpiani@mdp.edu.ar; gocastro@mdp.edu.ar; astarloa@mdp.edu.ar
[2]*Museum of New Zealand Te Papa Tongarewa, PO Box 467, Wellington, New Zealand. E-mail: AndrewS@tepapa.govt.nz*
[3]*Corresponding author*

Abstract

The high intraspecific variation among and the conservative external morphology of *Merluccius* spp. have resulted in serious identification difficulties. Four hundred and twenty fresh and preserved specimens of *Merluccius* were analyzed, including the type series of *Merluccius australis*, *M. tasmanicus* and *M. hubbsi*; specimens of *M. hubbsi* from Argentina, Brazil and Uruguay, and individuals of *M. australis* from Argentina and New Zealand were examined. The nomenclatural status of the type specimens of *M. australis* is discussed and the designation of a lectotype and a paralectotype is proposed. The comparative study of morphology, meristic, traditional and landmark-based morphometry, both external and internal, and through DNA-based Barcoding molecular tools demonstrates that *Merluccius tasmanicus* is a junior synonym of *Merluccius australis*. Meristic and morphometric characters of types of *M. tasmanicus* completely overlap those of *M. australis*, whereas *M. hubbsi* show fewer scales along the lateral line, total vertebrae, second dorsal and anal-fin rays. A trend of a longer snout and wider head in *M. australis* and *M. tasmanicus,* and larger eyes and longer pelvic fins, in *M. hubbsi* was observed. While discriminant characters were found in the internal elements (hyomandibula, urohyal and sagitta otolith) between *M. hubbsi* and *M. australis*, none were observed between *M. australis* and those reported for *M. tasmanicus*. DNA barcoding analyses found no evidence of the existence of other species of *Merluccius* besides *M. hubbsi* and *M. australis*.

Key words: *Merluccius* spp., meristics, morphometry, DNA barcoding, Argentina, New Zealand, lectotype, paralectotype

Introduction

The Genus *Merluccius* is one of the most heavily exploited demersal fishes worldwide (Whitaker 1980; Inada 1981*a*; Cohen *et al*. 1990; Pitcher & Alheit 1995; Moyle & Cech 1996; Lloris *et al*. 2003). In Argentinean waters, *Merluccius* spp. have been one of the most valuable fishery resource (Bezzi & Dato 1995), representing about 40% of the total fish catch in recent years (MAGyP 2010; 2011), and currently regarded as overexploited (FAO 2010; Vaz-dos-Santos *et al*. 2010). The New Zealand hake fishery has traditionally consisted of bycatch of the much larger hoki (*Macruronus novaezealandiae* (Hector) fishery (Colman 1995), but in recent years it has also become an important target fishery (Ballara 2012).

The correct specific identification is essential for most biological studies (Vecchione & Collette 1996; Lleonart *et al*. 2006), and is necessary to design effective fishery management strategies (Stauffer & Kocovsky 2007). Incorrect identifications, the use of outdated names, or the application of misleading names can have considerable economic and environmental consequences (Fischer 2013). Several detailed taxonomic studies of merluccids have been published (Inada 1981*a*; Cohen *et al*. 1990; Lloris *et al*. 2003). Nevertheless, the high intraspecific variation

Accepted by W. Holleman: 11 Mar. 2015; published: 7 May 2015

among and the conservative external morphology of the species have also resulted in identification (Inada 1981*a*; Lloris *et al*. 2003) and differentiation difficulties, usually of two or more congeneric species from the same region (Lloris & Matallanas 2003; Lloris *et al*. 2003). These problems are mainly due to many characters traditionally employed to classify merluccids having overlapping values across several species (Ho 1989; Lloris *et al*. 2003) or showing a high variability, depending on the degree of stomach fullness, sexual maturity (Svetovidov 1948), size (Ginsburg 1954) or state of preservation (Cousseau & Cotrina 1980). This can be compounded by the apparent distinctions between some species that are based on small numbers of specimens examined (Svetovidov 1948).

The genus *Merluccius* in Argentine waters has long been recognized as comprising two nominal species: *Merluccius hubbsi* Marini 1933 and *Merluccius australis* (Hutton 1872) (Cousseau 2010). However, two new species were described and/or cited for Argentinean Patagonian waters *Merluccius patagonicus* Lloris & Matallanas 2003 and *Merluccius tasmanicus* Matallanas & Lloris 2006. Díaz de Astarloa *et al*. (2011) analyzed the internal and external morphology and head/body shape of *M. patagonicus*, *M. hubbsi* and *M. australis* and found no evidence supporting the validity of *M. patagonicus*, concluding that this species was a junior synonym of *M. hubbsi*.

Matallanas and Lloris (2006) redescribed *M. australis* and described *M. tasmanicus* sp.n. from New Zealand waters, differentiating them mainly by non-overlapping morphometric characters (*i.e.* body depth and eye diameter) and secondarily on morphological features (*i.e.* shape of the upper profile of the head; eye position; shape of the lateral line, and length of the pectoral fin). Based on these diagnostic characters the authors stated that many specimens of *M. tasmanicus* off New Zealand, Argentinean and Chilean waters have been misidentified as *M. australis* by several authors (Waite 1911; Norman 1937; Ginsburg 1954; Cousseau & Perrotta 2004; Inada 1981*a*; Cohen *et al*. 1990; Lloris & Matallanas 2003). In this context Matallanas and Lloris (2006:198) stated the holotype of Ginsburg's *Merluccius polylepis* belonged to *M. tasmanicus* which would automatically make their new species a junior synonym of *M. polylepis*. They consider *M. polylepis* to be a junior synonym of *M. australis* but a valid subspecies *polylepis* (Lloris *et al*. 2003:22, Matallanas & Lloris 2006:197). Following their taxonomy, this would make *M. tasmanicus* a junior synonym of *M. australis*. Eschmeyer (2014), in the online *Calatog of Fishes*, states that the description of *Merluccius tasmanicus* was unwarranted, but did not provide any supporting information. A complete taxonomical study of *M. tasmanicus* types compared with hake species that share the same geographic area was needed to determine the validity of a new species of *Merluccius* in New Zealand and Argentina waters.

The purpose of this paper was to assess the specific validity of *M. tasmanicus* and its taxonomic relationship with *M. australis* (Argentina and New Zealand) and with *M. hubbsi*. To achieve this, we employed a combined comparison of morphometric, meristic, osteological and genetic characters of type and non-type specimens.

Material and methods

Sample collection. Fresh specimens of *Merluccius* were collected between the years 2008–2009, from the Argentine Sea (36°45' to 55°04' S), from 80 to 500 m depth, on board the R. V. *Dr. Eduardo L. Holmberg* of the Instituto Nacional de Investigación y Desarrollo Pesquero (INIDEP) (EH-02/08, EH-04/09 and EH-02/09), and by the commercial fishing fleets of ports of Mar del Plata and Ushuaia (Figure 1). Samples comprised 229 fresh individuals of *M. hubbsi* and 163 of *M. australis*. All the types (the holotype and three paratypes) of *M. tasmanicus*, the six paratypes of *M. hubbsi* and the lectotype and paralectotype (herein designed) of *M. australis* were analyzed. In addition, 16 alcohol-preserved specimens from Argentina, Brazil, Uruguay and New Zealand of *M. hubbsi* and *M. australis*, were included in the analyses.

Alcohol-preserved material. Institutional abbreviations are as follows (Sabaj Pérez 2013): UMMZ, University of Michigan Museum of Zoology, Ann Arbor, Michigan; USNM, United States National Museum, Washington D.C; BMNH, British Museum (Natural History), London, UK; MNHN, Muséum national d'Histoire naturelle, Paris, France; MACN, Museo Argentino de Ciencias Naturales "Bernardino Rivadavia", Buenos Aires, Argentina; MOVI, Museu Oceanográfico do Vale do Itajaí, Santa Catarina, Brazil; NMNZ, Museum of New Zealand Te Papa Tongarewa, Wellington, New Zealand.

Merluccius hubbsi. UMMZ 95461, one specimen (730 mm SL), paratype, Buenos Aires, Argentina. USNM 77291, three specimens (159–178 mm SL), paratypes, 45°22' S, 64°20' W, Argentina. BMNH 1935.8.29.14–15,

two specimens (96–140 mm SL), paratypes, 36° 47' S, 122° 09' W. MNHN 1975-0254, one specimen (102 mm SL), 34°30' S, 52°51' W, Uruguay, 33 m depth, Dec 1961. MNHN 1975-0245, six specimens (189–267 mm SL), 34°30' S, 52°51' W, Uruguay, 33 m depth, Dec 1961. MNHN 1975-0250, one specimen (228 mm SL), 35°0' S, 55°0' W, Uruguay, 7 m depth, Dec 1961. MNHN 1975-0249, one specimen (195 mm SL), 23°25' S, 44°36' W, Brazil, 36 m depth, Dec 1961. MNHN 1975-0252, one specimen (174 mm SL), 24°6' S, 45°28' W, Brazil, 48 m depth, Dec 1961. MNHN 1989-0376, one specimen (235 mm SL), 21°34' S, 40°6' W, 262 m, Brazil, 10 May 1987. MNHN 1989-0377, one specimen (141 mm SL), 23°36' S, 42°1' W, Brazil, 200 m depth,1 Jun 1987. [The holotype, possibly conserved in UMMZ or in MACN, is presumed lost (Eschmeyer 2014)].

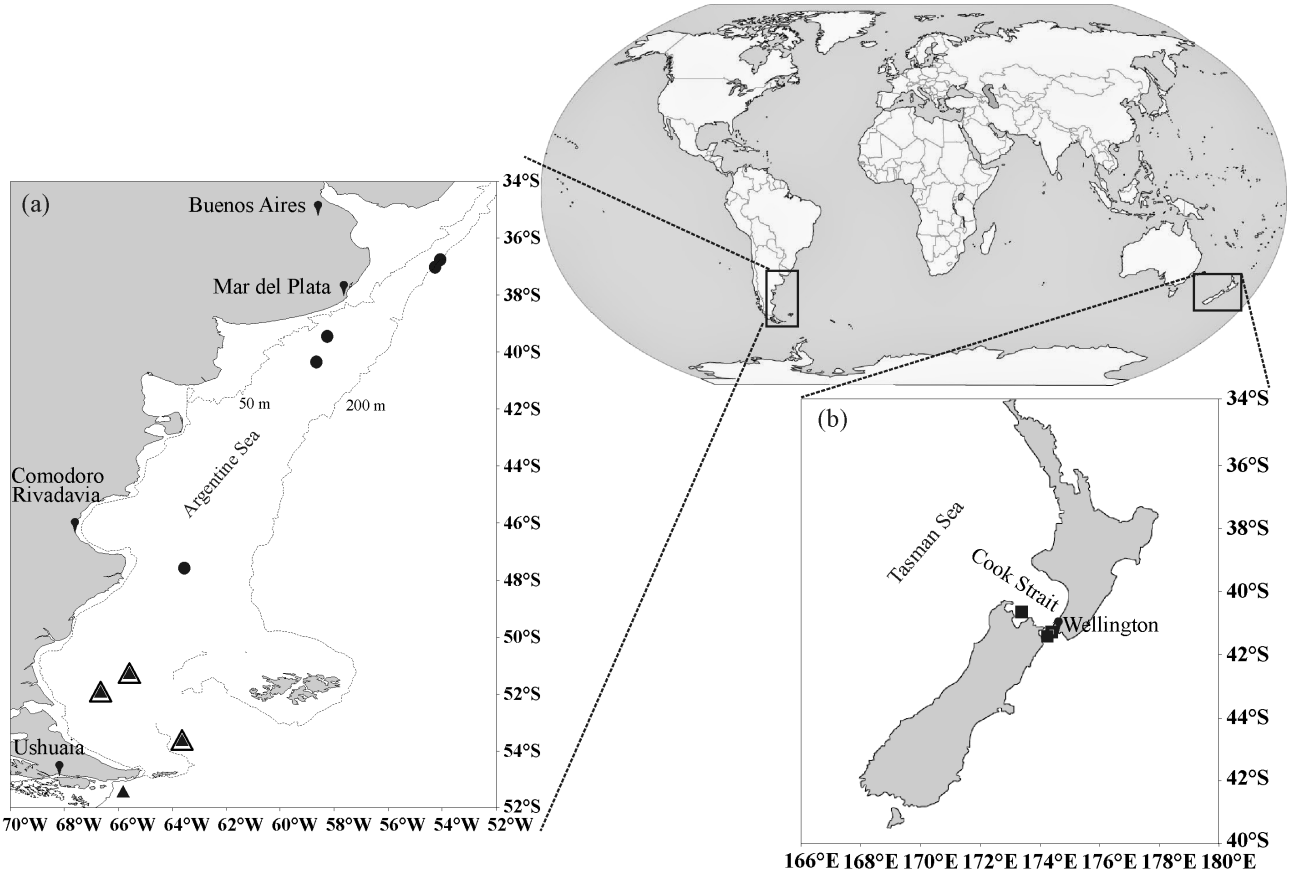

FIGURE 1. Map showing the localities from (a) Argentinean and (b) New Zealand waters where specimens of *Merluccius* were collected: solid circle, specimens of *Merluccius hubbsi*; solid triangle, specimens of *M. australis*; open triangle, specimens of *M. australis* with characters of *M. tasmanicus*, solid square, holotype of *M. tasmanicus* and open squares, paratypes of *M. tasmanicus*.

Merluccius australis. BMNH 1872.4.26.8, one specimen (323 mm SL), lectotype (herein designated), Wellington, New Zealand. BMNH 1905.11.30.38, one specimen (84 mm SL), paralectotype (herein designated), Wellington, New Zealand. BMNH 1886.11.18.79, one specimen (312 mm SL), New Zealand. MOVI 27492-93, two specimens (370–405 mm SL), 46°03' S, 166°20' E, Chalkey Intel, Fiordland, New Zealand, 370 m depth, 23 Oct 1982. NMNZ P.13122, one specimen (382 mm SL), 46°03' S, 166°20' E, 370 m, Chalkey Intel, Fiordland, New Zealand, 23 Oct 1982. [Holotype presumed lost and the syntype status of the Hutton´s specimens are discussed below].

Merluccius tasmanicus. NMNZ P.5566, one specimen (343 mm SL), holotype, 40°52' S, 173°08' E, Tasman Bay, New Zealand, 36 m depth, 7 Jan 1972. MOVI 27490-91, two specimens (237–249 mm SL), paratypes, 41°44' S, 174°16' E, Cape Campbell, New Zealand, 36 m depth, 20 Nov 1952. NMNZ P.3963, one specimen (377 mm SL), paratype, 41°30' S, 174°30' E, Cook Strait, New Zealand, 91 m depth, Mar 1964. [The paratype MOVI 27491 was partially dissected (Matallanas & Lloris 2006) and poorly preserved, therefore only some meristic and morphological characters could be obtained].

Meristic counts. Meristic standards followed Inada (1981*a*) and Lloris *et al.* (2003) and were taken from the left side of each specimen (Table 1). The lateral line scale counts began with the scale immediately posterior to the lateral line arch above the pectoral-fin rays and ended with the scale at the base of the caudal fin. Gill raker counts did not include rudiments. Vertebral counts of alcohol-preserved specimens were obtained from radiographs, and those of fresh specimens were taken by dissection. Total number of vertebrae was counted and includes the hypural plate. The abdominal vertebrae (those without haemal spines) were divided into two parts: the first vertebrae without parapophyses, the cervical vertebrae, and the other vertebrae that bear well developed parapophyses. Abbreviations of meristic features are given in Table 1. To assess patterns of multivariate meristic variation Principal Component Analysis (PCA) was used. Vertebral counts were not considered in the PCA analysis because it was not possible to obtain them from the types of *M. hubbsi* and *M. australis*. To correct the linearity between the variables, a square-root transformation was used and PCA was performed based on the correlation matrix (Quinn & Keough 2002). Differences in counts of meristic characters were tested using the nonparametric test Kruskal-Wallis ANOVA by rank (Sheskin 2004), followed by pairwise comparisons with Dunn's test (Zar 1984).

External morphology and morphometric measurements. The external morphology of fresh and preserved specimens were analyzed, covering the diagnostic qualitative characters used to differentiate species of *Merluccius* by Cousseau and Cotrina (1980), Lloris and Matallanas (2003) and Matallanas and Lloris (2006): body shape; shape of the upper profile of the head; position of the upper edge of the eye with respect to the dorsal profile of the head; condition of the dorsal border of the opercular membrane in relation to the lateral line; lateral line shape over the pectoral fin and in the caudal region; position of the lateral line between the dorsal and ventral body profiles, on the caudal region; relation between the pectoral fin distal extreme and the anal fin origin in specimens longer than 400 mm TL; and caudal fin shape.

For morphometric analysis two types of variables were employed (Figure 2): 1) linear morphometrics measurements (LMMs) and 2) inter-landmark distances (IlDs). Accordingly, two different morphometric approaches were carried out.

Thirteen LMMs (Figure 2a) were measured on the left side of the fish, to the nearest 0.1 mm with a digital caliper and a metal ruler. Body morphometrics are shown in Table 1, expressed either as percentages of standard length (SL) or head length (HL). Methods follow Hubbs and Lagler (1958) and Inada (1981*a*), except for the eye diameter (ED) that was taken from the external border of the eye. Total length (TL) and SL were measured from the anterior point of the lower jaw to the posterior end of caudal fin and to the base of the caudal fin (posterior end of the hypural plate) respectively. HL and lower snout lengths (LSL) were measured from the same anterior point to the posterior end of the opercular membrane and to the anterior fleshy edge of the eye, respectively. Upper snout length (USL) was taken from the upper lip to the anterior fleshy edge of the eye. Body depth (BD) was the maximum depth. Pectoral-fin and pelvic-fin lengths (PL and PeL) were taken as the longest ray of each fin, respectively. Interorbital width (IW) was the least bony width between the eyes. Prepectoral (PD), predorsal 1 (DD1) and preanal (AD) distances were measured from the anterior point of the lower jaw to the origin of pectoral, first dorsal and anal fins, respectively.

IlDs were obtained by a truss network protocol (Strauss & Bookstein 1982) based on 8 homologous anatomical points, following Díaz de Astarloa *et al.* (2011) (Figure 2b). Sixteen morphometric variables were taken as interlandmark distances over the left side of the head of each fresh individual of *M. hubbsi* and *M. australis* and of type specimens of *M. tasmanicus,* using a digital caliper (±0.05 mm).

LMMs and IlDs were transformed using the normalization technique to scale data that exhibited allometric growth detailed by Lleonart *et al.* (2000). SL and HL were taken as independent variables for LMMs and IlDs, respectively, while the remaining measurements were regarded as dependent variables. The reference values of size to which all individuals were reduced or amplified (X0) for LMMs analysis was 410 mm SL and for IlDs study was 120 mm HL (Lombarte & Lleonart 1993; González-Castro *et al*. 2008; González-Castro *et al*. 2012). After transformation, new matrices with the normalized data were constructed containing the corrected matrices for each species, and Principal Component Analyses (PCA) for the two datasets were performed. PCs were extracted from the correlation matrix (Quinn & Keough 2002). Finally, Discriminant Function Analyses (DFA) were conducted on LMMs and IlDs data of *M. hubbsi* and *M. australis* in order to establish the relative significance of the characters used in distinguishing between species and to build a predictive model of species membership. The assumptions of DFA were previously tested according to Zuur *et al*. (2007).

TABLE 1. Linear morphometric measurements and meristic characters of *Merluccius*. n, number of specimens; Mo, modal; SD, standard deviation; SL, standard length; HL, head length; *lectotype; () number of specimens different of n.

Character	*Merluccius australis*									*Merluccius tasmanicus*			
	Lectotype and paralectotype n = 2	Non-type specimens from Argentina n = 163				Non-type specimens from New Zealand n = 4				Holotype and paratypes n = 4			
	Range	Range	Mean	Mo	S.D.	Range	Mean	Mo	S.D.	Range	Mean	Mo	S.D.
Standard length	84–323	418–716				312–405				239–377			
As % HL													
Lower snout length	33.6–38.3	31.7–41.3	36.4		1.75	34.3–35.8	34.8		0.69	35.9–36.5(3)	36.2		0.31
Upper snout length	28.6	28.8–36.4	32.8		1.42	30.6–33.9(3)	32.3		1.64	32.6–34.3(3)	33.6		0.86
Eye diameter	13.9–14.8	12.3–19.4	14.7		1.10	17.1–19.3	18.3		0.96	16.7–17.9(3)	17.3		0.64
Interorbital width	23.5–27.2	23.9–30.4	27.1		1.28	26.8–27.9	27.2		0.54	25.2–28.1(3)	27.1		1.64
As % SL													
Head length	25.5–28.2	26.2–30.5	28.2		0.91	26.5–27.8	26.9		0.60	26.7–28.2(3)	27.3		0.77
Body depth		12.8–18.8	15.8		1.26	14.5–16.0(3)	15.2		0.75	14.2–18.9(3)	17.4		2.54
Pectoral-fin length	17.5–17.8	15.9–20.5	18.2		0.89	17.0–20.0	18.7		1.30	17.7–18.7(3)	18.2		0.50
Pelvic-fin length	10.7–11.8	10.1–13.9	12.0		0.73	12.0–13.4	12.7		0.54	12.2–13.4(3)	12.7		0.63
Prepectoral distance	29.1	25.3–29.6	27.4		0.91	27.3–28.8(3)	28.2		0.78	26.2–28.5(3)	27.5		1.17
Predorsal 1 distance	31.5	29.0–33.0	31.1		0.84	29.0–30.0(3)	29.4		0.53	30.0–32.0(3)	30.7		1.14
Preanal distance	44.8	42.7–49.5	46.2		1.35	44.3–46.0(3)	45.0		0.92	44.4–45.5(3)	45.1		0.60
First dorsal-fin rays	11–12	9–12	11.2	11	0.7	10–12	11.0	11	0.8	11–12	11.8	12	0.5
Second dorsal-fin rays	42–44	39–45	42.0	42	1.5	40–42	40.5	40	1.0	42	42.0	42	0.0
Anal-fin rays	43–44	40–46	42.6	43	1.5	40–45	42.8	43	2.1	42(3)	42.0	42	0.0
Pectoral-fin rays	14	13–16	13.9	14	0.7	14–15	14.8	15	0.5	13–15	14.0		1.1
Lateral line scales	153–168	148–173	164.2		5.6	156–160(3)	158.0		2.0	147–152(3)	150.3	152	2.9
Total gill rakers	13–14	11–15(158)	12.9	13	0.9	12–14	12.8	12	1.0	12–13	12.5		0.6
Upper gill rakers	2–3	2–4(158)	3.0	3	0.4	2–3	2.8	3	0.5	3	3.0	3	0.0
Lower gill rakers	10–12	9–12(158)	9.9	10	0.7	9–11	10.0	10	0.8	9–10	9.5		0.6
Total vertebrae		54–58(157)	56.1	56	0.8	54–55(2)	54.5		0.7	55–56	55.5		0.6
Abdominal vertebrae		19–22(157)	20.9	21	0.5	19–20(2)	19.5		0.7	20–21	20.3	20	0.5
Caudal vertebrae		27–31(157)	29.2	29	0.7	29(2)	29.0	29	0.0	29–30	29.3	29	0.5

……*continued on the next page*

TABLE 1. (Continued)

Character	*Merluccius hubbsi*							
	Paratypes n = 6				Non-type specimens n = 241			
	Range	Mean	Mo	S.D.	Range	Mean	Mo	S.D.
Standard length	96–730				138–620			
As % HL								
Lower snout length	31.0–36.7	34.0		2.16	28.2–35.7	31.7		1.52
Upper snout length	28.8–32.2(5)	30.0		1.31	25.9–32.8	29.1		1.46
Eye diameter	15.6–23.4	21.1		2.82	14.9–23.5	19.4		1.82
Interorbital width	23.1–27.8	25.5		1.60	21.3–27.1	24.2		1.13
As % SL								
Head length	28.1–31.6	29.8		1.34	26.2–32.1	28.7		1.17
Body depth					11.8–18.4	15.1		1.39
Pectoral-fin length	14.7–18.5(5)	17.2		1.54	15.8–20.9	18.4		1.09
Pelvic-fin length	11.8–17.1	14.9		2.03	11.5–17.7	14.6		1.31
Prepectoral distance	27.7–31.4(5)	29.3		1.60	25.5–31.7	28.3		1.22
Predorsal 1 distance	31.1–32.4(5)	31.7		0.38	28.0–33.2	30.6		1.04
Preanal distance	44.6–49.3(5)	46.9		1.96	43.2–49.2	45.8		1.27
First dorsal-fin rays	11–12	11.7	12	0.5	10–13	11.7	12	0.6
Second dorsal-fin rays	36–38	37.0		0.9	35–40	37.6	38	1.1
Anal-fin rays	35–38	36.3		1.2	35–40(236)	38.3	38	1.1
Pectoral-fin rays	13–15	14.0	14	0.6	13–16	14.1	14	0.5
Lateral line scales	99–120(3)	112.3		11.6	121–144(229)	133.8	133	4.7
Total gill rakers	14–15(5)	14.2	14	0.4	12–16(239)	14.1	14	0.9
Upper gill rakers	3–4(5)	3.4	3	0.5	3–4(239)	3.5	3	0.5
Lower gill rakers	10–11(5)	10.8	11	0.4	9–12(239)	10.6	11	0.7
Total vertebrae					50–53(225)	51.2	51	0.7
Abdominal vertebrae					17–20(225)	18.3	18	0.5
Caudal vertebrae					26–28(225)	26.9	27	0.5

Internal morphology and morphometric measurements. Methods for preparing disarticulated skeletons followed Deli Antoni *et al*. (2008). Twelve measurements were taken on the hyomandibula (Figure 3a) with a digital caliper (±0.01 mm), under a stereomicroscope following the protocol outlined by Díaz de Astarloa *et al.* (2011). On the urohial six IlDs were taken based on four anatomical landmarks (Figure 3b), according to Díaz de Astarloa *et al*. (2011). For both data sets, all the size effects due to allometric growth (Lleonart *et al*. 2000) were removed using the normalization technique of Lleonart *et al*. (2000) as explained above, with a reference value of size (X0) of 120 mm HL. After transformation, Principal Component Analysis (PCA) based on correlation matrix for the two datasets were performed. Terminology of the sagitta otolith morphological description follows Hecht (1987) and Volpedo and Echeverría (2000). The outline of each sagitta was observed and two morphometric variables were measured (± 0.01 mm) with the aid of a camera lucida and a stereomicroscope (Figure 3c): otolith maximum length (OL), as the distance between anterior and posterior ends; and the excisura ostii (which separates the rostrum and the anti-rostrum) length (EL), as the distance between the excisura innermost end and the anti-rostrum end.

Genetic analysis. A total of fourteen individuals were used for genetic analysis, eight *M. hubbsi* and six *M.*

australis. Of the later, four specimens exhibited diagnostic characters described by Matallanas & Lloris (2006) for *M. tasmanicus* (*i.e.* the lateral line bowed over the pectoral fin, the upper profile of the head concave and the pectoral fin end not reaching the anal fin origin).

A sample of white muscle tissue was removed from each specimen and preserved in 100% ethanol at -20°C. The specimens were labelled, photographed, formalin fixed (with further alcohol long-term preservation) and deposited as vouchers in the fish collection of the Universidad Nacional de Mar del Plata, Argentina or their photographs were retained as e-vouchers (Monk & Baker 2001). DNA extraction, polymerase chain reaction (PCR) and sequencing of the COI gene were performed according to with standard DNA barcoding protocols (Ivanova *et al*. 2006) and primer cocktails developed for fishes (Ivanova *et al*. 2007). Extraction and amplification was undertaken at the International Barcode of Life Argentinean reference Barcode Laboratory of CONICET at the Museo Argentino de Ciencias Naturales, Buenos Aires, Argentina. Sequencing was accomplished in the Canadian Centre for DNA Barcoding (CCDB) in Ontario, Canada. All sequence assemblies, as well as electropherogram (trace) files, primer sequences and specimen data were deposited in "Southwestern Atlantic Hakes" (code SAH) project at http://www.boldsystems.org (BOLD, Ratnasingham & Hebert 2007) (Process IDs in Table 3). Also the sequences of *M. hubbsi* are available on GenBank with Accession numbers HM421964–71 and those of *M. australis* under the Acc. Nos. KM255097, KM255101–103 and KM255105–106.

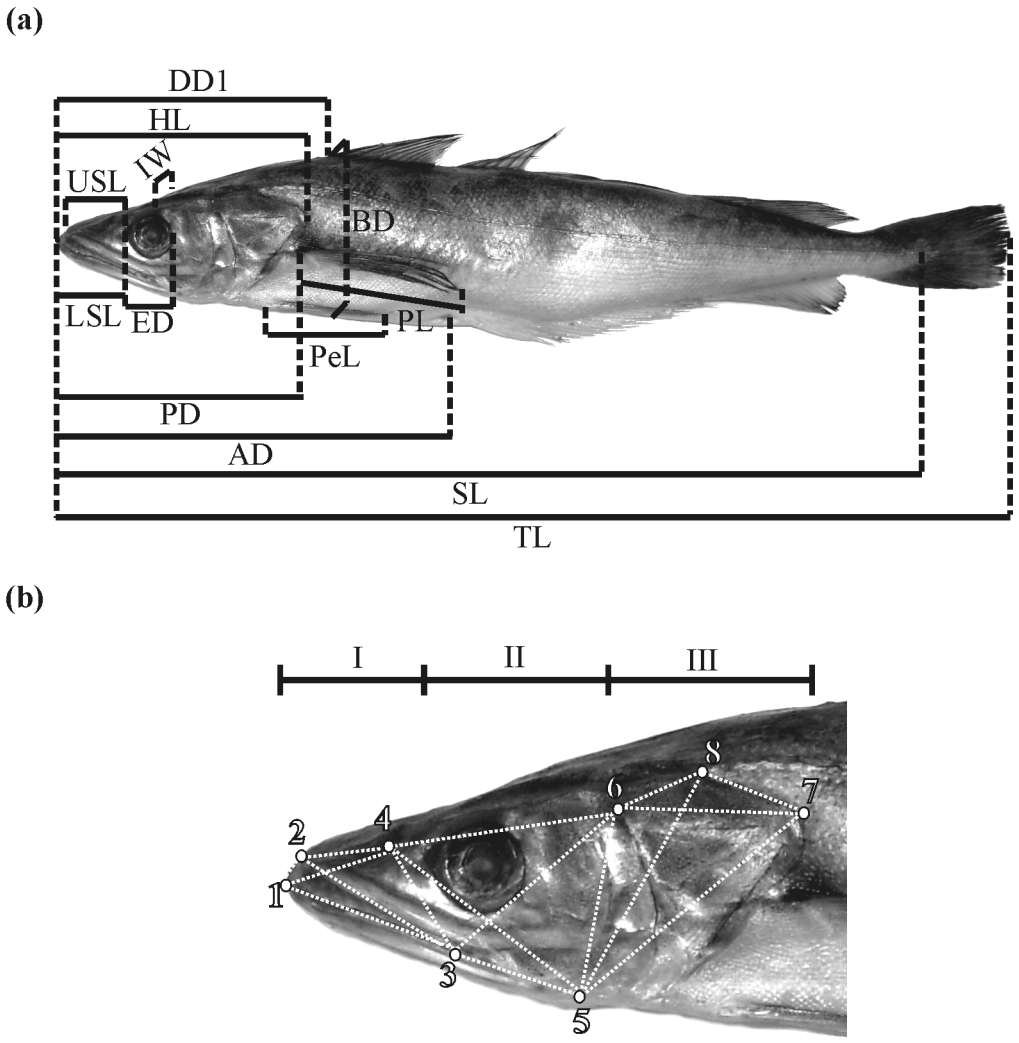

FIGURE 2. Morphometric variables employed. (a) Linear morphometrics measurements (LMMs): TL, total length; SL, standard length; HL, head length; LSL, lower snout length; USL, upper snout length; ED, eye diameter; IW, interorbital width; BD, body depth; PL, pectoral-fin length; PeL, pelvic-fin length; PD, prepectoral distance; DD1, predorsal 1 distance; AD, preanal distance. (b) Inter-landmark distances (IlDs) obtain from three Box-truss (Roman numerals) based on 8 anatomical landmarks: 1. lower tip of the snout; 2. upper tip of the snout; 3. corner of the jaws; 4. anterior nare; 5. lower tip of preopercular membrane; 6. upper tip of preopercular membrane; 7. posterior margin of opercular membrane; 8. upper margin of opercular membrane.

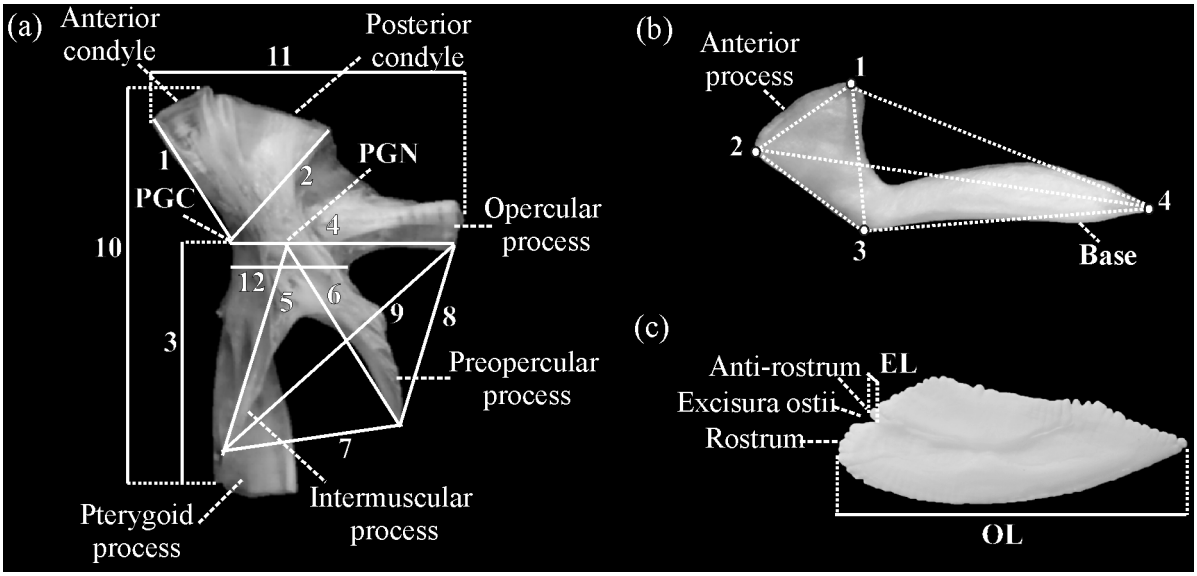

FIGURE 3. Morphometric measurements recorded on lateral side of the (a) hyomandibula, (b) urohial and (c) otolith of the studied species. PGN, point of greatest neckline; PGC, point of greatest curvature; EL, excisura length; OL, otolith length; (1) anterior vertex of the anterior process, (2) posterior vertex of the anterior process, (3) upper vertex of the anterior process and (4) posterior vertex of the base.

The sequences obtained in this work were compared with those of ten specimens of *M. hubbsi* from Argentina waters (GenBank Acc. No. EU074469–78) and seven individuals of *M. australis* from Argentina (GenBank Acc. Nos. EU074468; KM255096) and New Zealand waters (GenBank Acc. Nos. KM255095; KM255098–100; KM255104). Those sequences are available in the public access Project "Fishes of Argentina" (FARG) in the Barcode of Life Data Systems (BOLD, http://www.boldsystems.org) (Process IDs in Table 3). The COI sequence of one specimen of *Gadus morhua* Linnaeus (SCAFB104-07| KC015378) was extracted from BOLD and added to the analysis as an outgroup.

DNA sequences were aligned and posterior analyses were performed with MEGA 5.0 software (Tamura *et al*. 2011). Inter and intra specific sequence divergences were calculated based on *p*-distances, preferably when sequences are short and derived from closely related species (Nei & Kumar 2005; Srivathsan & Meier 2012). Additionally, the sequences divergences were analyzed with Kimura two-parameter (K2P) (Kimura 1980) and Tamura-Nei distance models (Tamura & Nei 1993), for comparisons purposes. A Neighbour-joining (NJ) tree (Saitou & Nei 1987) based on *p*-distances was created, to provide a graphic representation of divergences between species. Robustness of the tree was tested using bootstrap analysis (Felsenstein 1985) with 1000 replicates. Finally, with the Barcode Index Number (BIN) tool (Ratnasingham & Hebert 2013) available in BOLD (which includes every compliant COI record, public and unpublished) the concordance between BINs and species identifications were evaluated. For each BIN mean intraspecific, maximum intraspecific and Nearest-Neighbour (average distance to the most closely related species) *p*-distances were estimated and the presence of a "barcode gap", a disjunction between levels of intraspecific and interspecific variability was checked. Sequences were automatically assigned to a BIN on the BOLD Workbench v. 3.6 (http://www.boldsystems.org; analyses performed on 21 Jul 2014).

Results

Meristic counts. Meristic ranges are given in Table 1. Number of cervical vertebrae was constant (6) in all the studied groups. Most of the ranges of the meristic characters of *M. tasmanicus* overlap with those of the types of *M. australis*. Moreover, all the meristic features are completely contained within those of the Argentinean specimens of *M. australis*, and for most of them (except for the lateral line scales), in those of the specimens of *M. australis* from New Zealand (Table 1). Conversely, *M. hubbsi* show non-overlapping ranges and lower values of lateral line scales and total vertebrae than *M. australis* and *M. tasmanicus*. Second, mean numbers of second dorsal-fin and anal-fin rays are significantly higher in *M. australis* and *M. tasmanicus* than in *M. hubbsi* (Table 1). PCA of the

correlation matrix of the meristic data produces two PCs with eigenvalues >1, which explains 63% of variance in the data. In PC1 vs PC2 plane, two separated groups are evident (Figure 4a). The first group of type and fresh specimens of *M. hubbsi*, shifts towards negative values of PC1, and is characterized by higher numbers of gill rakers on both limbs (Figures 4a and b). The second group is represented by the lectotype, paralectotype and non-types of *M. australis* from Argentina and New Zealand and the holotype and paratypes of *M. tasmanicus*, had negative PC1 values, and has more lateral line scales and anal-fin and second dorsal-fin rays (Figures 4a and b). Kruskall-Wallis test showed significant differences among the three putative species in all the studied meristic characters, except for the number of pectoral-fin rays (Table 2). Post hoc Dunn multiple pairwise comparisons shows significant differences in all the considered characters between *M. hubbsi* and *M. australis* and, except for the number of first dorsal-fin rays, between *M. tasmanicus* and *M. hubbsi*. Conversely, non-significant differences were found between *M. tasmanicus* and *M. australis* (Table 2).

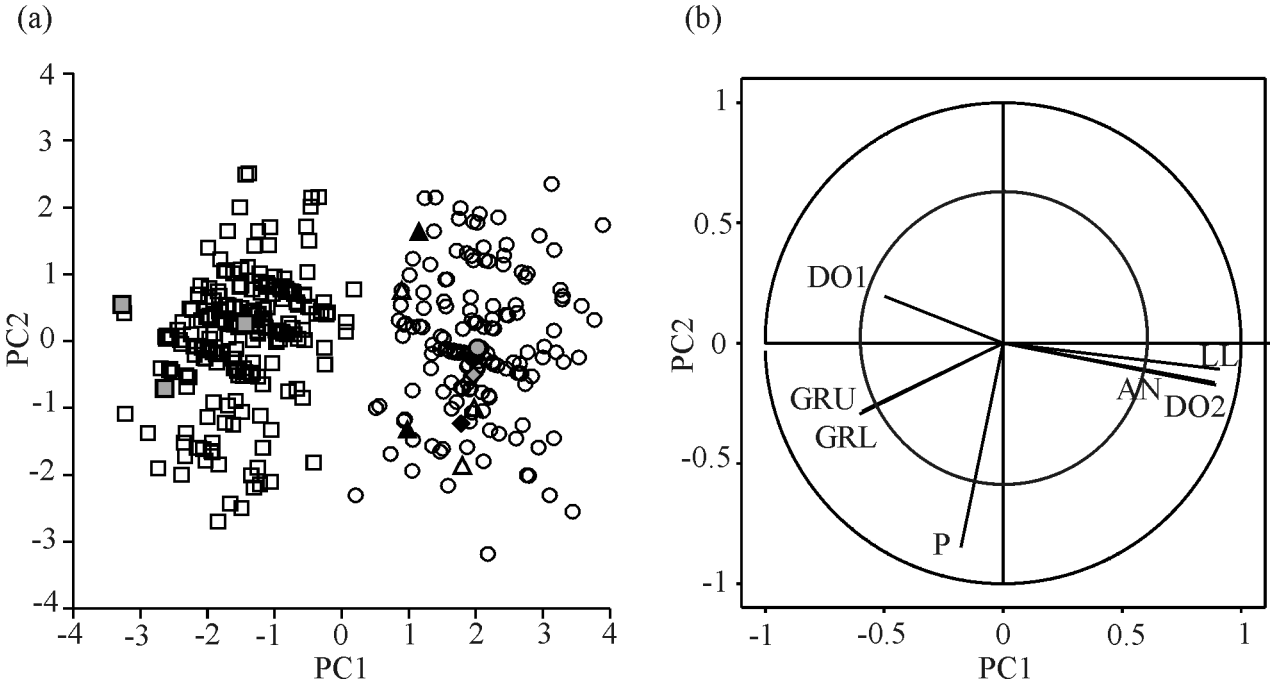

FIGURE 4. Principal Component Analysis (PCA) based on meristic data: (a) Ordination produced by the first two PCs, of the studied specimens of *Merluccius hubbsi* (solid grey square, paratypes; open square, non-type), *M. australis* (solid grey rhomb, lectotype; solid grey circle, paralectotype; open circle, non-type from Argentina; open triangle, non-type from New Zealand) and *M. tasmanicus* (solid black rhomb, holotype; solid triangle, paratypes); and (b) correlation between the first two PCs and meristic characters.

External morphology and morphometric measurements. Some features are the same in all species analyzed. In all cases the position of the lateral line on the caudal peduncle is equidistant from the dorsal and ventral body outlines, and the shape of the lateral line is straight. The other morphological characters studied show a great intraspecific variability and not one is distinctive of any of the three species of *Merluccius* (Figures 5 and 6). The body is robust in most specimens of *M. australis* (n = 137), and slender in those of *M. hubbsi* (n = 214). The holotype and one paratype of *M. tasmanicus* have a robust body and the other type specimens have slender bodies (Figure 6a). The dorsal profile of the head is straight in all the types of *M. tasmanicus* as well as in the majority specimens of *M. hubbsi* (n = 181) and *M. australis* from Argentina (n = 147) and New Zealand (n = 3). It is slightly concave in the remaining specimens of *M. hubbsi* and *M. australis* (Figure 6b). Similarly, the eye does not reach the dorsal profile of the head in all the specimens of *M. tasmanicus* and in many *M. hubbsi* (*n* = 149) and *M. australis* from Argentina (n = 162) and New Zealand (n = 2) (Figure 6c). The upper pectoral-fin ray inserts at level of the ventral edge of the eye in most specimens of *M. hubbsi* (n = 189) and *M. australis* (n = 140) and in all specimens of *M. tasmanicus* (Figure 6d). The pectoral fin inserts at level of the middle of the eye in the remaining studied specimens. The upper margin of the opercular membrane is oblique to the lateral line in 157 individuals from Argentina and 1 from New Zealand of *M. australis*, in 89 specimens of *M. hubbsi* and in all type specimens of *M. tasmanicus*. All other studied specimens have an opercular membrane parallel to the lateral line (Figures 6e).

The lateral line is bowed over the pectoral fin in all the specimens of *M. tasmanicus*, in most of *M. australis* from Argentina (n = 147) and New Zealand (n = 2) and in about half *M. hubbsi* (n = 129). It is straight in all other specimens of *M. hubbsi* and *M. australis* (Figures 6f). The pectoral-fin does not reach the anal-fin origin in specimens >400 mm TL in, one paratype of *M. tasmanicus*, 32 specimens of *M. hubbsi* and 70 of *M. australis* (Figure 6g). The posterior margin of the caudal-fin is truncate in most specimens of *M. tasmanicus* (n = 3), *M. hubbsi* (n = 154) and *M. australis* from Argentina (n = 146) and New Zealand (n = 2). The remaining specimen caudal-fins are convex to concave (Figure 6h).

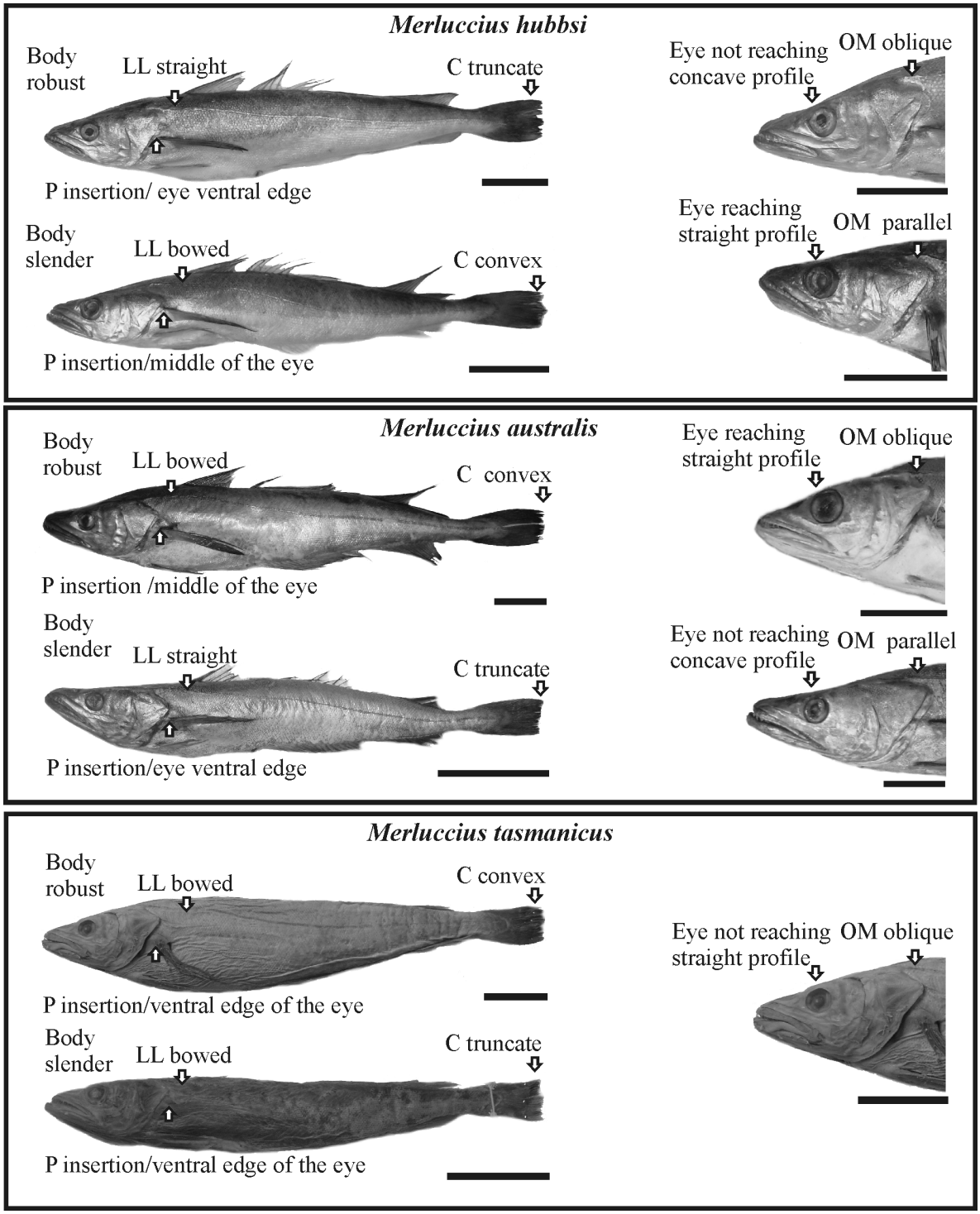

FIGURE 5. Morphological variation observed in the tree species of *Merluccius* studied. LL, lateral line; C, caudal-fin; P, pectoral-fin; OM, opercular membrane. Scale bar: 50 mm

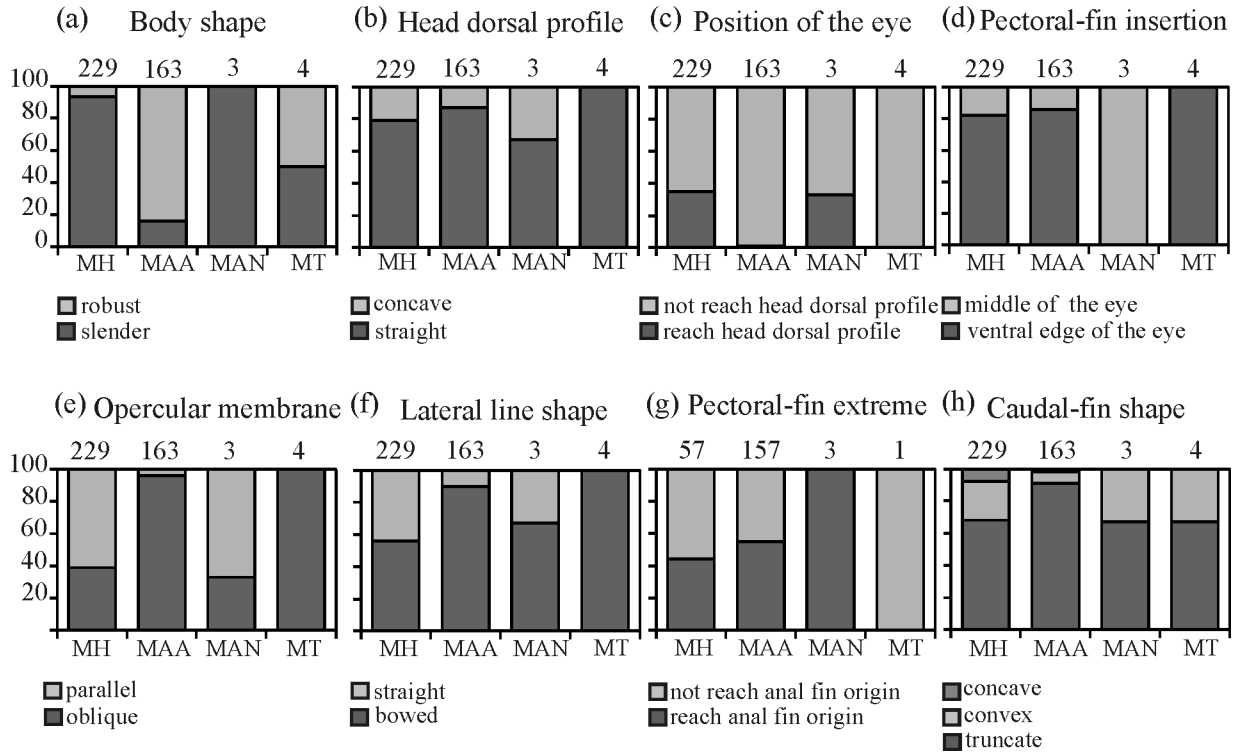

FIGURE 6. Frequency distribution (percentage) of the studied morphological characters in three species of *Merluccius*. MH, *M. hubbsi*; MAA, *M. australis* from Argentina; MAN, *M. australis* from New Zealand; MT, *M. tasmanicus*. Number of specimens analyzed above columns.

TABLE 2. Results of Kruskal-Wallis test and Dunn´s multiple comparison test. *H*-statistic. *Z*-statistic and results for meristic characters: *, significant ($P < 0.05$); NS, non-significant ($P > 0.05$).

Meristic features	H	Z		
		M. hubbsi vs. *M. australis*	*M. hubbsi* vs. *M. tasmanicus*	*M. australis* vs. *M. tasmanicus*
First dorsal-fin rays	61.90 *	7.0885 *	0.1516 NS	1.5498 NS
Second dorsal-fin rays	301.22 *	17.0473 *	3.4673 *	0.0906 NS
Pectoral-fin rays	5.41 NS			
Anal-fin rays	296.83 *	16.9968 *	2.5537 *	0.3783 NS
Lateral line scales	295.56 *	17.1692 *	2.7847 *	1.2107 NS
Total gill rakers	119.15 *	10.2876 *	2.8849 *	0.8209 NS
Upper gill rakers	81.42 *	7.4570 *	2.5413 *	0.0501 NS
Lower gill rakers	81.69 *	8.1682 *	2.6007 *	0.9607 NS
Total vertebrae	300.68 *	16.7707 *	2.8239 *	0.6184 NS
Abdominal vertebrae	307.96 *	16.6783 *	2.5414 *	0.8810 NS
Caudal vertebrae	296.85 *	16.3306 *	3.4674 *	0.1128 NS

Morphometric values of *Merluccius* are given in Table 1. Most of the variable ranges of the type series of *M. tasmanicus* completely overlap those of the types of *M. australis* and non-types from New Zealand; and all of them are contained in the ranges of the specimens of *M. australis* from Argentina (Table 1). The mean values of most of the morphometric characters are similar between *M. tasmanicus* and *M. australis*, except for the mean upper snout length and body depth which are slightly greater in *M. tasmanicus* than in *M. australis*. The mean value for eye diameter is also greater in *M. tasmanicus* but it is similar to that of *M. australis* from New Zealand.

Many of the morphometric characters show a partial overlapping between *M. hubbsi* and *M. australis*. The mean values of lower and upper snout length and interorbital width are greater in *M. australis*, while the eye diameter and pelvic-fin length are greater in *M. hubbsi* (Table 1). PCA of the correlation matrix, generated by normalization procedure of LMMs from the type and fresh specimens of *M. australis*, *M. hubbsi* and *M. tasmanicus*, produces two PCs with eigenvalues > 1, which explain 61% of variance in the data. In the PCA plot, two narrowly overlapped groups are evident (Figure 7a). In this respect, type and fresh specimens of *M. hubbsi*, had mainly positive values for both PCs, and are characterized by higher values of eye diameter and pelvic-fin length (Figures 7a and b). The paralectotype and non-types of *M. australis* from Argentina and New Zealand and the types of *M. tasmanicus* constitute one group, determined by higher loadings of lower snout length, upper snout length and interorbital width (Figures 7a and b).

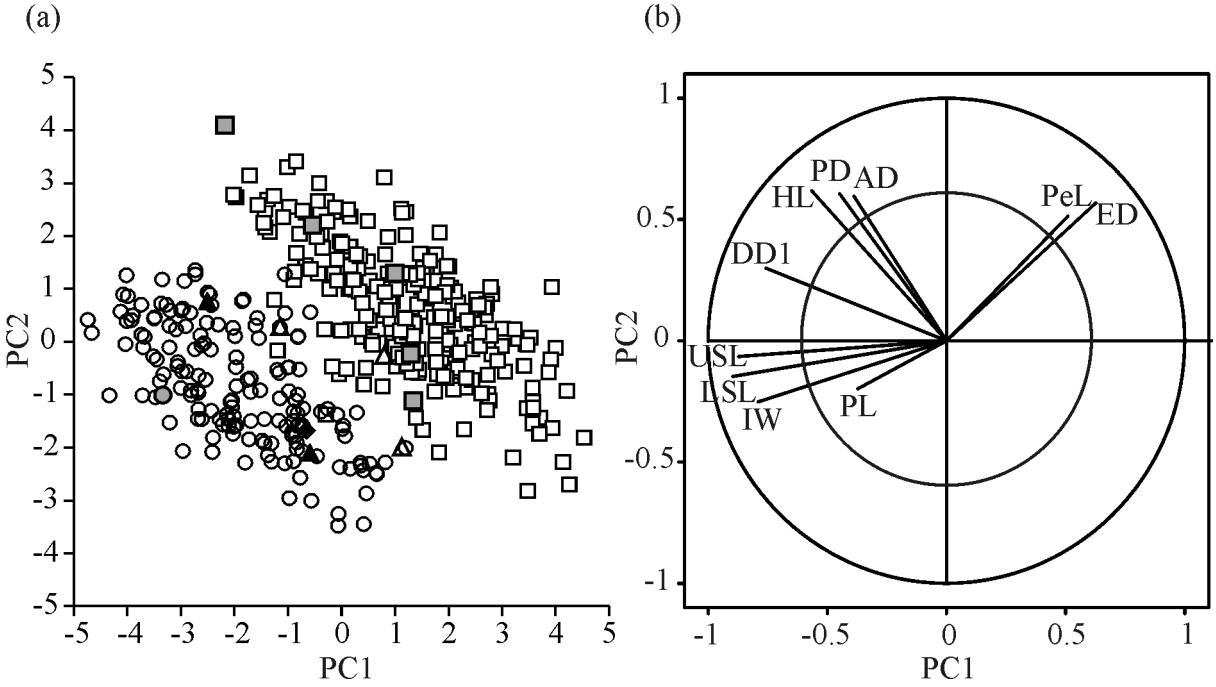

FIGURE 7. Principal Component Analysis (PCA) based on linear morphometric measurements: (a) Ordination produced by the first two PCs, of the studied specimens of *Merluccius hubbsi* (solid grey square, paratypes; open square, non-type), *M. australis* (solid grey circle, paralectotype; open circle, non-type from Argentina; open triangle, non-type from New Zealand) and *M. tasmanicus* (solid rhomb, holotype; solid triangle, paratypes); and (b) correlation between the first two PCs and traditional morphometric characters.

One single linear discriminant function (DF) was extracted from the discriminant analysis and shows highly significant interspecific differentiation (Wilk's $\lambda = 0.156$; $F_{10, 413} = 211.2$; $P < 0.00001$). The correct classification of individuals into their originals species was 99.2% for *M. hubbsi* and 98.8% for *M. australis*. Based on the classification function, the types of *M. tasmanicus* were classified as *M. australis*. Pooled within-group correlations between morphometric variables and the canonical function indicate high contributions from eye diameter, upper snout length, interorbital width, lower snout length and pelvic-fin length.

PCA analyses of the 16 normalized IlDs produces five eigenvalues >1 (results not shown). The first two PCs account for 54% of the variance in the data. Two clearly differentiated groups are shown in the PC plot, separated mainly by the first PC (Figure 8a). The specimens of *M. australis* from Argentina and New Zealand and the types of *M. tasmanicus*, grouped together at positive values of PC1, shows higher loadings for the IlDs of the first box truss (1–4, 2–3 and 2–4) which represents the snout length, longer in these specimens (Figures 2 and 8). *M. hubbsi* is characterized by higher loadings of the third box truss variables (5–7, 6–7 and 7–8), which represent the condition of the upper margin of the opercular membrane in relation to the lateral line, mainly parallel in *M. hubbsi* and oblique in *M. australis* and *M. tasmanicus* (Figures 2 and 8). Also, *M. australis* and *M. tasmanicus* present higher loading for the variables 4–5 and 3–5 (second box truss), associated with the lower end of the preopercular fold posterior in these species than in *M. hubbsi* (Figures 2 and 8).

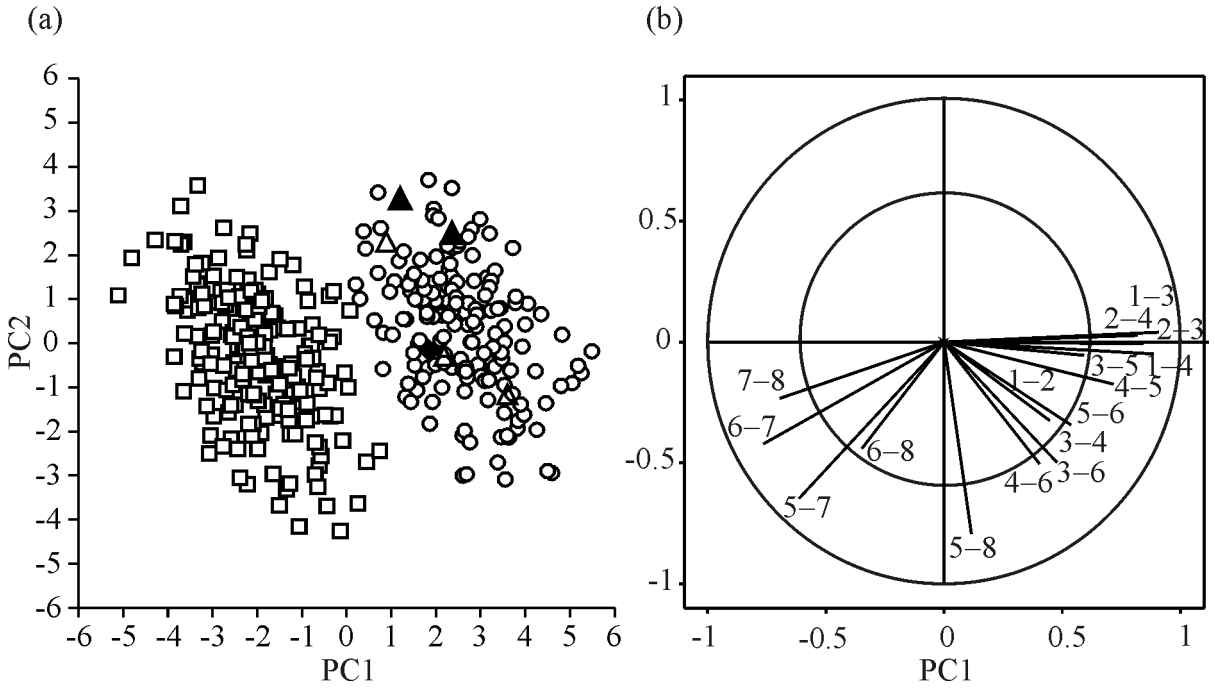

FIGURE 8. Principal Component Analysis (PCA) of inter-landmarks distances obtained from the head Box-truss protocol: (a) Ordination produced by the first to PCs, of the studied specimens of *Merluccius hubbsi* (open square, non-type), *M. australis* (open circle, non-type from Argentina; open triangle, non-type from New Zealand) and *M. tasmanicus* (solid rhomb, holotype; solid triangle, paratypes); and (b) correlation between the first to PCs and landmark-based morphometric characters.

The Discriminant Function Analyses of the landmark-based variables allows a significant differentiation of *M. hubbsi* and *M. australis* (Wilk's $\lambda = 0.10049$; $F = 201.4$; $p < 0.00001$). The overall correct classification into their originals groups was 100% and the types of *M. tasmanicus* were assigned to the *M. australis* cluster. The interlandmarks distances most correlated with the canonical function were 1–3, 1–4, 2–3, 2–4 and 6–7, corresponding to the snout length and to the condition of the opercular membrane.

Internal morphology and morphometric measurements. The hyomandibula is a large and irregular bone connecting the mandibular suspensorium and opercular bones to the neurocranium (Rojo 1976; Inada 1981*a*). Dorsally, it has two prominent condyles (anterior and posterior) (Figure 3a) which fit into fossae on the cranium, at the junction of the pterotic and sphenotic bones. Ventrally, a long, triangular and flattened pterygoid process (Figure 3a) constitutes the inferior region of the bone and articulates or connects with the metapterygoid, symplectic and the interhial. Posteriorly, a cylindrical shape opercular process (Figure 3a) articulates with the opercle. It is at a right angle to the vertical axis of the bone in *M. hubbsi*, while it shows a slight downward tilt in *M. australis* (Figure 9a). Two pointed processes originated near the base of the opercular process and project laterally: the preopercular process, which extends posteroventrally and is overlain by a flange of the preopercle, and the intermuscular process, situated anteriorly (Figure 3a). In *M. hubbsi*, the notch between both processes is shallow and ventrally to that between intermuscular and pterygoid processes, whereas in *M. australis*, it is deeper and dorsal or at the same level of the recess between the intermuscular and pterygoid processes (Figure 9a). The length and shape of the intermuscular process shows a great intraspecific variability and can be equal to, or larger than the preopercular process in both species (Figure 9a). Additionally, the intermuscular process is anteriorly curved or straight and divergent or parallel to the preopercular process in *M. hubbsi* and *M. australis* (Figure 9a). Principal component analysis was carried out on 12 measurements obtained from the hyomandibula of dissected specimens of *M. hubbsi* and *M. australis.* The first two PCs, with eigenvalues >1, explain 70% of the variance. Two distinct groups are produced with a narrow central area of overlapping values (Figure 10a). Specimens of *M. hubbsi*, mainly located at low values for both PCs, present high mean values of pterygoid and opercular processes lengths (3 and 4) and greatest distances between the opercular process and the intermuscular (9) and preopercular (8) processes (Figures 3a and 10a and b). *M. australis*, with positive values of both PCs, is characterized by greater distances between intermuscular and preopercular processes (7) and between anterior and posterior condyles to the

point of greatest curvature (2 and 1) and, to lesser extent, by a greater width of the pterygoid process (12) (Figures 3a and 10a and b).

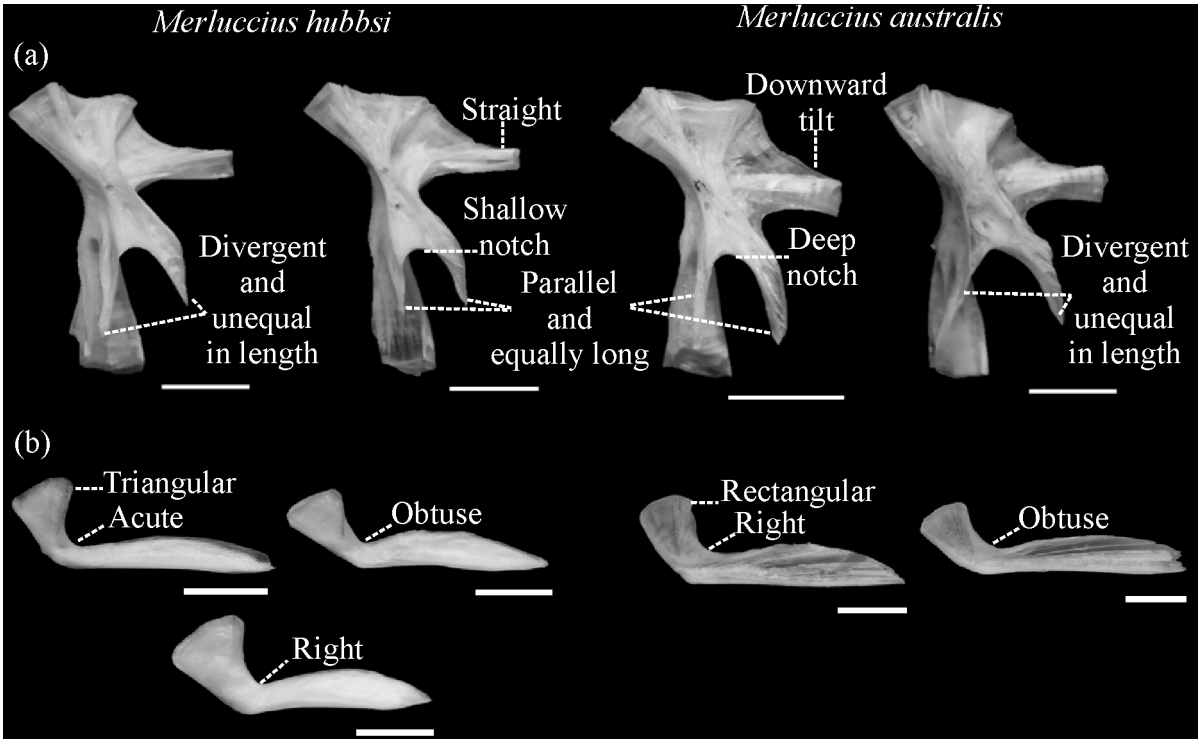

FIGURE 9. Inter and intraspecific variability in (a) the hyomadibula and (b) the urohyal morphology of *Merluccius* spp. Scale bar: 10 mm.

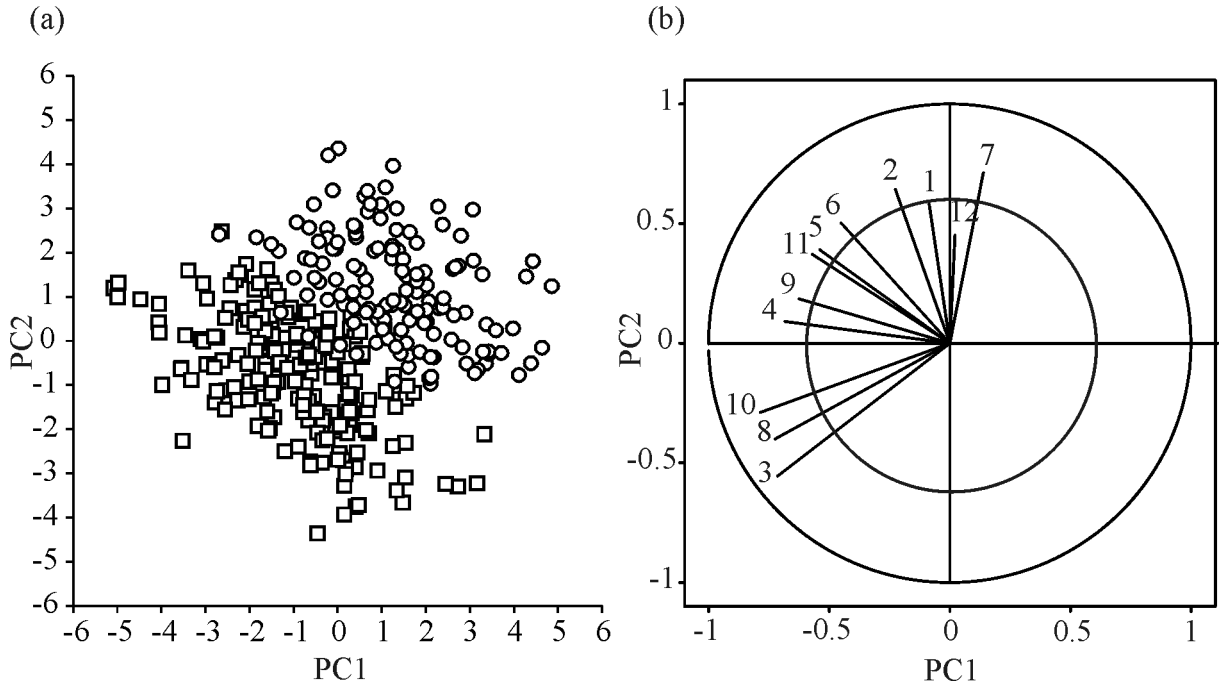

FIGURE 10. Principal Component Analysis (PCA) of linear morphometric measurements obtained from the hyomandibula: (a) Ordination produced by the first to PCs, of the studied specimens of *Merluccius hubbsi* (open square, non-type) and *M. australis* (open circle, non-type from Argentina) and (b) correlation between the first to PCs and morphometric characters.

The urohyal is an L-shaped bone in lateral view, connected anteriorly with the first basibranchial and the lower hypohyals, and posteriorly with the cleithrum (Rojo 1976; Inada 1981*a*). It has a long and laterally compressed anterodorsal process (Figure 3b). This process is dorsally wider in *M. hubbsi* than in *M. australis* and is roughly

triangular in the former and approximately rectangular in the latter (Figure 9b). The urohyal extends posteroventrally with a flat base ending in two points, and is expanded dorsally as a thin sail-like crest (Figure 3b). Great variation in the thickness of the urohyal in relation to its size was found in *M. hubbsi*. Specimens of less than ~330 mm TL have a very thin urohyal, whereas in larger fish it is heavily ossified. In *M. australis*, the urohyal is considerably thinner than in *M. hubbsi* at all sizes studied (~400–800 mm TL). Another variable character of the urohyal is the angle of inclination between the anterodorsal process and the bone's base. It can be slightly obtuse or square in both species, or even acute in *M. hubbsi* (Figure 9b). Principal component analysis performed on IlDs data set from urohyal produces two PCs with eigenvalues >1, which accounts for 80% of total variance. Two groups are defined in the plot of the first two PCs, separating principally on the PC1, with a central overlapping area (Figure 11a). Most of the specimens of *M. hubbsi* are distinguished by a longer margin length of the anterodorsal process (1–2) (Figures 3b and 11b); and *M. australis*, by a greatest base length (1–4, 2–4 and 3–4) (Figures 3b and 11b).

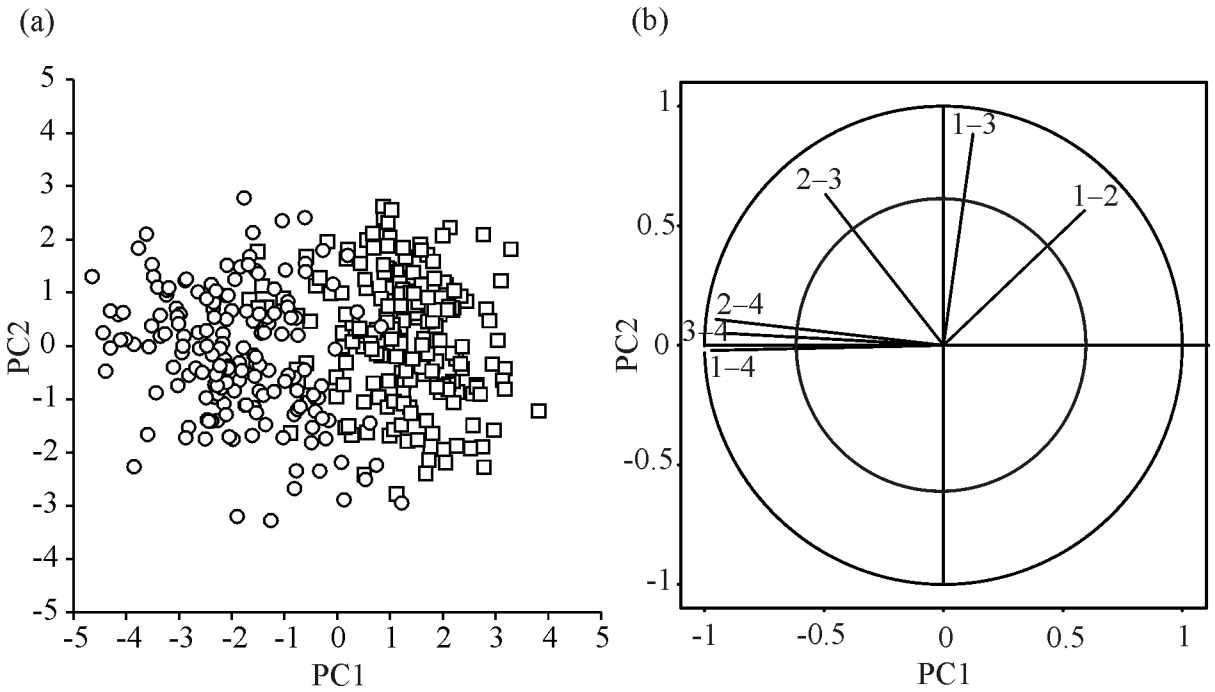

FIGURE 11. Principal Component Analysis (PCA) of inter-landmarks distances obtained from the urohyal: a) Ordination produced by the first to PCs, of the studied specimens of *Merluccius hubbsi* (open square, non-type) and *M. australis* (open circle, non-type from Argentina) and b) correlation between the first to PCs and landmark-based morphometric characters.

The sagitta is oblong with a rounded anterior and a pointed posterior end (Figure 3c). The outer face is slightly convex and the inner face is smooth and marked by a prominent sulcus acusticus. The dorsal margin is crenulate in both species, while the ventral border is smooth in its central portion in *M. hubbsi* and totally scalloped in *M. australis* (Figure 12a). There is a clear ontogenetic morphological variation in both species of *Merluccius* studied (Figure 12a). Juvenile specimens (<330 mm TL) of *M. hubbsi* tend to have a rounded otolith with a very short and wide posterior end. None of the 92 specimens examined <330 mm TL had an otolith with excisura ostii (Figure 12b). With increase in size, the posterior margin becomes longer and more tapered, becoming oblong. 92% (n = 118) of the specimens >330 mm TL of *M. hubbsi* have otoliths with excisura ostii, in which size did not show a clear relationship with specimen size (Figure 12b). In *M. australis*, the sagitta is more rounded and the posterodorsal margin gradually increase in height with specimen size (~400–800 mm LT) (Figure 12a). Also, 62 specimens (39%) of ~400–800 mm LT have otoliths with excisura ostii, in which size shows no clear relationship to specimen size (Figure 11b). Although both species show excisura ostii, comparing large specimens (>400 mm TL), this character presents a higher percentage (89%) in *M. hubbsi* than in *M. australis* (39%). In addition, the length of the excisura against the maximum length of the otolith has higher values in *M. hubbsi* (0–0.08 EL / OL) than *M. australis* (0–0.06 EL / OL) (Figure 12b).

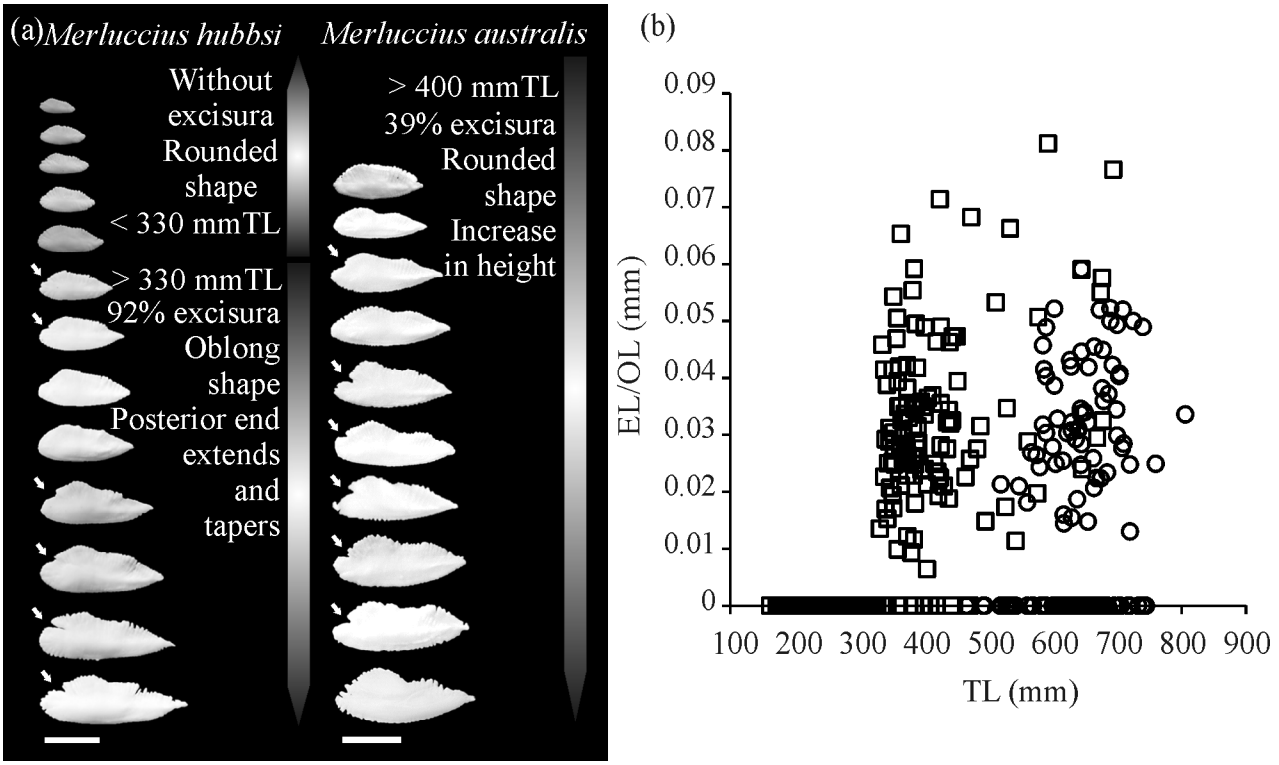

FIGURE 12. Intraspecific morphological variation with size of the otolith (a) and the excisura ostii (b) of *Merluccius hubbsi* (open square, non-type) and *M. australis* (open circle, non-type from Argentina). Scale bar: 10 mm.

DNA barcoding. COI sequences were obtained from the 14 specimens. Mean sequence length was 624 bp (range: 501–652 bp).

p, K2P and Tamura-Nei genetic distances between *M. hubbsi* and *M. australis* were high and also of similar magnitude (4.91%, 5.11% and 5.17%, respectively). Sequences revealed 29 variables sites between species (Table 3). Based on a *p*-distance / NJ tree (Figure 13), all specimens of *M. hubbsi*, studied in this paper and that of BOLD, cluster together. Also, all specimens of *M. australis* from Argentina and New Zealand, including ones with and without supposed diagnostic characters of *M. tasmanicus* grouped together (Figure 13). Moreover, all the specimens of *M. australis* exhibit a unique barcode haplotype (Table 3) and showed null *p*, K2P and Tamura-Nei intraspecific genetic distances. Three closely related haplotypes were found on *M. hubbsi*, two of them from specimens which sequences were obtained from BOLD (FARG 047–06 and FARG 249–06), differing by one nucleotide each and other haplotype shared by the remaining specimens (Table 3). However, *p*, K2P and Tamura-Nei genetic distances were very low (0.026% with all distance models) between the closely related haplotypes.

Finally, two operational taxonomic units (OTUs) were obtained by BIN analysis (Barcode Index Numbers; BOLD 3.0) of the sequences studied here and records of BOLD, which agree with the current taxonomic classification. Specimens of *M. australis*, including those with *M. tasmanicus* characters were assigned to the same BIN (BOLD: AAB2174) and specimens of *M. hubbsi* were placed in a unique BIN (BOLD: AAM2029). BIN compromising specimens of *M. australis* showed a within-BIN average and maximum *p*-distances of zero, while the distance to its nearest neighbor (*Gadomus* sp. Regan) was 2.95%. Within-BIN mean *p*-distance was 0.12% and maximum *p*-distance was 1.62% for *M. hubbsi*´s BIN, while the distance to its nearest neighbor (*Merluccius albidus* Mitchill) was of 3.25%. In both cases, therefore a "barcode gap" (discontinuity between intra- and interspecific divergences) is present.

Discussion

One of the main problems making fisheries management difficult is the inaccurate identification and/or the misidentification of the exploited species (Lleonart *et al*. 2006), which can seriously affect future conclusions

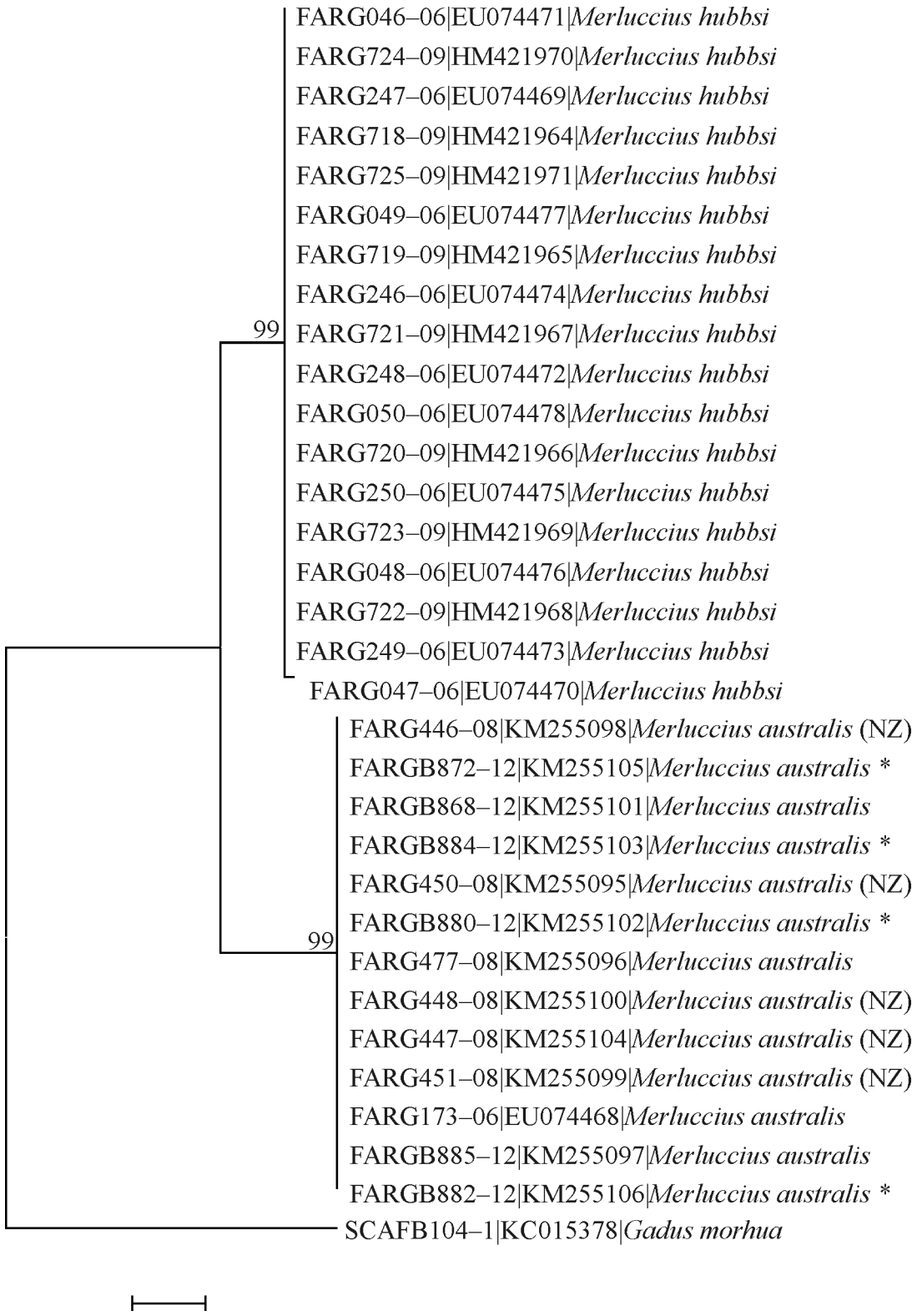

FIGURE 13. Neighbour-joining tree based on *p*-distances of *Merluccius* COI sequences from Argentina and New Zealand (NZ). Numbers at nodes represent bootstrap values. Code numbers represent BOLD process IDs and GenBank accession numbers. *specimens of *M. australis* with some diagnostic characters described for *M. tasmanicus*. Scale bar: 0.02 base substitutions per site.

TABLE 3. *Merluccius* spp. aligned COI sequences from Argentina and New Zealand (NZ); only variable nucleotide positions are shown. *, specimens of *M. australis* with some diagnostic characters described for *M. tasmanicus*; Dots, indicate identity with first sequence for *M. australis*, FARG450–08; -, missing sites.

BOLD process ID	Variable nucleotide positions																															
	7 3	8 2	1 4 4	1 9 9	2 2 6	2 3 2	2 4 1	2 5 9	2 6 5	2 7 1	2 9 5	3 0 1	3 1 3	3 1 9	3 3 4	3 7 1	3 9 4	4 1 3	4 2 7	4 6 3	4 6 6	4 8 4	5 2 6	5 3 2	5 4 1	5 5 6	5 7 7	6 2 2	6 2 5	6 2 8	6 4 9
M. australis																																
FARG450–08 (NZ)	T	A	T	C	A	T	G	G	C	A	T	G	G	C	T	C	C	A	G	A	G	A	C	T	C	G	G	A	G	A	C
FARG477–08	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-
FARGB885–12 (NZ)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
FARG446–08 (NZ)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
FARG451–08 (NZ)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
FARG448–08 (NZ)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
FARGB868–12	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	-	-	-
FARGB880–12 *	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
FARGB884–12 *	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-
FARG447–08	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
FARGB872–12 *	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
FARGB882–12	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
FARG173–06 *	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
M. hubbsi																																
FARG247–06	A	C	.	T	G	C	A	A	T	G	C	A	A	G	A	G	T	G	A	G	A	G	T	C	.	A	A	T	A	G	T
FARG047–06	A	C	A	T	G	C	A	A	T	G	C	A	A	G	A	G	T	G	A	G	A	G	T	C	.	A	A	T	A	G	T
FARG046–06	A	C	.	T	G	C	A	A	T	G	C	A	A	G	A	G	T	G	A	G	A	G	T	C	.	A	A	T	A	G	T
FARG248–06	A	C	.	T	G	C	A	A	T	G	C	A	A	G	A	G	T	G	A	G	A	G	T	C	.	A	A	T	A	G	T
FARG249–06	A	C	.	T	G	C	A	A	T	G	C	A	A	G	A	G	T	G	A	G	A	G	T	C	G	A	A	T	A	G	T
FARG246–06	A	C	.	T	G	C	A	A	T	G	C	A	A	G	A	G	T	G	A	G	A	G	T	C	.	A	A	T	A	G	T
FARG250–06	A	C	.	T	G	C	A	A	T	G	C	A	A	G	A	G	T	G	A	G	A	G	T	C	.	A	A	T	A	G	T
FARG048–06	A	C	.	T	G	C	A	A	T	G	C	A	A	G	A	G	T	G	A	G	A	G	T	C	.	A	A	T	A	G	T
FARG049–06	A	C	.	T	G	C	A	A	T	G	C	A	A	G	A	G	T	G	A	G	A	G	T	C	.	A	A	T	A	G	T
FARG050–06	A	C	.	T	G	C	A	A	T	G	C	A	A	G	A	G	T	G	A	G	A	G	T	C	.	A	A	T	A	G	T
FARG718–09	A	C	.	T	G	C	A	A	T	G	C	A	A	G	A	G	T	G	A	G	A	G	T	C	.	A	A	T	A	G	-
FARG719–09	A	C	.	T	G	C	A	A	T	G	C	A	A	G	A	G	T	G	A	G	A	G	T	C	.	A	A	T	A	G	T
FARG720–09	A	C	.	T	G	C	A	A	T	G	C	A	A	G	A	G	T	G	A	G	A	G	T	C	.	A	A	T	A	G	T
FARG721–09	A	C	.	T	G	C	A	A	T	G	C	A	A	G	A	G	T	G	A	G	A	G	T	C	.	A	A	T	A	G	T
FARG722–09	A	C	.	T	G	C	A	A	T	G	C	A	A	G	A	G	T	G	A	G	A	G	T	C	.	A	A	T	A	G	T
FARG723–09	A	C	.	T	G	C	A	A	T	G	C	A	A	G	A	G	T	G	A	G	A	G	T	C	.	A	A	T	A	G	T
FARG724–09	A	C	.	T	G	C	A	A	T	G	C	A	A	G	A	G	T	G	A	G	A	G	T	C	.	A	A	T	A	G	T
FARG725–09	A	C	.	T	G	C	A	A	T	G	C	A	A	G	A	G	T	G	A	G	A	G	T	C	.	A	A	T	A	G	T

(Vecchione *et al*. 2000). *Merluccius hubbsi* is the main fishery resource of Argentina, both for its role in the ecosystem as its social and economic importance (Gorini *et al.* 2010). On the other hand, commercial catches of *Merluccius australis* are relatively low, both in Argentina (Giussi *et al*. 2004; SSPyA 2012) and New Zealand (Colman 1995). Also, because of the difficulty of distinguishing between species of *Merluccius*, given their external morphological similarity, in Argentina overall fishery statistical data are presented for both species and they are not considered reliable (Csirke 1987).

Merluccius australis was first described by Hutton (1872) as *Gadus australis*, based on specimens from New Zealand, in a volume titled *Fishes of New Zealand* and comprising two papers: '*Catalogue with diagnoses of the species*' by F.W. Hutton, and '*Notes on the Edible Fishes of New Zealand*' by J. Hector. The species description is brief and somewhat typical of ones from that period, and reproduced here in its entirety:

D. 11–12 /19/22; A 19/22; V 7
Length equal to four and one-third times that of the head, or seven and a half times the height of the body; length of the head two and four-fifths that of the snout; diameter of the eye not much more than half the length of the snout; lower jaw longer; no barbel; strong teeth in both jaws, the outer series being shorter and fixed, the inner longer and capable of being folded back; strong teeth in a double series on the vomer, none on the palatine bones; upper profile of head straight, snout conical; head higher than broad; maxillary extending to beyond the middle of the eye; scales very small; vent rather nearer to the snout than to the end of the tail, below the commencement of the second dorsal; a space between the first and second dorsal, the second and third subcontinuous; a short space between the anals; proportions of the fins—

1D	2D	3D	1A	2A
1	2.7	1.9	2.4	2.1

Above purplish, sides and belly silvery; inside of the mouth white
Thrown up on the coast by heavy storms
It is said to attain a length of 4 feet
Cook Straits.

The number or size(s) of specimens of *M. australis* are not indicated in their original description (Hutton 1872; Russell 1996). At the back of the volume are 12 plates of wood-cut illustrations of many of the species covered by Hutton. A copy held at the Museum of New Zealand is annotated by the artist (Buchanan) linking Hutton's description with Plate VII Figure 72.

What has not been appreciated by many researchers is that Hector, in the following paper, refers back to Hutton's description, and notes that the species was '*...described by Captain Hutton from a few specimens that were cast up on the shore of Cook Strait after a heavy south-east gale.*' and that illustration is '*...one-sixth the natural size...*'. The wood-cut figure measures 107.14 mm TL (tip of lower jaw-caudal tip) which would mean the specimen it was drawn from a specimen 642.84 mm TL. This specimen was not found in the museum collections and is presumed lost.

In the online *Catalog of Fishes* (Eschmeyer 2014) the types of *M. australis* are recorded as BMNH 1872.4.26.8 and 1905.11.30.38 (one specimen each). According to the Natural History Museum zoology collection database both specimens come from Wellington and BMNH 1905.11.30.38 is labeled as "syntype?". Considering that BMNH 1872.4.26.8 was collected in the same year as Hutton´s description and that it in size (350 mm TL) close to the specimen of Plate VII Figure 72, we propose to designate this specimen as the lectotype of *M. australis*. We furthermore suggest that BMNH 1905.11.30.38 (donated by Hutton to the Natural History Museum) is designated as paralectotype.

Matallanas & Lloris (2006) examined BMNH 1905.11.30.38, and erroneously considered it as Hutton's holotype specimen. Based on this specimen and three catalogued specimens from off New Zealand waters (here re-examined as *M. australis*) these authors redescribed *M. australis*. Furthermore, the authors described *Merluccius tasmanicus* sp nov. based on four specimens from off New Zealand. This decision was done without discussion with the Curator of Fishes at The Museum of New Zealand, and is based on four moderate sized, rather soft and distorted specimens. The type locality of the holotype (NMNZ P.5566) is in Tasman Bay, only ~148 km in a direct line from the type location of *Merluccius australis*. In fact, one of the paratypes (NMNZ P.3963) is just 13 km off-shore of the coast where Hutton's *M. australis* specimens were washed up.

According to Matallanas & Lloris (2006) *M. tasmanicus* differs from all other congeneric species in a combination of several meristic, morphologic and morphometric characters. Meristic characters and proportional dimensions have been used in diagnostic keys for species of *Merluccius* from the first taxonomic revision (Marini 1933), to the most recent (Lloris *et al*. 2003). However, there have been difficulties because most of the characters exhibit high intraspecific variation and but minor interspecific differences (Inada 1981*a*; Lloris *et al*. 2003).
Matallanas & Lloris (2006) listed several meristic values to differentiate *M. tasmanicus* from its congenerics: numbers of first dorsal-fin, second dorsal-fin, anal-fin and pectoral-fin rays, lateral line scales and the upper and lower gill rakers. This study found no significant differences between *M. australis* and *M. tasmanicus* in any of the meristic characters (Table 2), including those listed by Matallanas & Lloris (2006). The ranges of meristic values obtained from the re-examined type specimens are similar and generally more limited than those cited in the original description of *M. tasmanicus*, except for the number of lateral line scales which is lower (>160 in Matallanas & Lloris (2006) cf. 147–152 in this study). This difference could be a result of scales lost in preserved specimens (Ginsburg 1954). Regardless, both counts overlap with the values obtained for *M. australis* (148–173) (Cohen *et al*. 1990; Lloris *et al*. 2003; Díaz de Astarloa *et al*. 2011; this study). We also found the mean values of lateral line scales of *M. tasmanicus* (150.3) are close to those of *M. australis* found by Díaz de Astarloa *et al.* (2011) (= 153.4) and Inada (1981*a*) (= 158.6).

The ranges and mean values of the remaining diagnostic counts of *M. tasmanicus* (number of first dorsal, second dorsal, anal and pectoral-fin rays and gill rakers on the upper and lower branches) are completely contained in those of *M. australis* from Argentina and New Zealand obtained in both this (Table 1) and previous studies (Ginsburg 1954; Wysokiński 1974; Cousseau & Cotrina 1980; Inada 1981*a* and *b*; Cohen *et al*. 1990; Lloris *et al*. 2003; Díaz de Astarloa *et al*. 2011). Of the other meristic characters studied, the ranges that overlap and have similar mean values between *M. australis* (from Argentina and New Zealand) and *M. tasmanicus* are the total number of gill rakers and total number of abdominal and caudal vertebrae (Table 1). This is also supported by previous studies (Ginsburg 1954; Angelescu *et al.* 1958; Wysokiński 1974; Cousseau & Cotrina 1980; Inada 1981*a* and *b*; Cohen *et al*. 1990; Lloris *et al*. 2003; Díaz de Astarloa *et al*. 2011).

On the other hand, our examination of meristic characters highlights significant differences between *M. hubbsi* and *M. australis* (Table 2), as obtained by Díaz de Astarloa *et al.* (2011). Meristic count ranges that do not overlap are the number of scales along the lateral line (99 to 144 vs. 148 to 173, respectively). These are widely used as a discriminating character of *Merluccius* spp. (Cohen *et al.* 1990; Lloris *et al*. 2003; Díaz de Astarloa *et al*. 2011). There is also clear separation in the total number of vertebrae (50 to 53 vs. 54 to 58, respectively) cited as a character of considerable importance by Wysokiński (1974) and Inada (1981*a*). Other meristic variables widely used in distinguishing between *M. australis* and *M. hubbsi* (Ginsburg 1954; Cousseau & Perrotta 2004; Díaz de Astarloa *et al*. 2011) are the number of second dorsal and anal-fin rays, which partially overlap between the species (Table 1). While the rest of the examined characters (number of first dorsal and pectoral-fin rays and gill rakers) overlap considerably between the species, lower mean values were observed for *M. australis* (Table 1), coinciding with the findings of Ginsburg (1954).

External morphological characters are most easily observed and are therefore useful for a quick and accurate diagnosis (Lloris *et al*. 2003). However, in the present study, the external morphological characters analyzed were not found to be unique to any of the three species of *Merluccius* (Figures 5 and 6). Morphometric characters traditionally used partially overlapped between *M. hubbsi* and *M. australis*, and completely overlapped between *M. tasmanicus* and *M. australis* (Table 1). This result was confirmed by multivariate analysis (Principal Component and Discriminant analyses) of LMMs and IlMs variables. These allowed significant differentiation of *M. hubbsi* and *M. australis* and supports synonymy of *M. tasmanicus* with *M. australis* (Figures 6 and 7). LMMs that allow better discrimination between *M. hubbsi* and *M. australis* are: eye diameter, lower and upper snout length, interorbital width and pelvic fin length. As was noted by Cousseau & Cotrina (1980), Cousseau & Perrotta (2004) and Díaz de Astarloa *et al.* (2011) the snout is longer in *M. australis*, while the diameter of the eye is larger in *M. hubbsi*. Cousseau & Cotrina (1980) found that interorbital width is greater in *M. australis* than in *M. hubbsi*. We found the length of the pelvic fin also a useful diagnostic feature that allows distinction between species. Cousseau & Cotrina (1980) cited as differentiating features the pectoral fin length and the preanal distance. These latter variables have largely overlapping ranges between species, with similar means (Table 1).

Matallanas & Lloris (2006) found some external morphological characteristics and morphometric proportions as diagnostic features for *M. tasmanicus*. According to these authors, *M. tasmanicus* has a more robust, shorter

body, with a greater body height than *M. australis*. We observed the type specimens of *M. tasmanicus* appear to be either robust or slender, as in *M. hubbsi* and *M. australis* (Figures 5 and 6). We found a wider range of body depth for *M. tasmanicus* (5.8–7.8 times in TL) than recorded by Matallanas & Lloris (2006). This proportion overlaps with what we found for that of *M. australis* from Argentina (6.0–8.5 times in TL) and New Zealand (6.9–7.6 times in TL). It also overlaps with what Matallanas & Lloris (2006) found for *M. australis* (7.3–7.5 times in TL). Moreover, Hutton (1872) in the original description of *M. australis* cited a body depth of 7.5 times in TL, value contained in the range of *M. tasmanicus*. Norman (1937) recorded *M. australis* with a body depth of 5–6 times TL, which also partially overlapped with *M. tasmanicus*. We consider that body depth is very subjective character as the types are somewhat distorted and soft, so cannot be relied on. Furthermore, this feature is highly variable and dependent (amongst other things) on the degree of stomach fullness and sexual maturity of each individual (Svetovidov 1948), and can also be affected by decompression during capture (Angelescu *et al*. 1958).

The shape of the dorsal margin of the head has also been used to differentiate *Merluccius* spp. (Lloris & Matallanas, 2003; Matallanas & Lloris, 2006). However, we observed that all types of *M. tasmanicus* have a straight head profile and, both *M. australis* (from Argentina and New Zealand) and *M. hubbsi*, exhibit both concave and straight head outlines of different proportions (Figures 5 and 6), proving to be highly variable and of poor taxonomic value, as also pointed out Díaz de Astarloa *et al*. (2011).

The size and position of the eye are described by Matallanas & Lloris (2006) as very small and distant to the dorsal margin of the head in *M. tasmanicus*, and large, reaching the dorsal margin of the head in *M. australis*. These features also show high intraspecific variation, with both smaller and distant or larger and closer eyes to the dorsal profile of the head in both *M. hubbsi* and *M. australis* (Figures 5 and 6). We also found lower values in eye diameter in head length (5.6–6.0 vs 6.1–7.1, respectively), in lower snout length (1.8–2.1 vs 2.1–2.2, respectively) and in interorbital width (1.4–1.7 vs 1.6–1.9, respectively) for *M. tasmanicus* than those published by Matallanas & Lloris (2006). These proportions are contained in of *M. australis* in this work from Argentina (5.1 to 8.1 HL, 1.5 to 2.8 LSL and 1.4 to 2.2 IW), and New Zealand (5.2 to 6.8 HL, 1.8 to 2.6 LSL and 1.4 to 1.8 IW), as well as in others studies (5.6 HL and < 2 LSL (Hutton 1872); 6 to 7 HL, > 2 LSL and 1.5 to 2 IW (Norman 1937)). The range of eye diameter (Table 1) also overlaps between *M. tasmanicus* and *M. australis* in the latest revisions of *Merluccius* spp. (Inada 1981*a*; Lloris *et al*. 2003). The mean value for eye diameter is greater in *M. tasmanicus* than in *M. australis* from Argentina, but is similar to that of *M. australis* from New Zealand (Table 1). This difference could the result of differential growth or allometry of the eye, which becomes evident when dissimilar group sizes are compared (264–410 mm TL in *M. tasmanicus* and 338–445 mm TL in *M. australis* from New Zealand vs 466–806 mm TL in *M. australis* from Argentina). Smaller specimens of *Merluccius* have proportionally larger eye diameter than larger ones (Angelescu *et al.* 1958). Analysis of normalized traditional morphometric characters shows that specimens of *M. tasmanicus* and *M. australis* from Argentina and New Zealand grouped together, characterized by an eye diameter smaller than for *M. hubbsi* (Figure 7).

Matallanas & Lloris (2006) reported that the shape of the lateral line over the pectoral fin and on the caudal peduncle is straight in *M. australis*, differing from *M. tasmanicus*. We found that the shape of the lateral line is either straight or curved over the pectoral-fin in both *M. hubbsi* and *M. australis* (from Argentina and New Zealand) and straight and equidistant from the dorsal and ventral body profiles on the caudal peduncle in all species studied (Figures 5 and 6). This is a highly variable character, very difficult to diagnose and cannot be considered of any taxonomic value. It appears curvilinear in hakes with full stomach and rectilinear in rigid and twisted preserved fish (Cousseau & Cotrina 1980).

Matallanas & Lloris (2006) also differentiate *M. tasmanicus* from *M. australis* on the basis of the distal end of the pectoral-fin not reaching the origin of the anal-fin in specimens of *M. tasmanicus* >400 mm TL, c.f. reaching or extending beyond the origin of the anal-fin in *M. australis*. In this study, we found the distal end of the pectoral-fin does not reach the origin of the anal-fin in about half of the specimens >400 mm TL, in both of *M. australis* and *M. hubbsi*. In addition, this characteristic depends largely on the size and/or sexual maturity of specimens (Inada 1981*a*). It was described as distinctive in *M. tasmanicus* by Matallanas & Lloris (2006), on the basis of a single specimen >400 mm TL.

The osteological elements of greatest diagnostic value for discriminating between *Merluccius* spp. are the hyomandibula, urohyal and the sagitta (Inada 1981*a*; Cohen *et al*. 1990; Lloris *et al*. 2003). Matallanas & Lloris (2006) included a figure of the hyomandibula, urohyal and otolith (Matallanas & Lloris 2006: Figure 2) of *M. tasmanicus*, with no further details of the catalogue/specimen number or the size of the specimens dissected. For

the hyomandibula, one of the most noticeable differences between *M. hubbsi* and *M. australis* is the shape of the opercular process (Figures 3a and 9a). It is nearly at a right angle to the vertical axis of the bone in the former, and slightly inclined downwards in the latter, coinciding with findings of Delpiani *et al.* (2012). Another distinguishing character of the hyomandibula found in this study, is the depth of the recess between intermuscular and preopercular processes (Figures 3a and 9a). This notch is greater and lies anterior to the recess between the intermuscular and the pterygoid processes in *M. australis*, but shallower and posterior to the recess between the intermuscular and pterygoid processes in *M. hubbsi*. It appears that the hyomandibula of *M. tasmanicus* (Matallanas & Lloris 2006: Figure 2) is very similar to that of *M. australis*, with the opercular process inclined downward and a deep recess between the intermuscular and the pterygoid processes.

The shape and relationship between the lengths of the intermuscular and preopercular processes of the hyomandibula have been used to separate species of *Merluccius* (Inada 1981*a*; Lloris & Matallanas 2003), but again we found that these characters show great intraspecific variability in *M. hubbsi* and *M. australis* (Figures 3a and 9a), as it was found by other authors (Díaz de Astarloa *et al*. 2011; Delpiani *et al*. 2012). Our finding is confirmed by Principal Component Analysis of the measurements obtained from the hyomandibula, where lengths of the two processes are variable and do not allow distinction between species (Figure 10).

Some of the rather interspecific distinctive features of the urohyal are the approximately triangular shape of the anterodorsal process in *M. hubbsi* and about rectangular shape, with a longer base in *M. australis* (Figures 3b and 9b). As seen in the plot of the first two PCs of the IlDs obtained from the urohial (Figure 10), these characters show some distinction between *M. hubbsi* and *M. australis*. From Figure 2 in Matallanas & Lloris (2006), the urohyal of *M. tasmanicus* has a rectangular anterodorsal process with a long base, like that of *M. australis*.

Lloris & Matallanas (2003) cited the angle between the anterodorsal process and the base of the urohyal as different between *Merluccius* spp. However, in this and other studies (Díaz de Astarloa *et al*. 2011; Delpiani *et al.* 2012) that angle is found to be obtuse or square in *M. hubbsi* and *M. australis*, and even acute in *M. hubbsi*. Inada (1981a) found no differences in the urohyal shape of *Merluccius* spp., but did find differences in the degree of ossification, which Cohen *et al.* (1990) incorporated into the identification key of *Merluccius*, discriminating the heavily ossified urohyal of *M. hubbsi* from other species. In the present study, as in Lloris & Matallanas (2003), the degree of ossification in the urohyal can vary greatly in relation to size in *M. hubbsi*. Specimens smaller than ~330 mm TL have a thin and poorly ossified urohyal, whereas in larger individuals it becomes thicker and heavily ossified. The TL of fish in which the change in the degree of ossification is observed, falls within the size range described for *M. hubbsi* at first maturity in both females (320–380 mm TL) and males (270–350 mm TL) (Pájaro *et al*. 2005; Macchi *et al*. 2007). In *M. australis*, all examined sizes (~ 400 –800 mm TL) had a thin urohyal as recorded in Inada (1981a), Cohen *et al*. (1990) and Matallanas & Lloris (2003).

The sagitta exhibits great variation in shape with size in the two studied species, coinciding with the findings for the genus *Merluccius* by other authors (Inada 1981*a*; Lombarte & Castellón 1991; Lombarte & Lleonart 1993; Torres *et al*. 2000). Differences were found, however, in the ventral margin of the otolith: entirely crenulate in *M. australis*, and smooth in its central portion in *M. hubbsi*. In *M. tasmanicus* the ventral margin of the otolith is totally crenulate as in *M. australis* (Matallanas & Lloris 2006: Figure 2). In *M. hubbsi*, specimens < 330 mm TL have a more oval otolith, with a rear end shorter and wider (Figure 12a). These individuals do not exhibit excisura ostii, as described by others (Díaz Astarloa *et al*. 2011; Delpiani *et al*. 2012). With increased size, the rear end of the sagitta becomes longer and more tapered, and most individuals (92%) present excisura (Figure 12a). In *M. australis*, a gradual increase in the height of the posterodorsal margin of the otolith is observed, and 62 specimens (39%) different sized specimens (~400–800 mm TL) show an excisura ostii (Figures 12a and b), different to that described by others authors (Díaz Astarloa *et al*. 2011; Delpiani *et al*. 2012). According to Lloris & Matallanas (2003) the presence of excisura ostii is a useful diagnostic character. Nevertheless, this character also shows great intraspecific variation, which could be related to changes in calcium metabolism during periods of reproductive activity (Morales-Nin *et al*. 1998), allometric growth (Lombarte & Castellón 1991) or environmental conditions, especially temperature variations (Lombarte & Lleonart 1993). No clear relationship was found between the excisura and fish length (Figure 12b), unlike that described by Díaz de Astarloa *et al.* (2011).

The analysis of COI sequences, based on *p*-distances with the topology created by the NJ tree was able to discriminate *M. hubbsi* from *M. australis* (Figure 13). All the specimens identified as *M. australis* from Argentina and New Zealand, with and without the diagnostic features of *M. tasmanicus* cluster together, sharing the same haplotype (Table 3), and are separate from *M. hubbsi* by a pronounce genetic distance (5%). Previous DNA

mitochondrial (cytochrome b, control region and 16S rDNA) studies already pointed out a large genetic distances between *M. hubbsi* and *M. australis* (K2P: 4.2% in Campo *et al.* (2007); Tamura-Nei: 1.7–4.8% in Silva-Segundo *et al.* (2011); Tamura-Nei: 7.8% in Quinteiro *et al*. (2000)). The intraspecific distances are very small in *M. hubbsi* (0.026%) or nule in *M. australis* (0%). In congruence, low divergences have been found between specimens in both species by the analysis of other DNA mitochondrial sequences (Quinteiro *et al*. 2000 (Tamura-Nei: 0.5%); Silva-Segundo *et al*. 2011 (Tamura-Nei: 0.2%)) and trough allozyme studies (Roldán 1991; Roldán *et al*. 1999; Grant & Leslie 2001; Roldán & Pla 2001).

The Barcode Index Number (BIN) system is a registry for animal OTUs (operational taxonomic units) recognized through sequence variation in the COI DNA barcode region (Ratnasingham & Hebert 2013). Since OTUs show high concordance with species, this system can be used to verify species identifications (Ratnasingham & Hebert 2013). BIN analysis recognizes two taxonomic units for *Merluccius hubbsi* and *M. australis* records, which agree with current taxonomic classification. Furthermore a barcode gap, which allows for successful identification, was detected for both species since low values of intraspecific divergences and high Nearest-Neighbour distances were found.

In conclusion, this integrative taxonomical study, using meristic, morphological, morphometric and DNA barcode analysis, found no evidence to support either the existence of another species of *Merluccius* in the New Zealand EEZ, or the occurrence of *M. tasmanicus* in Argentinean waters. The reported diagnostic characters of *M. tasmanicus* show overlapping values with those obtain of the examination of type and fresh specimens of *M. australis* in both this work and many other studies. Furthermore, DNA barcodes discriminate between two well-support groups, with no evidence of intraspecific variation between the specimens of *M. australis*. Therefore, the evidence irrefutably shows *M. tasmanicus* is a junior synonym of *M. australis*.

Acknowledgments

The authors are grateful to the crew and scientists of R.V. Dr. Eduardo Holmberg and from the Instituto Nacional de Investigación y Desarrollo Pesquero for assistance with sample collection. We are greatly indebted to Daniel Fernandez and Santiago Ceballos from the CADIC for their contribution to fieldwork logistic and laboratory facilities. We also thank Carlos Capiel and Daniela Ferrari and all the technicians of the Instituto Radiológico (Mar del Plata, Argentina). We are especially grateful to Pablo Tubaro and Darío Lijtmaer from Museo Argentino de Ciencias Naturales (MACN-CONICET) for molecular laboratory facilities. The senior author is much indebted to the Department Milieux et Peuplements Aquatiques (MNHN), especially Patrice Pruvost, Guy Duhamel, Romain Causse, Claude Ferrara and Zora Gabsi for the kind assistance during recent visits to the fish collection. Douglas Nelson from the UMMZ arranged for the loan of the paratype of *M. hubbsi*. Oliver Crimmen and Patrick Campbell (Natural History Museum, London) assisted in the examination of specimens curated in the fish collection. Michael Mincarone from the UFRJ assisted in sending the type specimens of *M. tasmanicus*. Gustavo Chiaramonte (MACN) provided helpful comments on the types of *M. hubbsi*. The Smithsonian Institution and the Muséum National d´histoire Naturelle, Paris supported the senior author by short-term visitor's grants for research. This research was partially funded by the Consejo Nacional de Investigaciones Científicas y Técnicas (Argentina IBOL grants), Universidad Nacional de Mar del Plata (Argentina), the International Development Research Centre of Canada (IDRC), the Canadian Barcode of Life Network from Genome (through the Ontario Genomics Institute) and Natural Sciences and Engineering Research Council of Canada. S.M.D. and M.Y.D.A. were supported by CONICET doctoral fellowships. A.S. was supported by the New Zealand National Institute of Water and Atmospheric Research Ltd., Core Funded Coasts and Oceans Programme 2: Biological Resources contract with the Museum of New Zealand Te Papa Tongarewa.

References

Angelescu, V., Gneri, F.S. & Nani, A. (1958) *La merluza del Mar Argentino. Biología y Taxonomía.* Servicio de Hidrografía Naval, Buenos Aires, 244 pp.

Ballara, S.L. (2012) Descriptive analysis of the fishery for hake (*Merluccius australis*) in HAK 1, 4 and 7 from 1989-90 to 2009-10, and a catch-per-unit-effort (CPUE) analysis for Sub-Antarctic hake. *New Zealand Fisheries Assessment Report*,

2012/02, 1–47.
Bezzi, S. & Dato, C. (1995) Conocimiento biológico pesquero del recurso merluza (*Merluccius hubbsi*) y su pesquería en la República Argentina. *Documento Científico del Instituto Nacional de Investigación y Desarrollo Pesquero* N° 4 (INIDEP), Mar del Plata 52 pp.
Campo, D., Machado Schiaffino, G., Pérez, J. & García Vázquez, E. (2007) Phylogeny of the genus *Merluccius* based on mitochondrial and nuclear genes. *Gene*, 406 (1–2), 171–179. http://dx.doi.org/10.1016/j.gene.2007.09.008
Cohen, D.M., Inada, T., Iwamoto, T. & Scialabba, N. (1990) FAO Species Catalogue. Gadiform fishes of the world (Order Gadiformes). An annotated and illustrated catalogue of cods, hakes, grenadiers and other gadiform fishes known to date**.** *FAO Fisheries Synopsis*, 10 (125), 1–442.
Colman, J.A. (1995) Biology and fisheries of New Zealand hake (*M. australis*). *In*: Alheit, J. & Pitcher, T.J. (Eds.), *Hake. Fisheries, ecology and markets*. Chapman & Hall, London, pp. 365–388.
Cousseau, M.B. (coord.) (2010) *Ictiología: aspectos fundamentales, la vida de los peces sudamericanos*. Editorial de la Universidad Nacional de Mar del Plata, Mar del Plata, 665 pp.
Cousseau, M.B. & Cotrina, C.P. (1980) Observaciones sobre diferencias morfométricas entre la merluza común (*Merluccius hubbsi*) y la merluza austral (*Merluccius polylepis*). *Revista de Investigación y Desarrollo Pesquero* 2, 47–56.
Cousseau, M.B. & Perrotta, R.G. (2004) *Peces marinos de Argentina. Biología, distribución, pesca*. Publicaciones especiales del Instituto Nacional de Investigación y Desarrollo Pesquero (INIDEP), Mar del Plata, 167 pp.
Csirke, J. (1987) Los recursos pesqueros patagónicos y las pesquerías de altura en el Atlántico sudoccidental. *FAO Documento Técnico de Pesca* 286, 1–78.
Deli Antoni, M.Y., González Castro, M. & Díaz de Astarloa, J.M. (2008) Análisis de la morfología ósea de peces de la laguna costera Mar Chiquita, Argentina. *Revista de biología marina y oceanografía*, 43 (2), 355–380. http://dx.doi.org/10.4067/S0718-19572008000200013
Delpiani, S.M., González Castro, M. & Díaz de Astarloa, J.M. (2012) El uso de otolitos y huesos de la cabeza para la identificación de dos especies del género *Merluccius*, en estudios de predador-presa. *Revista de biología marina y oceanografía*, 47 (2), 351–357. http://dx.doi.org/10.4067/s0718-19572012000200018
Díaz de Astarloa, J.M., Bezzi, S.I., González Castro, M., Mabragaña, E., Hernández, D., Delpiani, S.M., Figueroa, D.E., Cousseau, M.B., Deli Antoni, M.Y. & Tringali, L. (2011) Morphological, morphometric, meristic and osteological evidence for two species of hake (Actinopterygii: Gadiformes: *Merluccius*) in Argentinean waters. *Journal of Fish Biology*, 78 (5), 1336–1358. http://dx.doi.org/10.1111/j.1095-8649.2011.02937.x
Eschmeyer, W.N. (Ed.) (2014) *Catalog of Fishes*. California Academy of Sciences. Available from: http://research.calacademy.org/research/ichthyology/catalog/fishcatmain.asp (accessed 15 October 2014)
FAO (2010) *El estado mundial de la pesca y la acuicultura.* Departamento de Pesca y Acuicultura de la Organización de las Naciones Unidas para la agricultura y la alimentación, Roma, 219 pp.
Felsenstein, J. (1985) Confidence limits on phylogenies: An approach using the bootstrap. *Evolution*, 39, 783–791. http://dx.doi.org/10.2307/2408678
Fischer, J. (Ed.) (2013) Fish identification tools for biodiversity and fisheries assessments: review and guidance for decision-makers. *FAO Fisheries and Aquaculture Technical Paper*, 585, 1–107.
Ginsburg, I. (1954) Whitings on the coasts of the American continents. *Fishery Bulletin of the Fish and Wildlife Service*, 96 (56), 187–208.
Giussi, A.R., García de la Rosa, S.B. & Sánchez, M.F. (2004) Caracterización biológica y estado de explotación de la merluza austral (*Merluccius australis*). *In*: Sánchez, R.P. & Bezzi, S.I. (Eds.), *El Mar Argentino y sus recursos pesqueros. Tomo 4. Los peces marinos de interés pesquero. Caracterización biológica y evaluación del estado de explotación*. Publicaciones Especiales del Instituto Nacional de Investigación y Desarrollo Pesquero (INIDEP), Mar del Plata, pp. 307–320.
González-Castro, M., Heras, S., Cousseau, M.B. & Roldán, M.I. (2008) Assessing species validity of *Mugil platanus* Günther, 1880 in relation to *Mugil cephalus* Linnaeus, 1758 (Actinopterygii). *Italian Journal of Zoology*, 75 (3), 319–325. http://dx.doi.org/10.1080/11250000801886254
González-Castro, M., Ibáñez-Aguirre, A.L., Heras, S., Roldán, M.I. & Cousseau, M.B. (2012) Assessment of Lineal Versus Landmark-Based Morphometry for Discriminating Species of Mugilidae (Actinopterygii). *Zoological Studies*, 51 (8), 1515–1528.
Gorini, F.L., Abachian, V.E. & Giussi, A.R. (2010) Estimación de la edad y el crecimiento de la merluza austral *Merluccius australis* (Hutton, 1872) del Mar Argentino. *Revista de Investigación y Desarrollo Pesquero (INIDEP)*, 20, 65–73.
Grant, W.S. & Leslie, R.W. (2001) Inter-ocean dispersal is an important mechanism in the zoogeography of hakes (Pisces: *Merluccius* spp.) *Journal of Biogeography*, 28 (6), 699–721. http://dx.doi.org/10.1046/j.1365-2699.2001.00585.x
Hecht, T. (1987) A guide to the otoliths of southern ocean fishes. *South African Journal of Antartic Research*, 17, 1–87.
Hector, J. (1872) Notes on the edible fishes of New Zealand. *In*: Hutton, F.W. & Hector, J. (Eds.), *Fishes of New Zealand*. Colonial Museum and Geological Survey Department, Wellington, pp. 97–133.
Hubbs, C.L. & Lagler, K.F. (1958) Fishes of the Great Lakes region. *Cranbrook Institute of Science Bulletin*, 26, 1–213.

Hutton, F.W. (1872) Catalogue with Diagnoses of the Species. *In*: Hutton, F.W. & Hector, J. *Fishes of New Zealand*. Colonial Museum and Geological Survey Department, Wellington, pp. 1–93, Pl. I–XII.

Ho, J.S. (1990) Phylogeny and biogeography of hakes (*Merluccius*; Teleostei): a cladistic analysis. *Fishery Bulletin U.S.*, 88, 95–104.

Inada, T. (1981*a*) Studies on the Merlucciid Fishes. *Bulletin of the Far Seas Fisheries Research Laboratory*, 18, 1–172.

Inada, T. (1981*b*) Two Nominal Species of *Merluccius* from New Zealand and Southern South America. *Japanese Journal of Ichthyology*, 28 (1), 31–36.

Ivanova, N.V., deWaard, J.R. & Hebert, P.D.N. (2006) An inexpensive, automation-friendly protocol for recovering high–quality DNA. *Molecular Ecology Notes*, 6 (4), 998–1002.
http://dx.doi.org/10.1111/j.1471-8286.2006.01428.x

Ivanova, N.V., Zemlak, T.S., Hanner, R.H. & Hebert, P.D.N. (2007) Universal primer cocktails for fish DNA barcoding. *Molecular Ecology Notes*, 7 (4), 544–548.
http://dx.doi.org/10.1111/j.1471-8286.2007.01748.x

Kimura, M. (1980) A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, 16 (2), 111–120.
http://dx.doi.org/10.1007/bf01731581

Lleonart, J., Salat, J. & Torres, G.J. (2000) Removing allometric effects of body size in morphological analysis. *Journal of Theoretical Biology*, 205 (1), 85–93.
http://dx.doi.org/10.1006/jtbi.2000.2043

Lleonart, J., Taconet, M. & Lamboeuf, M. (2006) Integrating information on marine species identification for fishery purposes. *Marine Ecology Progress Series*, 316, 231–238.
http://dx.doi.org/10.3354/meps316231

Lloris, D. & Matallanas, J. (2003) Description of a new species of hake: *Merluccius patagonicus* sp. nov. (Gadiformes: Merlucciidae) from the waters of Argentina. *Scientia Marina*, 67 (3), 323–326.
http://dx.doi.org/10.3989/scimar.2003.67n3323

Lloris, D., Matallanas, J. & Oliver, P. (2003) *Merluzas del Mundo (Familia Merlucciidae). Catálogo comentado e ilustrado de las merluzas conocidas.* FAO Catálogo de Especies para los Fines de la Pesca N° 2, Roma, 57 pp.

Lombarte, A. & Castellón, A. (1991) Interspecific and intraspecific otolith variability in the genus *Merluccius* as determined by image analysis. *Canadian Journal of Zoology*, 69 (9), 2442–2449.
http://dx.doi.org/10.1139/z91-343

Lombarte, A. & Lleonart, J. (1993) Otolith size changes related with body growth, habitat depth and temperature. *Environmental Biology*, 37 (3), 297–306.
http://dx.doi.org/10.1007/bf00004637

Macchi, G.J., Pájaro, M. & Dato, C. (2007) Spatial variations of the Argentine hake (*Merluccius hubbsi* (Marini, 1933)) spawning shoals in the Patagonian area during a reproductive season. *Revista de Biología Marina y Oceanografía*, 42 (3), 345–356.
http://dx.doi.org/10.4067/s0718-19572007000300013

MAGyP (2010) República Argentina. Indicadores de la pesca marítima. Available from: http://www.minagri.gob.ar/site/pesca/pesca_maritima/04=informes/01-informes_de_coyuntura/_archivos/600004-2010/101201_Informe%20de%20coyuntura%20-%20Diciembre%202010.pdf (accessed 5 February 2014)

MAGyP (2011) República Argentina. Informe de coyuntura. Available from: http://www.minagri.gob.ar/site/pesca/pesca_maritima/04=informes/01-informes_de_coyuntura/_archivos/500005-2011/111201_Informe%20de%20coyuntura%20-%20Diciembre%202011.pdf (accessed 5 February 2014)

Marini, T.L. (1933) La merluza argentina. *Physis*, 11 (39), 321–326.

Matallanas, J. & Lloris, D. (2006) Description of *Merluccius tasmanicus* sp. nov. and redescription of *Merluccius australis* (Pisces: Merlucciidae). *Journal of the Marine Biological Association of the United Kingdom*, 86 (01), 193–199.
http://dx.doi.org/10.1017/s0025315406013038

Monk, R.R. & Baker, R.J. (2001) e-Vouchers and the use of digital imagery in natural history collections. *Museology*, 10, 1–8.

Morales-Nin, B., Torres, G.J., Lombarte, A. & Recasens, L. (1998) Otolith growth and age estimation in the European hake. *Journal of Fish Biology*, 53 (6), 1155–1168.
http://dx.doi.org/10.1111/j.1095-8649.1998.tb00239.x

Moyle, P.B. & Cech, J.J. Jr. (1996) *Fishes: an Introduction to Ichthyology*. Third Edition. Prentice Hall, New Jersey, 590 pp.

Nei, M. & Kumar, S. (2000) *Molecular Evolution and Phylogenetics*. Oxford University Press, New York, 333 pp.

Norman, J.R. (1937) Coast fishes. Part II. The Patagonian region. *Discovery Reports*, 16, 1–150.

Pájaro, M., Macchi, G.J. & Ehrlich, M. (2005) Reproductive pattern of the Patagonian stock of Argentine hake (*Merluccius hubbsi*). *Fisheries Research*, 72 (1), 97–108.
http://dx.doi.org/10.1016/j.fishres.2004.09.006

Pitcher, T.J. & Alheit, J. (1995) What makes a hake? A review of the critical biological features that substaint global hake fisheries. *In*: Alheit, J. & Pitcher, T.J., (Eds.), *Hake: Biology, fisheries and markets*. Chapman & Hall, Londres, pp. 89–124.

Quinn, G.P. & Keough, M.J. (2002) *Experimental design and data analysis for biologists.* Cambridge University Press,

Cambridge, 537 pp.
Quinteiro, J., Vidal, R. & Rey-Méndez, M. (2000) Phylogeny and biogeographic history of hake (genus *Merluccius*), inferred from mitochodrial DNA control–region sequences. *Marine Biology*, 136 (1), 163–174. http://dx.doi.org/10.1007/s002270050019
Ratnasingham, S. & Hebert, P.D.N. (2007) BOLD: The Barcode of Life Data Systems (www.barcodinglife.org). *Molecular Ecology Notes*, 7, 355–364. http://dx.doi.org/10.1111/j.1471-8286.2007.01678.x
Ratnasingham, S. & Hebert, P.D.N. (2013) A DNA-Based Registry for All Animal Species: The Barcode Index Number (BIN) System. *PLoS One*, 8, e66213. http://dx.doi.org/10.1371/journal.pone.0066213
Rojo, A. (1976) Osteología de la merluza argentina (*Merluccius hubbsi*, Marini 1933). *Boletín del Instituto Español de Oceanografía*, 219, 1–61.
Roldán, M.I. (1991) Enzymatic polymorphisms in the Argentinian hake, *Merluccius hubbsi* Marini, of the Argentinian continental shelf. *Journal of Fish Biology*, 39, 53–59. http://dx.doi.org/10.1111/j.1095-8649.1991.tb05067.x
Roldán, M.I., García-Marín, J.L., Utter, F.M. & Pla, C. (1999) Genetic relationships among *Merluccius* species. *Heredity*, 83, 79–86. http://dx.doi.org/10.1038/sj.hdy.6885300
Roldán, M.I. & Pla, C. (2001) Species identification of two sympatric hakes by allozymic markers. *Scientia Marina*, 65 (1), 81–84. http://dx.doi.org/10.3989/scimar.2001.65n181
Russell, B.C. (1996) Type specimens of New Zealand fishes described by Captain F.W. Hutton, F.R.S. (1836-1905). *Journal of the Royal Society of New Zealand*, 26 (2), 215–236. http://dx.doi.org/10.1080/03014223.1996.9517511
Sabaj Pérez, M.H. (Ed) (2013) Standard symbolic codes for institutional resource collections in herpetology and ichthyology: an Online Reference. Version 4.0. Available from: http://www.asih.org/sites/default/files/documents/resources/symbolic_codes_for_collections_v4.0_sabajperez_2013.pdf (accessed 28 June 2013)
Saitou, N. & Nei, M. (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, 4 (4), 406–425.
Sheskin, D.J. (2004) *Handbook of Parametric and Nonparametric Statistical Procedures*. *Third Edition.* Chapman & Hall/CRC, Boca Raton, 1193 pp.
Silva-Segundo, C.A., Brito-Chavarria, M., Balart, E., Barriga-Sosa, I., Rojas-Esquivel, R., Roldán, M., Murugan, G. & García-De León, F. (2011) Clarifying the taxonomic status of *Merluccius* spp. in the northeastern Pacific: a combined morphological and molecular approach. *Reviews in Fish Biology and Fisheries*, 21 (2), 259–282. http://dx.doi.org/10.1007/s11160-010-9166-6
Srivathsan, A. & Meier, R. (2012) On the inappropriate use of Kimura-2-parameter (K2P) divergences in the DNA-barcoding literature. *Cladistics*, 28, 190–194. http://dx.doi.org/10.1111/j.1096-0031.2011.00370.x
SSPyA (2012) Desembarques. Sistemas de la Subsecretaria de Pesca y Acuicultura, Ministerio de Agricultura, Ganadería y Pesca, Argentina. Available from: http://www.minagri.gob.ar/site/pesca/pesca_maritima/02-desembarques/index.php (accessed 5 February 2014)
Stauffer, J.R. & Kocovsky, P.M. (2007) Exploring links between systematics and fisheries management. *Transactions of the American Fisheries Society*, 136 (4), 1122–1125. http://dx.doi.org/10.1577/t06-166.1
Strauss, R.E. & Bookstein, F.L. (1982) The truss: body form reconstruction in morphometrics. *Systematic Zoology*, 31 (2), 113–135. http://dx.doi.org/10.2307/2413032
Svetovidov, A.N. (1948) Gadiformes. Fauna of the U. S.S.R, Fishes. *Zoological Institute of the Academy of Sciences of the USSR*, 9 (4), 1–222. [English translation 1962. Israel Program for Scientific Translations, Jerusalem 304 pp.]
Tamura K. & Nei M. (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution*, 10 (3), 512–526.
Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011) MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Molecular Biology and Evolution*, 28 (10), 2731–2739. http://dx.doi.org/10.1093/molbev/msr121
Torres, G.J., Lombarte, A. & Morales-Nin, B. (2000) Variability of the sulcus acusticus in the sagittal otolith of the genus *Merluccius* (Merlucciidae). *Fisheries Research*, 46 (1–3), 5–13. http://dx.doi.org/10.1016/s0165-7836(00)00128-4
Vaz-dos-Santos, A.M., Rossi-Wongtschowski, C.L.D.B., de Figueiredo, J.L. & Ávila-da-Silva, A.O. (2010) Threatened fishes of the world: *Merluccius hubbsi* Marini, 1933 (Merlucciidae). *Environmental Biology of Fishes*, 87 (4), 349–350. http://dx.doi.org/10.1007/s10641-010-9608-y

Vecchione, M. & Collette, B.B. (1996) The central role of systematics in marine biodiversity problems. *Oceanography*, 9 (1), 44–45.
http://dx.doi.org/10.5670/oceanog.1996.26
Vecchione, M., Mickevich, M.F., Fauchald, K., Collette, B.B., Williams, A.B., Munroe, T.A. & Young, R.E. (2000) Importance of assessing taxonomic adequacy in determining fishing effects on marine biodiversity. *ICES Journal of Marine Science*, 57 (3), 677–681.
http://dx.doi.org/10.1006/jmsc.2000.0707
Volpedo, A.V. & Echeverría, D.D. (2000) *Catálogo y claves de otolitos para la identificación de peces del mar argentino*. Editorial Dunken, Buenos Aires, 88 pp.
Waite, E.R. (1911) Scientific results of the New Zealand government trawling expedition, 1907. Pisces, Part II. *Records of the Canterbury Museum*, 1 (3), 157–272.
Whitaker, D.R. (1980) World utilization of hake. *Marine Fisheries Review*, 42, 4–7.
Wysokiński, A. (1974) Taxonomic position of hake, *Merluccius polylepis* Ginsburg, 1954, from the Falkland Islands and Tierra del Fuego Region. *Acta Ichthyologica et Piscatoria*, 4 (2), 19–31.
Zar, J.H. (1984) *Biostatistical Analysis*. *Second Edition*. Prentice Hall International Inc., Londres, 718 pp.
Zuur, A.F., Ieno, E.N. & Smith, G.M. (2007) *Analysing Ecological Data*. Springer Science + Business Media, LLC, New York, 672 pp.