Zootaxa 2876: 49–56 (2011)
www.mapress.com/zootaxa/

Article

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

Leptoderma macrophthalmum n.sp., a new species of smooth-head (Otocephala: Alepocephalidae) from the Mid Atlantic Ridge

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Abstract

A new species of Alepocephalidae, *Leptoderma macrophthalmum* **n.sp**., is described from one specimen caught in 2004 at about 2100 m depth at the Mid Atlantic Ridge, north of the Azores. Morphological and molecular evidence indicate a relationship closest to the Pacific species *Leptoderma lubricum*.

Key words: Alepocephalidae, *Leptoderma*, Mid Atlantic Ridge

Introduction

The smooth-heads, family Alepocephalidae, comprise 96 valid species of epibenthic deep-sea fishes, distributed among 22 genera (Eschmeyer & Fricke 2011). The genus *Leptoderma* is currently considered to contain four valid species, all of which lack scales and typically have a tapering, eel-like body. While three of the species, *Leptoderma retropinna* Fowler, 1943, *L. affinis* Alcock, 1899, and *L. lubricum* Abe, Marumo & Kawaguchi, 1965 are known from the Indian and Pacific oceans, *L. macrops* Vaillant, 1886 is an Atlantic species. A fifth nominal species, *L. springeri* Mead & Böhlke, 1953 has been synonymised with *L. macrops* (Krefft 1973, Markle & Quéro 1984, Markle & Sazonov 1990).

L. lubricum differs conspicuously from the three other species by having a distinct gap between the posterior ends of the dorsal and anal fins and the beginning of procurrent caudal finrays. A *Leptoderma* sp. with this characteristic has been reported from the Atlantic by Markle & Quero (1984) and Sazonov & Markle (1990). During the cruise of the Norwegian research vessel "G.O.Sars" on the Mid-Atlantic Ridge in the summer of 2004 for the project MAR-ECO (http://www.mar-eco.no/) a specimen of *Leptoderma* with a dorsal and anal fin configuration similar to that of *L. lubricum* was caught. Morphological and genetic examination revealed that it differed sufficiently from *L. lubricum* as well as from other known species of *Leptoderma* to be considered a new species. In this paper we present evidence for this and describe the new species.

Material and methods

The specimen was caught with a bottom otter trawl during the MAR-ECO cruise with the R/V "G.O. Sars" in 2004. A description of the gear can be found in Wenneck et al. (2008). The specimen was captured at an unknown location in the water column, but most probably at the bottom at about 2100 m depth, and labelled with the field number MAR-ECO 2577. It was stored frozen at sea until transferred to the Bergen Museum where it was preserved in 70% ethanol and given the catalogue number ZMUB 19686. The ethanol caused some shrinkage in the transversal plane of the fish.

Measurements and meristic characters follow those of Hubbs & Lagler (1967), and are shown in Fig. 1. The measurements were done several months after the specimen had been preserved, but weight was obtained from the fresh specimen shortly after it was caught. Comparisons were made with the following specimens of the four other

Accepted by M. Craig: 30 Mar. 2011; published: 12 May 2011

species of *Leptoderma*: *L. macrops* BMNH 1995.3.13.4-6 (3 specimens), BMNH 1996.8.7.5-10 (6 specimens), BMNH 1996.8.13.12-16 (5 specimens), BMNH 1998.2.3.102, BMNH 2001.2.1.60-61 (2 specimens), ZMUC P17720 (5 specimens), *L. retropinna* USNM 150073, USNM 150807, ZMUC P17378, *L. affinis* ZMUC P17375, and *L. lubricum* BMNH 2002.3.2.135, BSKU 57837, SIO 71-202-8, SIO 72-392, USNM 215602. Fin-ray counts and count of vertebrae were made from radiographed images.

In order to compare molecular distances within *Leptoderma*, we determined the complete mitochondrial sequence (mitogenome) of the holotype specimen for comparison to *L. lubricum* (BSKU 57837) and *L. retropinna* (NSMT 66397) both of which were included in a mitogenomic phylogeny of the Alepocephaliformes (Poulsen et al.2009). Mitogenome amplification were performed by using a long PCR technique (Cheng et al. 1994) including three primer pairs used for the initial mitogenome amplification and 18 primer pairs for the subsequent nested short PCR on the long PCR product (Table 1). PCR were performed according to Miya & Nishida (1999) and reactions were as described in Poulsen et al. (2009). The mitogenome sequence is deposited as AP011500 in the DDBJ/ EMBL/Genbank databases, and should be considered hologenetype, following the suggestion of Chakrabarty (2010).

TABLE 1. Primers used in long and subsequent nested PCR of *L. macrophthalmum.*

Long PCR primers 5′ → 3′	
Forward	
L2508_16S	CTCGGCAAACATAAGCCTCGCCTGTTTACCAAAAAC
L8346_Lys	AGCGTTGGCCTTTTAAGCTAAWGATWGGTG
L12329_Leu	CTCTTGGTGCAAMTCCAAGT
Reverse	
H12293_Leu	TTGCACCAAGAGTTTTTGGTTCCTAAGACC
H15149_Cytb	GGTGGCKCCTCAGAAGGACATTTGKCCTCA
H2990_16S	TGCACCATTRGGATGTCCTGATCCAACATC

Nested PCR primers 5′ → 3′			
Forward		Reverse	
L1083_12S	ACAAACTGGGATTAGATAC	H2590_16S	ACAAGTGATTGCGCTACCTT
L2510_16S	CGCCTGTTTACCAAAAACAT	H3084_16S	AGATAGAAACTGACCTGGAT
L2949_16S	GGGATAACAGCGCAATC	H4129_ND1	CGGAABCGGGGGTAKGARGC
L3686_ND1	TGAGCMTCWAATTCMAAATA	H5334_ND2	CGKAGGTAGAAGTAHAGGCT
L5261_ND2	CWGGTTTCRTRCCWAAATGA	H6855_CO1	AGTCAGCTGAAKACTTTTAC
L6730_CO1	TATATAGGAATRGTMTGAGC	H7892_CO2	TCGTAGCTTCAGTATCATTG
L7467_Ser	GAGAAAGGRAGGAATTGAACC	H8589_ATP8	AAGCTTAKTGTCATGGTCAGT
L8329_Lys	AGCGTTGGCCTTTTAAGC	H10019_Gly	CAAGACKGKGTGATTGGAAG
L9500_CO3	CACACVCCHCCYGTCCAAAA	H10677_ND4	AGGGCWAGGCCKGCGCTWGCTTC
L10267_ND3	TTTGAYCTAGAAATYGC	H11534_ND4	GCKAGGAYAATAAAKGGGTA
L11424_ND4	TGACTTCCWAAAGCCCATGTAGA	H12293_Leu	TTGCACCAAGAGTTTTTGGTTCCTAAGACC
L12329_Leu	CTCTTGGTGCAAMTCCAAGT	H13396_ND5	CCTATTTTTCGGATGTCTTG
L13280_ND5	CAACTWGGKCTAATAATAGT	H14714_Glu	TAGTTGAATAACAACGGTGGTT
L14504_ND6	GCCAAWGCTGCWGAATAMGCAAA	H15560_Cyt b	TAGGCRAATAGGAARTATCA
L14736_Glu	AACCACCGTTGTTATTCAACTA	H15913_Thr	CCGGTSTTCGGMTTACAAGACCG
L15172_Cyt b	TGAGGACAAATATCNTTYTGAGG	H16500_CR	GCCCTGAAATAGGAACCAGA
L15927_Thr	AGAGCGTCGGTCTTGTAAKCCG	H885_12S	TAACCGCGGYGGCTGGCACGA
L16517_CR	CATCTGGTTCCTATTTCAGG	H1358_12S	CGACGGCGGTATATAGGC

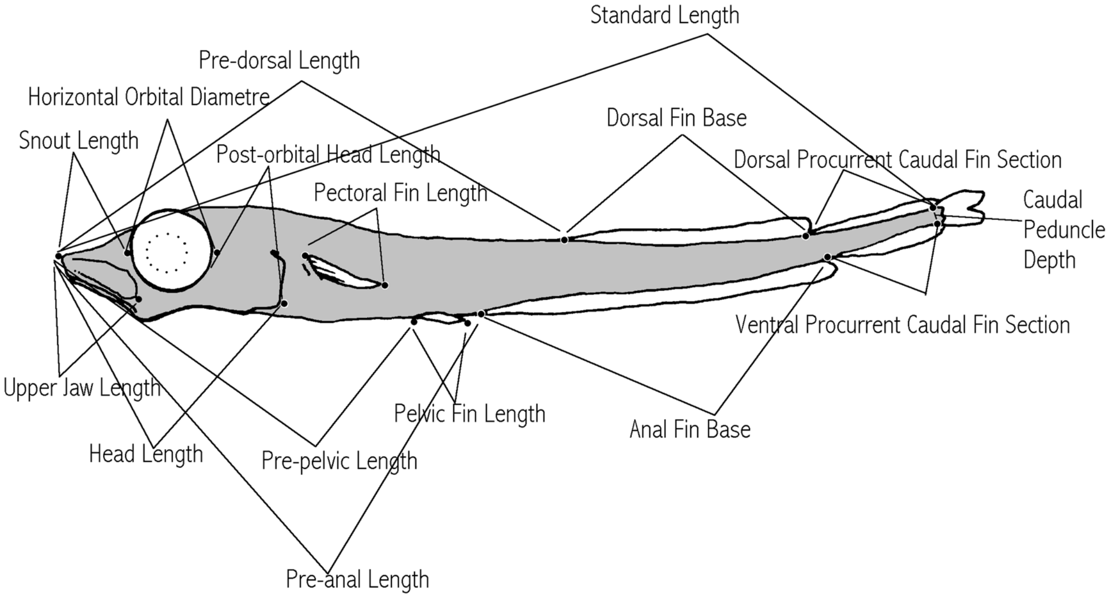

FIGURE 1. Measuring points for morphometric characters.

***Leptoderma macrophthalmum* sp.n.**

(Fig. 2–3)

Holotype. NORTH ATLANTIC, 42º47' N–29º38' W, 8 July 2004, 2107–2063 m gear depth, soft bottom, R/V "G.O. Sars", super station 42, local station 368, ser. 1150, bottom trawl (shrimp trawl). ZMUB 19686.

Diagnosis. The species differs from three of the four other congeneric species (*L. affinis, L. macrops* and *L. retropinna*) by having a distinct gap between the posterior ends of the dorsal and anal fins and the beginning of procurrent caudal finrays. This gap is absent in the three species mentioned, but present in the fourth (*L. lubricum*), from which *L. macrophthalmum* differs by having a lack of dermal papillae along the lateral line, pectoral fins lower on body, a larger eye relative to head dimensions, and a shorter pre-dorsal length.

Description. SL 151 mm, fresh weight 16 g. Dimensions as per cent of SL as follows: Pre-dorsal length 49.3, pre-anal length 45.7, pre-pelvic length 39.5, dorsal fin base 31.1, dorsal procurrent caudal fin section 15.5, anal procurrent caudal fin section 15.1, anal fin base 40.7, pectoral fin length (left) 7.3, pelvic fin length (left) 4.8, greatest body depth 12.8, caudal peduncle depth 2.1, maximal head width (behind eyes) 11.3, head length 23.2, snout length 7.0, horizontal orbital diameter 9.3, post-orbital head length 7.2, upper jaw length 7.9. Eye diameter as percent of head length 40.3. Fin-ray counts as follows: D 32, procurrent dorsal fin 21, A 47, procurrent anal fin 20, pectoral fins 8 (left and right), pelvic fins 8 (left and right), caudal fin 16. Number of gillrakers on first arch 19. Five branchiostegal rays on each side. Total number of vertebrae 67 (15 + 52).

No scales present. Body slender and compressed, greatest body depth a distance from the snout of 13.9 % of SL. Eyes large, upper margin protruding above the dorsal profile of the head. The dorsal profile of the forehead in the fresh specimen convex, but straightening over the snout (shrinkage caused by preservation has decreased the convexity of the forehead profile). Pectoral fins are set fairly low, their upper fin-ray in level with dorsal corner of the gill opening.

Pores along the lateral line about 48, difficult to observe. No dermal papillae found along the lateral line, on the head, or along the back. Three supraorbital pores, four suborbital pores, three preopercular pores, and four mandibular pores, all of these large. Nostrils with large longitudinal openings, partly covered by a dermal flap.

Mouth subterminal. Small, well separated conical teeth present in one row on premaxilla and dentary, the latter teeth of minute size. Supramaxilla slender, pear-shaped and curved.

Head and anterior part of body black, turning gradually lighter brownish towards posterior.

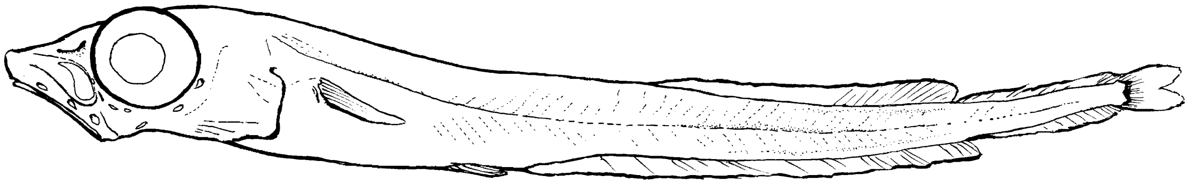

FIGURE 2. Drawing of *Leptoderma macrophthalmum*, holotype ZMUB 19686.

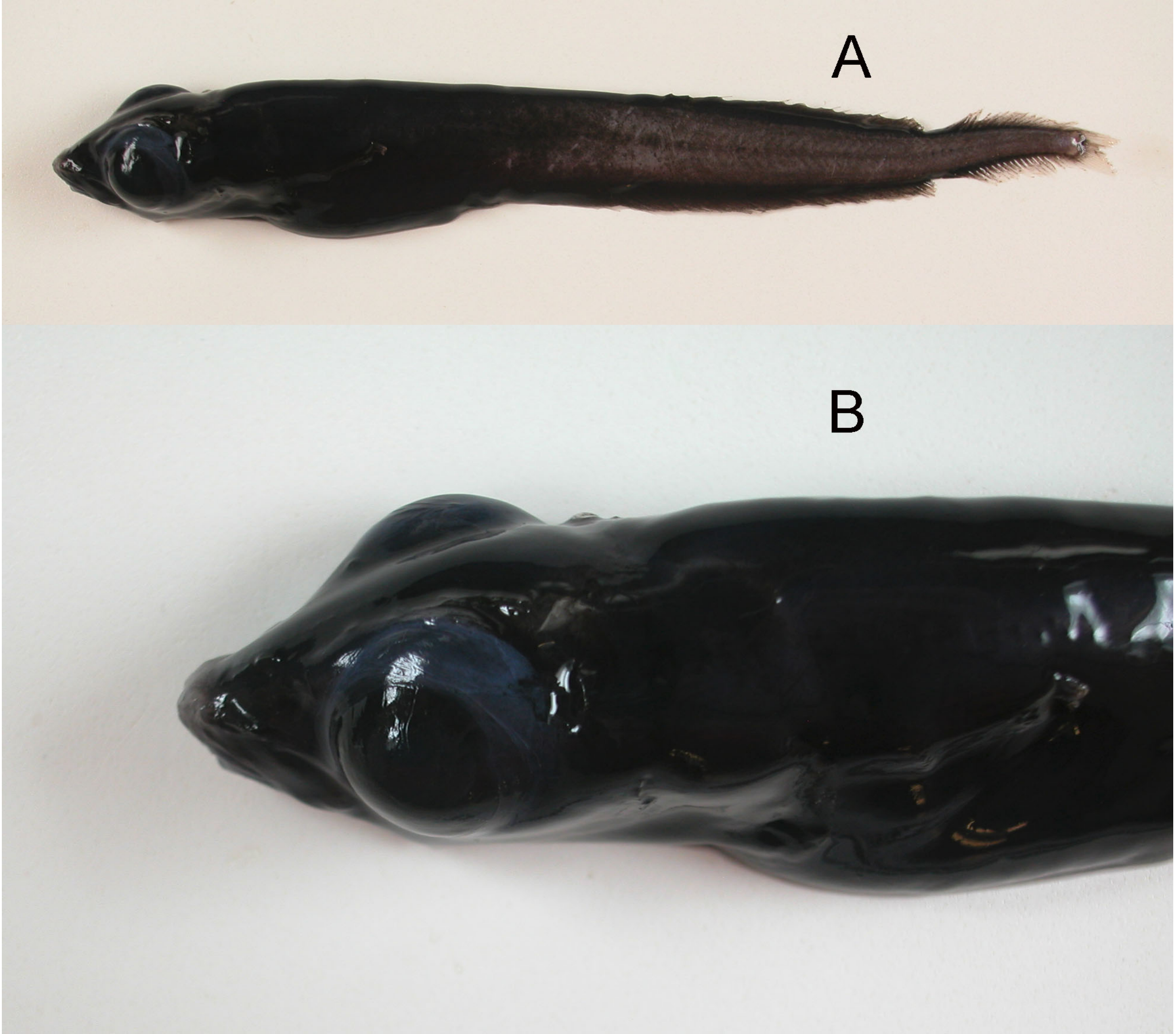

FIGURE 3. *Leptoderma macrophthalmum*, holotype ZMUB 19686, photographed newly caught, A showing specimen and B close-up of head.

Molecular characterization. The mitogenome sequence of the specimen is 16,607 bp long and shows a typical vertebrate gene order with L and H-strand coding of the 13 protein coding genes, 2 rRNA genes and 22 tRNA genes (Table 2). Compared to the other two species of *Leptoderma* available, the mitochondrial genome in this new species is 6 and 9 bp longer than *L. retropinna* and *L. lubricum*, respectively. Mitogenomic p-distances between the new species and congeners show a difference of 5.2% compared to *Leptoderma lubricum* and 6.1% compared to *L. retropinna* with polymorphisms distributed fairly equal throughout the genome. Most polymorphisms are found at third codon positions of protein coding genes and in the control region (Table 2).

Etymology. The specific epithet *macrophthalmum* (from Greek *makros*, large, and Greek *ophthalmos*, eye) refers to the large eyes found in this species.

TABLE 2. Mitogenome characterization of *Leptoderma macrophthalmum* (holotype) and codon positions, type of polymorphisms and genetic distances compared to *L. lubricum* (BSKU 57837) and *L. retropinna* (NSMT 66397), respectively. 1, 2 and 3 denote codon position in the 13 protein coding genes. Ts = transitions, Tv = transversions, P-dist. = Patristic distance. All coding region names follow standard mitochondrial nomenclature and genes encoded on the L-strand are shown in bold.

Mitogenome of *Leptoderma macrophthalmum*			Number and position of mt polymorphisms between *L. macrophthalmum* and[a]											
			L. lubricum						***L. retropinna***					
Region		bp	1	2	3	Ts	Tv	*P* (%)	1	2	3	Ts	Tv	*P* (%)
Phe	1–69	69				0	2	2.90				2	1	4.3
12S	70–1020	951				19	5	2.52				27	5	3.37
Val	1021–1092	72				0	0	0				1	0	1.39
16S	1093–2774	1682				24	11	2.08				27	12	2.32
$\mathrm{Leu}^{\mathrm{UUR}}$	2775–2849	75				3	1	5.33				3	1	5.33
ND1	2850–3824	975	4	0	50	42	12	5.54	5	2	59	51	15	6.77
Ile	3830–3899	70				2	0	2.86				3	0	4.29
Gln	3899–3969	71				0	0	0				1	0	1.41
Met	3969–4037	69				0	0	0				1	0	1.45
ND2	4038–5082	1045	14	3	59	60	16	7.27	14	3	65	64	18	7.85
Trp	5083–5152	70				1	0	1.47				2	0	2.94
Ala	5154–5222	69				1	1	2.90				0	1	1.45
Asn	5224–5296	73				0	0	0				0	0	0
O_{L}	5297–5329	33				2	0	6.06				3	0	9.09
Cys	5330–5396	67				2	1	4.55				2	1	4.55
Tyr	5395–5465	71				2	0	2.82				1	0	1.41
CO1	5467–7017	1551	4	0	78	68	14	5.29	2	0	83	70	15	5.48
$\mathrm{Ser}^{\mathrm{UCN}}$	7018–7088	71				6	0	8.45				5	0	7.04
Asp	7094–7164	71				3	0	4.62				4	0	6.25
CO2	7179–7869	691	3	0	29	24	8	4.63	2	0	40	33	9	6.08
Lys	7870–7943	74				0	0	0				0	0	0
ATP8	7945–8112	168	1	0	4	4	1	2.98	1	0	6	7	0	4.17
ATP6	8103–8786	684	8	2	38	33	15	7.02	11	2	41	42	12	7.89
CO3	8786–9571	786	6	1	36	36	7	5.47	7	2	40	42	7	6.23
Gly	9571–9641	71				0	0	0				0	1	1.43
ND3	9642–9990	349	5	0	21	22	4	7.45	7	0	21	23	5	8.02
Arg	9991–10059	69				1	0	1.45				2	0	2.94
ND4L	10060–10356	297	2	0	12	12	2	4.71	4	1	11	16	0	5.39
ND4	10350–11730	1381	16	2	84	87	15	7.39	20	3	101	101	23	8.98
His	11731–11798	68				2	0	2.90				2	0	2.94
$\mathrm{Ser}^{\mathrm{AGY}}$	11799–11866	68				2	1	4.41				3	0	4.41

continued next page

TABLE 2. (continued)

Mitogenome of *Leptoderma macrophthalmum*			Number and position of mt polymorphisms between *L. macrophthalmum* and[a]											
			L. lubricum						***L. retropinna***					
Region		bp	1	2	3	Ts	Tv	*P* (%)	1	2	3	Ts	Tv	*P* (%)
Leu$^{\text{CUN}}$	11868–11940	73				2	0	2.74				2	0	2.74
ND5	11941–13779	1839	12	4	107	101	22	6.69	12	4	121	113	24	7.45
ND6	13776–14297	522	4	3	28	27	8	6.70	5	3	24	21	11	6.13
Glu	14298–14366	69				1	0	1.45				1	0	1.45
Cytb	14372–15512	1141	11	5	49	53	12	5.70	11	5	67	69	14	7.27
Thr	15513–15584	72				0	0	0				0	1	1.39
Pro	15584–15653	70				0	0	0				2	0	2.90
CR	15654–16607	954				38	30	7.11				58	21	8.29
Mt total	1–16607	16607	90	25	595	682[b]	188	5.2	101	25	679	806[b]	197	6.1

[a] Gaps excluded from the data since only about 20 gaps are present in the alignments (most located in 16S and CR)

[b] Two additional transitions in intervening non-coding regions included

Discussion

Morphologically *L. lubricum* and *L. macrophthalmum* are similar in most meristic and morphometric characters (Table 3). From the examined specimens of *L. lubricum* and the description of Abe et al. (1965), the following differences can be pointed out. The predorsal length is 57–59% of SL in *L. lubricum*, compared to 49% in *L. macrophthalmum*. In *L. lubricum* the centre of the pectoral fin is level with the dorsal corner of the gill opening, whereas in *L. macrophthalmum* only the uppermost part of the pectoral fin base reaches that high. The procurrent part of the caudal fin dorsally counts 23–27 rays in *L. lubricum* while only 19 fin rays were counted in *L. macrophthalmum*. The eye diameter was found to be 40.3% of head length in *L. macrophthalmum* and 33.8–36.2% in *L. lubricum*. A characteristic feature of *L. lubricum* is rows of dermal papillae on parts of the head and, notably, along the lateral line. No such papillae were evident in the specimen of *L. macrophthalmum*, in spite of its skin being intact. Although the examination of more specimens of both species would be strongly desirable, the morphological differences indicate that the two species can readily be distinguished and identified morphologically.

The molecular results of the specimen corroborate morphology concerning a close relationship to *L. lubricum* although sufficiently distant from it to be considered a separate species (p-distance 5.2%). As expected, most polymorphisms between *Leptoderma* species were found in the third codon positions of the protein coding genes and the putative control region, indicating relative recent speciation events. Molecular distance standing alone is a difficult measure of species delimitation. However, combined with morphological or other sources of evidence, molecular distance provides good additional information and will undoubtedly prove valuable in future work concerning the problematic taxonomic status of *Leptoderma* species.

A *Leptoderma* sp. reported from the Azores, western Africa, eastern Pacific and the Southern Ocean by Markle & Quero (1984) and Sazonov & Markle (1990) looks very similar to *L. macrophthalmum*, but seems to differ in a longer predorsal distance, larger postorbital distance and a shorter snout, and may represent yet another species in the *L. macrophthalmum*-*L. lubricum* group.

TABLE 3. Characters of *L. macrophthalmum* (holotype) compared to the range in four specimens of *L. lubricum* (BMNH 2002.3.2.135; SIO 71-202-8; SIO 72-392; USNM 215 602). A fifth specimen examined of *L. lubricum* (BSKU 57837), too damaged to yield accurate measurements and fin-ray counts, is not included in the table, but the specimen did possess dermal papilla on the lateral line.

Character	*L. macrophthalmum*	*L. lubricum*
Standard length (mm)	151	119–210
Morphometric characters as per cent of standard length		
Pre-dorsal length	49.3	56.0–59.5
Pre-anal length	45.7	43.8–46.5
Pre-pelvic length	39.5	36.8–40.0
Dorsal fin base length	31.1	25.3–28.9
Dorsal procurrent caudal section length	15.5	15.0
Anal fin base length	40.7	36.6–40.4
Anal procurrent caudal fin section length	15.1	14.0
Pectoral fin length	7.3	5.0–11.4
Pelvic fin length	4.8	1.8–6.9
Greatest body depth	12.8	12.1–14.7
Caudal peduncle depth	2.1	2.0–2.8
Maximal head width (behind eyes)	11.3	8.8–12.1
Head length	23.2	23.8–25.7
Snout length	7.0	7.2–8.3
Horisontal orbital diameter	9.3	8.3–9.0
Interorbital distance	3.4	3.1–5.1
Upper jaw length	7.9	8.3–8.9
Meristic characters		
Number of finrays in dorsal fin	32	33–34
Number of finrays in dorsal procurrent caudal section	21	21–27
Number of finrays in anal fin	47	45–50
Number of finrays in anal procurrent caudal section	20	19–26
Number of finrays in pectoral fins	8	7–8
Number of finrays in pelvic fins	8	4–6
Number of finrays in caudal fin	16	18
Number of gillrakers on first arch	19	15–18
Lateral line pores	c.48	58–66
Branchiostegals	5	5
Vertebrae, visceral	15	15–17
Vertebrae, hemal (excl. urostyle)	51	49–55
Dermal papillae on lateral line	no	yes

Acknowledgements

We would like to thank M. Miya, M. Nishida and ORI lab-members for collaboration, P. Campbell Natural History Museum, London, H. Endo, Laboratory of Marine Biology, Kuchi, C. Klepadlo, Scripps Institution of Oceanography, La Jolla, P.R. Møller and J. G. Nielsen, Zoological Museum Copenhagen, G. Shinohara, National Science

Museum of Tokyo, and J.T. Williams, Smithsonian Institution, Washington DC for loan of specimens, H. Haflidason and D. Rees for help with radiographing specimens, T. de L. Wenneck for allowing us to use his photos for Fig. 3 and R. Santos for his astute preliminary diagnosis of the genus *Leptoderma* during the 2004 MAR-ECO cruise. We owe a special thanks to S. Lavoué (BMNH) concerning methodology. Lastly we are grateful for the contributions of the late Yuri Sazonov, an expert on the family and former MAR-ECO partner. D. Markle and an anonymous reviewer made valuable suggestions and comments on the manuscript.

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