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A new species of moray eel (Anguilliformes: Muraenidae) from Taiwan, with comments on related elongate unpatterned species

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Abstract

The following nine elongate unpatterned muraenid species of the subfamily Muraeninae, including one new species, are recognized from Taiwan and adjacent waters: *Gymnothorax albimarginatus* (Temminck & Schlegel), *G. dorsalis* Seale, *G. melanosomatus* Loh, Shao & Chen, *G. phasmatodes* (Smith), *G. prolatus* Sasaki & Amaoka, *G. sagmacephalus* Böhlke, *Pseudechidna brummeri* (Bleeker), *Strophidon sathete* (Hamilton) and *G. pseudomelanosomatus* new species, described from two specimens. This new moray eel is distinguished from its similar species, *G. melanosomatus*, by the following features: grey brown body (vs. black), snout length 20.5% (vs. 17.8%) of head length, smaller eye diameter 8.2% (vs. 10.0%) of head length; preanal length 49.5% (vs. 58.5%) total length, and preanal vertebrae 89–89 (vs. 105–109). Phylogenetic relationships of the nine species were examined using nucleotide sequence data from partial sequences of mitochondrial ND5 gene (600 bp), and seven species form COI (600 bp). The genetic analyses suggest that *G. pseudomelanosomatus* is distinct from *G. melanosomatus* and the other six species of *Gymnothorax*. Morphological features and mitogenetic affinities strongly suggest that "*G.*" *dorsalis* should be placed in *Strophidon* rather than in *Gymnothorax*. The results also suggest that employment of ND5 and COI gene sequences are rather useful for identification of species and for obtaining reasonable insights into the phylogeny of the muraenid species.

Key words: Muraenidae, taxonomy, new species, phylogeny relationships, ND5

Introduction

The Muraenidae is a diverse family of eels found around the world, about 15 genera and 197 species (Smith, 2012). More than 40 species of the family Muraenidae are indigenous to Taiwan (Chen et al., 1994; Shao et al., 2008; Loh et al., 2014). During his research into some elongate unpatterned morays, the first author (KHL) discovered two specimens similar to *Gymnothorax melanosomatus* Loh, Shao & Chen (2011) but with some observed differences. These specimens had similar but distinctly different body proportions from *G. melanosomatus* and the other elongate unpatterned Indo-Pacific muraenid species (Böhlke, 1997). Molecular analyses based on partial sequences of the mitochondrial COI and ND5 genes show differences from other similar moray species. Based on distinct morphological differences, as well as molecular analyses, we propose and describe it here as a new species in Taiwan.

Materials and methods

All moray specimens were collected by longline. The fresh specimens were stored in refrigerator for transfer to the

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laboratory after capture. Specimens used for morphological studies were fixed in 10 % formalin for a week before being transferred to 70 % ethanol for long-term preservation. The methods of measurements follow Böhlke and Randall (2000). Proportional measurements of the specimens of the moray eels are expressed as percentage of the total length (TL) or the head length (HL). Body depth is measured at the gill openings (DGO) and at the anus (DA) and does not include the fins; snout length is measured from the snout tip to the anterior margin of the eye; upper jaw (UJ) length is from the snout tip to the mouth corner, lower jaw (LJ) length from the lower jaw tip to mouth corner. Counts for the vertebral formula are obtained from radiographs, as explained in Böhlke (1982); the mean vertebral formula (MVF) gives the mean values for predorsal-preanal-total vertebrae counts. Teeth counts referred to Bohlke and Randall (2000) are approximate and include sockets of missing teeth. Gonadal type was determined by gross and histological examination of the muraenid gonads. All specimens were deposited in the collections of the Laboratory of Aquatic Ecology, Department of Aquaculture, National Taiwan Ocean University (TOU-AE).

The fresh specimens of muraenid species to be used for molecular analyses were directly preserved in 95% ethanol when caught, frozen and transferred to the laboratory. A total of 20 specimens, comprising three genera and nine species were used in the molecular analyses and phylogenetic studies.

All DNA extractions of were carried out according to the general protocols of the High Pure PCR Template Preparation Kit (Roche) method. The fragment of COI and ND5gene region which consisted of 600 bp, were amplified by polymerase chain reaction (PCR) using primers: (MECOF, MECOR, SQMRleuD1, MRGluR1, ND5-MR, ND5-leu) were used to amplify the complete mitochondrial ND5 genes by polymerase chain reaction (PCR) and sequencing. The sequences of the 6 sets of primers were: MECOF 5'-TAC CTG TGG CAA TYA CCC GTT-3'; MECOR 5'-TGT TGR TAD ARR ATW GGG TC-3';SQMRleuD1: 5'ACT CTT GTT GCA ACT CCA AG-3'; MRGluR1: 5'-ATA GTT GAA TTA CAA CGR TGG TTT TTC-3'; ND5-MR: 5'CCT ATT TTK CGG ATG TCY TG-3'; ND5-leu: 5'-GAA CCA AAA ACT CTT GGT GCA ACT-3'.

PCR was carried out using an ABI Model 2720 thermal cycler (Applied Biosystems) set for 35-50 cycles. The 50 uL reaction volume contained 33.5 uL of sterile distilled water, 5.0 uL of 10x PCR buffer (Takara), 4.0 uL of dNTP (2.5 mM each), 1.0 uL of each primer (10 uM), 0.5 uL of 2 unit Ex *T*aq Super-therm DNA polymerase and 2.0 uL of template. The thermal cycle profile was as follows: denaturation at 94ºC for 60 seconds, annealing at 48–52 ºC for 60 seconds and extension at 72 ºC for 90 seconds. A negative control without template DNA was carried out for each run of PCR. The PCR products were run on a 1.0% agarose gel (0.5x TBE buffer) gel electrophoresis and stained with ethidium bromide for band characterization under ultraviolet trans-illumination. Double-stranded PCR products were purified using a High Pure PCR Product Purification Kit (Roche), before undergoing direct cycle sequencing with dye labeled terminators (ABI Big-Dye kit). All sequencing reactions were performed according to the manufacturer's instructions and sequenced commercially by First BASE.

The raw sequences were assembled and edited in the program ChromasPro ver. 1.42. Consensus sequences were preliminarily aligned in ClustalX v. 2.0.8 (Thompson et al., 1997; Larkin et al., 2007) and subsequently manually revised in Bioedit v. 7.0.9.0 (Hall, 1999). In order to search for the best model to be used for maximum likelihood (ML) and Bayesian Inference (BI) analyses, Kakusan v. 3 (Tanabe, 2007) was used. ML analyses were carried out using output file of Akaike Information Criterion (AIC) criterion and was performed using Treefinder version October (Jobb et al., 2004) with 1,000 ML bootstrap replicates. The best selected model used were J2+G for COI and TN+G for ND5. BI analyses were carried out using the output file of Bayesian Information Criterion (BIC) by Kakusan v.3 in using MrBayes 3.1.2 (Huelsenbeck & Ronquist, 2001). Bayesian analyses were initiated with a random starting tree and two parallel runs, each of which consisted of running four chains of Markov chain Monte Carlo (MCMC) iterations for $6x10^6$ generations by sampling the trees in each chain every 200th generation. The first 30,000 trees (where the likelihood values were stabilized prior before they were discarded) were discarded to avoid trees that were sampled prior to convergence, and the remaining trees after burn-in were used to calculate posterior probabilities using the "sumt" command.

For maximum-parsimony (MP) and neighbor-joining (NJ) analyses, PAUP* 4.0b10 (Swofford, 2002) was used. The MP analyses was set with the heuristic search option, 100 random sequences additions, tree bisection reconnection (TBR) branch swapping, and unordered and unweighted characters. Bootstrap percentage (BP) was computed with 1000 replications. NJ bootstrap values were estimated u with Kimura's two-parameter model of substitution (K2P distance) evolution model with 1000 replicates.

Results

Gymnothorax pseudomelanosomatus Loh, Shao & Chen, new species

English name: False black body moray eel
Fig. 1, Table 1)

FIGURE 1. *Gymnothorax pseudomelanosomatus* sp. nov., holotype, TOU-AE 5302, 675 mm TL.

Holotype: TOU-AE 5302, male, 675 mm TL, 12 September 2008, off-shore from Changbin, Taitung county, Taiwan, longline, caught by J.-S. Chiou.

Paratype: TOU-AE 5301, female, 736^{+} mm TL, 11 December 2008; are from Changbin, Taitung county, longline, caught by J.-S. Chiou.

Diagnosis. An elongate and unpatterned moray eel of the genus *Gymnothorax* with body and fins uniformly greyish brown; anus just before mid-body, preanal length 2.0 in TL; head length 11.3 in TL; depth at gill opening 27.8 in TL. Teeth uniserial, few, long and needle-like. Mean vertebral formula 7-89-206.

Description. Morphometric and meristic data are provided in Table 1. Following values are provided for holotype, followed by paratype in parentheses. Proportions in percent of total length: preanal length 49.3 (49.7), depth at gill opening 3.6 (3.4), depth at anus 2.4 (2.3), predorsal length 6.1 (6.0), head length 8.9 (8.8). Proportions in percent of head length: length of upper jaw 43.6 (40.9), length of lower jaw 42.6 (37.9), interorbital width 13.3 (12.2), snout length 20.5 (20.6), eye diameter 8.6 (7.8).

TABLE 1. Morphometric and meristic data of *G. pseudomelanosomatus* sp. nov. and eight other unpatterned species of moray eels. Range (mean) values are provided for morphometric values and vertebral counts.

	G. pseudomelanosomatus sp. nov.		*G. albimarginatus*	*G. melanosomatus*	*G. phasmatodes*
	Holotype	Paratype			
TL (mm)	675	736+	675–1060 (=6)	407–658 (n=8)	268–382 (n=6)
In % TL					
Preanal length	49.3	49.7	53.3–55.2 (53.9)	56.6–59.5 (58.5)	50.4–54.5 (52.7)
Head length	8.9	8.8	10.0–12.6 (11.6)	9.3–10.5 (9.7)	9.6–10.2 (9.9)
Predorsal length	6.1	6.0	5.6–7.4 (6.7)	5.2–7.4 (6.3)	6.2–7.5 (6.8)
Depth at GO	3.6	3.4	4.8–6.7 (5.9)	2.2–3.7 (3.1)	2.7–4.2 (3.4)
Depth at anus	2.4	2.3	3.6–4.8 (4.1)	2.1–2.8 (2.4)	2.4–3.2 (2.8)
In % HL					
Upper jaw	43.6	40.9	31.6–41.6 (37.6)	32.4–41.0 (35.6)	35.2–40.7 (37.6)
Lower jaw	42.6	37.9	28.4–39.3 (33.2)	31.3–37.8 (34.2)	32.6–40.4 (35.7)
Interobital width	13.3	12.2	11.2–15.2 (13.2)	9.2–14.1 (12.3)	10.9–17.3 (13.9)
Snout length	20.5	20.6	16.3–18.4 (17.5)	14.9–20.5 (17.8)	16.0–19.1 (18.0)
Eye diameter	8.6	7.8	8.4–11.0 (9.6)	8.2–11.7 (10.0)	10.8–11.5 (11.3)
Teeth					
Intermaxillary	6–6	6–6	3–6	4–8	6–6
Median	1	4	0–2	0–3	0–3
Maxillary-inner	-	-	-	-	-
Maxillary-outer	8–8	10–10	7–11	6–7	7–12
Vomerine	5	6	1–5	1–7	4–9
Dentary-inner	-	-	-	-	-
Dentary-outer	14–14	14–15	11–15	10–12	12–17
Vertebrae					
Predorsal vert.	7	5	5–6 (5)	4–7 (6)	5–6 (5)
Preanal vert.	89	89	83–90 (86)	105–109 (108)	73–79 (76)
Total vert.	206	202+	184–189 (187)	201–211 (207)	163–168 (167)

TABLE 1 (Continued). Morphometric and meristic data of *G. pseudomelanosomatus* sp. nov. and eight other unpatterned species of moray eels. Range (mean) data are provided for morphometric values and vertebral counts.

	G. prolatus	*G. sagmacephalus*	*P. brummeri*	*S. dorsalis*	*S. sathete*
TL (mm)	310–490 (n=5)	375–512 (n=12)	548–730 (n=3)	415–1050 (n=5)	516–1470 (n=12)
In % TL					
Preanal length	46.6–50.4 (48.8)	50.4–52.8 (51.2)	46.8–52.6 (49.7)	41.0–44.3 (42.9)	42.2–45.4 (43.7)
Head length	9.7–10.3 (10.0)	9.7–11.5 (10.6)	7.1–7.5 (7.3)	9.5–11.1 (10.4)	8.0–10.9 (9.4)
Predorsal length	6.7–7.4 (7.2)	6.4–10.0 (8.1)	3.8–4.2 (4.0)	8.1–9.6 (8.8)	6.1–10.2 (7.3)
Depth at GO	2.9–3.8 (3.3)	2.9–3.8 (3.4)	1.8–2.4 (2.1)	3.5–4.0 (3.7)	1.8–2.9 (2.4)
Depth at anus	2.6–3.3 (2.9)	2.2–3.6 (2.8)	1.4–2.2 (1.8)	3.2–3.6 (3.4)	1.9–2.4 (2.1)
In % HL					
Upper jaw	37.4–41.9 (39.7)	34.0–45.3 (38.2)	25.4–27.1 (26.1)	32.0–36.7 (34.2)	29.6–39.6 (33.0)
Lower jaw	35.9–40.7 (38.4)	32.2–44.9 (37.4)	25.0–26.9 (25.9)	30.6–35.9 (33.4)	27.5–38.1 (32.7)
Interobital width	10.7–17.8 (14.9)	12.2–16.7 (14.4)	6.1–12.2 (10.0)	9.9–13.7 (12.0)	7.3–11.4 (9.0)

...Continued on next page

TABLE 1. (Continued)

	G. prolatus	*G. sagmacephalus*	*P. brummeri*	*S. dorsalis*	*S. sathete*
Snout length	19.4–21.4 (20.5)	15.5–19.7 (18.0)	10.0–13.8 (12.3)	12.5–13.4 (13.0)	9.7–13.9 (11.1)
Eye diameter	9.2–11.0 (10.1)	9.2–11.8 (10.3)	5.2–6.3 (5.6)	5.4–5.9 (5.7)	2.9–6.8 (4.8)
Teeth					
Intermaxillary	5–6	5–7	5–6	5–7	5–6
Median	1–3	0–3	0–1	3	2–4
Maxillary-inner	5–6	-	-	2–5	4–9
Maxillary-outer	15–24	6–10	6–7	15–18	19–27
Vomerine	1–5	3–8	0–3	3–7	3–8
Dentary-inner	-	-	-	2–5	3–4
Dentary-outer	13–23	9–18	11–16	18–23	23–33
Vertebrae					
Predorsal vert.	4–6 (6)	6–7 (6)	4–6 (4)	8–9 (9)	7–10 (9)
Preanal vert.	74–84 (78)	74–78 (76)	90–98 (94)	66–69 (68)	78–83 (81)
Total vert.	174–183 (179)	170–176 (173)	191^{+}–210 (210)	164–167 (166)	188–200 (194)

Teeth uniserial. The following values are provided for holotype, followed by paratype in parentheses. Median intermaxillary teeth 1(4); maxillary teeth uniserial about 8/8(10/10); intermaxillary teeth 6/6(6/6); vomerine teeth uniserial 5(6); dentary teeth uniserial about 14/14 (14/15) (Fig. 2). Head pores typical, three supraorbital pores, four infraorbital pores, six mandibular pores. Two to three branchial pores, branchial pores small before gill opening. Gill opening below mid-side. Total vertebrae 202–206, predorsal vertebrae 5–7, preanal vertebrae 89–89, MVF 6-89-206.

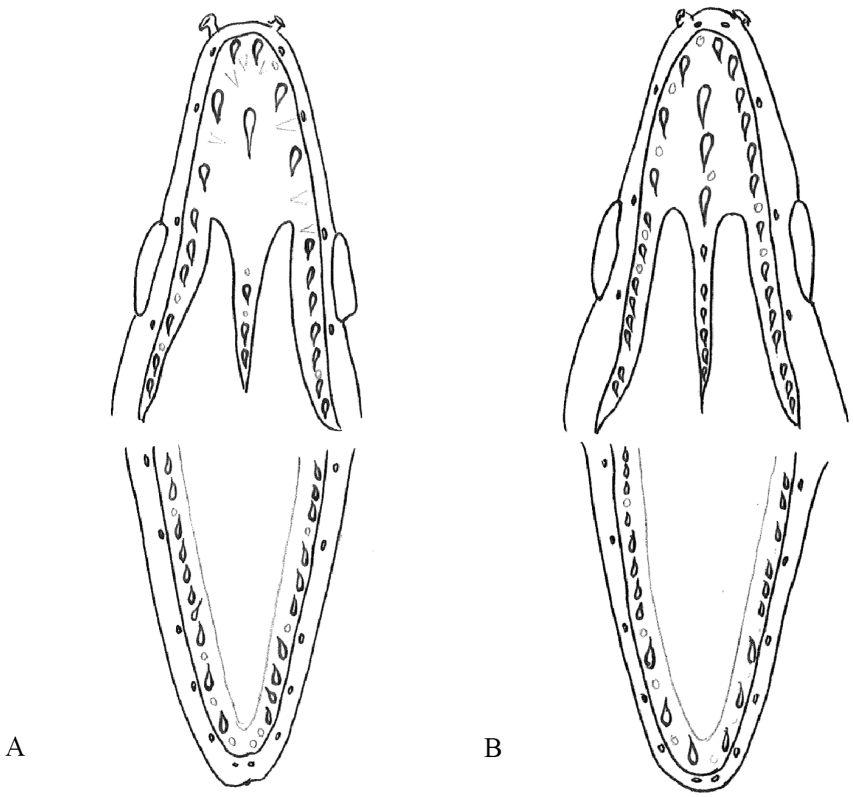

FIGURE 2. Diagrams of dentition and placement of jaw pores from the type specimens of *Gymnothorax pseudomelanosomatus*.sp. nov.: A, Holotype, male, TOU-AE 5302, 675 mm TL; B, paratype, female, TOU-AE 5301, 736^{+} mm TL.

Distribution. Known from the type series collected from southeastern Taiwan off Changbin, Taitung County at depth ca.200 m.

Biology. A small species, maximun length 675 mm TL in male, 736 mm TL in female (gravid, distended with 1.0–1.2 mm eggs).

Etymology. From the Greek *pseudes* (false) and *melanosomatus*, the name for the black body moray that it superficially resembles and has previously been identified as. To be treated as a noun in apposition.

Remarks. *Gymnothorax pseudomelanosomatus* sp. nov. can be distinguished from the similar species, *G. melanosomatus* Loh, Shao & Chen 2011, by the following features (Table 2): (1) grey brown body (vs. black) (Fig 3C); (2) longer snout length 20.5 % (vs. 17.8 %) of head length; (3) smaller eye diameter 8.2% (vs. 10.0%) of head length; (4) smaller preanal length 49.5 % (vs. 58.5 %) of total length; and (5) preanal vertebrae 89–89 (vs. 105–109).

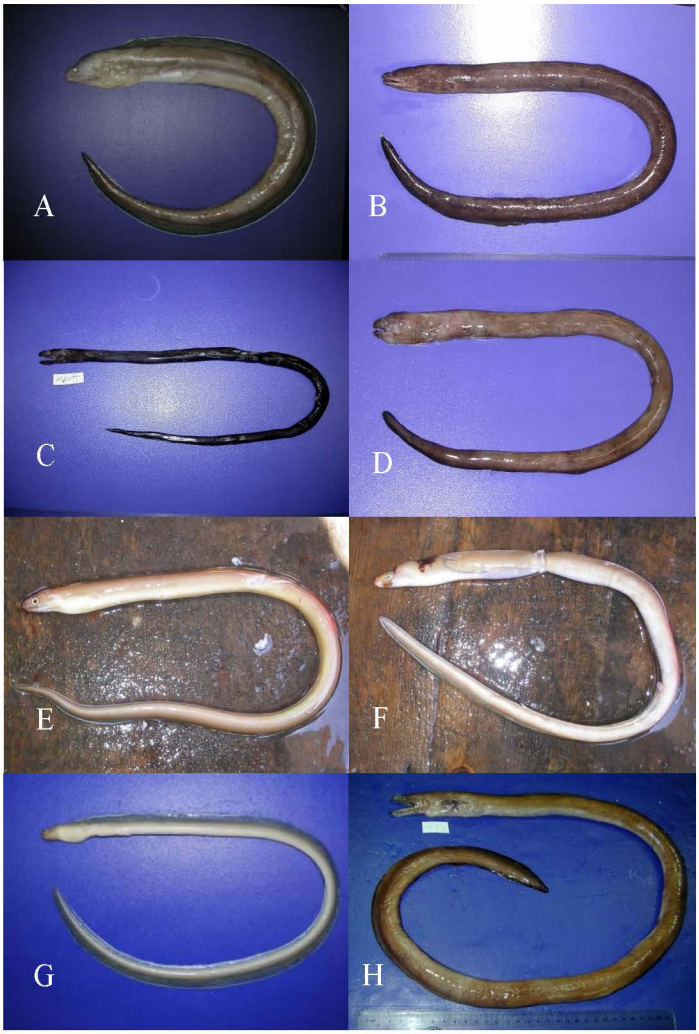

FIGURE 3. Other eight elongate unpatterned moray eels used in this study. A. *Gymnothorax albimarginatus*, TOU-AE 4220, 799 mm TL; B. "*G.*" *dorsalis*, TOU-AE 4834, 619 mm TL; C. *G. melanosomatus*, TOU-AE 5095, 447 mm TL; D. *G. prolatus*, TOU-AE 4833, 490 mm TL; E. *G. phasmatodes*, TOU-AE 227, 382 mm TL; F. *G. sagmacephalus*, TOU-AE 226, 464 mm TL; G. *Pseudechidna brummeri*, TOU-AE 5137, 592 mm TL; H. *Strophidon sathete*, TOU-AE 3028 , 1261 mm TL.

It also differs from *G. prolatus* Sasaki & Amaoka, 1991 (Fig 3D) by the smaller eye diameter 8.2 % (vs. 10.1 %) of head length; smaller predorsal length 6.0 % (vs. 7.2 %) of total length, and more preanal vertebrae 89 (vs. 78), and more total vertebrae 206 (vs. 179); and from *G. polyspondylus* Böhlke & Randall, 2000 by the longer preanal length 49.5 % (vs. 43.3 %) of total length, and in the total vertebrae 206 (vs. 233).

TABLE 2. The collecting localities and catalogue numbers of the elongate unpatterned morays for mtDNA analysis.

OTUs	Scientific name	Locality	Specimen number	TL	Wt	Sex
				(mm)	(g)	
Galb1	*Gymnothorax albimarginatus*	Changbin	TOU-AE 4220	799	521.1	M
Galb2	*G. albimarginatus*	Hopingdao	TOU-AE 5583	964	738.7	F
Gmel1	*G. melanosomatus*	Changbin	TOU-AE 5095	447	21.9	F
Gmel2	*G. melanosomatus*	Changbin	TOU-AE 1991	496	36.9	M
Gmel3	*G. melanosomatus*	Shihtiping	TOU-AE 3775	504	23.8	F
Gpha1	*G. phasmatodes*	Chengkung	TOU-AE 3270	268	13.4	F
Gpha2	*G. phasmatodes*	Chengkung	TOU-AE 3271	334	15.3	M
Gpro1	*G. prolatus*	Dasi	TOU-AE 4833	490	44.7	F
Gpro2	*G. prolatus*	Dasi	TOU-AE 5304	401	30.0	M
Gsp2-1	*G. pseudomelanosomatus*	Changbin	TOU-AE 5301	736	88.4	F
Gsp2-2	*G. pseudomelanosomatus*	Changbin	TOU-AE 5302	675	85.7	M
Gsag1	*G. sagmacephalus*	Changbin	TOU-AE 2728	375	28.1	M
Gsag2	*G. sagmacephalus*	Changbin	TOU-AE 2730	482	42.3	M
Pbru1	*Pseudechidna brummeri*	Philippines	TOU-AE 5137	592	23.9	M
Pbru2	*P. brummeri*	Philippines	TOU-AE 5138	548	31.2	M
Gdor1	*Strophidon dorsalis*	Vietnam	TOU-AE 786	763	596.5	M
Gdor2	*S. dorsalis*	Vietnam	TOU-AE 787	695	523.8	M
Ssat1	*S. sathete*	Dasi	TOU-AE 3027	1081	416.7	F
Ssat2	*S. sathete*	Dasi	TOU-AE 5306	992	356.7	F
Ssat3	*S. sathete*	Dasi	TOU-AE 4832	722	87.7	M

Molecular phylogenetic analysis

COI

The COI mitochondrial gene with 600 bp was amplified successfully for all the seven species of subfamily Muraeninae samples from a total of 16 individuals (Table 2, Figs. 3A–H). The COI gene is with 200 amino acid codings, and the aligned sequences with 218 divergent sites were variable in all seven species of Muraeninae. The average frequencies of nucleotides for all taxa of Muraeninae are as follows: A = 21.4 %; C = 25.5 %; G = 19.1 %; T = 31.3 %. Nucleotide sequences in COI gene of Muraeninae are slightly AT-rich (53.3–57.7 %) (Table 3). The numbers of transitions (Ti) were higher than those of transversions (Tv), and the average ratio of Ti / Tv was 1.8.

Phylogenetic trees on COI gene sequences for the seven elongate unpatterned moray eels, with the methods A) Maximum likelihood (ML); B) Bayesian Information (BI); C) Neighbor-joining (NJ; D) Maximum Parsimony (MP) are shown in the Figs. 4A–D. All trees of NJ, MP and ML, BI analyses based on J2+G model distance in molecular phylogenetic analyses are with *Anguilla japonica* and *Conger myriaster* as outgroups. The results of PAUP pair-wise genetic affinity suggests that *Gymnothorax pseudomelanosomatus* is distinct from the other four *Gymnothorax* species, and it can be supported genetically as a new species.

ND5

The ND5 mitochondrial gene with 600 bp was amplified successfully for all the nine species of subfamily Muraeninae samples from a total of 20 individuals (Table 2, Figs. 3A–H). The ND5 gene is with 200 amino acid codings, and the aligned sequences with 268 divergent sites were variable in all nine species of Muraeninae. The average frequencies of nucleotides for all taxa of Muraeninae are as follows: A = 29.8 %; C = 24.1 %; G = 17.2 %; T = 28.9 %. Nucleotide sequences in ND5 gene of Muraeninae are slightly AT-rich (53.9–61.8 %) (Table 3). The numbers of transitions (Ti) were higher than those of transversions (Tv), and the average ratio of Ti / Tv was 2.1.

TABLE 3. Percentages of base composition of nucleotide substitution of mtDNA ND5/ COI sequence data of nine unpatterned muraenid species.

Taxon	Nucleotide (%) of ND5/ COI					
	A	T	C	G	A+T	C+G
*Gymnothorax albimarginatus**	27.1/ 23.6	26.8/ 30.4	27.9/ 26.8	18.2/ 19.2	53.9/ 54.0	46.1/ 46.0
*G. melanosomatus***	28.8/ 23.8	28.6/ 32.7	24.1/ 23.8	18.5/ 19.7	57.4/ 56.5	42.6/ 43.5
*G. phasmatodes**	28.7/--	30.2/--	22.9/--	18.2/--	58.9/--	41.1/--
*G. prolatus**	28.6/ 24.0	30.2/ 33.7	23.2/ 23.7	18.0/ 18.6	58.8/ 57.7	41.2/ 42.3
*G. pseudomelanosomatus**	29.1/ 24.0	29.4/ 31.7	23.8/ 26.0	17.7/ 18.3	58.5/ 55.7	41.5/ 44.3
*G. sagmacephalus**	29.8/--	28.7/--	24.3/--	17.2/--	58.5/--	41.5/--
*Pseudechidna brummeri**	30.2/ 25.6	28.5/ 29.6	24.0/ 25.9	17.3/ 18.9	58.7/ 55.2	41.3/ 44.8
*Strophidon dorsalis**	33.2/ 24.2	28.6/ 30.8	23.2/ 25.4	15.0/ 19.6	61.8/ 55.0	38.2/ 45.0
*S. sathete***	32.6/ 23.5	28.9/ 29.8	23.6/ 27.5	15.0/ 19.2	61.4/ 53.3	38.6/ 46.7
Mean	29.8/ 21.4	28.9/ 31.3	24.1/ 25.5	17.2/ 19.1	58.7/ 55.4	41.3/ 44.6
Anguilla japonica	30.2/ 25.7	28.8/ 29.8	23.9/ 26.0	17.1/ 18.5	59.0/ 55.5	41.0/ 44.5
Conger myriaster	33.5/ 27.0	30.0/ 32.0	21.2/ 23.7	15.3/ 17.3	63.5/ 59.0	36.5/ 41.0

The species marked with an * are the average value (n=2); ** are the average value (n=3).

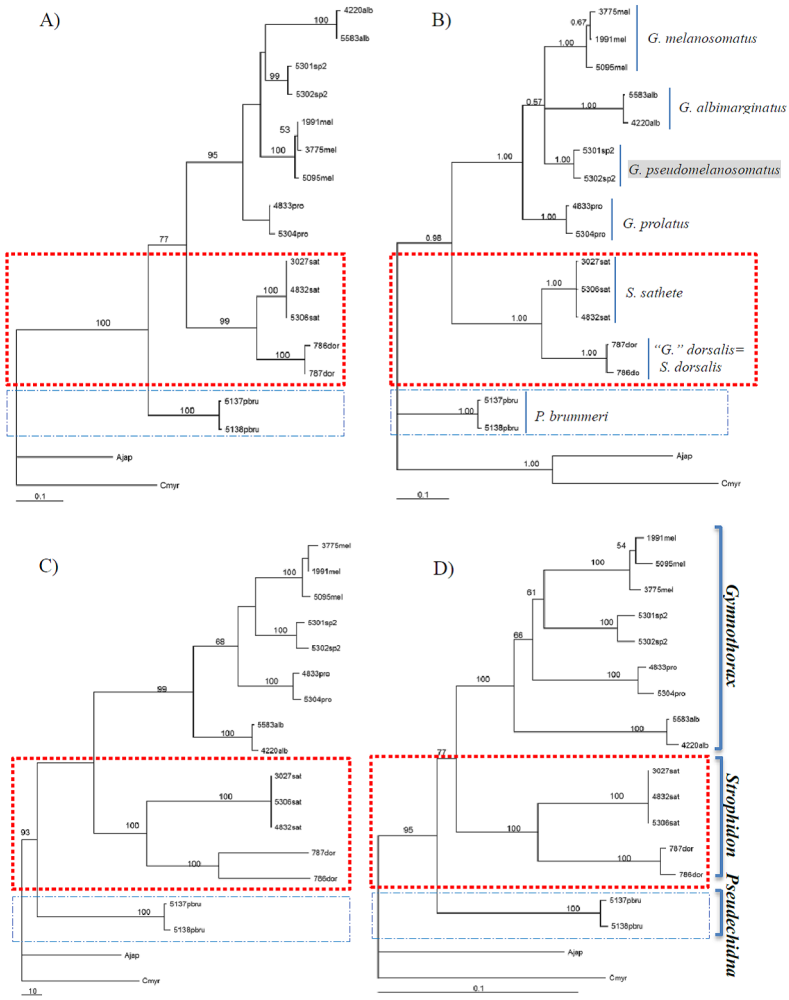

FIGURE 4. Phylogenetic inferred based on COI gene sequences (600 bp) for the elongate unpatterned moray eels. The bootstrap values and posterior probability higher than 50% are shown at the branching points. Methods A) ML; B) BI; C) NJ; D) MP.

Phylogenetic trees on ND5 gene sequences for the nine elongate unpatterned moray eels, with the methods A) Maximum likelihood (ML); B) Bayesian Information (BI); C) Neighbor-joining (NJ); D) Maximum Parsimony (MP) are shown in the Figs. 5A–D. All trees of NJ, MP and ML, BI analyses based on TN+G model distance in molecular phylogenetic analyses are with *Anguilla japonica* and *Conger myriaster* as outgroups. The results of PAUP pair-wise genetic affinity also suggest that *Gymnothorax pseudomelanosomatus* is distinct from the other six *Gymnothorax* species, and it is supported genetically as a new species.

Maximum likelihood (**ML**) based on J2+G model for COI (Fig. 4A) and TN+G model for ND5 (Fig. 5A) indicated that *Pseudechidna* is the basal group among the nine species analyzed and grouped as an independent clade with *Gymnothorax* and *Strophidon*, with the species *P. brummeri* by confidence levels 58-77 % of bootstrap replications. *Strophidon sathete* and "*G.*" *dorsalis* were sister species, supported with high confidence levels in 97–99% of bootstrap replications. Both trees of COI and ND5 distance in molecular phylogenetic analyses are with *Anguilla japonica* and *Conger myriaster* as outgroups.

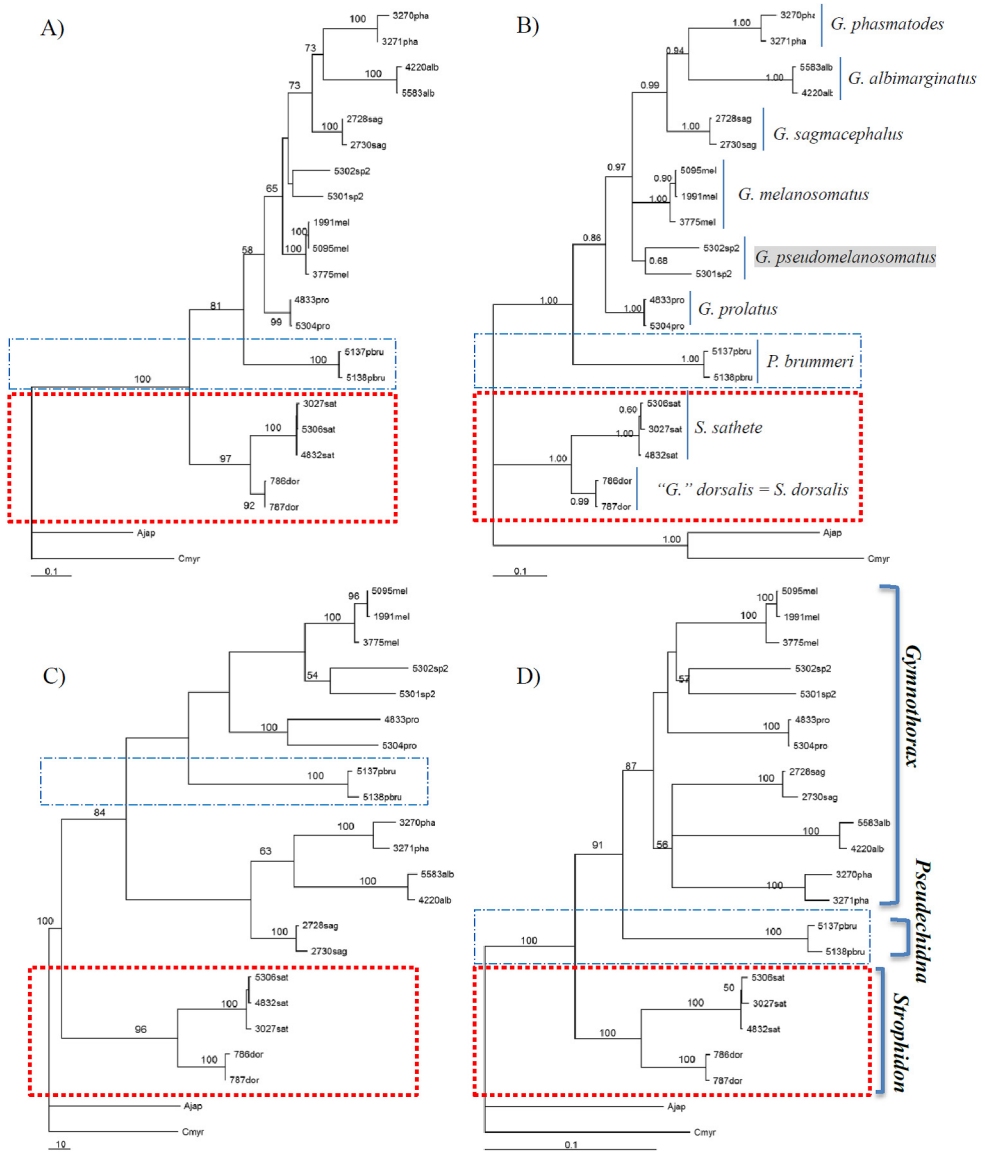

FIGURE 5.Phylogenetic inferred based on ND5 gene sequences (600 bp) for the elongate unpatterned moray eels. The bootstrap values and posterior probability higher than 50% are shown at the branching points. Methods A) ML; B) BI; C) NJ; D) MP.

Bayesian analyses (**BI**) based on J2+G model for COI (Fig. 4B) and TN+G model for ND5 (Fig. 5B) indicated that *Strophidon sathete* and "*G.*" *dorsalis* were sister species, supported with high Bayesian posterior probability of

1.00. The genetic analyses suggest that *G. pseudomelanosomatus* is distinct from *G. melanosomatus* and the other six species of *Gymnothorax*. Both trees of COI and ND5 distance in molecular phylogenetic analyses are with *Anguilla japonica* and *Conger myriaster* as outgroups.

Neighbor-joining analysis (**NJ**) supported the different muraenid species (Figs. 4C, 5C). Results shows that *Gymnothorax pseudomelanosomatus* is closely related to *G. melanosomatus*, *G. prolatus*, *G. albimarginatus*, *G. phasmatodes* and *G. sagmacephalus.* Apparently "*G.*" *dorsalis* does not belong the genus *Gymnothorax*. Both trees of COI and ND5 distance in molecular phylogenetic analyses are with *Anguilla japonica* and *Conger myriaster* as outgroups.

Maximum-parsimony (**MP**) analysis are shown in Fig. 4D and Fig. 5D. The distinction of different genera within the Muraenidae is well supported by confidence level 77–100% of bootstrap replications. The species of *Gymnothorax prolatus*, *G. pseudomelanosomatus* and *G. melanosomatus* are sister species. Both trees of COI and ND5 distance in molecular phylogenetic analyses are with *Anguilla japonica* and *Conger myriaster* as outgroups.

Based on the common conclusion of ML, NJ, MP and Bayesian analyses (Figs. 4A–D, 5A–D), eight phylogenetic trees share congruent support for the monophyly of the muraenid species in relation to *Pseudechidna brummeri* by confidence levels 58–100 % of bootstrap replications (ML, MP and NJ), and 0.86–0.98 posterior probability (Bayesian analysis). *Gymnothorax phasmatodes*, *G. pseudomelanosomatus*, *G. albimarginatus*, *G. melanosomatus*, *G. sagmacephalus* and *G. prolatus* fall within the same clade (*Gymnothorax*) by confidence levels 54–100 % of bootstrap replications (MP), and 0.57–0.99 posterior probability (Bayesian analysis).

Discussion

Based on the outgroup assignment of *Anguilla japonica* and *Conger myriaster*, the phylogenetic analysis seems to gather the congruent results of overall topology. According to the ML, NJ, MP and Bayesian analyses, all the phylogenetic trees (Figs 4–5) show that *Pseudechidna* is the basal group among the species, and grouped as an independent clade with *Gymnothorax* and *Strophidon*.

Based on the molecular analysis using ND5 and COI, "*G.*" *dorsalis* was grouped in the clade of *Strophidon* (Figs 4–5). That the species of *Strophidon sathete* and *Strophidon dorsalis* (previously referred to "*G.*" *dorsalis*) are sister species within the same genus is supported by the morphological common features of the body slender and very elongate, anus well before midlength, preanal length > 2.2 in total length, snout very short > 7.2 in head length, eye diameter > 14.6 in head length. The results strongly suggest that "*G.*" *dorsalis* should be placed in *Strophidon* rather than in *Gymnothorax*.

Loh et al., (2008) reported that the employment of the 1842 bp fragment of the complete mitochondrial ND5 gene is rather useful for identification of species and for obtaining reasonable insights into the phylogeny of the subfamily Uropterygiinae. Here, we assess the 600 bp fragment of the ND5 and COI gene for identification of species and for obtaining reasonable insights into the phylogeny of the subfamily Muraeninae.

Comparative materials

Gymnothorax albimarginatus: males: TOU-AE 104, 1638, 1813, from Bisha fishes market, Keelung; TOU-AE 1034, 4220, Changbin, Taitung; females: TOU-AE 5583, From Hopingdao. *Gymnothorax melanosomatus*: males: ASIZP0072170, TOU-AE 1991, 0627, 1879, 3774, Changbin; females: ASIZP 0072171, Changbin; TOU-AE 3775, 5095, Shihtiping, Hualien. *Gymnothorax phasmatodes*: males: TOU-AE 227, Kenting; TOU-AE 3271, Chengkung; TOU-AE 3684, 4263, Changbin; females: TOU-AE 1269, 3270, 4264, Changbin. *Gymnothorax prolatus*: males: ASIZP 55652, Dong-gang, Pintung; TOU-AE 5304, Daxi; females: TOU-AE 4267, Changbin; TOU- AE 4833, Daxi; ASIZP 58435, Dong-Sha Island. *Gymnothorax sagmacephalus*: males: TOU-AE 2728, 2730, 2732, 5099, 5100, Changbin; females: TOU-AE 226, Kenting, TOU-AE 1407, 1409, 1410, 2726, 2729, 2731, Taitung. *Pseudechidna brummeri*: males: TOU-AE 5137, 5138, Philippines. ASIZP 56685, Lanyu. *Strophidon sathete*: males: TOU-AE 1868, 3458, Changbin; TOU-AE 3026, 4832, 5305, Daxi; females: TOU-AE 628, Changbin; TOU-AE 3027, 3028, 3990, 4478, 4563, 5306, Daxi. *Strophidon dorsalis*: females: TOU-AE 157, 4834, 5303, Daxi; males: TOU-AE 786, 787, Nha Trang, Vietnam.

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References

Böhlke, E.B. (1982) Vertebral formulae for type specimens of eels (Pisces: Anguilliformes). *Proceedings of the Academy of Natural Sciences of Philadelphia*, 134, 31–49.

Böhlke, E.B. (1997) Notes on the identity of elongate unpatterned Indo-Pacific morays, with description of a new species (Muraenidae, Subfamily Muraeninae). *Proceedings of the Academy of Natural Sciences of Philadelphia*, 147, 89–109.

Böhlke, E.B. & Randall, J.E. (2000) A review of the moray eels (Anguilliformes: Muraenidae) of the Hawaiian Islands, with descriptions of two new species. *Proceedings of the Academy of Natural Sciences of Philadelphia*, 150, 203–278.

Chen, H.M., Shao, K.T. & Chen, C.T. (1994) A review of the muraenid eels (Family Muraenidae) from Taiwan with descriptions of twelve new records. *Zoological Studies*, 33 (1), 44–64.

Hall, T.A. (1999) *BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT.*

Huelsenbeck, J.P. & Ronquist, F. (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics*, 17, 754–755. http://dx.doi.org/10.1093/bioinformatics/17.8.754

Jobb, G., von Haeseler, A. & Strimmer, K. (2004) Treefinder: a powerful graphical analysis environment for molecular phylogenetics. *BMC Evolutionary Biology*, 4, 18. http://dx.doi.org/10.1186/1471-2148-4-18

Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H. & Higgins, D.G. (2007) Clustal W and Clustal X version 2.0. *Bioinformatics*, 23, 2947–2948. http://dx.doi.org/10.1093/bioinformatics/btm404

Loh, K.H., Shao, K.T. & Chen, H.M. (2011) *Gymnothorax melanosomatus*, a new moray eel (Teleostei: Anguilliformes: Muraenidae) from southeastern Taiwan. *Zootaxa*, 3134, 43–52.

Loh, K.H., Chen, I.S., Randall, J.E. & Chen, H.M. (2008) A review and molecular phylogeny of the moray eel subfamily Uropterygiinae (Anguilliformes: Muraenidae) from Taiwan, with description of a new species. *The Raffles Bulletin of Zoology*, 19 (Supplement), 135–150.

Loh, K.H., Shao, K.T., Chong, V.C. & Chen, H.M. (2014) Additions to the Taiwan eel fauna with five newly recorded species of moray eel (Anguilliformes: Muraenidae), and redescription of *Gymnothorax sagmacephalus*. *Journal of Marine Science and Technology*, 20140704, 1–7. [published online] http://dx.doi.org/10.6119/JMST-013-1227-1

Sasaki, K. & Amaoka, K. (1991) *Gymnothorax prolatus*, a new moray eel from Taiwan. *Japanese Journal of Ichthyology*, 38 (1), 7–10.

Shao, K.T., Ho, H.C., Lin, P.L., Lee, P.F., Lee, M.Y., Tsai, C.Y., Liao, Y.C., Lin, Y.C., Chen, J.P. & Yeh, H.M. (2008) A checklist of the fishes of southern Taiwan, northern South China Sea. *The Raffles Bulletin of Zoology*, 19 (Supplement), 233–271.

Smith, D.G. (2012) A checklist of the moray eels of the world (Teleostei: Anguilliformes: Muraenidae). *Zootaxa*, 3474, 1–64.

Swofford, D.L. (2002) PAUP*. Phylogenetic Analysis Using Parsimony (* and Other Methods), version 4.b10. Sinauer Associates, Sunderland, Massachusetts.

Tanabe, A.S. (2007) Kakusan: a computer program to automate the selection of a nucleotide substitution model and the configuration of a mixed model on multilocus data. *Molecular Ecology Notes*, 7, 962–964. http://dx.doi.org/10.1111/j.1471-8286.2007.01807.x

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. & Higgins, D.G. (1997) The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research*, 24, 4876–4882. http://dx.doi.org/10.1093/nar/25.24.4876