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Additional description on morphology of the Misol snake eel from Taiwan, with four verified barcodes of life sequences

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Abstract

An additional description of the Misol snake eel *Yirrkala misolensis* (Günther, 1872) is reported on the basis of 9 specimens collected from Dong-gang and Ke-tzu-liao, southwestern Taiwan. The species was previously reported from Indonesia and Australia and then extends northward to Taiwan and Japan, and was lacking adequate characterization on morphology. A detail description, fine condition of fresh photographs and 4 partial CO1 sequences are provided for the first time.

Key words: Ophichthidae, Anguilliformes, barcode of life, CO1 sequence, mitochondrial DNA

Introduction

The snake eel family Ophichthidae is the most diverse Anguilliform fishes comprising a total of 2 subfamilies 59 genera and 313 species worldwide. Of these, 2 subfamilies 20 genera and 69 species can be found in Taiwan (Chiu *et al.*, 2013; Ho *et al.*, 2015; Shao, 2022).

Ophichthid eels occupy a variety of tropical and subtropical habitats, including freshwater, estuaries, shallow coastal waters to mid waters, reefs, rubble, sand and mud substrates, usually at depth less than 400 m, although some species of genus *Ophichthus* are reported from depth 1,300 m (McCosker *et al.*, 2012). They are common bycatch from bottom trawlers in Taiwan.

The genus *Yirrkala* was named for the type locality Yirrkala, northern Australia by Whtley (1940) with an assignation of type species *Y. chaselingi*. There are 15 valid species of congeners which are widely distributed from the central Pacific to the western India ocean and the Red Sea (Froese & Pauly, 2022), but the specimens were rare, lacking of details on morphological variation and molecular information.

In this study, an additional description on *Yirrkala misolensis* collected from Dong-gang fish market and Ke-tzu-liao fish markets, southwestern Taiwan, were provided. The species was mentioned as a new record genus and species in Ho *et al.* (2015), but without descriptions on the two voucher specimens. Here, photos of specimens in fresh condition, morphological description and barcodes of CO1 data were presented.

Materials and methods

Abbreviations and terms used in diagnosis and description follow McCosker (1977) and McCosker *et al.* (1989), with the additional measurements of anterior nostril (AN), and posterior nostril (PN).

Counts and measurements follow Böhlke (1989). Measurements include a straight-line with a 300 mm ruler for total length (TL), tail length (Tail), trunk length (TR), head length (HL), predorsal length (PD), preanal length

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(PA), and recorded to the nearest 0.5 mm and with a dial caliper for body depth at gill opening (DGO), body depth at anus (DA), interorbital width (IOW), eye diameter (ED), snout length (S), gill opening length (GO) and pectoral-fin length (PL) recorded to the nearest 0.1 mm.

The terminology of cephalic pores follows McCosker *et al*. (1989). Supraorbital pores (SO) are expressed as ethmoidal pore + pores in its supraorbital canal; infraorbital pores (IO) are expressed as pores along the upper jaw + those in vertical part of the canal behind the eye (the postorbital pores); the preoperculo-mandibular pores (POM) include those on the lower jaw and preopercular canal (preopercular pores, POP); the supratemporal pores (ST) are those on the dorsolateral side of the head pore before the lateral line pores. Frontal pore (F) is expressed as the pore in the position of transverse frontal commissure. Lateral-line pores (LL) are expressed as pre-gill-opening lateral-line pores (PGLL), predorsal lateral-line pores (PDLL), preanal lateral-line pores (PALL), and total lateral-line pores (TLL).

Vertebral counts (hypural included) were taken from radiographs. The mean vertebral formula (MVF) follows Böhlke (1982) and it is expressed as the average of predorsal (PDV), preanal (PAV) and total vertebrae (TV).

The terminology of dentition generally follows Hatooka (1986). All species belonging to Anguilliformes have a premaxillo-ethmo-prevomer complex where the premaxilla, ethmoid and prevomer is almost fused together, nonetheless, it is preferential to use the words premaxillary, middle-premaxillary and vomer (Chiu *et al.,* 2013).

Cytochrome C Oxidase 1 (CO1) barcoding method follow Chang *et al.* (2016). DNA was extracted from 4 of the 9 specimens mentioned in this report by using QuickGene DNA tissue kit S (KURABO, Japan). PCR amplification of partial mitochondria gene (about 650bp) were performed in a total volume of 25 μL containing a mixture of 3 μL DNA template, 1 μL (5μM) of each forward and reverse primer, forward: FishF1+2 (5'-TCR ACY AAY CAY AAA GAY ATY GGC AC-3'); reverse: FishR1 (5'-TAG ACT TCT GGG TGG CCA AAG AAT CA-3'), and FishR2 (5'-ACT TCA GGG TGA CCG AAG AAT CAG AA-3'). These universal primers were modified based on Ward *et al*. (2005), using 12.5 μL 2X SuperRed PCR Master Mix with loading dye (BIOTOOLS CO. Ldt.) and 7.5 μL deionized water. PCR (Polymerase chain reaction) started with the first step at 94℃ for 4 min followed by 35 cycles of denaturation at 94℃ for 30 seconds, 47℃ for 30 seconds and 72℃ for 30 seconds, and ended with once extension step at 72℃ for 7 min. The successfully amplified DNAs were purified and sequenced by MISSION BIOTECH and sequences were aligned (Clustal W), trimmed, constructed and saved as FASTA format by using BioEdit ver. 7.2.5 (Hall, 1999), then constructed a Neighbor-joining tree with 10,000 bootstrap-replicated K2P distance by using MEGA ver. 10.0.5 (Kumar *et al*., 2018). Four sequences of this study and one congener *Y. tenuis* (GenBank: KF930537) were used as the ingroup, and one of *Myrophis punctatus* (GenBank: JQ841945) was used as the outgroup.

All the specimens were deposited from the collections of Laboratory of Aquatic Ecology, National Taiwan Ocean University, Taiwan (TOU-AE), National Museum of Marine Biology, Taiwan (NMMB-P) and Biodiversity Research Center, Academia Sinica, Taiwan (ASIZP).

Taxonomy

Family Ophichthidae

Genus *Yirrkala* Whitley, 1940

Yirrkala Whitley, 1940: 410 (Type species: *Yirrkala chaselingi* Whitley, 1940; by the original designation). McCosker, 1977: 69; McCosker & Castle, 1986: 185; McCosker *et al.*, 1989: 297; McCosker, 1999: 583; McCosker, 2011: 46; Kottelat, 2013: 47.

Pantonora Smith, 1964: 719 (Type species: *Ophichthys tenuis* Günther, 1870; by the original designation).

Diagnosis. A genus of Ophichthinae with branchiosteagal rays overlapped and caudal fin absent. Very elongate, slender and cylindrical body, snout and tail tip both pointed; anal position placed in the medium of body or slightly near the head side, in some case tail shorter than preanal length; median fins low; dorsal fin origin above or behind gill opening; pectoral fin absent; tubular anterior nostrils (AN) and posterior nostrils (PN) within upper jaw; gill opening ventrolateral to lateral; conical, pointed, and generally equal-uniserial teeth; the second temporal pore usually present (McCosker, 1977; McCosker, 2011).

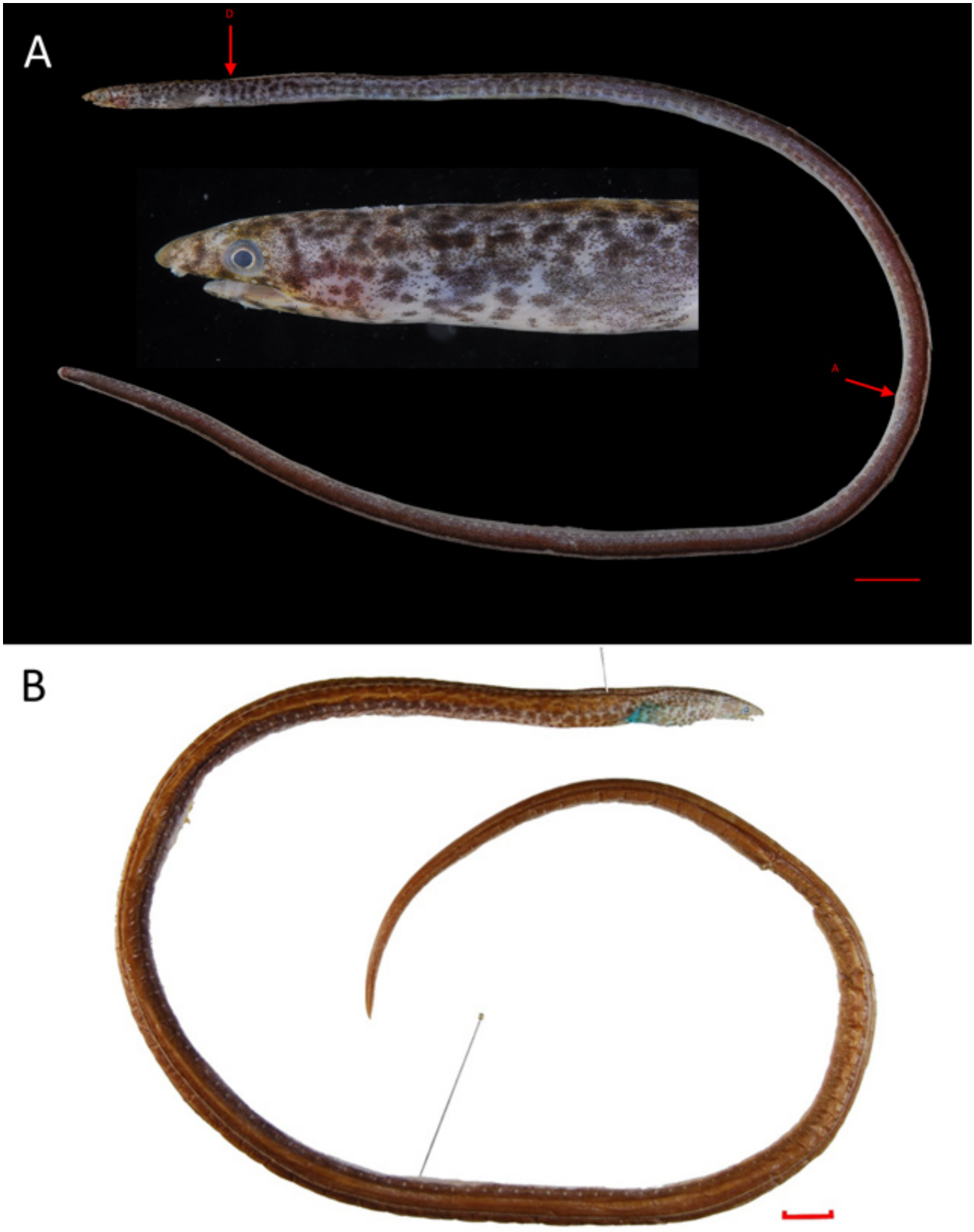

FIGURE 1. Lateral view of *Yirrkala misolensis.* (A) Before fixing, TOU-AE7866, 351 mm TL with broken tail, Ke-tzu-liao fish market, Kaohsiung, Taiwan. Scale represents 10 mm. The position of dorsal fin origin and vent marked with red arrows. The center is a close-up view of cephalic part (Photo by W. -C. Huang). (B) When preserved, NMMB-P12003, 486 mm TL, mature female, Dong-gang fish market, Ping-tung, Taiwan, the position of dorsal fin origin and vent marked with pins.

***Yirrkala misolensis* (Günther, 1872)**
Fig. 1A–B, Fig. 2 A–B

Ophichthys misolensis Günther, 1872: 426 (Type locality: Misol Island, Indonesia). McCosker, 1977: 69.
Yirrkala misolensis (Günther, 1872): Smith & McCosker, 1999: 1669; McCosker *et al.*, 2006: 277; Bucol *et al.*, 2010: 98; McCosker, 2011: 47; Chiu, 2014: 30; McCosker, 2014: 339; Ho *et al.*, 2015: 177; Hibino *et al.*, 2021: 22.

Material examined. ASIZP 0080303 (1 specimen, 383 mm TL) and ASIZP 0080574 (1 specimen, 411 mm TL), 7 May 2016; ASIZP 0080575 (1specimen, 512 mm TL), 5 July 2016; TOU-AE7866 (1 specimen, 351 mm TL with broken tail), 4 September 2020, off Ke-tzu-liao fish market. NMMB-P12003 (1 specimen, 486 mm TL, mature female), 4 Sep. 2010; NMMB-P13735 (1 specimen, 455 mm TL), 20 July 2011; NMMB-P17506 (1 specimen, 478 mm TL), 4 Sep. 2010; NMMB-P24400 (1 specimen, 397 mm TL), 24 August 2016; TOU-AE7843 (1 specimen, 479 mm TL), 17 August 2020, off Dong-gang fish market, southwestern Taiwan. **Other locality.** NMMB-P12495 (5 specimens, 261–401 mm TL), 15 April 2011, Phýờng Mũi Né, Việt Nam.

Diagnosis. Cephalic pores: SO 1 + 3, IO 4 + 2, POM 4 or 5 + 2, 3 temporal pores, single frontal pore (Fig. 2A). MVF: 9.8–77.4–173.3. Uniserial teeth on jaws and vomer (Fig. 2B). Lateral-line pores composition: PGLL 8.1, PDLL 11.1, PALL 79.4, TLL 164.3. Body gray to brownish in background, irregular patterns on dorsal side, pale ventrally.

Description. Well cylindrical body without compressed tail. Eye located from middle to one third of upper jaw near rictus; Eye small, length just about half of snout or shorter. Tubular anterior nostril moderately developed, downward from lateral view with each of AN have 2 short flaps inside; posterior nostril just a hole from the front edge to midpoint of eyes hind in mouth. Gill opening ventral, the margin of upper side oblique posteriorly.

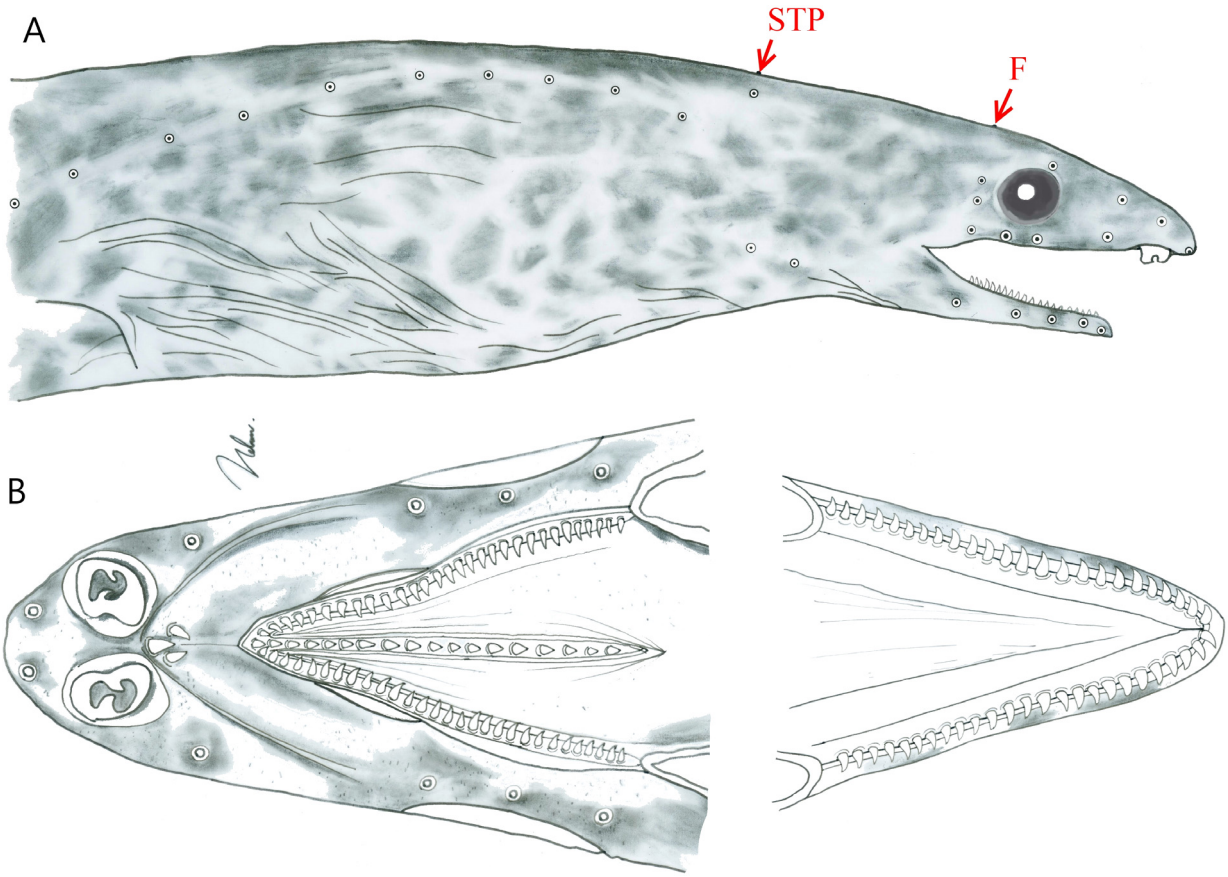

FIGURE 2. Illustration of *Yirrklala misolensis*, NMMB-P12003, 486 mm TL, mature female, Dong-gang fish market, Ping-tung, Taiwan. (A) Lateral view of head, arrows show the position of frontal pore and supratemporal pore. (B) Dentition.

TABLE 1. Proportions (percent) and counts comparison of *Yirrkala misolensis*. Every ratio relates to total count or measurement are excluding TOU-AE7866, which tail is broken. (*: Holotype of *Yirrkala misolensis*, personal communication from J. E. McCosker)

Specimen sources	Present study (n = 9)		HOLOTYPE
Locality	Taiwan		Indonesia
	Mean	Range	*BMNH 1870.8.3:12
TL(mm)		351–512	283
HL/TL	5.3	4.4–6.0	6.0
PA/TL	48.2	46.5–50.1	50.5
Tail/TL	51.8	49.9–53.5	49.5
DGO/TL	1.4	1.1–1.6	1.8
PD/TL	6.6	5.6–7.8	8.3
Upper-Jaw/HL	24.8	21.0–29.5	26.5
S/HL	14.3	11.8–18.2	14.7
ED/HL	6.5	4.8–8.7	5.9
SO	1 + 3	-	1 + 3
IO	4 + 2	-	4 + 2
POM	4 + 2	-	4 + 2
ST	3	-	3
PGLL	8.1	7–9	-
PDLL	11.1	10–12	-
PALL	79.4	76–85	-
TLL	164.3	156–175	-
PDV	9.8	9–11	11
PAV	77.4	74–82	74
TV	173.3	165–180	166

Dorsal fin origin starts about 23.5% HL after gill opening. Cephalic pores are obvious in the mottled background without any pattern around: single ethmoid pore before AN, 2nd SO pore placed in the central of snout, other SO pores and IO pores arranged near posterior side of eye. Head and trunk slightly shorter than tail (48.6% TL in average). Pectoral fin absent.

Dentition on both jaws uniserial, neatly arranged; 3 large teeth on middle-premaxilla arranged in an inverted "V" shape, visible when mouth closing; large teeth followed by a gap, then 18 vomerine teeth; 29–30 in both side of maxilla and the last 5 become smaller gradually; 49–50 on dentary in total, all teeth shape somewhat recurved (Fig. 3).

Lateral line pores minute, difficult to distinguish: 7–9 before gill opening, 10–12 before dorsal fin origin, 76–85 before vent, and about 156–175 in total (the last c.a. 2.6% TL from tail tip invisible).

Body coloration. Pearl whitish in background and canary yellow covered when fresh, with mottled irregular patterns cover the whole head and the dorsal side of anterior 23.8% part of total length, followed by uniform brown or dark with regular white spot till the end of lateral line, each of spot corresponding to one lateral line pore; belly and median fin pale, no pattern covered (Fig.1A). Body overall white to yellowish when preserved, other pigmentation the same with fresh sample (Fig. 1B).

Distribution. Widespread in the western Pacific Ocean. North to Ryukyu Island, Japan, including Makiya and Teruma Beach, Okinawa (Hibino *et al*., 2021); Southern Taiwan, including Kaohsiung (this study) and Ping-tung, Taiwan (Ho *et al.*, 2015); Nha Trang and Mui Ne, Vietnam (Ho *et al.*, 2015); west to Nicobar Island, India; Misol Island, Indonesia (Günther, 1872); Philippines (Bucol *et al.*, 2010, McCosker, 2014); Fiji (Dr. J.E. McCosker, pers.

comm.); and east to Queensland, Australia (McCosker *et al.*, 2006). Taiwanese specimens were caught at the range of 200–400 m by bottom trawlers, McCosker (2006) noted that they are benthic, burrowing species and live in coral rubble bottom, confirming the ecological information is still needed.

Genetic features. A Neighbor-Joining tree constructed by partial CO1 gene sequences (552 bp after processed by BioEdit software) of four voucher specimens in this study and the other two CO1 sequences obtained from NCBI (National Center for Biotechnology Information) supported the separation of these species (Fig. 3). The GenBank accession numbers of 4 specimens examined in this study were attached following the voucher, in addition, the K2P distance matrix reveals that the distance ranged from 0.004 to 0.011 within *Y. misolensis*, 0.146 to 0.151 between *Y. misolensis* and *Y. tenuis*, and 0.182 to 0.213 between *Yirrkala* spp. and the outgroup (Table 2).

TABLE 2. Matrix of Kimura-2-parameter distances of the 6 CO1 sequences used to construct NJ tree in the present study. (1) to (4) are 4 specimens of *Yirrkala misolensis*, (5) is the other congener *Y. tenuis*, with a *Myrophis punctatus* as an outgroup.

	(1)	(2)	(3)	(4)	(5)
(1)ASIZP0080303 (GenBank: KX954875)					
(2)ASIZP0080574 (GenBank: KX954876)	0.011				
(3)TOU-AE7866 (GenBank: ON332487)	0.005	0.009			
(4)TOU-AE7843 (GenBank: ON332488)	0.005	0.009	0.004		
(5)*Yirrkala_tenuis* (GenBank: KF930537)	0.150	0.151	0.148	0.146	
(6)*Myrophis_punctatus*_(GenBank: JQ841945)	0.189	0.182	0.189	0.191	0.213

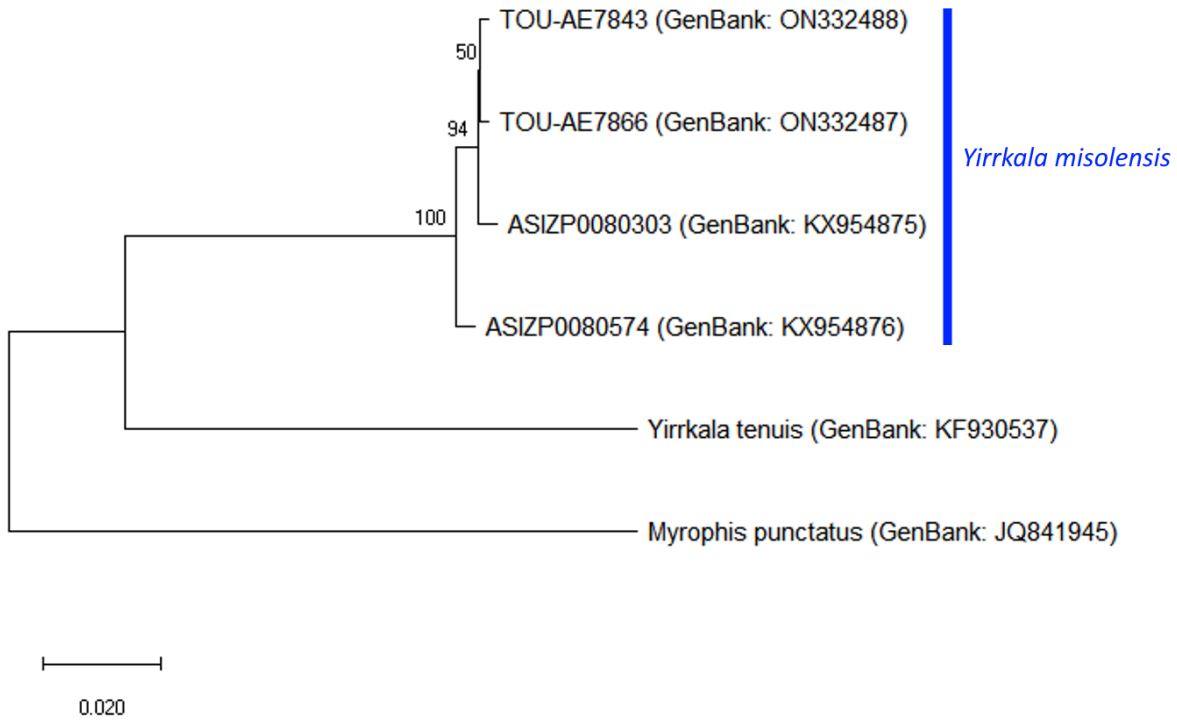

FIGURE 3. Neighbor-joining tree based on CO1 sequences, constructed using the specimens mentioned in the present study and 1 congener from NCBI. The bar indicates the evolutionary distances which were computed using the Kimura 2-parameter method with 10,000 bootstrap-replicated.

Discussion

A key to identify snake eels from the Western Central Pacific was provided by Smith & McCosker (1999). We could identify the specimens following this key by some diagnostics characters: tail tip finless; pectoral fin absent; anal fin present; dorsal fin begins over or slightly behind in front of gill opening; dorsal fin origin near level of gill opening.

Bucol *et al.* (2010) pointed out that the coloration of *Yirrkala misolensis* is very similar to *Lamnostoma mindora*, however, they can still be separated from their higher total vertebral count (165–180, vs. 141–150), much slender on body depth (1.1–1.8, vs. 3.0 in TL), different shapes on snout (sharp, vs. lightly slender) and the position of eye (located from middle to 1/3 of upper jaw near rictus, vs. placed near 1/3 of upper jaw length from the tip of snout) (Chiu *et al*., 2018) (Fig. 1B; Fig. 2A).

Proportions (%) and counts comparison of holotype and Taiwan's *Yirrkala misolensis* shown in Table. 1. The Taiwanese specimens differ slightly from the holotype by having a shorter head (4.4–6.0% TL, vs. 6% in holotype); a slightly longer tail, tail length longer than trunk length (49.9–53.5% TL, vs. 49.5%); 3 middle-premaxilla teeth are distinctly larger than all other teeth (vs. all teeth similar in size); relatively more total vertebrae (165–180, vs. 166). The body is uniformly pale in holotype (McCosker, pers. comm) which might be a result of poor condition of the holotype. All other morphological conditions are consistent with the original description.

The coloration of *Yirrkala* is usually pale without patterns or reticulations, except *Y. misolensis* and *Y. calyptra*, the only two species with patterns covered on body. *Yirrkala misolensis* differs from the latter by having irregular spots on the head and dorsal side of body (vs. distinctive black facial slash between eye and rictus) (Fig. 1A–B).

The first record of genus *Yirrkala* in South China Sea was *Y. kuro* (Shao *et al.*, 2008: 239), but its generic status was subsequently changed to *Callechelys* (McCosker, 2011: 272). More recently, Ho *et al.*, (2015) recorded *Y. misolensis* with 2 vouchers from Dong-gang, but no description based on those specimens was provided. Our study provided the first description and genetic information of *Y. misolensis* in Taiwan, which contribute to future taxonomic works.

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