

First record of butterflyfish, *Roa haraguchiae* (Actinopterygii: Perciformes: Chaetodontidae), from northeast Taiwan

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Abstract

A recently-described butterflyfish, *Roa haraguchiae* Uejo, Senou et Motomura, 2020, is herewith for the first time reported from northeast Taiwan. In Taiwan, the genus *Roa* has been known represented by a single species, *Roa modesta* (Temminck et Schlegel, 1844). This study presents a comparison of *R. haraguchiae* with its congeners and includes diagnostic characters on the basis of morphology and genetic differences by life-barcoding. Our specimens have some differences that may be attributed to the individual variations, which are compared and discussed.

Keywords

CO1 sequence, mitochondrial DNA, morphology, taxonomy, white-spine butterflyfish

Introduction

The family Chaetodontidae is commonly referred to as butterflyfishes and its representatives inhabit tropical and subtropical sea areas, which are mainly distributed in the Indo–West Pacific. They live on coral reef substrate and are characterized by their colorful skin. According to Nelson et al. (2016), the family Chaetodontidae is represented by 12 genera and 129 species; whereas, according to Fricke et al. (2022), the family currently contains 12 genera and about 136 species in the world with seven genera and 46 species records in Taiwan (Shao 2022).

The genus *Roa* Jordan, 1923 represents the family Chaetodontidae and it can be separated from other butterflyfishes by having three distinct bands on the body, with the first band passing behind the eye and ending at the lower edge of the preopercle. According to Matsunuma and Motomura (2022), the genus includes the following

eight valid species: *Roa modesta* (Temminck et Schlegel, 1844); *Roa excelsa* (Jordan, 1921); *Roa jayakari* (Norman, 1939); *Roa australis* Kuiter, 2004; *Roa rumsfeldi* Rocha, Pinheiro, Wandell, Rocha et Shepherd, 2017; *Roa haraguchiae* Uejo, Senou et Motomura, 2020; *Roa semilunaris* Matsunuma et Motomura, 2022; and *Roa uejoi* Matsunuma et Motomura, 2022. The genus *Roa* was originally described as *Loa* with a type species *Loa excelsa* Jordan, 1921. Quite soon, however, Jordan (1923) changed the genus name to *Roa* because of its homonymy with *Loa* Stiles in Stiles et Hassall, 1905, which is a genus of nematodes belonging to Filariidae. For a long time, the majority of authors have considered the genus *Roa* to be a subgenus of the genus *Chaetodon* Linnaeus, 1758. Kuiter (2004) was the first researcher to confirm the generic rank of *Roa* following an unpublished thesis of Blum, mentioned in Blum (1989). He also described *Roa australis* Kuiter, 2004 as the only *Roa* species distributed

in the Southern Hemisphere. He also moved *Chaetodon modestus* to *Roa* as *R. modesta*, which is the only *Roa* species reported from Taiwan by Shen (1993). Recently, Matsunuma and Motomura (2022) described two new species of *Roa*—*Roa semilunaris* and *Roa uejoi*, which are respectively distributed in the southwestern Indian Ocean and the Mariana Islands.

Species of the genus *Roa* inhabit waters deeper than 200 m and are often caught by bottom trawl nets. Those fishes have been recorded from different environments. The ecosystems where *Roa rumsfeldi* was found vary from sheltered rocky outcroppings heavily covered by fine sediment to areas exposed to strong currents (Rocha et al. 2017). Uejo et al. (2020) mentioned that the *Roa* specimens from the East China Sea were all taken using bottom trawls, indicating a sandy bottom habitat, while all of the underwater photographs were taken in rocky bottom areas in fairly deep water.

The type locality of *Roa haraguchiae* is in the East China Sea, Japan (146–162 m), with a paratype collected at Iloilo, Panay Island, the Philippines. It was also found in the Izu Peninsula, the Pacific coast of Japan; Suruga Bay and Sakurajima in Kagoshima Bay, southern Kyushu (37–70 m) (Uejo et al. 2020).

In this study, the first record of *Roa haraguchiae* in Taiwan has been described, including the diagnostic characters on the basis of morphology, genetic differences from its congeners and a key for species of the genus *Roa*. The specimens collected in Taiwan have some differences that may be attributed to the individual variations, which are also compared and discussed.

Methods

Counts, measurements, and terminology generally follow Pyle and Kosaki (2016) and Uejo et al. (2020). Body depth is measured as a vertical straight line from the front edge of the pelvic-fin spine base to the insertion point of the first dorsal-fin spine. Postorbital length is the distance from the posterior edge of the bony orbit to the posterior edge of the fleshy flap near the end of the gill opening.

The radiographs on the skeleton of butterflyfish specimens were obtained using the X-ray equipment of the National Marine Museum of Biology and Aquarium (NMMB, Taiwan). All specimens were deposited at the Laboratory of Aquatic Ecology of the National Taiwan Ocean University (TOU-AE).

The cytochrome *c* oxidase subunit 1 (CO1) barcoding method follows Chang et al. (2016). PCR amplification of the 5' region of the CO1 gene (approximately 650 bp) was performed and all the successfully amplified sequences were aligned (Clustal W), trimmed, constructed, and saved as FASTA format by using BioEdit ver. 7.2.5 (Hall 1999), followed by the construction of a Neighbor-Joining (NJ) tree with 10 000 bootstrap-replicated K2P distance using MEGA ver. 10.0.5 (Kumar et al.

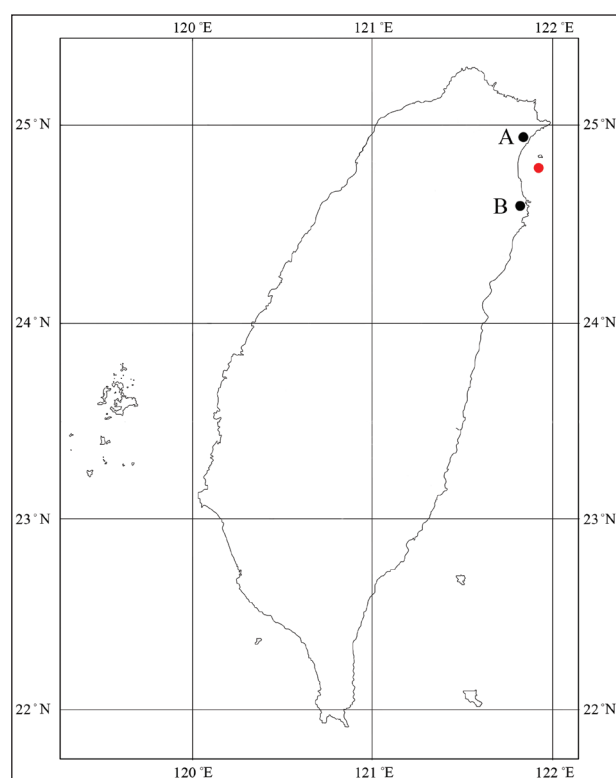


Figure 1. Distribution records of *Roa haraguchiae* from Taiwan. Black point symbols collection port, A = Da-xi, B = Nan-fang-ao. Red point means fishing location.

2018). Nine sequences from five species were used as the ingroup and one of *Chaetodon octofasciatus* Bloch, 1787 (KU944212) served as the outgroup. All of the accession numbers are listed in Table 1. Due to some differences in the appearance of these four specimens and given that they are the only vouchers with muscle tissue from this batch, we can only use them as the materials for the molecular analysis.

Our *Roa haraguchiae* specimens were all collected by bottom trawls at a depth of 100–300 m from northeast Taiwan (Fig. 1), including sandy and rocky bottom habitats.

Table 1. List of accession numbers of the species of *Roa* and *Chaetodon* (as outgroup) in GenBank and one specimen in BOLD.

Specimen No.	Scientific name	Accession number	
TOU-AE8100	<i>Roa haraguchiae</i>	OM365890	This study
TOU-AE8354	<i>Roa haraguchiae</i>	OM365891	This study
TOU-AE8355	<i>Roa haraguchiae</i>	OM365892	This study
TOU-AE8379	<i>Roa haraguchiae</i>	OM365893	This study
PNM15198	<i>Roa rumsfeldi</i>	MF995631	Rocha et al. 2017
CIFE:FGB-RJ-001	<i>Roa jayakari</i>	KF268176	
ASIZP0805725	<i>Roa modesta</i>	KU944230	Chang et al. 2016
ASIZP0802360	<i>Chaetodon octofasciatus</i>	KU944212	Chang et al. 2016
NMV A 29675-001	<i>Roa australis</i>	FOAG413-08. COI-5P	

Bold font denotes sequence ID from BOLD.

Results

Taxonomical status

Family Chaetodontidae Rafinesque, 1815

Roa Jordan, 1923

Roa haraguchiae Uejo, Senou et Motomura, 2020

Figs. 1–2, Table 2

English name: white-spine butterflyfish

New Chinese name: 原口氏羅蝶魚

Specimens examined. (six specimens) TOU-AE8100, 72.22 mm SL, Da-xi, NE Taiwan, bottom trawl, 8 May

2021; TOU-AE8354, 96.27 mm SL, Nan-fang-ao, NE Taiwan, bottom trawl, 1 Aug 2021; TOU-AE8355, 108.59 mm SL, sharing the same collecting information with TOU-AE8354; TOU-AE8379, 96.78 mm SL, Da-xi, NE Taiwan, bottom trawl, 13 Aug 2021; TOU-AE8491, 90.21 mm SL, Da-xi, NE Taiwan, bottom trawl, 26 Oct 2021; TOU-AE8492, 98.25 mm SL, sharing the same collecting information with TOU-AE8491.

Comparative material. *Roa modesta* (26 specimens): TOU-AE7862, 89.70 mm SL, Da-xi, NE Taiwan, date unknown; TOU-AE7863, 96.11 mm SL, Da-xi, NE Taiwan, date unknown; TOU-AE7876, 76.96 mm SL, Ba-dou-zi, NE Taiwan, 15 Aug 2020; TOU-AE7877, 84.71 mm SL and TOU-AE7878, 90.11 mm SL sharing the same

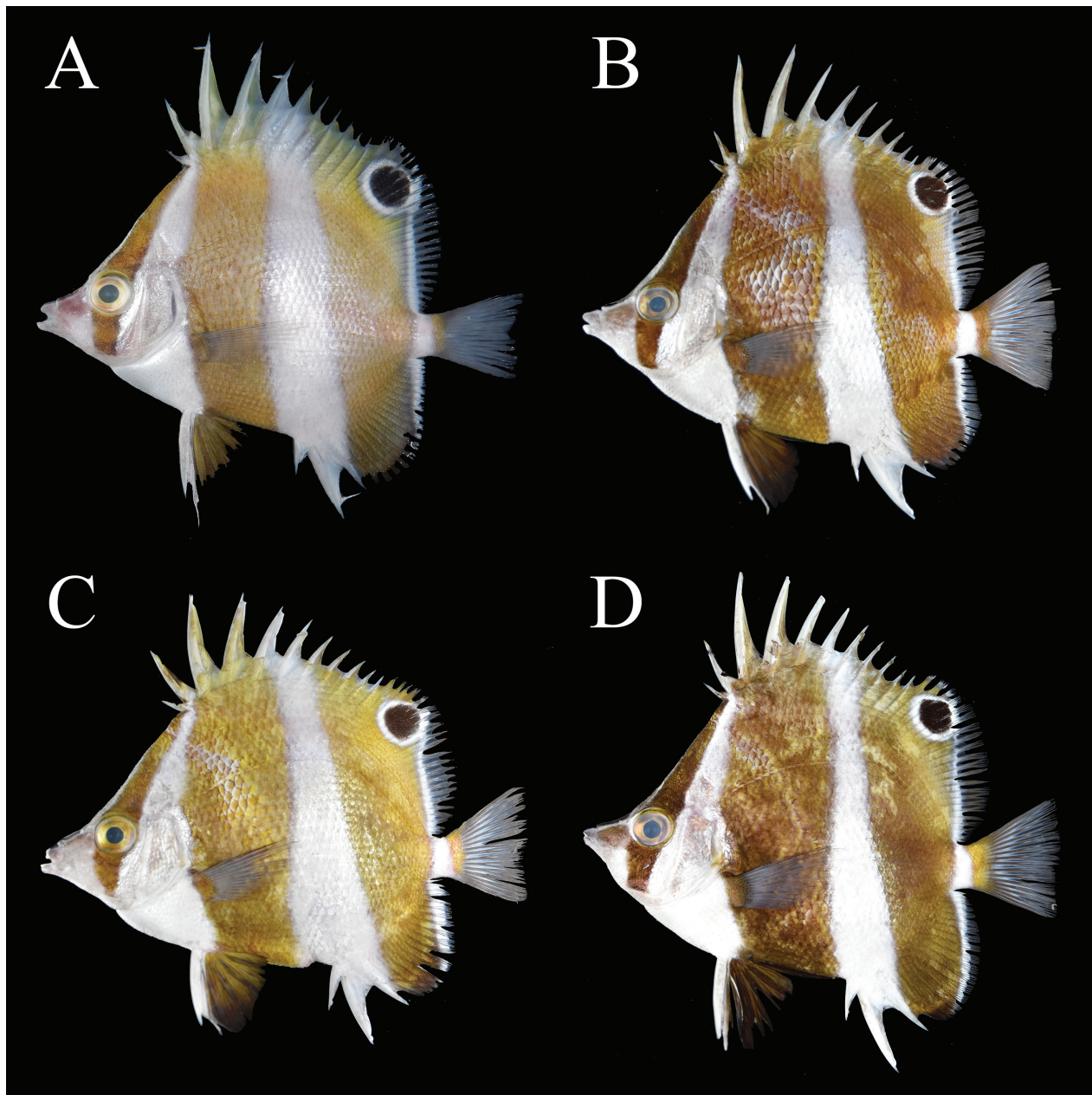


Figure 2. Comparison of the morphological differences of the fresh specimens of *Roa haraguchiae* in Taiwan, for which CO1 genes were sequenced. A. TOU-AE 8100, 72.22 mm SL; B. TOU-AE 8354, 96.27 mm SL; C. TOU-AE8355, 108.59 mm SL; D. TOU-AE 8379, 96.78 mm SL. Photo by J.-F. Huang.

collecting information with TOU-AE 7876 ; TOU-AE7995, 80.30 mm SL, Dong-gang, S Taiwan, 2 Jan 2021; TOU-AE7999, 86.50 mm SL, Dong-gang, S Taiwan, 23 Jan 2021; TOU-AE8000, 75.58 mm SL, Ke-tzu-liao, S Taiwan, 24 Jan 2021; TOU-AE8011, 43.91 mm SL sharing the same collecting information with TOU-AE8000; TOU-AE8109, 86.78 mm SL, Kan-zi-ding Fish Market, N Taiwan, 27 Apr 2021; TOU-AE8110, 92.98 mm SL sharing the same collecting information with TOU-AE8109; TOU-AE8131, 95.76 mm SL, Da-xi, NE Taiwan, 26 Jul 2020; TOU-AE8132, 91.31 mm SL sharing the same collecting information with TOU-AE8131; TOU-AE8133, 97.54 mm SL, Da-xi, NE Taiwan 1 May 2021; TOU-AE8241, 63.40 mm SL, Ke-tzu-liao, S Taiwan, 24 Jan 2021; TOU-AE8242, 54.87 mm SL, Ke-tzu-liao, S Taiwan, date unknown; TOU-AE8243, 72.98 mm SL and TOU-AE8244, 79.78 mm SL, Ke-tzu-liao, S Taiwan, date unknown; TOU-AE8287, 91.96 mm SL, Da-xi, NE Taiwan, 26 Jul 2021; TOU-AE8288, 90.82 mm SL sharing the same collecting information with TOU-AE8287; TOU-AE8322, 98.68 mm SL, Nan-fang-ao, NE Taiwan, 1 Aug 2021; TOU-AE8323, 94.74 mm SL and TOU-AE8324, 98.55 mm SL sharing the same collecting information with TOU-AE8322; TOU-AE8372, 74.76 mm SL, Ba-dou-zi, NE Taiwan, 2 Aug 2021; TOU-AE8373, 71.30 mm SL sharing the same collecting information with TOU-AE8372; TOU-AE8426, 77.42 mm SL, Da-xi, NE Taiwan, 13 Aug 2021.

Diagnosis. Specimens of *Roa haraguchiae* in Taiwan with the following combination of characters: pored lateral-line scales 39–42; non-pored lateral-line scales 4–6; scale rows above lateral line 11–13, scale rows below lateral line 20–24; scale rows under longer axis of black blotch on dorsal-fin soft-rayed portion 10–12; longer snout length 2.8–3.0 in HL; shorter caudal-peduncle depth 8.8–10 in SL; shorter dorsal-fin soft-rayed portion base length 2.7–2.9 in SL; longer 2nd anal-fin spine length 3.7–4.0 in SL; anterior margin of second body band not reaching anteroventrally to pelvic-fin spine base; first pelvic-fin soft ray white and extended; membranes associated with first and second dorsal-fin spines are respectively blackish completely and distally.

Description of Taiwanese specimens. Counts and proportional measurements as a percentage of SL and HL are given in Table 2. Data for the specimens in Taiwan, followed by data for the other congeners. Dorsal-fin XI, 20–21; anal-fin III, 16–17; pelvic-fin rays I, 5; pectoral-fin rays 14; pored lateral-line scales 39–42; Non-pored lateral-line scales 4–6; Scale rows above lateral line 11–13; Scale rows below lateral line 20–23; Scale rows in longer axil of black blotch on dorsal-fin soft-rayed portion 11–12; gill rakers 3–4 + 11–12 (14–16); vertebrae 11 + 13 = 24; caudal rays 12 + 11 = 23.

Body strongly deep and compressed, its depth 1.5–1.7 in SL and width 6.4–7.2 in SL; head length 2.6–2.8 in SL; orbit diameter slightly shorter than snout length, its length 3.0–3.3 in HL; snout length 2.8–3.0 in HL; postorbital length 2.8–3.1 in HL; interorbital region narrow, bony width 3.5–

4.0 in HL. Mouth small, terminal, and slightly protractile. Numerous bristle-like teeth in both jaws. Opercular membranes narrowly attaching to isthmus, slightly projecting at posterior margin of operculum; gill rakers short. Two pairs of nostrils closely symmetric, anterior to eye.

Scales on body ctenoid, also scattered on head, abdomen, and part of upper jaw, except lower jaw. Lateral line scales ascending from posterior edge of gill opening to divide between white and brown bands below base of eleventh dorsal-fin spine and then gradually declining to end of dorsal-fin.

Dorsal-fin spinous portion base length 2.5–2.7 in SL, soft-rayed portion base length 2.7–2.9 in SL; origin of dorsal-fin at vertical through base of pectoral-fin, well at origin of pelvic-fin. First dorsal-fin spine shortest, its length 11.9–14.7 in SL; second dorsal-fin spine length 5.1–8.3 in SL; third dorsal-fin spine slightly shorter than fourth spine, its length 2.9–3.2 in SL; fourth dorsal-fin spine longest, its length 2.8–3.2 in SL; after that, spine length becomes shorter when spine number increasing.

First dorsal-fin soft ray length 4.1–4.9 in SL. Pectoral-fin moderately long, its length 3.1–3.5 in SL, first ray not segmented, second or third ray longest and reaching through posterior edge of second band. Pelvic-fin origin below pectoral-fin base origin, its spine length 3.7–4.7 in SL; its soft ray length 3.1–4.0 in SL. Second anal-fin spine longest, its length 3.7–4.0 in SL; longest anal-fin soft ray length 4.0–4.6 in SL. Caudal-fin truncated, its length 4.1–5.1 in SL.

Coloration. In fresh specimens (Figs. 2A–D): head and body white, with three main brown bands. First band starting from origin of dorsal fin and descending through eye to lower edge of preopercle, with width slightly shorter than orbit diameter. Second band extending from base of second to seventh dorsal-fin spines down to base of pelvic-fin soft rays, posterior margin to anus. Third band starting from base of tenth dorsal-fin spine to terminal end of dorsal-fin, downwards almost covering soft anal fin, between third or fourth soft rays and terminal end of anal-fin. Two additional narrow brown bands also visible; one on anterior margin of body, extending from tip of upper snout through interorbital, approximately half of predorsal-fin length. Another narrow band on posterior margin of caudal-peduncle in form of black blotch with white edge between first and seventh dorsal-fin soft rays. Dorsal-fin spines and soft rays whitish, except for those blackish banded areas and membranes associated with first and second dorsal-fin spines. Anal-fin spines and soft rays whitish, except those banded areas. Caudal-fin and pectoral-fin greyish. Pelvic-fin spine and first soft ray whitish, while others brownish.

When preserved (Fig. 3): Head and body change from whitish to slightly yellowish, with three brown bands becoming lighter. Opercle brownish; opercular membrane yellowish; membranes associated with first and second dorsal-fin spines blackish; blotch on soft-rayed portion blackish, with still obvious whitish margin. The fin color is approximately the same as described above in color when fresh.

Table 2. Counts and measurements of *Roa haraguchiae*, *Roa modesta*, and *Roa rumsfeldi*.

Character	<i>Roa haraguchiae</i>		<i>Roa modesta</i>	<i>Roa rumsfeldi</i>
	This study <i>n</i> = 6	Uejo et al. 2020 <i>n</i> = 7	This study <i>n</i> = 26	Uejo et al. 2020 ASIZP 68098
Standard length [mm]	72.2–108.6	63.2–92.8	43.9–98.7	51.2
Counts				
Dorsal-fin rays	XI, 20–21	X–XI, 21	XI, 21–23	XI, 20
Anal-fin rays	III, 16–17	III, 16–17	III, 16–18	III, 17
Pelvic-fin rays	I, 5	I, 5	I, 5	I, 5
Pectoral-fin rays	14	13–15	14–15	15
Caudal-fin rays	12+11		12+11	
Pored lateral-line scales	39–42	38–42	40–44	32
Non-pored lateral-line scales	4–6	5–7	3–5	13
Scale rows above lateral line	11–13	11–12	12–13	8
Scale rows below lateral line	20–23	22–28	24–25	19
Scale rows in longer axil of black blotch on dorsal-fin soft-rayed portion	11–12	10–12	9–11	8
Gill rakers (upper + lower limbs)	3–4 + 11–12	3–5 + 9–11	3–4+9–10	3+9
Vertebrae (precaudal + caudal)	11+13		11+13	
Measurements [% SL]				
Body depth	60.1–66.9	56.5–66.6	64.1–70.6	60.2
Body width	13.8–15.7	14.5–16.7	13.4–17.4	15.4
Head length	35.9–38.0	34.3–38.7	32.3–39.8	39.3
Head depth	47.8–53.2	46.2–54.9	49.4–55.5	51.6
Predorsal-fin length	49.5–53.7	47.1–54.8	46.6–53.5	53.1
Prepelvic-fin length	42.0–48.5	39.7–46.2	43.1–49.1	44.9
Preanal length	65.0–66.7	67.3–72.0	61.0–67.1	69.1
Preanal-fin length	72.7–78.5	72.3–77.0	69.3–75.8	73.8
Snout length	12.4–13.8	12.6–13.6	9.2–12.4	12.7
Orbit diameter	10.8–13.3	11.1–13.5	10.7–14.1	14.6
Postorbital length	12.4–13.4	12.1–14.1	10.6–14.1	13.7
Interorbital width	9.2–11.3	9.2–11.3	9.3–11.6	10.2
Caudal-peduncle depth	10.0–11.4	10.8–11.3	11.1–13.9	10.0
Caudal-peduncle length	4.3–5.3	5.4–8.0	3.3–6.3	7.8
Caudal-fin length	19.7–24.5	20.1–24.2	20.1–25.0	23.8
Pectoral-fin length	28.7–31.8	27.9–30.5	26.2–32.8	32.4
Dorsal-fin spinous portion base length	37.4–40.4	34.0–39.9	35.7–40.1	33.4
Dorsal-fin soft-rayed portion base length	34.7–36.4	29.4–34.8	36.8–40.1	28.5
1 st dorsal-fin spine length	6.8–8.4	8.6–10.3	6.1–11.7	9.6
2 nd dorsal-fin spine length	12.0–19.8	19.0–25.0	13.6–24.4	22.9
3 rd dorsal-fin spine length	30.9–34.9	31.6–39.5	24.6–33.9	35.4
4 th dorsal-fin spine length	31.6–35.8	34.4–41.0	27.5–36.3	37.5
5 th dorsal-fin spine length	30.2–33.9	29.9–36.2	28.4–35.6	35.5
6 th dorsal-fin spine length	28.8–32.0	29.2–34.0	27.7–35.6	33.0
7 th dorsal-fin spine length	26.3–30.5	25.6–30.5	25.7–33.8	NA
8 th dorsal-fin spine length	23.9–27.8	24.0–27.6	22.7–31.2	29.9
9 th dorsal-fin spine length	21.9–25.4	22.2–26.4	20.6–29.5	26.2
10 th dorsal-fin spine length	20.3–22.9	21.5–24.5	20.9–27.2	NA
11 th dorsal-fin spine length	19.3–21.8	21.5–24.5	19.3–26.8	18.8
1 st dorsal-fin soft ray length	20.4–24.5	20.9–27.4	19.4–27.9	25.0
Anal-fin base length	33.4–37.0	20.6–34.8	34.5–41.6	32.6
1 st anal-fin spine length	10.7–12.8	11.6–15.0	9.7–13.4	11.3
2 nd anal-fin spine length	25.2–27.1	26.8–30.1	17.9–23.7	25.4
3 rd anal-fin spine length	20.5–24.3	20.4–26.2	17.9–23.1	24.4
Longest anal-fin soft ray length	21.8–24.9	21.8–29.4	18.2–24.2	NA
Pelvic-fin spine length	21.9–25.9	23.7–27.2	19.1–26.0	27.0
Pelvic-fin length	24.8–32.3	28.0–38.3	25.5–36.6	38.5
Measurements [% HL]				
Body depth	162.8–177.7	163.6–174.7	172.5–208.7	153.2
Body width	37.4–43.6	39.3–45.2	33.5–51.8	39.3
Head height	131.5–139.8	133.9–143.4	139.4–163.8	131.3
Predorsal-fin length	131.8–141.7	129.4–143.1	129.5–155.8	135.3
Prepelvic-fin length	113.6–128.8	112.6–121.3	122.9–139.3	114.4
Preanal length	166.1–184.2	178.4–203.8	161.5–199.2	176.1
Preanal-fin length	188.4–211.4	189.5–224.2	182.1–228.2	188.1
Snout length	33.4–36.3	33.2–38.7	25.3–33.4	32.3
Orbit diameter	30.0–33.7	31.4–35.0	30.9–37.1	37.3
Postorbital length	32.6–36.3	33.3–36.3	32.7–37.9	34.8
Interorbital width	24.9–28.2	25.0–29.2	27.1–33.7	25.9



Figure 3. Preserved specimens of *Roa haraguchiae*. The band coloration turns slightly brownish and the blackish part has remained.

Discussion

Roa haraguchiae, compared to *Roa modesta*, have a longer snout length 33.4–36.3 (vs. 25.3–33.4) in HL; a shorter caudal-peduncle depth of 10.0–11.4 (vs. 11.1–13.9) in SL; shorter dorsal-fin soft-rayed portion base length 34.7–36.4 (vs. 36.8–40.1) in SL; longer 2nd anal-fin spine length 25.2–27.1 (vs. 17.9–23.7) in SL. The second band extends from the base of the second to the seventh dorsal-fin spines (fourth to eighth). Shorter 2nd/3rd dorsal-fin spine length 53.1% (vs. 65.9%); pelvic first soft ray whitish and

others darkish (vs. all yellow). The main three bands on the lateral side are brownish without blackish margin (vs. yellowish with blackish margin); the eye band is equal to the eye diameter (vs. narrower than the eye diameter); the anterior margin of the second band reaching the gill opening where the lateral-line scale begins (vs. not reaching, through the fifth lateral-line scale) (Fig. 4).

A comparison of the four specimens in Taiwan shows the following: according to the results of CO1 sequencing, it was confirmed that TOU-AE 8100, TOU-AE 8354, TOU-AE 8355, and TOU-AE 8379 are the same species. An NJ tree constructed by partial CO1 gene sequences (552 bp after being processed by BioEdit software) of six species (Table 1) supports the separation of these species (Fig. 5). Besides, in Table 3, the K2P distance matrix reveals that there is no great distance amongst the same species, the distance ranging from 0.038 to 0.129 amongst the five *Roa* congeners and the outgroup shows the distance ranging from 0.191 to 0.220. The width of the eye band is equal to the eye diameter, except that TOU-AE 8355 narrows down. The second dorsal-fin spine of TOU-AE 8354 is shorter than the others (12% compared to the mean value of 17.4%) and the membrane-associated with second dorsal-fin spines is blackish completely (others are blackish distally). The band color from yellowish-brown to dark brown in fresh specimens has been observed to be the same in the preserved specimen.

The other six *Roa* species are not mentioned herein for they can be clearly separated from morphological features. Compared to *R. australis*, the second band reaching the posterior edge of the opercular membrane (vs. not reaching), is shown to be brownish (yellowish) in color. Compared to *R. excelsa*, the longest dorsal-fin

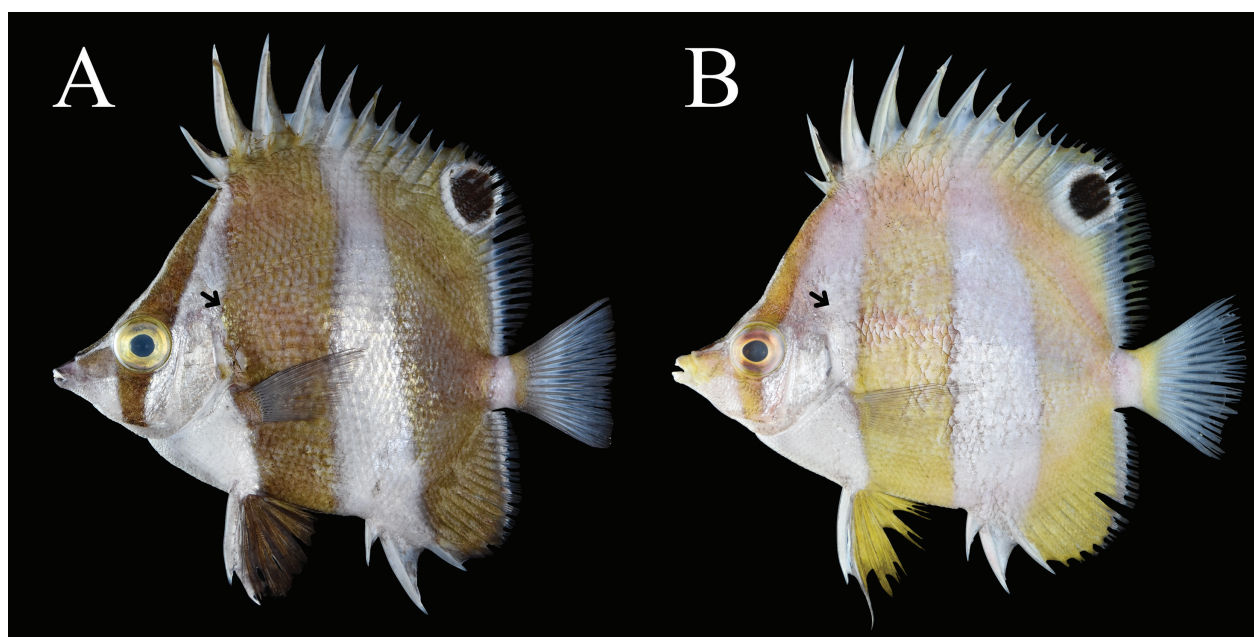
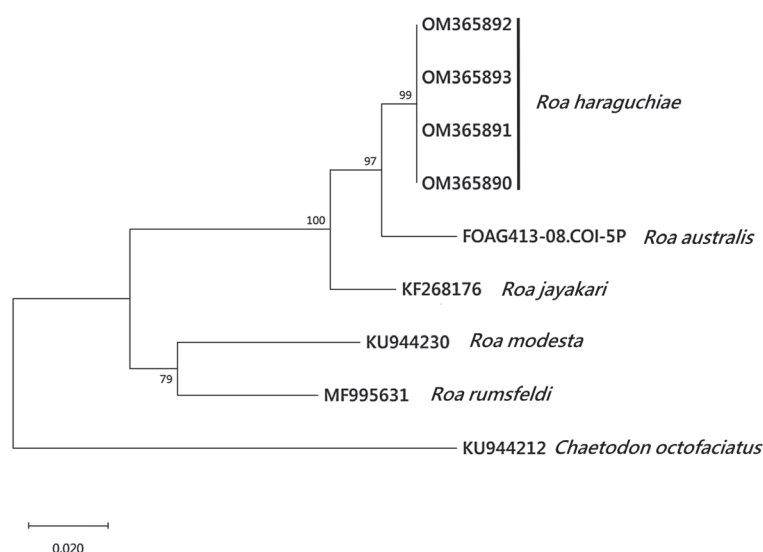


Figure 4. Comparison of the dorsal-fin spine and band coloration of two species in Taiwan. A. *Roa haraguchiae*, TOU-AE 8491, 90.21 mm SL; B. *R. modesta*, TOU-AE 8426, 77.42 mm SL. The arrow shows the differences where the second band reaches the anterior edge.

Table 3. Matrix of Kimura-2-parameter distances of the 9 CO1 sequences used to construct the NJ tree in the presently reported study. (1) to (8) are 8 specimens of five *Roa* congeners and (9) was taken as an outgroup, respectively.

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
(1) <i>Roa haraguchiae</i> (TOU-AE8100)									
(2) <i>Roa haraguchiae</i> (TOU-AE8354)	0.000								
(3) <i>Roa haraguchiae</i> (TOU-AE8355)	0.000	0.000							
(4) <i>Roa haraguchiae</i> (TOU-AE8379)	0.000	0.000	0.000						
(5) <i>Roa rumsfeldi</i> (MF995631)	0.113	0.113	0.113	0.113					
(6) <i>Roa jayakari</i> (KF268176)	0.038	0.038	0.038	0.038	0.124				
(7) <i>Roa modesta</i> (KU944230)	0.129	0.129	0.129	0.129	0.077	0.122			
(8) <i>Roa australis</i> (FOAG413-08.COI-5P)	0.027	0.027	0.027	0.027	0.129	0.048	0.138		
(9) <i>Chaetodon octofasciatus</i> (KU944212)	0.213	0.213	0.213	0.213	0.194	0.205	0.191	0.220	

Bold font denotes sequence ID from BOLD.

**Figure 5.** NJ tree, based on CO1 sequences, constructed using four specimens mentioned in the presently reported study. The bar indicates the evolutionary distances which were computed using the Kimura 2-parameter method with 10 000 bootstrap-replicated.

spine is the fourth (third) and membranes associated with the first and second dorsal-fin spines are blackish (vs. whitish or yellowish). Compared to *R. jayakari*, the eye band under the eye is equal to the eye diameter (vs. narrower than the eye diameter). Compared to *R. rumsfeldi*, the specimens have more pored lateral-line scales 39–42 (32) (Table 2) and the anterior margin of the sec-

ond band does not reach anteroventrally to the pelvic-fin spine base (vs. reaching). Compared to *R. semilunaris*, the origin of the second band is from the base of the second to the seventh dorsal-fin spines (vs. third to sixth). Compared to *R. uejoi*, membranes associated with the first and second dorsal-fin spines are blackish (vs. whitish or yellowish).

Key to species of genus *Roa*

- 1a Second body band approximately same width as eye diameter, its anterior edge not passing through pectoral-fin base *R. australis*
- 1b Second body band two or more times wider than eye diameter, its anterior edge reaching to pectoral-fin base ...2
- 2a Pored lateral-line scales 26–32; pelvic fin spine brownish *R. rumsfeldi*
- 2b Pored lateral-line scales 36–41; pelvic-fin spine whitish3
- 3a Body bands yellowish with distinct dark edges *R. modesta*
- 3b Body bands entirely blackish without dark edges4
- 4a Whitish or yellowish membranes associated with first and second dorsal-fin spines; longest dorsal-fin spine on third5
- 4b Blackish membranes associated with first and second dorsal-fin spines; longest dorsal-fin spine on fourth6

- 5a Ratio of third and second dorsal-fin spine length in SL more than 2.0 (include 2.0) *R. excelsa*
 5b Ratio of third and second dorsal-fin spine length in SL less than 2.0 *R. uejoi*
 6a First band under eye equal to eye diameter *R. haraguchiae*
 6b First band under eye narrower than eye diameter 7
 7a Second body band broad, whitish space between second and third bands narrow, with 3–5 pored lateral-line scales at lateral line level *R. jayakari*
 7b Second body band narrow, whitish space between second and third bands broad, with 9 or 10 pored lateral-line scales at lateral line level *R. semilunaris*

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