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Betta mahachaiensis, a new species of bubble-nesting fighting fish (Teleostei: Osphronemidae) from Samut Sakhon Province, Thailand

CHANON KOWASUPAT[1], BHINYO PANIJPAN[2*], PINTIP RUENWONGSA[2] & NAMKANG SRIWATTANAROTHAI[1]

[1]*Institute for Innovative Learning, Mahidol University, 999 Phuttamonthon 4 Road, Salaya, Nakhon Pathom 73170, Thailand*
E-mail: chanonnon@hotmail.com, namkang.sri@mahidol.ac.th,
[2]*Faculty of Science, Mahidol University, 272 Rama 6 Road, Phayathai, Bangkok 10400, Thailand*
Email: bhinyo.pan@mahidol.ac.th, pintip.rue@mahidol.ac.th
** Author to whom correspondence should be addressed; email: bhinyo.pan@mahidol.ac.th*

Abstract

Betta mahachaiensis, a new species of fighting fish belonging to the *Betta splendens* group, is described. The fish inhabits specifically sites with brackish water and nipa palms in the Samut Sakhon Province, Thailand. It is distinguishable from other members of the *B. splendens* group in having an iridescent green/bluish-green appearance on the brown-to-black body background. The opercular membrane has brown-to-black colour without red streaks or patches. The opercle has two parallel iridescent green or bluish-green vertical bars. Its dorsal, caudal, and anal fin rays are brown to black, contrasting with the iridescent green or bluish-green of the interradial membrane. Black transverse bars are present on at least the proximal two-thirds of the dorsal fin, but not on the caudal fin. The colour of the pelvic fins is brown to black with an iridescent green/bluish-green front margin and a white tip. Females possess similar characters but are less colourful than males.

Key words: *Betta mahachaiensis,* Bubble-nesting fighting fish, Thailand

Introduction

Many people familiar with the fighting fishes of the genus *Betta* recognize members of the four well known wild-type bubble-nesters in the *B. splendens* group: *B. splendens* Regan, 1910, *B. smaragdina* Ladiges, 1972, *B. imbellis* Ladiges, 1975, and *B. stiktos* Tan & Ng, 2005, as distinct species by their distinguishing external features such as iridescence and colour of the operculum, body coloration, bars and patterns on the fins, and colour and iridescence of body scales (Witte & Schmidt, 1992; Tan & Ng, 2005). The species group used herein refers to an assemblage of species sharing a set of diagnostic characters (Tan & Ng, 2005). Regional distribution in thirty-three provinces of Thailand of the wild bubble-nesting bettas, classified according to the above general appearance and morphological measurements, has been reported (Lertpanich, 2007; Lertpanich & Aranyavalai, 2007; Lertpanich & Aranyavalai, 2010). An article on RAPD (randomly amplified polymorphic DNA) identification of a limited number of fish and their locations was also published by Tanpitayacoop & Na-Nakorn (2005). To obtain a more up-to-date and comprehensive understanding of the fish's natural distribution, we began a more extensive countrywide survey of these fishes in 2007 (Monvises *et al*., 2009; Sriwattanarothai *et al.*, 2010). We focused particularly on an iridescent green/bluish-green betta fighting fish, *B.* sp. Mahachai. It has become the centre of our interest and also of ichthyologists and breeders because of its attractiveness and dwindling habitats (Panitvong, 2002; Lertpanich, 2007; Somadee & Kühne, 2012). The habitats of this fish are generally the brackish waters west of Bangkok, where some parts have nipa palms (*Nypa fruticans*) whose bracts hold phytotelmata that may host the parental pair and their bubble nest. The type locality of the new species is near the Maha Chai subdistrict of Samut Sakhon province, west of Bangkok. Because of human activities (e.g., industrial pollution and incursion by housing developments), this fish's biotopes have diminished and become less habitable. In fact, it is a likely candidate for extinction, considering the small area to which the species is now restricted (Vidthayanon, 2005; Griffin, 2005).

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The possibility of the Maha Chai fish being a distinct species was investigated by both morphology and the barcoding of mitochondrial (mt) DNA of the cytochrome *c* oxidase subunit 1 (COI) gene coupled with the 16S RNA gene. *Betta* sp. Mahachai was thus demonstrated to be a new species of the *B. splendens* group (Sriwattanarothai *et al*., 2010), so is *B. stiktos* (in preparation). In our recent studies, *B.* sp. Mahachai specimens were additionally collected from their natural habitats, and also acquired from a market and a fighting fish gambling den. The wild-caught ones were distinguishable as a distinct species. Hybrids between *B.* sp. Mahachai and *B. splendens* were found in some specimens bought from the market. The aim of this paper is to formally describe *B.* sp. Mahachai based on morphometric and meristic data, as well as other distinguishing external characters.

Materials and methods

Fighting fish specimens were preserved in 70% ethanol for long-term storage. The left side of all specimens was used for morphometric measurements point to point using digital Vernier caliper readings to the nearest 0.1 mm according to the method of Schindler and Schmidt (2006) and Tan & Ng (2005), both modified from Witte & Schmidt (1992). Ratios are given in percent standard length or percent head length. The terminology and general format of description is based on Witte & Schmidt (1992), Tan & Kottelat (1998), and Tan & Ng (2005). Meristic counts were carried out as described by Witte and Schmidt (1992) except for the number of predorsal scales, which were counted continuously following Ng & Kottelat (1994). The phylogenetic species concept is used in this study (Cracraft, 1989; see Tan & Ng, 2005).

All statistical analyses were performed using SPSS Version 20. To avoid undesirable effects of using ratios in statistical analyses, the logarithms of these ratios (LGRAT) were used (Atchley & Anderson, 1978). The LGRATs of each trait of each species were tested for normality using the Shapiro-Wilk test. For nonnormal traits, the Kruskal-Wallis one-way ANOVA together with Mann-Whitney U test were used to compare means. Otherwise, the Levene's test was used to test for homogeneity of variances. If the variances were equal, the one-way ANOVA together with the Tukey post-hoc test were used to compare means. Conversely, the Welch one-way ANOVA and the Games-Howell post-hoc test were used. Significant level was adjusted by the Bonferroni correction.

Specimens examined have been deposited at the Thailand Natural History Museum, Pathum Thani (THNHM) and also kept in the authors' own collection for the Thailand Betta Project (TBP). Non-type specimens will also be deposited at one or more specialized museums outside Thailand. Abbreviations used are SL—standard length, HL—head length, and TL—total length.

Betta mahachaiensis, new species

Pla Kad Pa Mahachai
(Figures 1–6)

Holotype: THNHM-F-01630 (37.41 mm SL), Thailand, Samut Sakhon province, Muang district, Tha Sai subdistrict, behind the Pattarachai food factory (13° 34' N, 100° 15' E); coll. C. Kowasupat *et al.*, 30 Apr. 2011.

Paratypes: THNHM-F-01617, 12 ex., same locality as holotype; coll. N. Sriwattanarothai *et al.*, 10 Nov. 2008.—THNHM-F-01622, 2 ex., same locality as holotype; coll. C. Kowasupat *et al.*, 17 Dec. 2009.—THNHM-F-01623, 3 ex., same locality as holotype; coll. C. Kowasupat *et al.*, 30 Apr. 2011.—THNHM-F-01821, 3 ex., same locality as holotype; coll. C. Kowasupat *et al.*, 2 Sep. 2012.—THNHM-F-01616, 1 ex., Thailand, Samut Sakhon province, Muang district, Tha Sai subdistrict, Tong Tham Mikaram temple (13° 34' N, 100° 15' E); coll. P. Suadech *et al.*, 10 Nov. 2008.—THNHM-F-01822, 3 ex., Thailand, Samut Sakhon province, Muang district, Tha Sai subdistrict, beside the Thai Prasit Textile factory (13° 34' N, 100° 16' E); coll. C. Kowasupat, 2 Sep. 2012.—THNHM-F-01632, 5 ex., Thailand, Samut Sakhon province, Muang district, Tha Sai subdistrict, inside Mahachai Villa housing estate (13° 34' N, 100° 15' E); coll. C. Kowasupat *et al.*, 30 Apr. 2011.—THNHM-F-01633, 3 ex., Thailand, Samut Sakhon province, Muang district, Tha Sai subdistrict, across the canal from Mahachai Villa housing estate (13° 34' N, 100° 15' E); coll. T. Jeenthong *et al.*, 30 Apr. 2011.—THNHM-F-01621, 1 ex., Thailand, Samut Sakhon province, Muang district, Chai Mongkhon subdistrict, the road to Nuam Ka Non temple (13° 33' N, 100° 11' E); coll. B. Panijpan et al., 17 Dec. 2009.—THNHM-F-01661, 1 ex., Thailand, Samut

Sakhon province, Muang district, Chai Mongkhon subdistrict, Nuam Ka Non temple (13° 33' N, 100° 11' E); coll. T. Jeenthong *et al.*, 16 May. 2011.—THNHM-F-01662, 1 ex., Thailand, Samut Sakhon province, Muang district, Chai Mongkhon subdistrict, Nuam Ka Non temple (13° 33' N, 100° 11' E); coll. S. Petchjeed, 16 May. 2011.—THNHM-F-01658, 3 ex., Thailand, Samut Sakhon province, Muang district, Chai Mongkhon subdistrict, Bang Koi temple (13° 31' N, 100° 10' E); coll. B. Panijpan *et al.*, 16 May. 2011.—THNHM-F-01659, 2 ex., Thailand, Samut Sakhon province, Muang district, Chai Mongkhon subdistrict, Pak Bo temple (13° 33' N, 100° 11' E); coll. B. Panijpan *et al.*, 16 May. 2011.—THNHM-F-01660, 2 ex., Thailand, Samut Sakhon province, Muang district, Chai Mongkhon subdistrict, Nuam Ka Non temple (13° 33' N, 100° 11' E); coll. P. Ruenwongsa *et al.*, 16 May. 2011.—THNHM-F-01657, 5 ex., Thailand, Samut Sakhon province, Muang district, Ban Bo subdistrict, 100 m from Ta Satit Toonklao bridge (13° 31' N, 100° 11' E); coll. C. Kowasupat *et al.*, 29 May. 2011.—THNHM-F-01655, 3 ex., Thailand, Samut Sakhon province, Muang district, Bang Krachao subdistrict, Wisut Taram temple (13° 31' N, 100° 12' E); coll. C. Kowasupat *et al.*, 29 May. 2011.—THNHM-F-01656, 5 ex., Thailand, Samut Sakhon province, Muang district, Bang Krachao subdistrict, Sri Wanaram temple (13° 30' N, 100° 31' E); coll. C. Kowasupat *et al.*, 29 May. 2011.—THNHM-F-01611, 17 ex., Thailand, Samut Sakhon province, Muang district, Maha Chai subdistrict, Area 1 (13° 33' N, 100° 16' E); coll. P. Suadech *et al.*, 7 Aug. 2008.—THNHM-F-01612, 2 ex., Thailand, Samut Sakhon province, Muang district, Maha Chai subdistrict, Area 2 (13° 33' N, 100° 16' E); coll. P. Suadech *et al.*, 25 Jul. 2008.—THNHM-F-01634, 4 ex., Thailand, Samut Sakhon province, Muang district, Khok Kham subdistrict, along the railway opposite the Kaem Ling project (13° 33' N, 100° 18' E); coll. C. Kowasupat *et al.*, 30 Apr. 2011.—THNHM-F-01629, 2 ex., Thailand, Samut Sakhon province, Muang district, Khok Kham subdistrict, near Sophon Naram temple (13° 34' N, 100° 18' E); coll. T. Jeenthong *et al.*, 30 Apr. 2011.—THNHM-F-01635, 4 ex., Thailand, Samut Sakhon province, Muang district, Khok Kham subdistrict, Sophon Naram temple (13° 33' N, 100° 18' E); coll. S. Kruttha & B. Puangmalai, 30 Apr. 2011.—THNHM-F-01652, 2 ex., Thailand, Samut Sakhon province, Muang district, Khok Kham subdistrict, Khom village (13° 34' N, 100° 18' E); coll. S. Petchjeed, 1 May. 2011.—THNHM-F-01653, 1 ex., Thailand, Samut Sakhon province, Muang district, Khok Kham subdistrict, Ban Khom school (13° 33' N, 100° 18' E); coll. S. Petchjeed, 1 May. 2011.—THNHM-F-01615, 1 ex., Thailand, Samut Sakhon province, Muang district, Na Di subdistrict, Ba Lee (13° 35' N, 100° 17' E); coll. S. Suadech *et al.*, 10 Nov. 2008.—THNHM-F-01623, 1 ex., Thailand, Samut Sakhon province, Muang district, Na Di subdistrict, behind Index factory (13° 34' N, 100° 17' E); coll. A. Rungreungwuttikul *et al.*, 26 Feb. 2011.—THNHM-F-01624, 1 ex., Thailand, Samut Sakhon province, Muang district, Na Di subdistrict, behind Index factory (13° 34' N, 100° 17' E); coll. V. Pinkaew, 5 Mar. 2011.—THNHM-F-01666, 1 ex., Thailand, Samut Sakhon province, Muang district, Na Di subdistrict, 500 m from Makham Wan restaurant (13° 35' N, 100° 17' E); coll. S. Petchjeed, 26 May. 2011.—THNHM-F-01626, 1 ex., Thailand, Samut Sakhon province, Muang district, Ban Ko subdistrict, Bang Pla (13° 34' N, 100° 13' E); coll. A. Rungreungwuttikul *et al.*, 12 Dec. 2010.—THNHM-F-01628, 3 ex., Thailand, Samut Sakhon province, Muang district, Khok Krabue subdistrict, beside SL plastic factory (13° 34' N, 100° 20' E); coll. C. Kowasupat *et al.*, 30 Apr. 2011.—THNHM-F-01627, 1 ex., Thailand, Samut Sakhon province, Muang district, Phan Thai Norasing subdistrict, about 300 m from Phan Thai Norasing shrine (13° 31' N, 100° 22' E); coll. A. Rungreungwuttikul *et al.*, 12 Dec. 2010.—THNHM-F-01625, 1 ex., Thailand, Samut Sakhon province, Ban Phaeo district (13° 35' N, 100° 6' E); coll. A. Rungreungwuttikul *et al.*, 14 Nov. 2010.—THNHM-F-01667, 2 ex., Thailand, Samut Sakhon province, Ban Phaeo district (13° 35' N, 100° 6' E); coll. P. Ruenwongsa *et al.*, 2 Sep. 2011.—THNHM-F-01664, 5 ex., Thailand, Bangkok province, Bang Khun Thian district, Samae Dam subdistrict (13° 36' N, 100° 24' E); coll. S. Kruttha *et al.*, 14 May. 2011.—THNHM-F-01665, 1 ex., Thailand, Bangkok province, Bang Khun Thian district, Samae Dam subdistrict, Bang Kradi 43 (13° 33' N, 100° 11' E); coll. C. Sibsam, 1 Jun. 2010.—THNHM-F-01666, 1 ex., Thailand, Bangkok province, Bang Khun Thian district, Samae Dam subdistrict, Bang Kradi 43 (13° 33' N, 100° 11' E); coll. P. Ruenwongsa *et al.*, 29 May. 2011.

Diagnosis: *Betta mahachaiensis* is distinguishable from other congeners in the *B. splendens* group by the combination of the following characters: an iridescent green to bluish-green appearance on the brown-to-black body background; two parallel iridescent green to bluish-green vertical bars on opercle; brown-to-black opercular membrane without red streaks or patches; dorsal, caudal, and anal fin rays brown to black contrasting with iridescent green or bluish-green interradial membranes; dorsal fin with at least its proximal two-thirds having transverse black bars; absence of dark transverse bars on caudal fin, small black rounded marks flanking the internal rays on interradial membranes; pelvic fin brown to black with iridescent green/bluish-green front margin and white tip; head length 24.9–31.2% SL; postdorsal length 17.1–25.2% SL; length of dorsal fin base 12.2–19.3% SL; pelvic fin length 21.2–49.6%; pectoral fin length 15.4–21.3% SL; orbit diameter 22.8–29.7% SL.

FIGURE 1. *Betta mahachaiensis*, THNHM-F-01630, male, holotype, 37.4 mm SL, GenBank Accession Numbers: JQ818561 (COI), JQ818652 (ITS1).

TABLE 1. Morphometric data of *Betta mahachaiensis*, *Betta splendens*, and *Betta smaragdina*.

SPECIES	***Betta mahachaiensis***				***Betta splendens***			***Betta smaragdina***		
	Holotype	**Paratype**								
Number of specimens	1	40			40			40		
		Min.–Max.	**Mean**	**S.D. (±)**	**Min.–Max.**	**Mean**	**S.D. (±)**	**Min.–Max.**	**Mean**	**S.D. (±)**
Standard length (mm)	37.4	29.7–43.7	34.9	3.3	19.9–35.0	28.0	4.2	24.3–36.3	22.0	2.5
(%SL)										
Total length*	132.3	123.7–144.6	134.5	4.5	123.8–139.1	132.2	3.8	128.4–149.5	134.5	4.4
Predorsal length	64.2	59.1–68.2	63.1	2.2	57.1–68.2	62.9	2.4	58.1–67.2	62.6	2.1
Postdorsal length	20.5	17.1–25.2	22.0[b]	1.9	19.1–27.2	22.6	2.1	20.2–27.6	23.5[b]	2.0
Preanal length	40.4	29.6–44.5	37.7	3.7	32.1–44.5	37.4	2.8	34.2–42.0	38.8	2.0
Body depth	27.3	21.6–31.4	25.9[b]	2.3	22.9–31.3	26.6	2.0	24.5–30.8	27.9[b]	1.6
Caudal peduncle depth*	15.8	14.9–20.2	17.1[b]	1.1	13.9–21.3	17.2	1.7	16.0–20.4	18.2[b]	1.1
Dorsal fin base length	16.0	12.2–19.3	15.8[b]	1.7	12.1–19.3	15.8	1.7	14.6–19.9	17.1[b]	1.3
Anal fin base length	63.2	56.1–69.8	63.4	3.1	53.3–70.1	61.9	3.9	56.2–70..8	62.2	3.6
Pectoral fin length	17.8	15.4–21.3	18.5[b]	1.5	15.3–24.4	19.4	2.0	17.3–24.6	20.8[b]	1.9
Pelvic fin length	31.6	21.2–49.6	35.4[b]	5.9	21.2–55.0	35.5	8.0	28.3–57.2	41.3[b]	7.0
Head length	28.8	24.9–31.2	28.0[a,b]	1.5	27.7–33.1	30.4[a]	1.4	26.6–32.2	29.6[b]	1.2
(%HL)										
Snout length	21.9	19.7–30.2	24.1	2.3	18.1–28.6	23.1	2.3	18.5–28.3	22.5	2.3
Orbit diameter	27.0	22.8–29.7	26.2[b]	1.8	23.9–28.9	26.5	1.4	23.0–31.4	28.0[b]	2.0
Interorbital length*	10.1	8.0–15.1	11.4	1.8	8.6–13.7	10.6	1.5	8.9–14.7	12.0	1.4
Postorbital length	52.8	46.0–59.5	52.5[a]	2.9	46.1–55.3	50.4[a]	2.3	46.4–56.1	51.3	2.6

* Nonnormal distribution by using the Shapiro-Wilk test for normality (α=0.05)

[a] *B. mahachaiensis* is significantly different from *B. splendens* (α=0.05, Bonferroni-adjusted)

[b] *B. mahachaiensis* is significantly different from *B. smaragdina* (α=0.05, Bonferroni-adjusted)

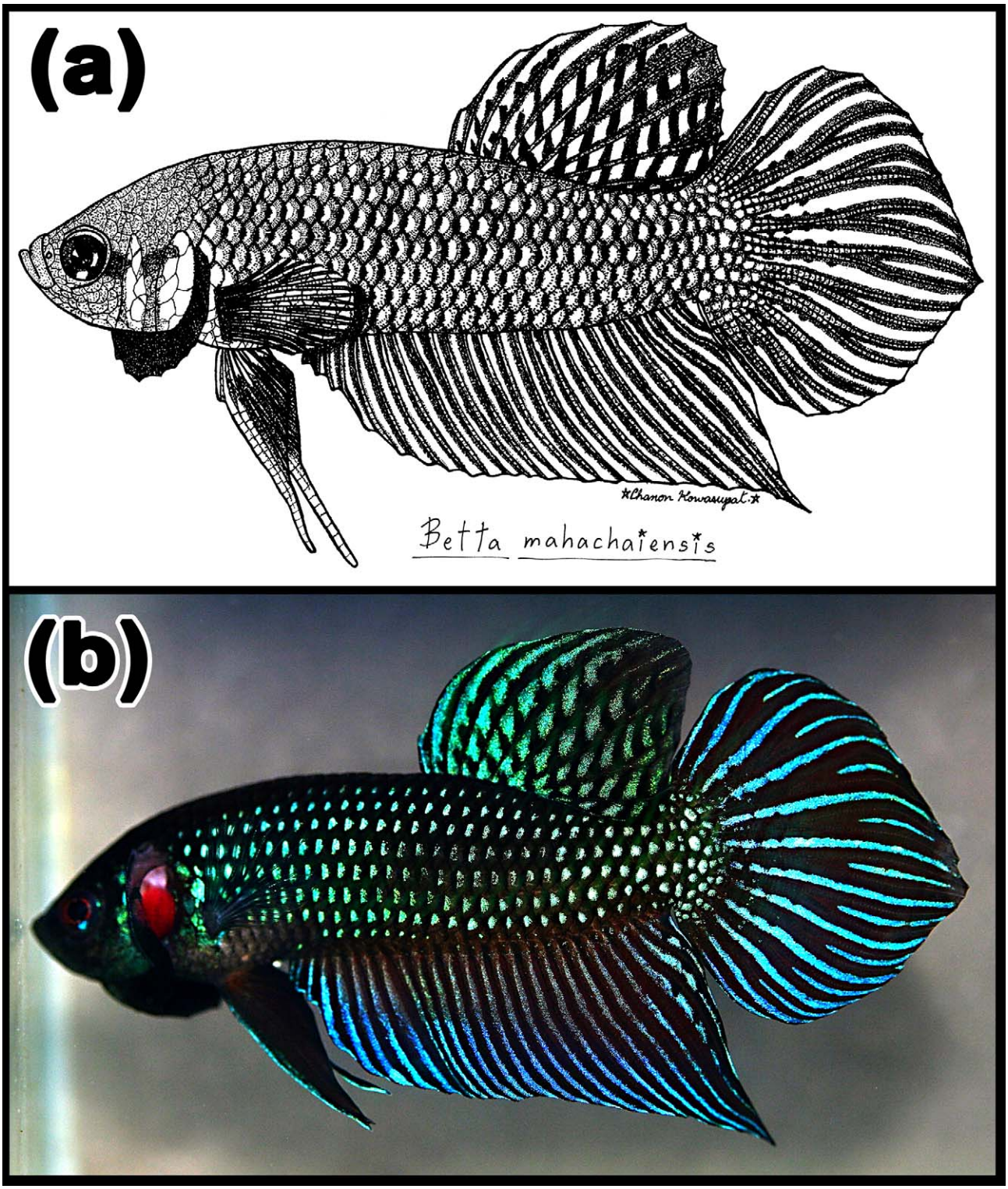

FIGURE 2. *Betta mahachaiensis*: (a) schematic drawing of a specimen, an adult male, paratype, THNHM-F-01622, 35.2 mm SL; (b) photograph of live adult male displaying aggression, paratype, THNHM-F-01623, 36.7 mm SL (note that gill covers are open, displaying the red gills, which are not the red bars on the opercle).

Description: General body shape and appearance illustrated in Figs. 2–3. Head small, short (head length 24.9–31.2 %SL), eyes large (orbital diameter 22.8–29.7 %SL); adult males with convex hump at posterior of head, immediately behind eye; body slender (body depth 21.6–31.4 %SL); opercles parallel when head viewed dorsally; dorsal fin positioned after mid-body (predorsal length 59.1–68.2 %SL); caudal fin posterior margin rounded to medially pointed; dorsal fin distal margin rounded; pelvic fins falcate; anal fin pointed. Meristic and morphometric data are summarized in Tables 1 and 2. Maximum size: 43.7 mm SL (THNHM-F-01623).

Coloration: See Fig. 2 for coloration in life. Male with dark-brown to black head, with iridescent green to bluish-green scales. Opercles with two parallel iridescent green to bluish-green vertical bars, opercular membrane brown to black, eyes with iridescent green to bluish-green patches at bottom and posterior regions. Body dark brown to black with iridescent green to bluish-green scales arranged in regular rows, with a similar iridescent area on each scale. Dorsal, caudal, and anal fin rays brown to black, contrasting with iridescent green to bluish-green interradial membranes; dorsal fin with at least proximal two-thirds having black transverse bars; small black rounded marks flanking internal rays on interradial membranes in most specimens (60 out of total 65); pelvic fin brown to black, with iridescent green to bluish-green front margin, white tip; pectoral fins hyaline. Female less colourful (see Fig. 3): head and body yellowish to light-brown with iridescent green to bluish-green scales; opercles pale gold with two faint iridescent green to bluish-green bars; throat with 'chin-bar'. Body with distinct upper central stripe nearly parallel to central stripe, at level of eye; caudal-peduncle black spot present. Dorsal, caudal, anal, and pelvic fin rays yellowish on faint iridescent green to bluish-green interradial membranes. Other characters similar to male.

TABLE 2. Meristic data of *Betta mahachaiensis*, *Betta splendens*, and *Betta smaragdina*.

SPECIES	***Betta mahachaiensis***			***Betta splendens***		***Betta smaragdina***	
	Holotype	**Paratype**					
Number of specimens	1	40		40		40	
		Min.–Max.	**Mode**	**Min.–Max.**	**Mode**	**Min.–Max.**	**Mode**
Subdorsal scales	6	5–7	6	5–7	6	5–8	6
Transverse scales	9	8–9	8	8–9	9	8–9	9
Lateral scales	30	26.5–32	30	28.5–32	30	29–33	31
Predorsal scales	24	20–27	25	20–27	24	20–26	23
Postdorsal scales	10	8–11	10	8–12	10	7–13	9
Lateral scales below dorsal–fin origin	16	14–17	15	14–17	16	14–16	15
Lateral scales below anal–fin origin	6	5–8	7	4–7	6	5–7.5	6
Anal fin rays (spines)	4	3–4	4	3–5	4	3–5	4
Anal fin rays (articulated)	23	22–26	23	22–26	23	22–27	25
Dorsal fin rays (spines)	1	0–2	1	1–2	1	1–2	1
Dorsal fin rays (articulated)	9	8–11	9	7–10	9	8–10	9
Pectoral fin rays	12	12–13	13	11–14	13	11–15	13

Comparative notes: *Betta mahachaiensis* is distinguishable from other congeners in *B. splendens* group by having the opercle with two parallel iridescent green to bluish-green vertical bars (vs. parallel red vertical bars in *B. splendens* and green iridescent scales in *B. smaragdina* and *B. stiktos*) (see Fig. 4); brown-to-black opercular membrane without red streaks or patches (vs. presence of red streaks in *B. splendens*, *B. imbellis*, *B. smaragdina*, and *B. stiktos*); caudal fin rays brown to black, contrasting with iridescent green to bluish-green interradial membranes (vs. presence of distal red crescent with a thin black edge in *B. splendens* and *B. imbellis*); absence of dark transverse bars on caudal fin (vs. presence in *B. stiktos*); anal fin rays brown to black with iridescent green to bluish-green on interradial membranes (vs. distal half of posterior anal fin rays red with a small red patch at distal tip in *B. splendens* and *B. imbellis*); pelvic fin brown to black with iridescent green to bluish-green front margin and white tip (vs. black and red from proximal pelvic fin base to the white tip in *B. splendens* and *B. imbellis*); smaller head length than *B. splendens* and *B. smaragdina* (24.9–31.2% SL vs. 26.6–33.1); smaller postdorsal length than *B. splendens* and *B. smaragdina* (17.1–25.2% SL vs. 19.1–27.6); smaller length of dorsal fin base than *B. smaragdina* (12.2–19.3% SL vs. 14.6–19.9); smaller pelvic fin length than *B. smaragdina* (21.2–49.6% SL vs. 28.3–25.2); smaller pectoral fin length than *B. smaragdina* (15.4–21.3% SL vs. 15.3–24.4); smaller orbit diameter than *B. smaragdina* (22.8–29.7% SL vs. 23.0–31.4).

FIGURE 3. *Betta mahachaiensis*: (a) live male (acclimatized), paratype, THNHM-F-01658, 35.5 mm SL; (b) live female (acclimatized), paratype, THNHM-F-01554, 27.1 mm SL.

Distribution: Based on our survey of fighting fish during 2007–2012, the wild *B. mahachaiensis* was found only in two provinces in Thailand: 28 catch sites in Samut Sakhon Province and 2 sites in western Bangkok bordering Samut Sakhon Province (Fig. 5). The catch sites were in Samut Sakhon Province's Muang district encompassing Mahachai and other subdistricts, viz. Tha Sai, Na Di, Khok Kham, and nearby districts including Ban Phaeo. We have not been able to collect any wild *B. mahachaiensis* in Samut Prakan and Samut Songkhram, as cited in earlier reports (see Monvises *et al.*, 2009). Nevertheless people in the villages of this area claim to have caught specimens in Samut Songkhram Province adjacent to Samut Sakhon.

Reproductive behaviour: During spawning, the females, while upside-down or nearly so, have been observed to cradle some of the eggs emerging from the vent of the ovipositor with the pelvic and pectoral fins before the

falling eggs are picked up by the mate. As with other members of the *B. splendens* group, the female fish also help in picking up eggs and placing them in the bubble-nest.

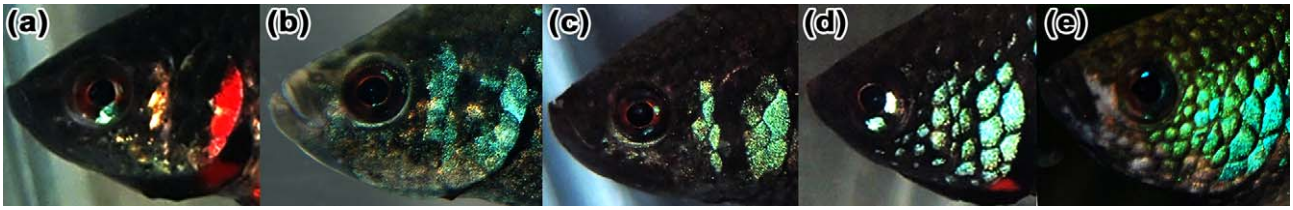

FIGURE 4. Close-ups of heads & opercle patterns of live males: (a) *Betta splendens*, THNHM-F-01703; (b) *Betta imbellis*, THNHM-F-01560; (c) *Betta mahachaiensis*, THNHM-F-01632; (d) *Betta smaragdina*, THNHM-F-01778; (e) *Betta stiktos*, THNHM-F-01824.

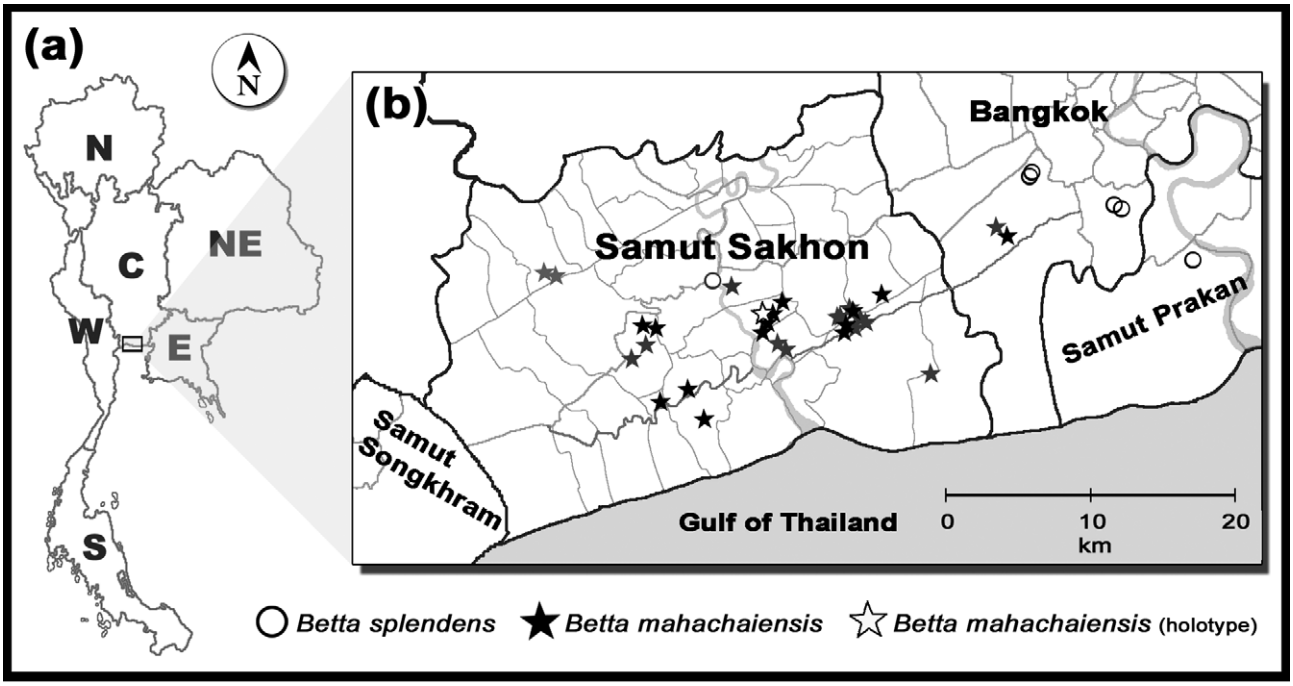

FIGURE 5. (a) Map of Thailand showing *Betta mahachaiensis* catch sites (encompassed by the small rectangle in the middle) in the central part of Thailand (C-Central, N-North, S-South, W-West, E-East, and NE-Northeast); (b) Catch (stars) of *Betta mahachaiensis* between years 2007 and 2012, twenty-eight sites in Samut Sakhon province and two sites in west of Bangkok.

Field Notes: *Betta mahachaiensis* lives in brackish waters, some quite heavily polluted; pH at catch sites 6.87–7.80, salinity 1.1–10.6 ppt. The species was found in nipa palm (*Nypa fruticans*) swamps, especially, in the water-holding phytotelma of the palm bract, which the pair use for bubble-nest building, courtship, and hatching of fry (see Fig. 6). Some syntopic fish species, although not found in the bract's watery pocket, are *Trichopsis vittata*, *Trichopodus trichopterus*, *Anabas testudineus*, *Aplocheilus panchax*, *Oryzias javanicus*, *Dermogenys siamensis*, *Channa striata* and *Boraras urophthalmoides*.

Etymology: The name *Betta mahachaiensis* is derived from its common Thai name for a subdistrict of Samut Sakhon Province, viz. Maha Chai, the first publicised collecting site.

Remarks: *B. mahachaiensis* has been found only in Thailand, while other members of *B. splendens* group occur also in three neigbouring countries, namely Laos, Cambodia, and Malaysia. Additional support for *B. mahachaiensis* as a distinct species comes from our nuclear DNA sequencing of wild-caught and tank-bred specimens. Our previous results from mitochondrial DNA barcoding of cytochrome c oxidase I (COI) (gene bank accession no. GQ911877-904) and 16S rRNA (GQ912150-85) (*Sriwattanarothai et al.* 2010) additional COI barcoding sequence (JQ818643-64, JQ818675-90, and JQ818693) and nuclear gene (internal transcribed spacer 1 or ITS1) (JQ818556-63 and JQ818568-74) support the taxonomic status of *B. mahachaiensis* as an independent lineage. The Kimura 2-parameter (K2P) distances estimated from COI between *B. mahachaiensis* and *B. splendens*

is 0.110 (about 11%) and that between *B. mahachaiensis* and *B. smaragdina* is 0.147 (about 14%) (in preparation). The accession numbers (www.ncbi.nlm.nih.gov) of the holotype *B. mahachaiensis* are JQ818652 (COI) and JQ818561 (ITS1).

One fish breeder (Suwit Suadech) of long standing has bred *B. mahachaiensis* for more than 10 years and many more generations and this fish have always bred true, i.e., no occasional morphologically *B. splendens*-like fish or *B. smaragdina*-like ones have ever emerged. Our DNA barcoding results of all *B. mahachaiensis* from his farm are unique to the *B. mahachaiensis* with none having COI barcoding characteristics of *B. splendens* or other fish in the *B. splendens* group. We are confident therefore, that *B. mahachaiensis* is neither of hybrid origin nor a cultivated form. On the contrary, some fish bought from the Chatuchak market have been found to be hybrids with DNA barcoding characters of *B. splendens* despite their general appearance being very much like that of *B. mahachaiensis*.

The new DNA sequences of *B. splendens*, *B. imbellis*, and *B. smaragdina* cited above not included in Sriwatanarothai *et al*. 2010 are: JQ818724-8, JQ818784-5, JQ818797-806 for COI gene of *B. splendens*; JQ818637-40, JQ818608-9 for ITS1 gene of *B. splendens*; JQ818776-81, JQ818783, JQ818786-96 for COI gene of *B. imbellis*; JQ818594-605, JQ818607, JQ818610-19 for ITS 1 gene of *B. imbellis*; JQ818744-9, JQ818753-75 for COI gene of *B. smaragdina*; and JQ818581-3, JQ818588-93 for ITS1 gene of *B. smaragdina.*

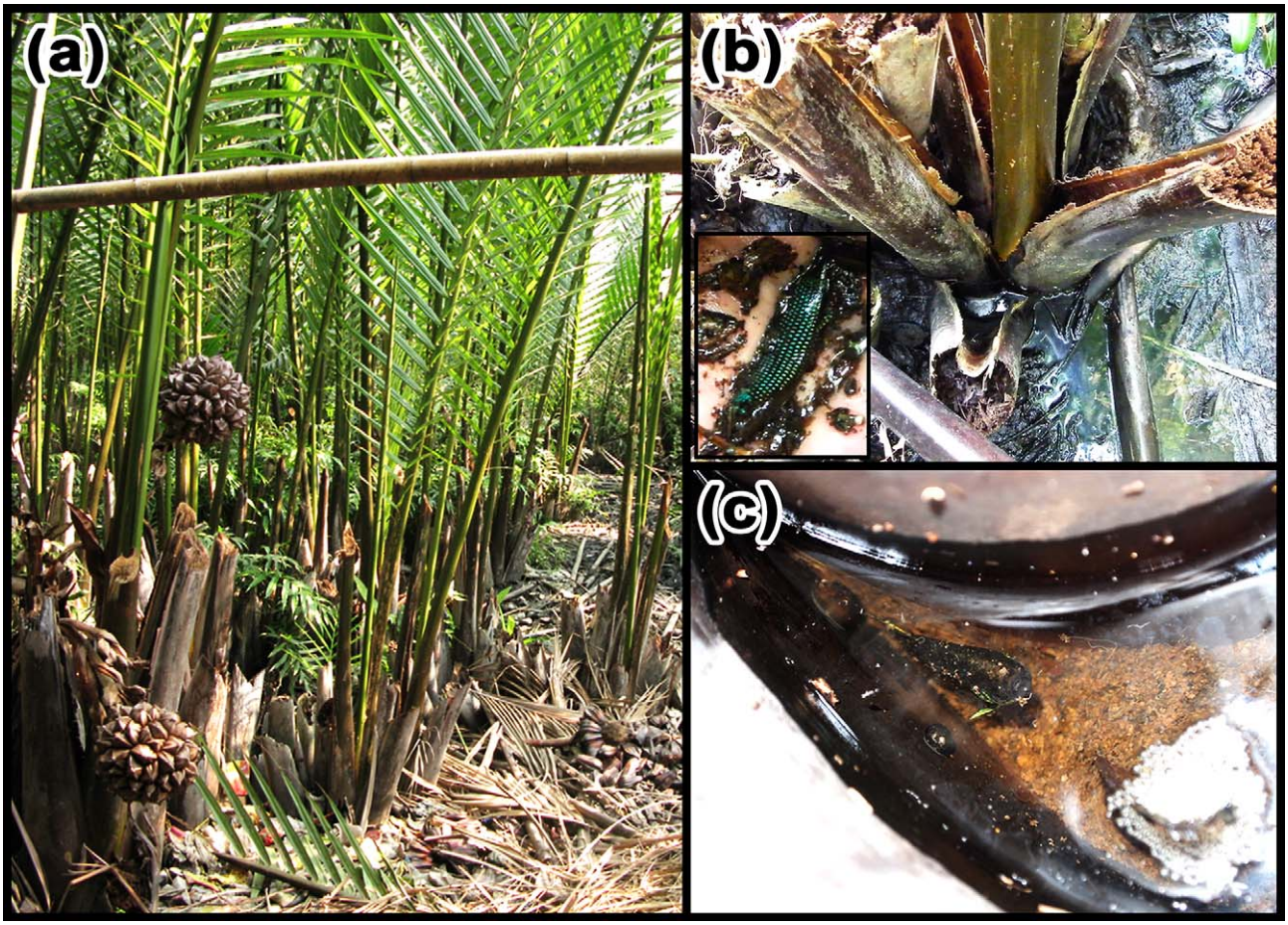

FIGURE 6. Habitat of *Betta mahachaiensis* in Samut Sakhon province, Thailand: (a) Nipa palm forest behind a food factory (Type Locality) (30 Apr. 2011); (b) Water held in the pocket phytotelma of the nipa palm bract as spawning space and the fish (THNHM-F-01632) caught from it (30 Apr. 2011); (c) The fish (THNHM-F-01655) and bubble nest in a pocket of a nipa palm bract (29 May 2011).

Discussion

Although the new species shares common characters with other members of the *B. splendens* group, it is easily distinguished from congeners. *Betta mahachaiensis* has an iridescent green to bluish-green appearance on the

brown-to-black body background together with two parallel iridescent green to bluish-green vertical bars on the opercle. Other distinct characters are also mentioned above. These data arise from close examination of about two hundred specimens from 30 catch sites in Samut Sakhon and Bangkok. That *Betta mahachaiensis* is a new species is further supported by evidence from DNA barcoding of mitochondrial cytochrome c oxidase I (COI) and sequencing results from mitochondrial 16S rRNA (Sriwattanarothai *et al.*, 2010). The relative position of *Betta mahachaiensis* on the phylogenetic tree is a distinct branch closely related (sister clade) to that of *B. splendens*, well separated from other members of the *B. splendens* group. As supporting evidence, the mitochondrial COI can help discriminate between most closely related species. In fact, according to proponents of the procedure, valid species-level diagnosis can be routinely done using COI analysis (Hebert *et al.*, 2003; Ratnasingham & Hebert, 2007). Incidentally, next generation sequencing with high potential for generating large volumes of genomic data (at a reasonable cost) may eventually supplant COI barcoding as a tool for species identification (Taylor & Harris, 2012).

We recognize that there are reported uncertainties about using mitochondrial COI as the sole tool in identification of animal species (Dasmahapatra *et al.*, 2010; Krishnamurthy & Francis, 2012). But it should be mentioned here that our preliminary work using the nuclear ITS1 (internal transcribed spacer 1) is also consistent with the COI cladogram showing the relationships between *B. mahachaiensis* and other members of *B. splendens* group (in preparation). There are researchers who maintain that both morphology and molecular phylogeny are essential for taxonomy, i.e., neither of them provides infallible proof of relationship: indeed they are complementary (Hillis & Wiens, 2000). Tan & Ng (2005) commented that it is difficult to use meristic characteristics and morphological data to differentiate between species within the same group due to rather conservative characteristics (see also Tan & Ng, 1996; Tan & Tan, 1996). Nevertheless, our meristic and morphometric data in Tables 1 and 2 show that some of these values of *B. mahachaiensis* are sufficiently different to distinguish the fish from other members of *B. splendens* group. In fact these data revealed that *B. mahachaiensis* is more distinct from *B. smaragdina* than from *B. splendens*. This is consistent with our results on phylogenetic tree (Sriwattanarothai *et al.*, 2010). Thus both morphological and molecular phylogenetic data support the taxonomic status of *B. mahachaiensis* as a new species.

That *B. mahachaiensis* is recognized as a new species is important for conservation. Unlike other nest-building bettas, *B. mahachaiensis* habitats are confined to a relatively small area west of Bangkok, Thailand, and nowhere else in the country nor in neighbouring countries. We found the species only in the Samut Sakhon province of Thailand and in western Bangkok, and not even in the nearby provinces with similar nipa palm groves, e.g., Samut Songkhram and Samut Prakan, after an extended and exhaustive search. In spite of its ability to tolerate and thrive in brackish and polluted waters, the habitats of *B. mahachaiensis* are being further threatened by human activities. Recent annual or even semi-annual floods in 1995, 2006, and 2011 (Ziegler *et al.*, 2012) may make matters worse. In addition to sweeping away some bettas, the floods reduced the habitat water's salinity, a possible protection for this restricted-niche fish. Fresher water may bring along large aquatic predators that may threaten the resident fishes and even newly reestablished fish after the floods. Fortunately, as of the early months of 2012, the fish is still found in some of the earlier catch sites. Because of large-scale housing and industrial developments around suburban Bangkok and landfill of salt marshes, its favoured habitats are in a very precarious position, e.g., one catch site in 2011 was partially filled three months after our previous visit. High volume orders for wild-caught *B. mahachaiensis* may diminish the population yet further. Conserving this threatened species may have to involve at least a dedicated local government agency (e.g., Department of Fisheries) tasked with rearing populations of the fish that breed true, for eventual release if and when warranted. Other agencies having environmentally conscious professional breeders may also be involved in the conservation. For papers addressing fish commercial trading and conservation see Ng & Tan (1997) and Nijman (2010).

Comparative materials: *B. splendens* caught from sixteen provinces from Central, Northern, Eastern, Southern, and Western Thailand, one province in Laos, and one province in Cambodia (THNHM-F-01673, THNHM-F-01676-8, THNHM-F-01680-1, THNHM-F-01684, THNHM-F-01710, THNHM-F-01712-3, THNHM-F-01716, THNHM-F-01718, THNHM-F-01719, THNHM-F-01691-2, THNHM-F-01696, THNHM-F-01698-704, THNHM-F-01707-8, THNHM-F-01691-2, THNHM-F-01734-5, THNHM-F-01753, THNHM-F-01788, THNHM-F-01790, THNHM-F-01820) and *B. smaragdina* from eight provinces of Northeastern Thailand (THNHM-F-01740, THNHM-F-01743, THNHM-F-01745-8, THNHM-F-01780, THNHM-F-01782-3, THNHM-F-01787-90, THNHM-F-01792-3, THNHM-F-01807, THNHM-F-01813-5, THNHM-F-01817-9) were compared for morphometric and meristic characters well as general appearance and body shape.

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