

Biodiversity of Bubble-Nest Building and Mouth-Brooding Fighting Fish Species of the Genus *Betta* in Southeast Asia

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ABSTRACT:- Wild fighting fish species belonging to the genus *Betta* inhabit fresh water bodies of Southeast Asian countries and are found in the Indochinese mainland, the islands of the Kingdom of Brunei, Indonesia and Malaysia. The best known species of the genus is *Betta splendens*, the colourful Siamese fighting fish, from which ornamental variants are bred and have proved to be a commercial success internationally. These fishes display territorial aggressiveness. Two modalities of paternal care are employed by the species, nest-building and mouth-brooding, whilst caring for fertilized eggs and newly hatched fry. Both types of fishes are found in most of these ASEAN countries mentioned above. With recent taxonomic studies, there are now 73 wild valid species in total; 18 that are bubble-nest builders and 55 mouth brooding species, a number of which have been listed as endangered. These fishes have adapted to various habitat conditions and can be found in a variety of water types. *Betta* habitats can be waters that are very still or slow running, soft, slightly brackish or even acidic. Though generally preferring the tropical climate, some can survive temperatures below 20°C. Species identities have so far been established mainly based on morphological criteria, but some have now been identified morphologically as well as genetically. Recent DNA works indicate that the validity of some species and their phylogenetic relationships should be re-examined. This article aims to make an updated review of all *Betta* species in terms of biodiversity, phylogenetic relationships and distribution in Southeast Asia.

KEY WORDS: *Betta* fighting fish, Biodiversity, DNA barcoding, Phylogenetic tree, Siamese fighting fish, Southeast Asian countries, Thailand

INTRODUCTION

The Southeast Asian region encompasses; island countries such as Brunei, Indonesia and Singapore, Malaysian eastern states (Sarawak and Sabah (on Borneo Island)) and

its western peninsular states and Indochinese countries, Cambodia, Lao PDR, Thailand and Vietnam. From the nations above there have been reports (Kottelat, 1994; Tan and Ng, 2005a, 2005b; Panijpan *et al.*, 2014) of wild native species in the genus *Betta*

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Bleeker, 1849 whose members prefer freshwater. One prominent species of the genus *Betta* is *B. splendens* Regan, 1910, the Siamese fighting fish, which hobbyists and aquarists have bred to produce variants used for fighting and ornamental purposes (Fig. 1). The ornamental variants have appealing appearances in terms of fin shapes and colour patterns and thus there is a thriving international trade of these varieties in and out of Thailand as well as elsewhere in the world (Monvises *et al.*, 2009).

The species of *Betta* are divided into two types according to the modality of paternal care for the fertilized eggs and the newly-hatched fry (Witte and Schmidt, 1992; Britz, 2001). The ‘nester’ male builds a bubble-nest shaped like a 3D convex lens into which the fertilized eggs and the fry are put. The other, the ‘brooder’ male keeps the fertilized eggs and hatchlings in its mouth ensuring the release of relatively strong fry. Observation shows, due to the presence of the almost lung-like labyrinth organ, these fish can gulp air in addition to using their gills to supply oxygen to the rest of the body enabling them to survive in waters with near-zero dissolved oxygen.

The bubble-nest builders prefer living in

shallow and still or very slow-running waters, whereas the mouth-brooders are found more predominantly in slow-running waters. But in Thailand there are places where both types cohabit (our observation).

These fishes, especially members of the *B. splendens* group, are very territorial, e.g., for food sources, potential mates and display aggressiveness, especially the males, by flaring the opercular covers and also unfurling fully the anal, caudal and dorsal fins (Simpson, 1968). Body colours also become more intense with the display, during which the flickering of the pelvic fins is also noticeable in some species members (Simpson, 1968).

Some of the better known wild nesters are *B. splendens*, *B. smaragdina* Ladiges, 1972, *B. imbellis* Ladiges, 1975, *B. mahachaiensis* Kowasupat, Panijpan, Ruenwongsa & Sriwattanarothai, 2012, *B. bellica* Sauvage, 1884, *B. coccinea* Vierke, 1979, *B. rutilans* Witte & Kottelat, 1991 and *B. simorum* Tan & Ng, 1996. As for some of the wild brooders, the following are recognized, e.g., *B. prima* Kottelat, 1994, *B. pugnax* Cantor, 1849, *B. simplex* Kottelat, 1994, *B. pi* Tan, 1998, *B. kuehnei* Schindler & Schmidt, 2008 and *B. albimarginata* Kottelat & Ng, 1994.



(a)



(b)



(c)

FIGURE 1. Variants of *Betta splendens* (a) wild *B. splendens*; (b) farm-bred fighter *B. splendens*; (c) farm-bred ornamental (double tail) *B. splendens*.

Generally, the species of *Betta* have been identified and differentiated according to external appearance, morphological measurements and meristics. Recently, many species of nesters and brooders have been identified by the combined morphological and DNA criteria. By the latter means more critical evidence has arisen that should lead to further research into species status of several wild species and their ancestral relationships (Panijpan *et al.*, 2014).

The nest builders in Thailand, Lao PDR, Cambodia and Vietnam (e.g., *B. splendens*, *B. smaragdina*, *B. siamorientalis* Kowasupat, Panijpan, Ruenwongsa & Jeenthong, 2012) are generally more colourful than the mouth brooders (for e.g., *B. prima*, *B. stigmosa* Tan & Ng, 2005). The body of the latter two appears dull brownish in colour; however, the *B. simplex* (a mouth brooder) appears quite attractive by opercular and anal fin colour. Elsewhere mouth brooders can be quite colourful, e.g., *B. macrostoma* Regan, 1910, *B. channoides* Kottelat & Ng, 1994, *B. albimarginata*.

In terms of total body length (mouth tip to caudal fin tip) the mature males of *B. livida* Ng & Kottelat, 1992 (mouth brooder, approximately 3 cm) are quite short relative to *B. splendens* (nest builder, approximately 5 cm). Generally the mouth brooders are quite long, e.g., *B. enisae* Kottelat, 1995 (approximately 10 cm), *B. pi* (approximately 12 cm), *B. unimaculata* Popta, 1905 (approximately 8 cm). But some brooders are not as long for e.g., *B. channoides* (approximately 3 cm), *B. simplex* (approximately 4 cm).

So far there have been only limited reports on species numbers and distribution of nesters and brooders in Thailand as well as elsewhere in Southeast Asia. The most recent article reviewed the species but in a limited way,

was in 2006 (Schindler and Schmidt, 2006). Here we aim to provide an updated and more comprehensive review of the biodiversity all species of *Betta*, nesters and brooders, discovered and identified in the last 30 years, and their phylogenetic relationships and distribution in Southeast Asia, their native habitats. For brevity references to earlier works cited in more recent articles have been omitted. For more detailed information about *Betta* fishes in Thailand (and some of its neighboring countries) from the group of the authors involved in this research, please refer to Panijpan *et al.*, 2014. (For the uninitiated on fish biology, conventionally the word “fish” refers to one or more piscine animals in general, whereas “fishes” is used to emphasize animals from more than one species.)

MATERIALS AND METHODS

From 2007-2014, the research group covered 71 out of the 77 provinces in Thailand (the number of Thai provinces in 2014), several times and in different seasons and found *Betta* in 66 provinces, in each of which, many sites were explored. We also caught and obtained fishes from other countries.

To facilitate locating the habitats, Figs. 2, 3 and 4 show the locations of all wild species of *Betta*, the nest builders and the mouth brooders, identified based on DNA (Kowasupat *et al.*, 2014; Panijpan *et al.*, 2014) and morphology in most Southeast Asian countries. For a more detailed Southeast Asian distribution of all the fish species and water characteristics of their habitats, readers are recommended to read the following: Tables 1a, 1b and the latest authoritative and updated book “Labyrinth Fish World” by Linke (2014) which has colour photographs of all the species mentioned here and some are yet to be identified. This article provides tables which should facilitate finding the

fish species in ASEAN countries and the locations therein. Maps of Southeast Asian countries harbouring species identified by morphological and DNA criteria are provided as well (Figs. 2, 3 and 4).

RESULTS AND DISCUSSION

Wild Bubble-Nest Builders

With their coverage of such a vast expanse of land and various environmental conditions it is to be expected that there is such a diverse speciosity in members of the genus *Betta*, see Figs. 2, 3 and 4.

Among the wild nest builders, *Betta mahachaiensis* is endemic to Thailand (Kowasupat *et al.*, 2012b). *Betta smaragdina* has been reported mainly in the northeast of Thailand (Kowasupat *et al.*, 2014). A location in the northern part of this region, Bueng Khong Long on the Mekong River has *B. smaragdina* cryptic species where the DNA sequence deviates a great deal from the type locality one. *Betta splendens* spreads widely from the north to central, east, west and upper peninsulas of Thailand (Lertpanich and Aranyavalai, 2007). Lao PDR has *B. smaragdina* and *B. splendens* as well. *Betta imbellis* is only found in the lower peninsula of Thailand and also adjacent states of Malaysia. Eastern provinces of Thailand have the *B. siamorientalis* which can also be found in Cambodia and south Vietnam (Kowasupat *et al.*, 2012a). The latter region also has *B. imbellis*. The Malaysian peninsula has *B. imbellis*, *B. bellica*, *B. coccina*, *B. livida*, *B. persephone* Schaller, 1986, *B. tussyae* Schaller, 1985, whereas eastern Malaysia (Sarawak) and Indonesia (Kalimantan part of the Borneo Island) have many nest builders (Tan and Ng, 2005a, 2005b), *B. brownorum* Witte & Schmidt, 1992, *B. rutilans*, *B. hendra* Schindler & Linke, 2013, *B. uberis* Tan & Ng, 2006. *Betta simorum* was also found in

Jambi, Sumatra Island of Indonesia (Tan and Ng, 1996).

Wild Mouth Brooders

Betta simplex is endemic to Thailand (Vidthayanon, 2005). The *Betta prima* lives in the eastern region of Thailand. The Vietnamese *B. prima*-like fish appears to be slightly different from the Thailand *B. prima* and the Cambodia fish: the latter two are identical by DNA criteria (Panijpan *et al.*, 2014). *Betta pallida* Schindler & Schmidt, 2004, *B. pugnax*, *B. ferox* Schindler & Schmidt, 2006, *B. apollon* Schindler & Schmidt, 2006 are found in the peninsulas of Thailand. *Betta pi* has been reported in the southern tip of Thailand and this fish and its group members e.g. *B. chloropharynx* (see Table 1b) with similar appearances are found in neighbouring Malaysia. Brunei and the Malaysian Sarawak state both have *B. akarensis* Regan, 1910 and *B. macrostoma* Regan, 1910. In Kalimantan, many brooders, e.g., *B. dimidiata* Roberts, 1989, *B. foerschi* Vierke, 1979, *B. pugnax*, have been reported (Tan and Ng, 2005a, 2005b; Schindler and Schmidt, 2006). Sumatra has *B. raja* Tan & Ng, 2005, *B. renata* Tan, 1998 and *B. falx* Tan & Kottelat, 1998.

Habitat Characteristics

Species populations of the genus *Betta* have evolved and adapted to fit the environment they occupy (Tables 1a, 1b). Although they generally prefer the warm climate, they are found from latitudes 8°S to 20°N, indeed *B. splendens* have been reported in northern Thailand up where sub-temperate temperatures can be reached in the winter. One aquarist has reported finding the fish in a Myanmar town bordering northern Thailand (at about 20°N) (personal communication). Most of *Betta* in Cambodia and Vietnam are found in the subtropical south.

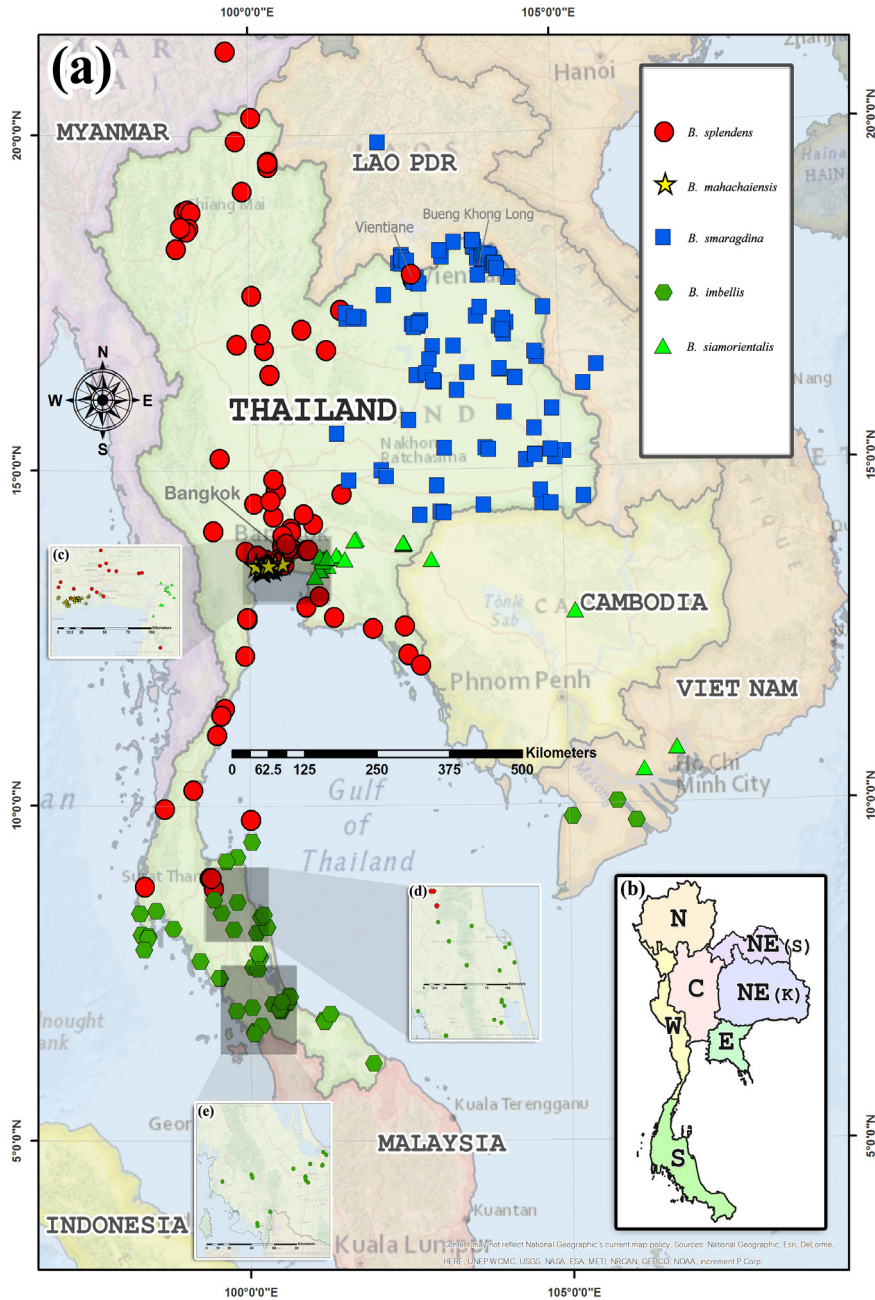


FIGURE 2. (a) Habitat locations (marked by symbols) of various bubble-nester species as identified by DNA sequences in the genus *Betta* in Cambodia, Lao PDR, Myanmar, Thailand, Vietnam: arrows pointing to Bangkok, Vientiane and Bueng Khong Long; (b) Thailand and its regional divisions: N-north, NE(S)-northeast (Sakon Nakhon basin), NE(K)-northeast (Khorat basin), C-central, E-east, W-west, S-south); (c) central and eastern, (d) and (e) southern regions magnified. The map was constructed by using the ArcGIS version 10.1.

The bubble nesters prefer shallow waters with covering vegetation to spawn and to avoid predators. Usually, the labyrinth fishes of the genus *Trichopsis* are found cohabiting with the bubble nesters in Thailand. The mouth brooders also live in shallow, slow running waters, e.g., streams running off waterfalls in Thailand with rocks and scattered leaves as hiding places. Not only do these fishes inhabit the mainland, they can be found on relatively small islands, e.g., Tarutao and Samui in southern Thailand. Unlike those nest builders in Thailand, which inhabit relatively clear near-neutral water bodies, some fish in Malaysia and Indonesia live in dark-coloured water bodies which are very acidic because of tannins and humic acid in the area, e.g., the nest builder *B. rutilans*, *B. coccina*, *B. hendra*. Among the nesters, *B. mahachaiensis* is endemic to a small provincial subdistrict (Mahachai) near Bangkok (habitat area is approximately 100 square kilometers) (Kowasupat *et al.*, 2012b) so is *B. hendra* which is endemic to a small peat swamp in central Kalimantan (Schindler and Linke, 2013). *B. mahachaiensis* (central Thailand) and *B. imbellis* (southern Thailand) can be found in brackish water with slight salinity, although both can live in normal soft water as well. Among the brooders, *B. simplex* is found in translucent (turbid) calcified, i.e. hard and neutral water of small ponds and small streams near Krabi in Thailand (Panijpan *et al.*, 2014). *Betta chini* Ng, 1993 (in Sabah) and *B. aurigans* Tan & Lim, 2004 (in Natuna Besar Island) are endemic species. Also *B. ibanorum* Tan & Ng, 2004 is found only in a peat swamp of Sarawak, east Malaysia, *B. pinguis* Tan & Kottelat, 1998 live in acidic tannin-stained water in Kapuas basin (Kalimantan, Indonesia). But there are others that live in clear water, e.g., *B. falx* and *B. cracens* Tan & Ng, 2005 in Sumatra.

Some *Betta* fish are quite widespread, e.g.,

the nest builder *B. splendens* covers a large area in Thailand. The mouth brooder *B. edithae* Vierke, 1984 is widespread in Kalimantan and Sumatra. Nest builders that live near human populations are losing habitats fast, due to industrialization, pollution, real estate development and road building, etc.

High volumes of trade of some *Betta*, e.g., *B. mahachaiensis* (nest builder) and *B. simplex* (mouth brooder), are also threatening their populations. The situation is exacerbated by the fact that both have very small habitats. Also threatened is *B. persephone* (nest builder, Malaysia), *B. miniopinna* Tan & Tan, 1994 (nest builder, Indonesia), *B. spilotogeta* Ng & Kottelat, 1994 (mouth brooder, Indonesia). In fact all three of the above, are cited in the IUCN (the International Union for Conservation of Nature) red list (www.iucnredlist.org) as critically endangered species as the *B. simplex* (Kottelat, 1996a, 1996b, 1996c; Vidthayanon, 2013). Some habitats that have the threatened species should be at least, partially preserved in situ or some of the fish should be carefully farmed as pure species to conserve the gene pool.

There are sites in Malaysia, Thailand and Vietnam where more than one species of *Betta* cohabit, nesters with nesters, brooders with brooders and even nesters with brooders, e.g., *B. imbellis* with *B. ferox* and *B. pugnax* (southern Thailand); *B. imbellis*, *B. tussya* with *B. waseri* Krummenacher, 1986 (peninsular Malaysia) (see Figs 2, 3, 4 and Table 2).

It is to be noted that no brooders have been reported between eastern Thailand provinces (where *B. prima* brooders are found) overland all the way to the provinces of Nakhon Si Thammarat, near the lower peninsular Thailand, a distance of about 850 km (see Figs 2 and 3). Incidentally, so far, we have

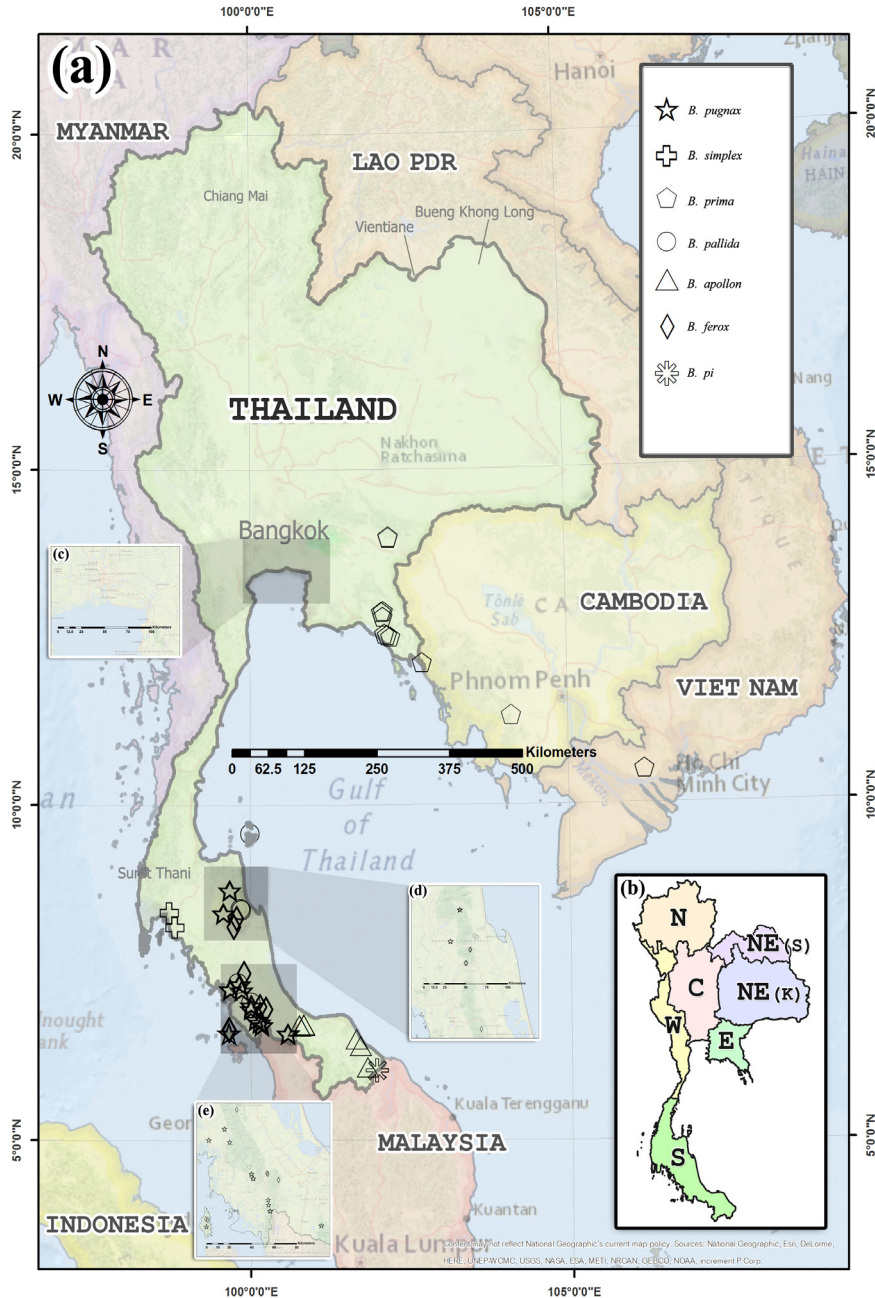


FIGURE 3. (a) Habitat locations (marked by symbols) of various brooder species as identified by DNA sequences in the genus *Betta* in Cambodia, Thailand, Vietnam: arrows pointing to Bangkok, Vientiane and Bueng Khong Long; (b) Thailand and its regional divisions: N-north, NE(S)-northeast (Sakon Nakhon basin), NE(K)-northeast (Khorat basin), C-central, E-east, W-west, S-south); (c) central and eastern, (d) and (e) southern regions magnified. The map was constructed by using the ArcGIS version 10.1.

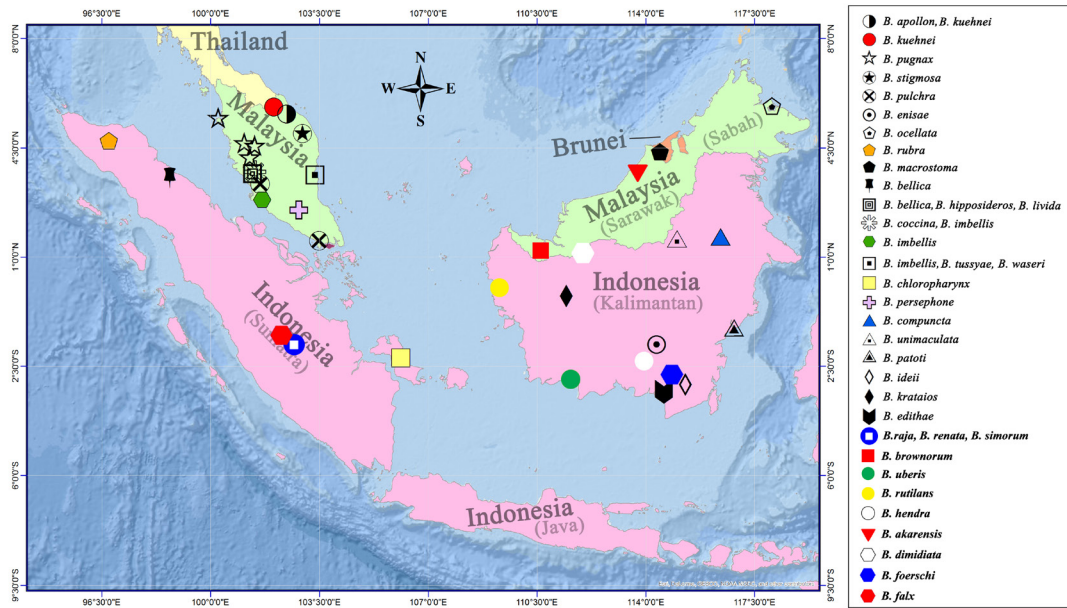


FIGURE 4. Habitat locations of various species (nesters and brooders as identified by DNA sequences) in the genus *Betta* in east and west Malaysia, Indonesia, and Brunei. The map was constructed by using the ArcGIS version 10.1. Habitats of these fishes may occupy wider areas than marked by the symbols.

not been able to find hybrid populations in the wild, although we are capable of detecting hybrids with the maternal mitochondrial DNA (cytochrome c oxidase subunit 1, COI) and the paternal nuclear DNA (first internal transcribed spacer, ITS1) sequences and analyses (Kowasupat *et al.*, 2014; Panijpan *et al.*, 2014). In fact, we found hybrids in wild *Trichopsis* by sequencing such mitochondrial and nuclear DNAs (Panijpan *et al.*, 2015). Some *Betta* discovered in Bangkok's Chatuchak Market have been found by the researchers to be hybrids, e.g., between *B. mahachaiensis* and *B. splendens* (Kowasupat *et al.*, 2014). Also, in spite of their obvious differences in external appearance, the various ornamental and fighting fish, bred in Thailand farms and elsewhere, have mainly been found to be variants of *B. splendens* species (Sriwattanarohtai *et al.*, 2010).

Phylogenetic Relationship

Based mainly on external traits (not osteology) and behavioural criteria attempts have been made to put both paternal-care types of *Betta* species into various groups, e.g., the *B. splendens* group, the *B. picta* group, the *B. waseri* group (Witte and Schmidt, 1992; Tan and Ng, 2005a). By combining morphology with additional DNA evidence, a phylogenetic tree had been proposed previously to show their ancestral relationships (Rüber *et al.*, 2004). However, more recent COI and ITS1 results (Kowasupat *et al.*, 2014; Panijpan *et al.*, 2014), based on a more accurate analysis, have produced a new phylogenetic tree which agrees reasonably well with the above; however, there are some significant differences. The new DNA-based phylogenetic tree (Fig. 5) shows slightly different species relationships from the previous tree and grouping (Panijpan *et al.*, 2014). For example, *B. macrostoma* should not be a member of the *B. unimaculata* group.

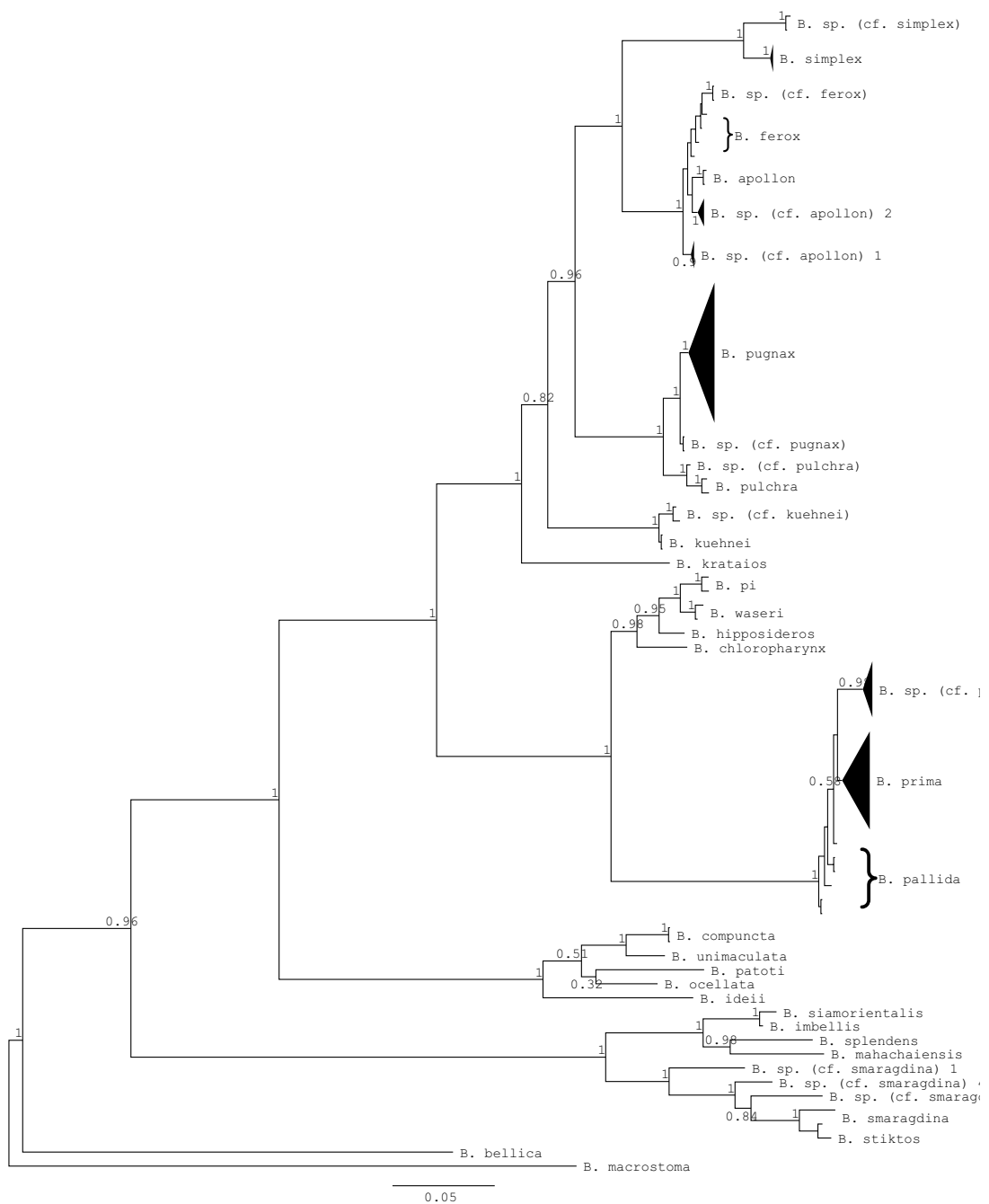


FIGURE 5. New phylogenetic tree reconstructed from the alignment of 83 COI and ITS1 sequences as reported by Panijpan *et al.* (2014): the posterior probabilities are indicated next to the nodes.

Betta bellica group, *B. splendens* group and *B. smaragdina* group should have speciated from other fishes much earlier. Also *B. unimaculata* group did not speciate before the nesters such as *B. bellica* and *B. splendens*. Nevertheless, the latest tree supports an earlier conclusion that paternal care modality can change from one type to the other and back again. These new results should lead to further investigation into the species status and the phylogenetic relationship of at least some species of *Betta*. For example, it was found that, genetically, two differently named species, *B. prima* and *B. pallida* to be virtually identical and *B. ferox* and *B. apollon* to be virtually the same (Panijpan *et al.*, 2014).

Biogeography

The emergence of the Sundaland from the sea is estimated to have happened some 20,000 years ago during the last ice-age. The present central and eastern Thailand / Cambodia land mass and peninsular Malaysia were all connected to a number of islands which are now in the Gulf of Thailand as well as some Indonesian islands via land bridges (Voris, 2000; Woodruff and Turner, 2009; de Bruyn *et al.*, 2013). After sea water rose with the melting ice, the land bridges disappeared and most species of *Betta* could have been isolated. Some of these species had been more closely linked genetically before then and might have evolved their separate ways after the thaw. Thus, now, we witness the eastern Thailand *B. siamorientalis* (nest builder) to be genetically very close to *B. imbellis* (nest builder) in the lower peninsular Thailand. This latter species has been reported in south Vietnam together with *B. siamorientalis*. The two populations (southern *B. imbellis* and eastern *B. siamorientalis*) are separated at present by water in the Gulf of Thailand by more than 400 km. Another case (as mentioned above) is the virtual identity of

B. prima (mouth brooder) of eastern Thailand with the *B. pallida* (mouth brooder) of lower peninsular Thailand. Yet, as mentioned above no brooders are found from the western edge of the eastern region of Thailand overland all the way to the southern Nakhon Si Thammarat province. Thus the brooders must not have migrated over present land mass. One country in the region with no *Betta* is the Philippines, possibly because even during the last ice age with the Sundaland connecting most of the Southeast Asian land masses, for a long time the Philippines remained separated by the great depth of sea (more-or-less) along Wallace's line.

In the northeast Thailand, *B. smaragdina* and some species of *Trichopsis* seem to be genetically very different from other members of their respective genus. Past geological events, e.g., the last ice age and the geological formations in the north-eastern Thailand (Smith and Stokes, 1997), may have contributed to the unusual populations of *Betta* and *Trichopsis* species (Panijpan *et al.*, 2015). It is interesting to find that two particular cryptic species (Kowasupat *et al.*, 2014) of *B. spp.* (cf. *smaragdina*) 1 and 4, and also one of *T. schalleri* are genetically different from their neighbouring species to the south of the region to warrant new species status for each of them (Panijpan *et al.*, 2015). The northeast of Thailand is 'hemmed in' by mountain ranges in the west of the region, more-or-less cutting it off from the northern and central plains of the Chao Phraya River basin. The southern part of this region is also separated by mountain ranges from the eastern part of Thailand. These geographical features may help explain why *B. smaragdina* fish have been found to be different and encompass more cryptic species than other species of the nest-building *Betta* in Thailand. The new phylogenetic tree shows the *B. smaragdinas* to be on a different branch from other nest-building *Betta*

(Kowasupat *et al.*, 2014).

Future Research

With the research shown in this paper, it is suggested that genetic research based on high-throughput DNA sequencing should be used more frequently to identify and differentiate species of *Betta*. Identification of genes responsible for various desirable features in the ornamental fish should be pursued, especially, in *B. splendens*. The aim should be to integrate DNA technology with classical know-how accumulated from long-standing breeding culture in South East Asia, based on Mendelian genetics to produce commercially attractive fish with as few genetic defects as possible, as some of these classically bred fish have been found to be weak and to have missing body parts.

In Thailand, government agencies or concerned private organizations should come together to pass laws to reserve large enough areas near the present habitats to allow the fish populations to grow as naturally as possible. Globally, ethical issues about producing and breeding fish with extremely deviant and debilitating appearances should be discussed and problems rectified and with official ratification.

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Table 1a (Continue)

Species group	Fighting fish species	Singapore	Myanmar	Viet Nam	Cambodia	Lao PDR	Thailand					Malaysia			Indonesia								Habitat water characteristics*	
							Northeast	North & West	Central	East	South	Malay Peninsula	Sarwak (Central)	Sarwak (South)	Kalimantan (West)	Kalimantan (Central)	Riau Archipelago	Sumatra (South)	Bangka-Belitung Islands	Sumatra (West)	Sumatra (Central & East)	Sumatra (North)		
<i>B. smaragdina</i>	<i>B. siamorientalis</i> Kowasupat, Panijpan, Ruenwongsa & Jeenthong, 2012			+	+				+															c, n
	<i>B. splendens</i> Regan, 1910		+			+	+	+	+	+														c, n
	<i>B. smaragdina</i> Ladiges, 1972					+																		c, n
	<i>B. stiktos</i> Tan & Ng, 2005				+																			c, n

- All fish species have been grouped according to their similarities in appearance and behavior (Witte and Schmidt, 1992; Tan and Ng, 2005a; Schindler and Schmidt, 2006; Kowasupat *et al.*, 2014)
- Collection sites of established species can be found in the articles cited in this Table and those published subsequently (Cardot, 2010; Linke, 2014)
- Habitat water characteristics are taken from our survey, articles and Linke's book cited here*; a – acidic, c – clear/transparent, d – dark, n – neutral (pH 7±1), soft water and ? – when water characteristics have not been well described.
- *B. imbellis* and *B. mahachaiensis* can live in fresh to brackish water.

Table 1b Countries and regions of all published mouth-brooding *Betta* fish species (allocated into groups) in Southeast Asia

Species group	Fighting fish species	Singapore	Viet Nam	Cambodia	Lao PDR	Thailand		Malaysia						Brunei	Indonesia												Habitat water characteristics *
						East	South	Malay Peninsula	Sarawak (West)	Sarawak (North & East)	Sarawak (Central)	Sarawak (South)	Sabah	Kalimantan (East)	Kalimantan (West)	Kalimantan (Central)	Kalimantan (South)	Riau Archipelago	Java	Sumatra (South)	Bangka-Bellitung Islands	Sumatra (West)	Sumatra (Central & East)	Sumatra (North)	Nias Island		
B. akarensis	B. akarensis Regan, 1910									+																	a, d
	B. antoni Tan & Ng, 2006																										a, d
	B. aurigans Tan & Lim, 2004																										a, d
	B. balunga Herre, 1940																										?
	B. chini Ng, 1993												+														a, d
	B. ibanorum Tan & Ng, 2004													+													a
B. albimarginata	B. obscura Tan & Ng, 2005																										d
	B. pinguis Tan & Kottelat, 1998																										a, d
	B. albimarginata Kottelat, 1998																										a, t
	B. channoides Kottelat & Ng, 1994																										a
B. anabatoides	B. anabatoides Bleeker, 1851																										a, d
	B. midas Tan, 2009													+													a, d
B. dimidiata	B. dimidiata Roberts, 1989																										a, d
	B. krataios Tan & Ng, 2006																										a, d
B. edithae	B. edithae Vierke, 1984																										a to n, d

Table 1b (Continue)

Species group	Fighting fish species	Singapore	Viet Nam	Cambodia	Lao PDR	Thailand		Malaysia						Brunei	Indonesia											Habitat water characteristics *
			East	South	Malay Peninsula	Sarawak (West)	Sarawak (North & East)	Sarawak (Central)	Sarawak (South)	Sabah		Kalimantan (East)	Kalimantan (West)	Kalimantan (Central)	Kalimantan (South)	Riau Archipelago	Java	Sumatra (South)	Bangka-Beliung Islands	Sumatra (West)	Sumatra (Central & East)	Sumatra (North)	Nias Island			
B. waseri	B. patoti Weber & de Beaufort, 1922																								n	
	B. unimaculata Popia, 1905																								?	
	B. chloropharynx Kottelat & Ng, 1994																		+						a, d	
	B. hipposideros Ng & Kottelat, 1994								+												+				a, d	
	B. pardalotos Tan, 2009																	+							?	
	B. pi Tan, 1998							+																	a	
	B. renata Tan, 1998																	+			+				a, d	
	B. spilotogeta Ng & Kottelat, 1994																+								?	
	B. tomi Ng & Kottelat, 1994	+							+																a, d	
	B. waseri Krummenacher 1986								+																	a, d

- All fish species have been grouped according to their similarities in appearance and behavior (Witte and Schmidt, 1992; Tan and Ng, 2005a; Schindler and Schmidt, 2006; Tan, 2013; Linke, 2014)

- Collection sites of established species can be found in the articles cited in this Table and those published subsequently (Kottelat, 2001; Cardot, 2010; Linke, 2014)

- Habitat water characteristics are taken from our survey, articles and Linke's book cited here*; a – acidic, c – clear/transparent, d – dark, n – neutral (pH 7±1), soft water, t – turbid and ? – when water characteristics have not been well described.

Table 2 Locations in Thailand (provinces), Vietnam (provinces), and Malaysia (states) where the authors caught / observed both bubble-nest building and mouth-brooding betta fighting fish species

Bubble-nest building betta species	Mouth-brooding betta species	Provinces/states in the region	Locations with cohabitation of both modality parental care
Viet Nam			
<i>B. siamorientalis</i>	<i>B. prima</i>	Tiền Giang	
Eastern Thailand			
<i>B. siamorientalis</i> <i>B. splendens</i>	<i>B. prima</i>	Chonburi	
<i>B. splendens</i>	<i>B. prima</i>	Chantaburi	
<i>B. siamorientalis</i>	<i>B. prima</i>	Sa Kaeo	
<i>B. splendens</i>	<i>B. prima</i>	Trat	
Southern Thailand			
<i>B. imbellis</i>	<i>B. simplex</i>	Krabi	
<i>B. imbellis</i>	<i>B. ferox</i> <i>B. pallida</i> <i>B. pugnax</i>	Nakhon Si Thammarat	*
<i>B. imbellis</i>	<i>B. apollon</i> <i>B. pi</i>	Narathiwat	
<i>B. imbellis</i>	<i>B. ferox</i> <i>B. pallida</i>	Phatthalung	
<i>B. imbellis</i>	<i>B. ferox</i> , <i>B. pugnax</i> <i>B. pallida</i>	Satun	*
<i>B. imbellis</i>	<i>B. apollon</i> <i>B. ferox</i>	Songkhla	
<i>B. imbellis</i> <i>B. splendens</i>	<i>B. pallida</i>	Surat Thani	
<i>B. imbellis</i>	<i>B. pallida</i> <i>B. pugnax</i>	Trang	
Malay Peninsula			
<i>B. imbellis</i> , <i>B. tussyae</i>	<i>B. waseri</i>	Pahang	*
<i>B. bellica</i> , <i>B. livida</i> <i>B. coccina</i> <i>B. imbellis</i>	<i>B. hipposideros</i>	Selangor	*

The asterisk (*) indicates cohabitation at the same site for only those *Betta* species of different modalities listed on the same horizontal line.

Remarks: 1. Cohabiting species of the same modality of paternal care (not mentioned in the Table) are as follows:

- In southern Thailand, *B. pugnax* cohabited with *B. ferox* in Satun, also did *B. pugnax* with *B. pallida*. In Nakhon Si Thammarat *B. ferox* and *B. pallida* were found in the same collection site.

- In Malay Peninsula, *B. coccina* and *B. imbellis* cohabited.

2. Two species of bubble-nest building fighting fish found in the same provinces but did not cohabit (not mentioned in the Table), are as follows:

- In central Thailand, *B. splendens* and *B. mahachaiensis* were found in Bangkok, Samut Prakan and Samut Sakhon provinces.

- In southern Thailand, *B. splendens* and *B. imbellis* were found in Phangnga province.

- In northeastern Thailand, *B. splendens* and *B. smaragdina* were found in Loei and Nakhon Ratchasima (Korat) provinces.

- In Lao PDR, *B. splendens* and *B. smaragdina* were found in Vientiane province.

3. In addition (not mentioned in the Table), both mouth-brooding fighting fishes *B. kuehnei* and *B. apollon* were found in Kelantan, Malay Peninsula but did not cohabit.