Wild *Betta* fighting fish species in Thailand and other Southeast Asian countries

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**ABSTRACT:** This review updates what is known about *Betta* fish species in Thailand and Southeast Asian countries. The text is made easy to read for an uninitiated audience. It covers wild nest-builders, wild mouthbrooders, their appearance, distribution, and habitats. A section on domesticated fish is included for the general public. Details for more serious readership can be found in photos, maps, a table, and a diagram. DNA analyses for species differentiation leading to a phylogenetic tree and ancestral relationships are discussed.

**KEYWORDS:** *Betta* spp., fighting fish, mouthbrooder, bubble-nest builder, species distribution, fish molecular taxonomy

**INTRODUCTION**
Ichthyologists know that many species of fish fight with their kinds and others to protect their territories \[1--3\], but the name fighting fish appears to be mainly attributed to a member of *Betta* genus, the Siamese fighting fish, *Betta splendens* Regan, 1910. It has been declared “the national aquatic animal” in 2019 by The National Legislative Assembly of Thailand (NLA). This formal declaration of the fish by the Thai Government may lead to better livelihood for smaller farmers and even high-volume exporters. Mahidol University delegates had contributed significant data, especially DNA analysis, during the three discussion sessions prior to the NLA historical announcement.

Before we describe wild *Betta* fish, we have to address the domesticated ones first.

**DOMESTICATED BETTA FISH**
Most people are familiar with domesticated (not wild-caught) fish bred by farmers and sold in the market for fish lovers both locally and abroad with high volumes. These fish mainly bred from wild *B. splendens* are desired mostly for their attractive appearance, body color and iridescence, fin shape and color pattern, and body size \[4--6\] (see some domesticated *Betta* fish in Fig. 1). These are products of careful and diligent breeders from overseas and in Thailand using mainly Mendelian genetics. These efforts often yield hit-and-miss results, but sometimes produce spectacular outcomes.

Our group did the DNA work at the Canadian Centre for DNA barcoding, Guelph, Canada proving that wild *B. splendens* and most of the farmed nest-builders shown here have basically the same barcoding sequences \[7\]. Our review of 2010 in this journal was published after the fact.

Naturally, fighters derived mainly from *B. splendens* are also produced nowadays but not as many as the ornamental ones described above (Fig. 1). The fighter has a stout body, short fins, and sharp teeth. Their trained endurance can cause some of them to fight to the death from injuries in gambling dens \[8, 9\]. Fighters are generally very hardy and not susceptible to pathogens. However, in general, both farm-bred and wild fish can be infected by parasites, bacteria, and other pathogenic agents \[10--12\].

**NEST BUILDERS AND MOUTHBROODERS**
In *Betta* fish, there are two separate modes of paternal care for fertilized eggs and young fry \[13--15\]. The nest-building male parent builds a raft of sticky bubbles shaped like a 3D convex lens, called a nest, on the water surface or underneath the aquatic plant leaves, to deposit newly fertilized eggs into each bubble to take atmospheric oxygen \[16--18\]. Once the eggs hatch, the male parent would try to retrieve some of the falling fry back into the nest \[19, 20\]. Bubble-nest builders prefer stagnant waters \[21\].

The mouthbrooding male takes fertilized eggs
to incubate in the mouth until the hatching stage. Even then, the male parent still keeps their young fry in the mouth for a while before releasing them to swim freely. Brooders prefer running waters, e.g. near waterfalls [22, 23]. The male nester parent uses the mouth to retrieve fertilized eggs and young hatchlings when it feels danger [17]. They both thus use their mouths in caring for their offspring. As can be seen later, they can evolve from one paternal care type to the other [15, 24].

_Betta_ females do not become pregnant after viewing desirable males. They simply produce more eggs that swell the ovary. Fertilization by male sperm occurs outside the female body when eggs are released.

All these freshwater _Betta_ fish have a common feature that is important for their survival in water with low oxygen content [25]: some can even survive in water with near-zero dissolved oxygen. They can gulp air to put molecular oxygen into the labyrinth organ inside the head region. Their distribution in Thailand is shown in Figs. 2 and 3. The nest builders occupy mainly the north and central plains, the upper south, and the west (_B. splendens_); the northeast (_B. smaragdina_ Ladiges, 1972); the eastern region (_B. siamorientalis_ Kowasupat, Panijpan, Ruenwongsa & Jeenthong, 2012); the upper and lower southern peninsular Thailand (_B. imbellis_ Ladiges, 1975); and the west of Bangkok, Samut Sakhon Province, and Samut Prakan Province (_B. mahachaiensis_ Kowasupat, Panijpan, Ruenwongsa & Sriwattanarothai, 2012) [21–23, 26–29]. Recently, large populations of _B. smaragdina_ were unexpectedly found in the

**Fig. 1** Examples of domesticated _Betta splendens_ sold in the market nowadays: (A) tricolor crown-tail fish possibly emblematic of flags of Thailand, France, the Netherlands etc.; (B) butterfly halfmoon; (C) veil tail or Pla Kat Jeen; (D) giant _Betta_ (Jumbo); (E) Dumbo short fin; (F) fighter or Pla Kat Mor. The pictures are not of the same scale.
Fig. 2 Distribution of selected nest-building *Betta* species in Thailand and neighboring countries.
Fig. 3 Distribution of selected wild mouthbrooding *Betta* species in Thailand and other ASEAN countries. No brooders are found at latitudes above 14.5 degrees north. Wild nesters are also found in Eastern Malaysia and Kalimantan part of the Borneo Island.

south region of Kanchanaburi Province [30].

The brooders are mainly found in the lower southern peninsular Thailand provinces (Fig. 3), including big islands, e.g. Samui and Pa Ngan [22, 29, 31]. These fish are found in streams with running water originating from waterfalls, which can be quite a distance away. Incidentally, Thailand has endemic species of the *Betta* genus, namely the brooders *B. simplex* Kottelat, 1994, *B. pallida* Schindler & Schmidt, 2004, and *B. prima* Kottelat, 1994, and the nest-builder *B. mahachaiensis*. *Betta prima* is found in the eastern region of Thailand. *B. simplex* and *B. mahachaiensis* are subjected to high-volume exports that will push them further into the critically endangered species list [27, 32, 33].

**WILD BETTA FISH IN THAILAND**

Five wild-type nest-building fish in Thailand are very colorful when they, especially males, display their full aggressive posture or stage courtship displays [8,34–36]. The body turns dark, fins are unfurled, and colors and color patterns of fins intensify. With the naked eye, some of their external body features of five nest-building *Betta* in Thailand that are sufficient for differentiating them from one
Myanmar and the Philippines. In fact, outside of the ASEAN countries mentioned above, no wild Betta fish are found. China has no native wild Betta fish. Pla Kat Jeen (China fighting fish) is called that because their fins are longer than usual, making them appear like Chinese opera characters wearing long loose clothing.

The upper peninsular Malaysia provinces share common species of both types of paternal care with those in lower peninsular Thailand (Figs. 2 and 3). Cambodia, Lao PDR, and Vietnam have Betta nest-building fish; some are common to those of Thailand [29]. Surprisingly, estuarial south Vietnam has B. imbellis identical with those of southern peninsular Thailand and Malaysia. As mentioned above, eastern coastal Thailand has a wild mouthbrooder species, B. prima, living along mountain streams (running water), whereas similar-looking wild mouthbrooders in south Vietnam live in paddy fields (stagnant water) (Fig. 3).

Wild Betta species in Southeast Asia occupy a variety of habitats: from fresh to brackish; from alkaline to acidic; from transparent to translucent; and even dark brown waters of the peat swamp [23, 29]. Not all Betta species are small. Some wild Betta are quite large. For example, the brooder B. pi Tan, 1998’s total length is about 12–14 cm and that of the nester B. bellica Sauvage, another are included in Table 1 and Fig. 4. Although our DNA analyses have found no natural hybrids, farm-bred hybrids of these nest-builders are possible. They have the same number of chromosomes (2n = 42) with identical karyotype [37–39], our unpublished results.

The total lengths of B. splendens, B. mahachaiensis, and a typical B. smaragdina are very similar (3.7–4 cm), whereas those of B. imbellis and B. siamorientalis are also similar, but shorter (2.7–3 cm) than the other three above [13, 26]. The farm-bred ones may become longer eventually.

The bodies and fins of mouthbrooders are usually dull in color, mostly brown with darker stripes, making it difficult for the untrained eye to distinguish one species from another [23]. One exception is B. simplex, which has a beautiful pattern on its anal fin and caudal fin (Fig. 5).

**Table 1.** Comparative table showing, as seen through the naked eye, some external characters of five wild nest-building Betta species of Thailand that sufficiently differentiate one species from another.

<table>
<thead>
<tr>
<th>Betta species</th>
<th>B. splendens</th>
<th>B. imbellis</th>
<th>B. siamorientalis</th>
<th>B. mahachaiensis</th>
<th>B. smaragdina</th>
</tr>
</thead>
<tbody>
<tr>
<td>Opercle</td>
<td>Two parallel vertical red bars</td>
<td>Two parallel vertical red bars</td>
<td>Two parallel red bars</td>
<td>Two to three parallel red bars</td>
<td>Multiple iridescent yellowish-green to bluish-green plates or scales</td>
</tr>
<tr>
<td>Caudal fin</td>
<td>Blush-green strips fanning out from caudal peduncle toward posterior</td>
<td>Blush-green strips fanning out from caudal peduncle toward posterior</td>
<td>Blush-green strips fanning out from caudal peduncle toward posterior</td>
<td>Dark strips fanning out from caudal peduncle toward posterior with bifurcation</td>
<td>Dark strips fanning out from caudal peduncle toward posterior with bifurcation</td>
</tr>
<tr>
<td>Anal fin</td>
<td>About 5% of red at distal tip</td>
<td>About 5% of red at distal tip</td>
<td>About 5% of red at distal tip</td>
<td>Presence of black dots flanking stripes</td>
<td>Presence of black dots flanking stripes</td>
</tr>
<tr>
<td>Pelvic fin</td>
<td>Red-brown to black and red from proximal pelvic fin base to the white tip</td>
<td>Red-brown to black and red from proximal pelvic fin base to the white tip</td>
<td>Black to black with iridescent green front margin and white tip</td>
<td>Brown to black and red from proximal pelvic fin base to the white tip (some populations having green to bluish-green front margin)</td>
<td>Brown to black and red from proximal pelvic fin base to the white tip</td>
</tr>
<tr>
<td>Body scale and background</td>
<td>Scale iridescence not prominent</td>
<td>Scale iridescence prominent</td>
<td>Scale iridescence prominent</td>
<td>Scale iridescence very prominent</td>
<td>Scale iridescence prominent</td>
</tr>
</tbody>
</table>

**WILD BETTA FISH IN OTHER ASEAN COUNTRIES**

Thailand is not the only country harboring the wild Betta fish species. Cambodia, Lao PDR, Vietnam, east and west Malaysia, and Indonesia islands, especially Borneo, also have native wild species [23, 29]. There are no reports of wild fish of this genus in Myanmar and the Philippines. In fact, outside of the

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Fig. 4 Photos of the five fully-grown and fully-aggressive male nest-builders in Thailand: (A) *B. mahachaiensis*; (B) *B. smaragdina*; (C) *B. siamorientalis*; (D) *B. imbellis*; (E) *B. splendens*; (F) a modified photo of the wild-caught Betta from Nakhon Si Thammarat Province mistaken for *B. splendens* in a publication by its authors [35].

1884 is about 7–8 cm. Some fish from Borneo can be quite colorful, e.g. *B. albimarginata* Kottelat & Ng, 1994 (mouthbrooder), *B. macrostoma* Regan, 1910 (mouthbrooder), *B. rubra* Perugia, 1893 (mouthbrooder), and *B. coccina* Vierke, 1979 (nest-builder).

Geological evidence shows that in the last Ice Age, about 20,000 years ago, the Gulf of Thailand was dry [40]. Betta fish might have been spreading and evolving among Southeast Asian countries, namely Thailand, Vietnam, Malaysia, and Indonesia, through freshwater rivers running along the Gulf of Thailand, the Sunda Strait, and Borneo [24].

ROLE OF SHORT PIECES OF MITOCHONDRIAL AND NUCLEAR DNA IN DIFFERENTIATING BETTA SPECIES

Up to now, most taxonomic works on Betta fish have been based on morphological criteria in grouping them accordingly. Past attempts using random amplified polymorphic DNA (RAPD) analysis have not been satisfactory [41].

Our group started using a more sophisticated mitochondrial DNA barcoding (based on cytochrome c oxidase subunit I, COI) and 16S ribosomal DNA (16S rDNA), together with the nuclear gene ITS1 (ribosomal internal transcribed
Fig. 5 Photos of the four fully-grown and fully-aggressive male mouthbrooders in Thailand: (A) B. apollon; (B) B. ferox; (C) B. prima; (D) B. simplex.

(spacer 1), to successfully identify B. mahachaiensis and B. siamorientalis (both nest builders of Thailand) as new species [7, 26, 27]. Another application is using DNA analyses to put the fish in their groupings, which turn out to correspond very well with those for fish of similar appearances of both types as shown in Fig. 6. Moreover, our DNA analyses show that eastern Thailand B. prima is identical with B. pallida, and the same for southern brooders B. apollon Schindler & Schmidt, 2006 and B. ferox Schindler & Schmidt, 2006 [31]. The number of valid Betta species is not definite because a few brooder pairs with different species names are suspected to be the same by evidence based on DNA analysis (B. pallida vs B. prima and B. apollon vs B. ferox) as well as their dubious morphological differences (B. rubra vs B. dennisyongi Tan, 2013). By the way, B. dennisyongi and B. omega Tan & Ahmad, 2018 (both brooders) are two latest additions to the previous list of species [42, 43].

MOLECULAR ANCESTRAL RELATIONSHIP

Another result of working with mitochondrial COI is that we reconstructed ancestral relationships among the nest-builders and brooders whose COI sequences were available for analysis. One interesting finding is that Betta fish could switch from one type of paternal care to the other (Fig. 6) [15, 24]. Another finding is that some species differentiation, especially that for the B. smaragdinas in the north-east of Thailand, corresponds to the timeline of geographical shifts of terrains. The estimated timeline for the northeastern “speciation” coincides well with the eastward tilt of the northeastern plateau causing the Chi and Mun rivers to flow eastward, opposite to their previous direction [44]. Two important basins (Korat and Sakon Nakhon) were likely created by this geobiological event.

FUTURE RESEARCH

After more than a decade researching Betta fish, we wish to suggest two research venues to exploit DNA technology to enhance the beauty and reduce aggressive behavior. Complete mitochondrial genome sequences of the bubble-nesters (B. splendens and B. mahachaiensis) and the mouthbrooders (B. apollon, B. pi, and B. simplex) have now been published [45–48].

(A) Exploiting molecular techniques to produce fish with desirable traits for fish lovers, for example fish pigment distribution on the body, fin shape, and body size [49–52], with more precise outcomes, unlike the present practice of hit-and-miss Mendelian genetics.
Fig. 6 The phylogenetic tree of selected *Betta* fishes reconstructed from their COI sequences. Reds indicate nest-building parental-care type while blues signify mouthbrooding one. Estimated speciation times can be read from the scale at the bottom. The numbers at some speciation nodes indicate the probabilities that the corresponding groupings are correct while those without any number are practically certain.

(B) Pinpoint *Betta* genes that are responsible for their aggressiveness with the aim of eventually reducing aggressive tendencies without affecting their essential behaviors such as courtship. Normally a litter of fish growing up together does no fight; they have their pecking orders. Nonetheless, fish from various sources will fight when put in the same tank. This kind of work may lead to suppressing aggressive tendencies in other animals including humans. Researchers have found hormones and neurotransmitters in fish that correspond to those found in higher animals [53, 54]. Perhaps exploiting CRISPR technology may help.

We have observed different degrees of aggressiveness among domesticated *Betta* fish populations, long-finned and short-finned alike. Perhaps these can be used to study genes responsible for differential aggressive behavior.

RESOURCES FOR POPULAR EASY-TO-READ INFORMATION


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