

The Siamese fighting fish: Well-known generally but little-known scientifically

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ABSTRACT: The Siamese fighting fish, of which *Betta splendens* is representative, is gaining popularity as judged by increasing export values and demands for novel types worldwide, especially the ornamental ones. However, relatively little is known about the bettas scientifically. In this review we cover what is known about the desired morphology and pigments, genetics, aquaculture, diseases and feeds involved in breeding bettas. We also propose breeding the bettas for domestic enjoyment and export by exploiting current knowledge of gene technology and molecular developmental biology in addition to the use hitherto of only classical genetics. Other aspects of fish culture such as feeds, disease prevention and water quality should also be scientifically studied and improved upon. Because of dwindling and worsening habitats in Thailand, more studies on biodiversity and work towards species conservation of the wild types are needed.

KEYWORDS: betta, pigments, genetics, diversity, conservation

INTRODUCTION

The Siamese fighting fish is found not only in Thailand but also in other neighbouring countries in SE Asia. Because of the male's pugnacious nature and beauty, the representative fish is called *Betta splendens* (the splendid battler). Traditionally, Thais love to watch and bet on two combating males, with fights sometimes resulting in death. The market for this type of fish, which has been ongoing since recorded history¹, has not changed much. Fish bred for fighting are selected for a large and strong body with hard scales (hard targets) but smaller fins (flimsy targets) as protection against bites of the opponent. Here colours and patterns are of secondary importance. Ability to bite vulnerable targets such as the swimmers and tail fins (for stability and kicking ability) is a very crucial asset.

Breeding the males as ornamental fish for local sale and exporting is also becoming increasingly lucrative. The features favoured by aficionados and amateurs alike are colour intensity, colour pattern, scale iridescence, body shape, and fin size. Changing the emphasis of breeding from fighting to ornamental bettas is preferable not only for ethical reasons but also for commercial reasons in that the export market for the latter is larger and has higher profit margins. According to the Department of Fisheries (2000–2005), bettas ranked among the top two ornamentals

in terms of number of fish and revenue. The latest official value (2005) for bettas is about 25 million Thai baht. This figure can be improved upon. In raising the fish for high quality mass export, water quality in the breeding jars, feeds, disease prevention, better transport conditions, and certification will be of utmost importance.

Although wild types are eagerly sought and command high prices as breeding stocks for the fighting bettas, it is the ones bred in captivity that bring outward varieties to other commercial bettas. Ornamental fish with novel colours, colour patterns, and fins are regularly imported and are in high demand until extensive local propagation brings the prices down from 10,000–20,000 to 10–20 Thai baht each. These varieties probably still arise from classical breeding and selection (personal communication). It appears that traditional betta breeders still base their thinking on classical Mendelian genetics in spite of constant demands for new features by the market. Atison Phumchoosri, a prominent betta expert, believes that breeders still exchange information and keep their own practice-based experiences and beliefs that may not have any scientific foundation.

Although this article is written by Thai biologists with experience mainly in Thailand, we recognize that other scientific communities, especially those in SE Asia, have similar challenges and problems and will benefit from all the aspects addressed in this



Fig. 1 Bettas found in Thailand: (top) *B. splendens*, (middle) *B. smaragdina*, (bottom) *B. smaragdina* male wrapped around female during spawning.

review. We would therefore like to persuade Thai scientists and others in the region to work more on their native fish for original scientific contributions, to innovate ways to make this fish more ornamental for enjoyment and export, and to conserve their habitats and diversity. We have selected certain topics deemed relevant to breeding for specific aesthetic purposes and commercialization at the expense of other topics. Proposals are made here to highlight aspects that we think should be prioritized by biologists and breeders

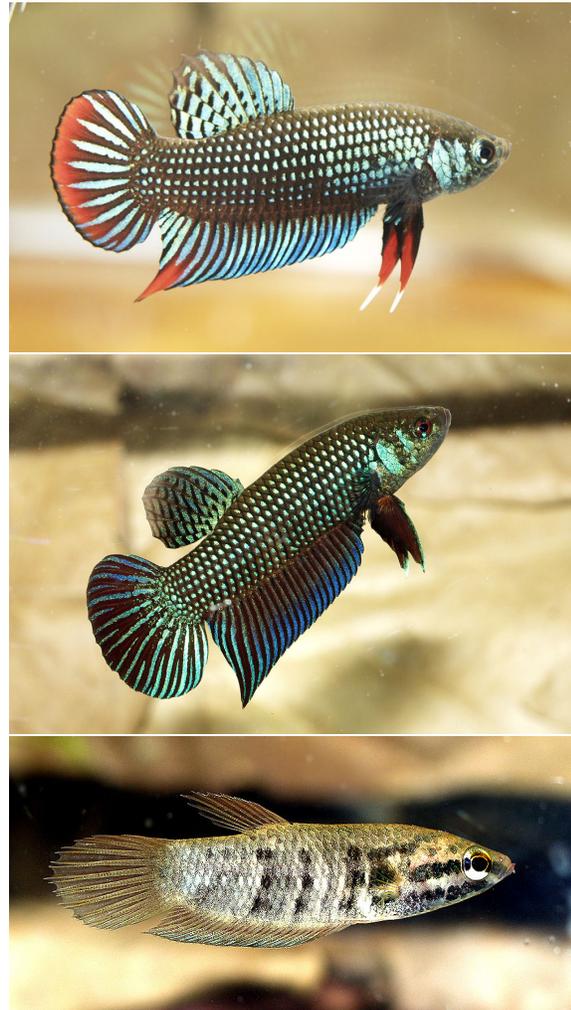


Fig. 2 Bettas found in Thailand: (top) *B. imbellis*, (middle) *Betta* sp. Mahachai, (bottom) *B. prima*.

who wish to make contributions for future knowledge as well as raising bettas.

CLASSIFICATION

The most recent classification of bettas from phylogenetic evidence²⁻⁴ places them in the Osphronemidae family (whose freshwater members are *Anabas* spp., *Trichogaster* spp., and *Trichopsis* spp.), Macropodusinae subfamily and genus *Betta*. Thus far 55 species of bettas have been described from Indochina⁵. Each one displays one of two different types of egg brooding care (nest building and mouth brooding). Of all the 10 wild-type species found in Thailand, 4 are bubble-nest builders and 6 are mouth brooders. Goldstein⁶ and Linke⁷ have written about the 4 species of wild-type bubble nest builders in Thailand as follows.



Fig. 3 *B. splendens* varieties bred in Thailand: (top) multicoloured crown tail, (middle) red double tail, (bottom) opaque white halfmoon.

B. splendens Regan, 1910 is the most well-known and the most widespread in the Central Plain. Its habitats are small bodies of water such as those in paddy fields, ponds, lagoons and marshes.

B. smaragdina Ladiges, 1972 is found throughout the Northeastern region, e.g., provinces of Nong Khai, Udon Thani, Khon Kaen, Loei and Ubon Ratchathani. Paddy fields, marshes, ponds and lagoons are places it can be found.



Fig. 4 *B. splendens* varieties bred in Thailand: (top) pineapple short tail, (middle) marble short tail, (bottom) orange short tail.

B. imbellis Ladiges, 1975 is ubiquitous in the Southern regions, e.g., provinces of Phuket, Surat Thani, Nakhon Si Thammarat and Songkhla, the Malay Peninsula and Penang Island. Shallow waters of paddy fields, marshes, ponds, lagoons, lakes and acidic swamps are the habitats. However, it can also be found in areas where the water is brackish due to the ebb and flow of the sea tide.

B. sp. Mahachai is distributed only in a narrow and specific area of Samut Sakhon, Samut Songkhram and Samut Prakarn. It is found in tidal brackish waters with thick vegetation. No other wild *Betta* species

have been found in the area. It has not yet been classified. The possibilities remain that it is a new and still unestablished species or a hybrid species bred true.⁸ The authors are studying it for diversity and conservation purposes.

As for the wild-type mouth brooders, Schindler and Schmidt⁵ made a survey in Thailand and reported 6 species as follows.

B. prima Kottelat, 1994 is widespread in the Eastern region, e.g., in Chonburi, Trat and Sa Kaeo. It is found in slow-flowing streams, lagoons and ponds.

B. simplex Kottelat, 1994 is found in the Krabi province in the South. However there are no reports so far from other provinces nearby. It can be found in spring-fed streams with slow flowing waters with dense vegetation.

B. pi Tan, 1998 is found in Su-ngai Kolok district and Tak Bai district of Narathiwat province in peat swamps and flooded areas on river banks together with decaying vegetation.

B. pallida Schindler & Schmidt, 2004 is found in the Ruso and Su-ngai Kolok districts of Narathiwat province and Ko Samui of Surat Thani province in shaded shallow waters with decaying vegetation.

B. apollon Schindler & Schmidt, 2006 was recently reclassified. It is ubiquitous in the Narathiwat province in shallow waters of mountain streams with a depth of about 20–30 cm.

B. ferox Schindler & Schmidt, 2006 was also recently reclassified. It can be found around Paribatra Fall, Hat Yai district, Songkhla province. It lives in slow flowing streams around tree roots and *Acacia* plants.

To conclude this section, we wish to mention the little known *B. sp.* Satun which is a less colourful mouth-brooder of southern Thailand.⁹ Its habitats are easy-flowing rivers in the hilly landscape northeast of Satun. It has glowing and vigorous turquoise green on the lower head. It may belong to the *B. pugnax* complex.

CONSERVATION

Foresight and better knowledge are needed to keep wild bettas in their habitats and to keep domesticated ones as diverse as possible while developing new varieties.

We and our colleagues have surveyed Thailand for habitats and diversity of bettas and our combined data are shown in Fig. 5. The last 20–30 years have witnessed a dwindling of habitats such as ponds, paddy fields and swamps due to urbanization and industrialization. In response, we need: (1) a detailed catalogue of all localities with *Betta* species



Fig. 5 Distribution of (top) four species of wild-type bubble nest builders and (bottom) six species of wild-type mouth brooders in Thailand.

or subspecies (2) gene banks and preserved specimen deposits for all the catalogued species or subspecies where scientists may conduct continuing research on the bettas (i.e. systematics) (3) institutes in which to conduct research on the natural history of bettas. This will allow for a species maintenance programme for vulnerable or threatened species (by locality not

phenotype). Also, as *B. splendens* has proven to be a good model for domestication and mass production contributing to a significant segment of the fisheries industry, it follows that such institutes should have a role in helping technology transfer to fish farmers for the aquaculture of all bettas. Incidentally, we should be careful about divulging the exact locations of rare species in case of poaching, although there has been no precedent for collecting bettas to extirpation. Nevertheless, several *Betta* species have variously been listed as critically endangered (*B. sp. Mahachai* and *B. simplex*), vulnerable (*B. prima* and *B. pi*) and threatened in situ (*B. imbellis*, *B. smaragdina*, and *B. splendens*)¹⁰. Thus far, a number of wild forms of bettas has been domesticated and incorporated into farming programmes to serve the export industry. The greatest demands are for *B. splendens*, also *B. smaragdina*, *B. sp. Mahachai*, *B. imbellis*, all nest building bettas. The mouth brooding species are in a small-demand niche market and not as popular. One particular population that is easily impacted is *B. simplex* as it is in a microhabitat. The main threats are from tourism, agriculture, elephant camps, and spring water spas. The protection of habitats has to be instituted soon.

CLASSICAL AND MOLECULAR GENETICS

To conserve and develop bettas, knowledge must be accumulated about their genomes and various phenotypes resulting from gene expression.

The classical genetic basis for the pigments of *B. splendens* was first published in 1934¹¹. Using statistical analysis of betta scale pigments, Wallbrunn¹² found that the body, fin, and scale colours of *B. splendens* were regulated by genes for melanin and iridescence pigments as well as the genes for pigment density. The gene for iridescence completely dominates that for melanin.

Khoo et al¹³ studied sarcoplasmic protein patterns of *B. splendens* by isoelectric focusing and found little differences, probably arising from low genetic diversity. After comparing hatchery stocks of *B. splendens* in Nakorn Pathom province by studying their allozymes, Meejui et al¹⁴ concluded that the genetic diversity of hatchery populations of Siamese fighting fish in Thailand was marked with a slight decline in allele diversity and proportion of polymorphic loci while heterozygosity increased. However, sufficient genetic diversity existed among populations and would greatly benefit genetic improvement programmes of this species. They recommended keeping genetic integrity of each population by minimizing stock translocation between genetically distinct pop-



Fig. 6 Fragments of mitochondrial (mt) DNA sequences of *Betta* spp., *Trichopsis*, and *Trichogaster*. The complete sequences include partial 12S (mt) ribosomal RNA, which is connected to the complete tRNA-Val and partial 16S ribosomal RNA sequences. Stars indicate identity positions.

ulations.

Recently work has begun on the taxonomy and population structure of bettas using genetic markers of mitochondrial and nuclear DNA sequences³. The studies are the ones that showed bettas to be in the Osphronemidae family. It was also found that the *B. splendens* nesters are genetically more closely related to *B. imbellis* than the others whereas the *B. pi* brooders are genetically close to *B. simplex* (see Fig. 6 and phylogenetic tree in Ref. 3) as supported by an RAPD study¹⁵.

REPRODUCTION AND PROPAGATION

Breeding fish to obtain the desired characteristics requires knowledge of the lineage of both male and female. The pair must be placed in a jar with proper dimensions in the presence of vegetation. The depth of water used should not exceed 15 cm so that the hatched fry that tend to sink can come up to the surface to breathe⁶. Yucca and tropical almond leaf (*Terminalia catappa*) extracts (the active ingredients in, e.g., Atison’s Betta spa, Ocean Nutrition) are used in the spawning vessel with the aim of ridding body waste and, in particular, ammonia.

Usually, in situ infusoria and purchased water fleas (*Daphnia* sp. and *Moina* sp.) are used as natural feeds for the fry. However, some fish tanks may not have sufficient infusoria, in which case artificial feeds have to be used. Artificial feeds for young fish (which may number up to 500 per litter) are selected by their particle size and nutritious value, e.g., yolk of boiled egg mixed with water or mixed with standard feeds sieved to obtain only particles small enough for them. Decapsulated *Artemia* cysts have to be ground before mixing with floating material for efficient use as feed. Feeds of different sizes are used at various stages of development to ensure good health and survival.

At about two months after hatching, males are morphologically distinguishable from females. To

keep the males from fighting, 4-month old males may have to be isolated. At the farm level, prices are then fixed for the beauty or fighting abilities of representatives of the litter. Because of their lacklustre colours and display, females are usually sold en masse at a very low price; only some are kept for breeding. Breeders then use some of these fish with the desired characteristics to further propagate the lines.

PIGMENTS AND BODY COLOURS

Pigments of bettas give rise to red, blue, green, and yellow body surface colours and also to scale iridescence. Pigments are compounds of the general structures such as melanins, carotenoids, xanthines and pterins whereas iridescent compounds on the scales and body surfaces are guanines and purines.¹⁶ These pigments are also common in other animals. However, fish have special pigment cells (chromatophores) which respond to neural signals for quick outward expression by deepening of colour intensity and changing of shades¹⁷. The genetic basis and synthetic routes to these pigments have not been well established, let alone the interactions between them. However, Braasch et al¹⁸ studied an ancient fish-specific genome duplication (FSGD) by using a comparative genomic approach for two major pigment synthesis pathways (melanin and pteridine) in teleost fish. Due to the FSGD, teleost fish apparently have a greater repertoire of pigment synthesis genes than other vertebrate groups. These results support the important role of the FSGD and other types of duplication in the evolution of pigmentation in fish. Therefore, to successfully and systematically breed bettas and/or to specifically modify bettas genetically using gene technology the relevant basic science has to be done. Developmental biology and molecular biology techniques should help to produce desired body colour patterns on demand.

The chromatophores of fish change colours in accordance with the habitat, the environment and stimuli.¹⁹ There are two types of chromatophore, those that absorb lights in the visible range (melanophores, erythrophores, xanthophores and cyanophores) and those that reflect light (leucophores and iridophores). The pigments are moved intracellularly by motor proteins such as tubulin, dynein, and kinesin¹⁷. The pigment cells respond to brightness, ultraviolet light, pressure, temperature, fish activity, pH, and chemicals via the neurohumoural and neurochemical systems producing melanophore-stimulating hormone, adrenocorticotrophic hormone, prolactin, somatolactin, melanin-concentrating hormone, melatonin, epinephrine and norepinephrine.^{17, 19-21}

In vertebrates, pigment cells develop from the neural crest of the embryo^{22, 23}. In the epidermis and dermis there are four pigment types: iridescent or blue, black, red, and yellow, which are made up of, respectively, blue and green pigment-containing iridocytes and guanophores, melanophores, erythrophores, and xanthophores. The pigmentation is controlled by genes giving rise to different types, quantities of pigments, and pigment distributions.

During development, one can easily observe changes in the outward appearance of the fish at different stages. The early hatchlings have no spots on the yolk sac which is attached ventrally. The pre-larval stages also have no or very few coloured body spots. Only at the post larval stage do the fry have dispersed colour spots all over the body²⁴.

AGGRESSIVE BEHAVIOUR

Another factor that may pose problems for big scale breeding and propagation is the excessive aggressive behaviour of bettas raised in a litter, especially for the ornamentals, because they need an appearance untouched by biting.

Bettas are social animals capable of living in groups with a pecking order²⁵ or in isolation. They are very territorial, especially after isolation or during courtship. Their aggressiveness is usually expressed by body colour intensity, expansion of fins, and opening of gill covers (opercula), which are all features preferred by the females²⁶⁻³¹. Although such expressions give rise to the splendours of the fish and the obsession of gamblers on results of their fighting³², people who raise them as ornamentals would prefer to have such beautiful displays with minimal fighting²⁶. Hence breeding out the aggressiveness or genetically modifying the fighting tendency would make keeping home-based bettas in large groups a better prospect, instead of only in isolation as generally practised. Success in this direction would enhance their position as truly ornamental. In trying to tone down the male aggressivity, one has to keep in mind that the male courtship display should still be effective.

Published results document attempts to enhance the number of males per litter or to make the females look more colourful³³⁻³⁵. In Thailand, breeders have used a variety of concoctions to enhance the male/female ratio, e.g., mangosteen (*Garcinia mangostana*) leaf extract.³⁶ Much work on betta aggression has looked into the general sociobiological and psychological aspects of behaviour rather than the fish themselves³⁷⁻⁴¹.

DISEASES AND THEIR PREVENTION

For long-term keeping and breeding and also for commercial transport, diseases due to microorganisms should be prevented. Male bettas (one per bag) being sent to local markets and for export often suffer from limited oxygen in their containers. Sometimes, individuals in plastic bags have about 10 ml of air per fish (water to air volume ratio, 1:3) for up to 5 days starting from packaging to shipment and sale. For the export markets, generally plastic bags containing 60 ml (for short fins) and 120–150 ml (for long fins) in Styrofoam boxes⁴² are used while maintaining the water to air ratio of 1:3 for 1–3 days or 3:5 for 4–7 days from preparation to destination (personal communication). These fish survive quite well due to the labyrinth above their gills, an organ which acts as an internal air reservoir and for the adjustment of their metabolism. Nevertheless, they still suffer from microbial and viral attacks^{43–45}. The Department of Fisheries of Thailand is developing a more rapid and specific diagnosis for pathogens in addition to the external examinations for parasites and bacteria. Generally, acriflavin, gentian violet, and sodium chloride are used to prevent infection during transport of other fishes. Some of these and other low-cost antibiotics have also been used for bettas being transported.⁴² Anaesthesia and reduction of metabolic rates are two technical possibilities being exploited in making the fish dormant. This, in combination with novel transport packaging, may reduce the overall losses and costs.⁴² For sedatives, tricaine methane sulphate (MS-222) at 60–70 ppm or quinaldine sulphate at 25 ppm are generally used. Tetracycline (5–20 ppm) and neutral acriflavin (3–10 ppm) are popular among Asian exporters. For stabilising water quality, buffers, zeolites, and activated carbon for capturing body waste such as ammonia are used. Temperature has to be controlled by ice or heat pack for transport. Bettas usually cannot survive well in a cold climate mostly because of increased disease susceptibility.

For export, the Aquatic Animal Health Research Institute of the Department of Fisheries, Thailand, has the duty of ensuring that the fighting fish be free from diseases such as ich (ick) or white spot disease (due to the protozoan *Ichthyophthirius multifiliis*), velvet or rust (due to *Oodinium* sp. protozoa), fin rot (due to *Pseudomonas fluorescens* bacterium) and tuberculosis (due to *Mycobacterium piscium*).

ARTIFICIAL FEEDS

Since natural foods are not widely available, for most betta lovers it is important to have artificial feeds

that are as close to the natural ones as possible. We therefore would like to encourage people to do research on this aspect.

Apart from artificial feeds for fry mentioned earlier, bettas favour live and moving prey, e.g., tiny infusorium organisms, water fleas, blood worms, and especially mosquito larvae^{6,46}. However, some of these are not easy to find in Thailand and some others transmit diseases. Mosquito larvae should not be kept just for the purpose of feeding these fish; Japanese encephalitis and dengue fever possibilities should constantly be kept in mind, even though the Ministry of Public Health has only warnings but no regulations against raising them for commercial purposes. The larvae are generally of the *Culex* and *Mansonia* sp. which are potentially less harmful as disease carriers than the *Aedes* and *Anopheles* sp.

Immobile feeds such as ant eggs and boiled egg yolk particles are not preferred by the fish, and these are not widely available nor do they keep well. Although older fish can be trained to feed on artificial feeds, there is still a need for artificial feeds which are disease free with a good complement of nutrients. These feeds should be widely available and cheap, keep well and make the fish grow healthily with normal body proportions⁶. Several commercial products, e.g., those of the Sakura, Tetra, and Hikari, are currently available but they are relatively expensive compared with feeds for other ornamental fishes such as guppy, molly, and goldfish. An ideal artificial feed for bettas should also be mobile on the water surface and/or sink very slowly.

RESEARCH IN THAILAND

There have been only a handful of publications in peer-reviewed journals by Thai scientists. Karyotyping of *B. splendens* has been done and there is a good agreement on the number of chromosomes ($2n = 42$) but less on the types of chromosome^{47,48}. The sex chromosomes are also not easily distinguishable from autosomes⁴⁹. A behavioural study of bettas in “natural” setting has also been reported. Two Thai scientists, M. and K. Jaroensutasinee,^{50–52} found that male body size and nest size influences the female choice of mate. Larger males are better at courtship display than their smaller counterparts. Nest size also reflects the body size. During the mating season and the parental care stage, the male is more aggressive. Two other pieces of work done overseas by Thai scientists on microbes responsible for betta disease are worth mentioning here^{44,45}.

As the subtitle of this review suggests, there is still much that is not known about bettas. We have

the fish locally yet most innovations and publications come from people overseas who do not have the fish in abundance. There is much opportunity for commercial as well as scientific ventures, in addition to which the fish is part of the heritage of the region, and so is worthy of the attention of the local scientific community.

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