

THE “CELESTIAL PEARL DANIO”, A NEW GENUS AND SPECIES OF COLOURFUL MINUTE CYPRINID FISH FROM MYANMAR (PISCES: CYPRINIFORMES)

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ABSTRACT. – *Celestichthys margaritatus*, a new genus and species of Danioinae, is described from a rapidly developing locality in the Salween basin about 70–80 km northeast of Inle Lake in northern Myanmar. Males and females are strikingly colourful. It is apparently most closely related to two danioins endemic to Inle, *Microrasbora rubescens* and “*Microrasbora*” *erythromicron*. The latter species may be congeneric with the new species. The new genus is identified as a danioin by specializations on its lower jaw and its numerous anal fin rays. The colouration, while highly distinctive, seems also to be characteristically danioin. The danioin notch (Roberts, 1986; Fang, 2003) is reduced or absent, but the danioin mandibular flap and bony knob (defined herein) are present. The anal fin has iii8½–10½ rays. In addition to its distinctive body spots and barred fins the new fish is distinguished from other species of danioins by the following combination of characters: snout and mouth extremely short; premaxillary with an elongate and very slender ascending process; mandible foreshortened; body deep, with rounded dorsal and anal fins; modal vertebral count 15+16=31; caudal fin moderately rather than deeply forked; principal caudal fin rays 9/8; scales vertically ovoid; and pharyngeal teeth conical, in three rows

KEY WORDS. – Hopong; principal caudal fin rays; danioin mandibular notch, knob, and pad; captive breeding.

INTRODUCTION

A tiny cyprinid fish of exquisite beauty discovered in Myanmar in August 2006 is being exported as a tropical aquarium fish. Photographs of it have appeared in numerous websites and Internet forums as “galaxy Rasbora” or “galaxy *Microrasbora*” It is here described as a new genus and species of the subfamily Danioinae. It appears to be most closely related to “*Microrasbora*” *erythromicron* Annandale, 1918, a tiny colourful species endemic to Inle Lake (Salween basin, central Myanmar). The fish lives in small heavily vegetated ponds apparently maintained by seepage in hilly grassland at an elevation of about 1,040 m (about 3,420 feet) near Hopong town, 30 km east of Taunggyi. This is in the Salween basin, about 70–80 km northeast of Inle Lake but not in the Inle drainage. It is apparently near the watershed or divide between two moderately large Salween tributaries, the northward flowing Nam Lang and the southward flowing Nam Pawn. The extraordinary worldwide demand for this fish and its intensive exploitation are cause for concern about the welfare of the species.

MATERIAL AND METHODS

Most of the specimens of the new fish utilized in this study are aquarium specimens provided by Bangkok aquarist Kamphol Udomritthiruj. The main series of specimens were already dead, dying, or in poor condition when they were preserved in ethyl alcohol. These were eventually supplemented by a small number of live specimens sent from Bangkok to Singapore and facilitated by Patrick Yap. Comparative material of *Microrasbora rubescens* and “*Microrasbora*” *erythromicron* was provided by Kamphol and the Zoological Reference Collection of the Raffles Museum for Biodiversity Research. Observations on colouration and chromatophores were made from photographs of both sexes taken by Kamphol, including those published with this article, and on specimens immediately after preservation in formalin.

Variation in colouration in a series of photographs of a highly-colourful fish such as *Celestichthys margaritatus* new genus, new species, may have as much to do with differences in lighting and photographic processing as to differences in individual colour of fish specimens. Individual variation in colouration of course may be recorded photographically, but

this should be done by photographing samples of several samples simultaneously under as near-identical conditions as possible. Even so it will be difficult to eliminate differences in colouration due to intensity or angle of the light source(s) and differences in chemical or electronic processing. The photographs published here represent the natural life colours of the species.

Observations of the jaws of *Celestichthys* and *Microrasbora* were made on specimens cleared and stained with alizarin. Vertebral counts were obtained from radiographs. Abdominal vertebrae are defined as all of the vertebrae with their hemal processes or spines anterior to the first elongate anal fin pterygiophores; this is actually the second anal fin pterygiophore (the first pterygiophore is very short). The rest of the vertebrae are post-abdominal (with hypural half centrum counted as one). The first elongate anal invariably extends anteriorly very clearly in front of the hemal process of the first post-abdominal vertebra as thus defined. Abdominal vertebra include the first four vertebra associated with the Weberian apparatus. Individual centra of the first four vertebrae are sometimes clearly distinguishable in good radiographs but more often not. Thus the vertebral count generally is made beginning with the first centrum bearing a normally formed pleural rib. In Danioninae, as in cyprinoids generally, this is vertebra 4 (Grande & Young, 2004). Observations on the Weberian apparatus and some other osteological features also were obtained from radiographs. Attempts to counterstain specimens of *Celestichthys*, *Microrasbora rubescens* and "*Microrasbora*" *erythromicron* with alcian blue and alizarin were uneven and otherwise unsatisfactory and therefore extensive osteological comparisons are not provided here.

Four males and two females 15.6–17.1 mm were prepared for scanning electron microscopy. The specimens were fixed in 10% formalin for several days then dehydrated for three days by passing them from distilled water through a series of increasing concentrations of ethyl alcohol beginning at 30% and ending with three changes of absolute alcohol over three days. After dehydration the specimens were dried in a critical point drier, mounted with silver paint on stubs, gold plated with an ion sputterer and carbon coated with a vacuum evaporator. Viewing was done with a JEOL JSM T220A scanning electron microscope.

The following institutional abbreviations are used: ZRC, Zoological Reference Collection, Raffles Museum for Biodiversity Research, National University of Singapore; USNM, Division of Fishes, Smithsonian Institution, Washington DC; and CAS, California Academy of Sciences, San Francisco. Lengths of specimens are standard lengths expressed in millimetres (mm).

Celestichthys, new genus

Type species. – *Celestichthys margaritatus*, new species, by monotypy.

Diagnosis. – *Celestichthys* differs from all previously-known Asian Cyprinidae in its distinctive head and body shape, small upturned mouth with shortened jaws, unique colouration, and 9/8 principal caudal fin rays (whereas *Microrasbora rubescens*, type species of the genus *Microrasbora*, has 10/9, the usual count for the family Cyprinidae; and "*Microrasbora*" *erythromicron* has 9/9). It is further distinguished by caudal fin moderately rather than deeply forked; pharyngeal teeth conical, three-rowed; scales in lateral series 25–26; and body without lateral line canal (no scales with lateral line pores).

In size and shape of head, jaws, body and fins it is most similar to another diminutive and highly colourful cyprinid, "*Microrasbora*" *erythromicron* Annandale, 1918, endemic to Inle Lake. That species, which may represent the same or another new genus, has entirely different colouration and 9/9 principal caudal fin rays. All other Asian cyprinids so far as known, including *Microrasbora rubescens* Annandale, 1918 (type species of *Microrasbora* Annandale, 1918) have 10/9 principal caudal fin rays except for *Danionella translucida* Roberts, 1986, and *D. mirifica* Britz, 2003, and "*Microrasbora*" *erythromicron* with 9/9 and *Paedocypris* Kottelat, Britz, Tan & Witte, 2005, with only 8/8. While they also are danioins, *Danionella* and *Paedocypris* apparently are not closely related to the new genus and species. They are relatively slender fish, even smaller than *Celestichthys*, but with increased numbers of post-abdominal vertebrae and elongate lower jaw.

A 24.8 mm *Microrasbora rubescens* has numerous fine tubercles of about equal size in three or four rows extending the length of the ventrolateral margin of the mandibles. The mandible in *Celestichthys* apparently is non-tuberculate. The anal fin of *M. rubescens*, with $ii11\frac{1}{2}$ rays, has a distinctly concave posterior margin, giving it almost a falcate shape. This differs from the anal fin of *Celestichthys*, which has a convex margin and only $ii8\frac{1}{2}$ – $9\frac{1}{2}$ rays. The caudal peduncle, similar in both sexes, is much more elongate than in *Celestichthys*. In these respects, "*Microrasbora*" *erythromicron* is again like *Celestichthys*.

Celestichthys margaritatus new species

(Figs. 1–3)

Material examined. – Holotype: male, 21.2 mm (ZRC 50706), Myanmar, isolated small, heavily-vegetated pools at the foot of a mountain near Hopong town 30 km east of Taunggyi, elevation 1,040 m (about 3,420 feet), coll. Kyaw Toe, Oct.2006.

Paratypes: Female, 20.5 mm (ZRC 50707), same collection data as holotype; 303 specimens: 14.0–19.6 mm (ZRC 50708), same collection data as holotype; 40 specimens: 14.1–19.3 mm (CAS 224434), same collection data as holotype; 40 specimens: 13.9–20.5 mm (USNM 388753), same collection data as holotype.

Description. – Dorsal fin rays $ii7\frac{1}{2}$ – $8\frac{1}{2}$, anal fin rays $iii8\frac{1}{2}$ – $10\frac{1}{2}$, caudal fin rays v – $i9/8v$, pectoral fin rays 11–12, pelvic fin rays 7. Trailing edge of second dorsal fin ray smooth, without serrations. Dorsal and anal fins with convex

margins. Caudal fin moderately forked; upper and lower lobes nearly equal, broadly rounded. Danioins have branched dorsal fin ray counts of 6–7 and 8–16, with no previously-described species spanning the interval 7–8 (Fang, 2003: 722). Dorsal fin origin far anterior to a vertical drawn through anal fin origin and distinctly posterior to a vertical line drawn through pelvic fin origin.

Scales in lateral series 24–25. No scales with lateral line pores. Dorsum scale count from head to dorsal fin origin 14–15 (first 3–4 scales bilateral, rest dorsomedian). Scale rows between dorsal and anal fin origin 7. Circumpeduncular scales 8. Scaly sheath of anal fin with a single row of 5–6 scales. The anterior and posterior circuli and the radii are similar in *M. rubescens*, "*M.*" *erythromicron* and *Celestichthys*, but in the latter two the shape of the scale is vertically more elongate than in *M. rubescens* (Fig. 2).

Gill rakers absent. Pharyngeal arches small but stout. Pharyngeal teeth in three rows with 2/3/5 teeth, as in many

Asian Cyprinidae, including *M. rubescens* and "*M.*" *erythromicron*. The teeth are conical.

Head rounded, its length 3.9–4.1 times in standard length. Snout very short, its length only one-half of eye diameter. Eye large, in anterior half of head. Horizontal eye diameter 10 times in standard length. Barbels absent (as in most Danioinae).

Mouth very small, jaws entirely in front of orbit (Fig. 3). When mouth is closed jaws are oriented at an angle of 45° upward. See discussion for remarks on "danioin notch."

Nostrils normally developed, anterior nostril with low rounded rim from the posterior margin of which arises the nasal flap (Fig. 2a). Area surrounding posterior nostril and immediately posterior to it largely covered with exposed elongate cilia (apparently olfactory cilia) (Fig. 2c-d). Such cilia are usually found only on the lamellae of the olfactory rosette inside the nasal capsule (largely covered over by the nostrils and nasal flap).

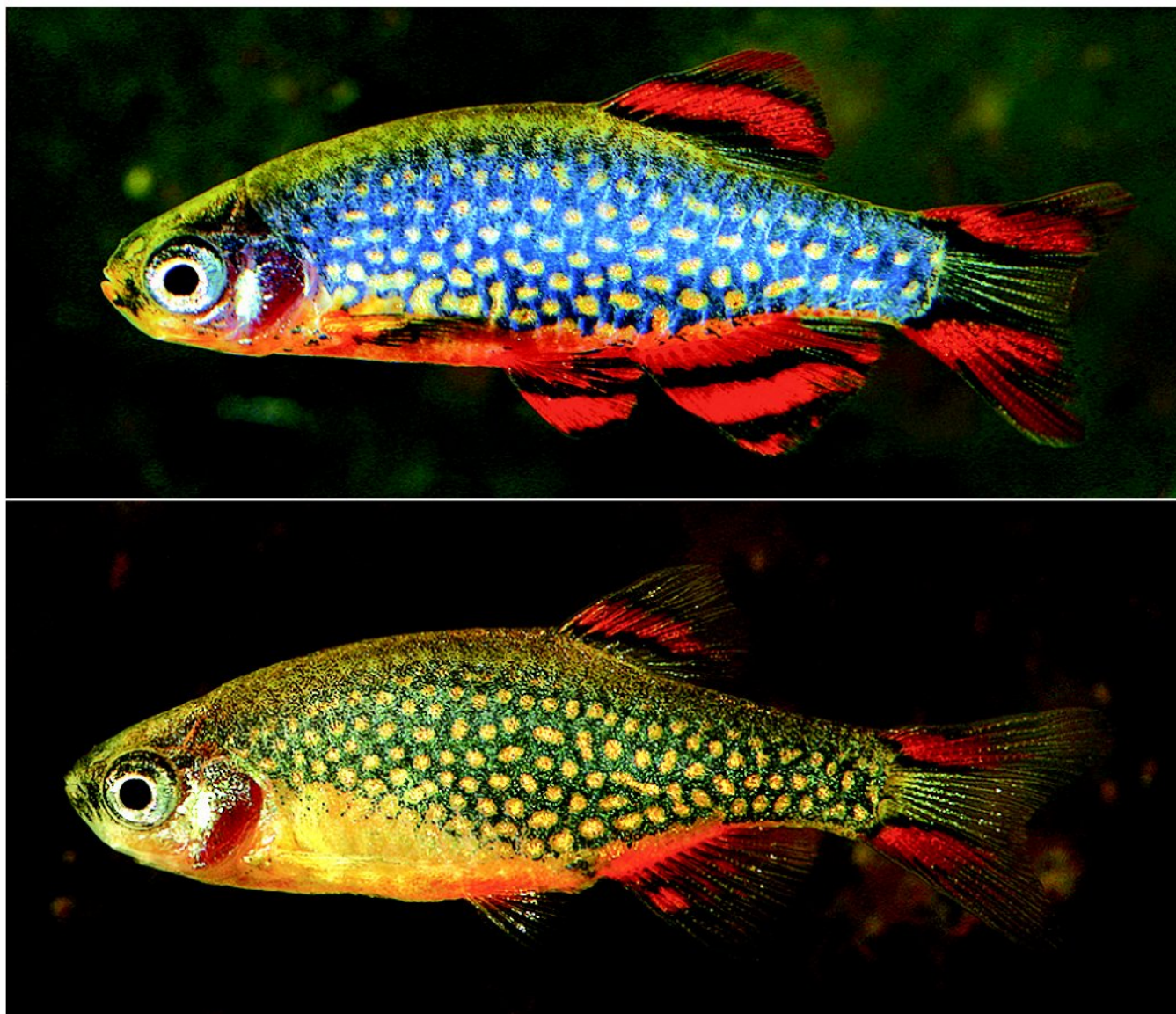


Fig. 1. *Celestichthys margaritatus*, new species. Above: holotype, 21.2 mm mature male (ZRC 50706); below: paratype, 20.5 mm mature female (ZRC 50707). Images: Kamphol Udomritthiruj.

Body deep and strongly compressed, much more so than in *M. rubescens* but similar to "*M.*" *erythromicron*. Greatest body depth (just anterior to dorsal fin) 3.0–3.1 times in SL. Caudal peduncle sexually dimorphic, length 4.8–5.2 times in SL in both sexes, height 6.5 times in SL in males but 7.3 times in SL in females.

Total vertebrae 30–32, with a strong mode at 31 (N=40). Frequencies 30(7), 31(26), 32(7). Abdominal and post-abdominal vertebrae often equal or nearly equal in number, with a modal count of 15+16 (n=13). Counts of abdominal vertebrae range from 13 to 16 and of post-abdominal vertebrae from 15 to 17. Post-abdominal vertebrae more numerous than abdominal by 2–3; abdominal vertebrae more numerous than post-abdominal by 1–2 in several specimens.

Tuberculation. – Multicellular keratinous tubercles occur on the head, jaws, dorsal surface of the paired fins and elsewhere on many cyprinids (especially bottom dwellers). Tubercles occur on the head and especially on the jaws including the mandibular flap ventrolateral margin of the mandibles in

many Danioinae. These may occur in both sexes (e.g. in *Danio rerio*), or only in males (e.g. in *Sundadanio axelrodi*) (Roberts, 1989: 69-70, Fig. 48). Tubercles have not been observed on the head or elsewhere in either sex of *Celestichthys margaritatus*. No tubercles were found on the mandibular flap any of the male or female specimens examined with SEM (Fig. 2b). Taste buds are often present in the sites occupied by tubercles in larger danioins and other cyprinids (Fig. 2c).

Unculi. – Most Cyprinidae (probably including many Danioinae) have unicellular keratinous projections or unculi on the ventral surfaces of the paired fins and on the lips and/or horny sheaths of both jaws. Unculi also often project from the surfaces of cyprinid multicellular tubercles. No unculi were found on any of the male or female specimens of *C. margaritatus* examined with SEM.

Eggs. – Dissection of several 18 to 19 mm females revealed eggs of different size classes. The largest eggs are 1.0–1.3 mm in diameter, slightly smaller than the pearl spots on the

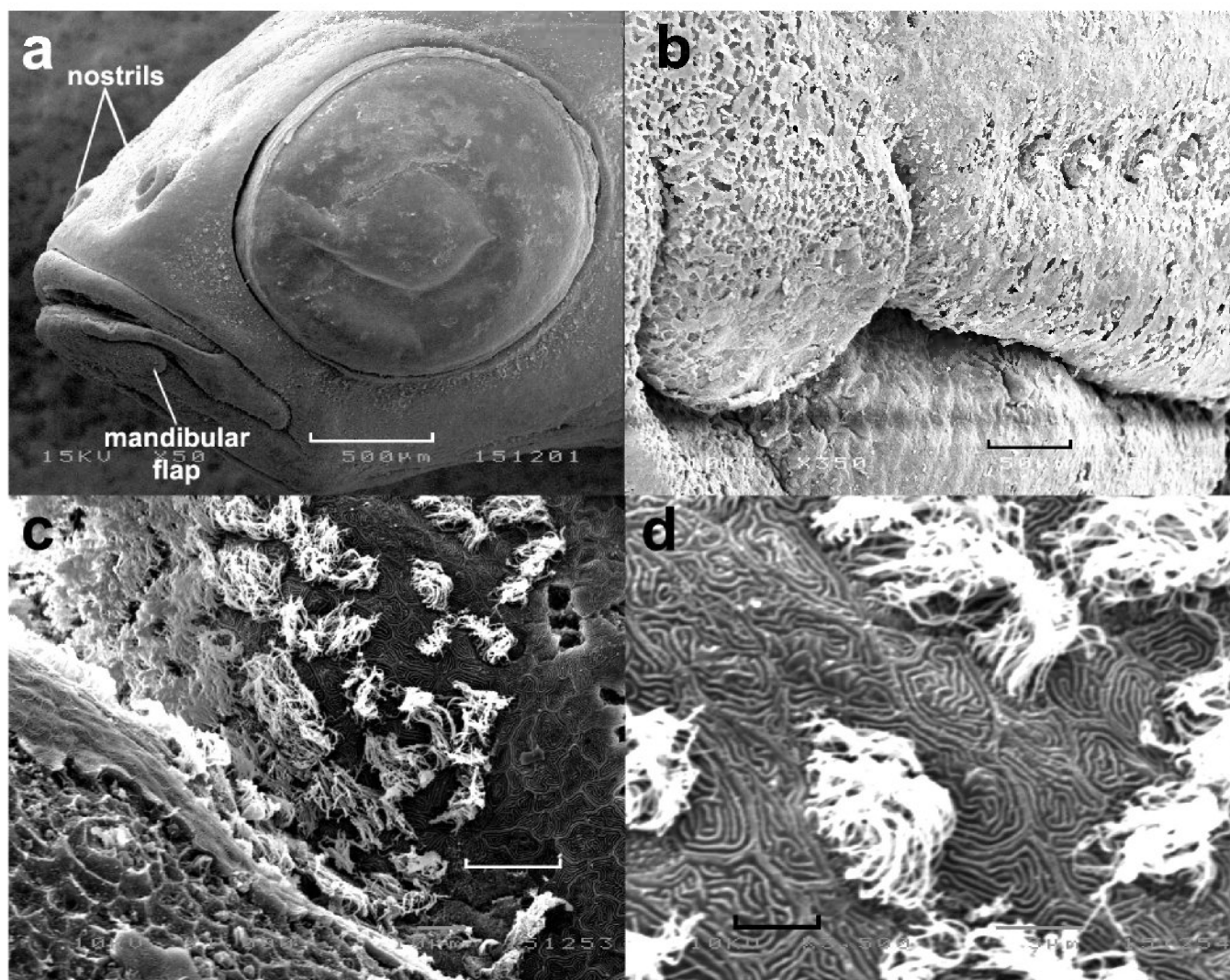


Fig. 2. *Celestichthys margaritatus*, male, 16.4 mm (scanning electron micrographs): a, head in side view showing nostrils, eye, and jaws (scale bar = 500 µm); b, left half of lower jaw with mandibular flap; c, row of taste buds on lower jaw (scale bar = 50 µm) (similar taste buds present on lips, barbels, and widely scattered on the head); c and d, exposed olfactory cilia in shallow depression posterior to nostrils (scale bars = 20 µm and 5 µm).

flanks of the fish. The number of eggs of the largest size is very few, about a half-dozen. If this is typical of wild fish in spawning condition, it indicates that spawning events are partial and sporadic or partial, with very few eggs fertilized at a time, and reproductive activity extends over a long period of time, perhaps throughout the year.

Vertebral column. – As elsewhere in the skeleton, stress on the vertebral column apparently results in significantly higher levels of calcification, which show up in radiographs as brightened areas. As usual in cyprinids, the anterior half of the centrum of each abdominal vertebra where it articulates with the ribs is particularly well mineralized (pers. obs.). Of more interest is that the centrum of the third post-abdominal vertebrae is often strongly calcified. This sample of 40 specimens includes six in which the radiograph shows that the third abdominal vertebrae is much denser than the rest of the vertebrae. One specimen has the posterior half of the centrum of the second abdominal vertebra denser, and one has the centrum of the seventh abdominal vertebra denser. None of the 40 specimens have the centrum of any other vertebrae similarly affected. Similarly modified post-abdominal vertebrae at about the same position occur in specimens examined of “*M.*” *erythromicron* but at lower frequencies. For vertebral counts in the latter two taxa see Discussion.

Colouration. – This new species is distinguishable from all other known species by its extraordinary colouration. Most of the body is deep blue, steely blue or greenish blue with numerous white, cream-coloured, pearly pink, or golden-iridescent oval spots. The dorsum, covered by the dorso-medial scale row and the first scale row immediately below it on either side, is bronze or greenish from the head to the end of the body. The ventrum, covered by the ventro-medial scale row and one or two scale rows on either side of it, is rosy or reddish from the head to the end of the body. In males, the white-spotted bluish colour of the flanks covers the sides

of the anterior part of the abdomen. In females, this part of the external body wall is white or cream. The dorsal, anal, and caudal fins in both sexes, and also the pelvic and to a lesser degree the pectoral fins of the males, have broad cinnabar red and black slashes. See Discussion for comparisons of colouration in other danioins.

Variation in the disposition of the spots, especially in somewhat smaller fish, indicates that they develop from (and therefore presumably evolved from) continuous or nearly-continuous longitudinal pale stripes of the sort seen in many danioins. In some of these danioins, the longitudinal stripes occasionally break up into smaller units suggestive of the numerous small oval spots in *Celestichthys* (but far fewer).

In reproductively active males the abdomen reddens. The dorsum of males has a pale stripe, and the prominence of this is enhanced when males are showing courtship colours. It is reminiscent of the “blaze” shown in courting male rainbowfishes, *Melanotaenia* spp., and appears to be used in a similar fashion during the “head-down” display, although whereas in *Melanotaenia* it is the “blaze” which becomes stronger in appearance, in the *C. margaritatus* it is the flanks which darken and therefore increase the contrast with this area (Peter Liptrot, pers. comm.). Even small males show intense breeding colour and court females.

Sexual dimorphism. – In addition to the differences in colouration, fin shapes, and caudal peduncle depth mentioned in the species description above, adult males have a darkly-pigmented “mandibular pad” with a rounded, weakly crenulate distal margin on the anteroventral surface of each mandible (Fig. 3). This apparently is homologous with a similar mandibular structure, sometimes bearing multicellular keratinous tubercles, present in both sexes or in males only of some danioins (e.g., *Danio rerio*, *Sundadanio axelrodi*). The structure is absent or much reduced and unpigmented in female *Celestichthys*. Scanning electron microscopy observations of the mandibular pad in a 16.4 mm male revealed that its free margin is non-tuberculate. Examination of a female revealed that the pad is either absent or considerably reduced.

Etymology. – The generic name *Celestichthys*, gender masculine, is from the Latin *caelestis*, “heavenly”; and Greek *ichthys*, masculine, “fish”. The species or trivial name *margaritatus* is Latin for “adorned with pearls.” Used as a noun in apposition.

DISCUSSION

Relationships

That *Celestichthys*, *M. rubescens* and “*M.*” *erythromicron* belong in Danioinae is indicated by the structure of the lower jaw, the numerous anal fin rays, and perhaps by the presence in all three of sexually dimorphic mandibular pad. The nature of the colouration, including spots evidently derived from longitudinal pale stripes and the essentially barred colouration

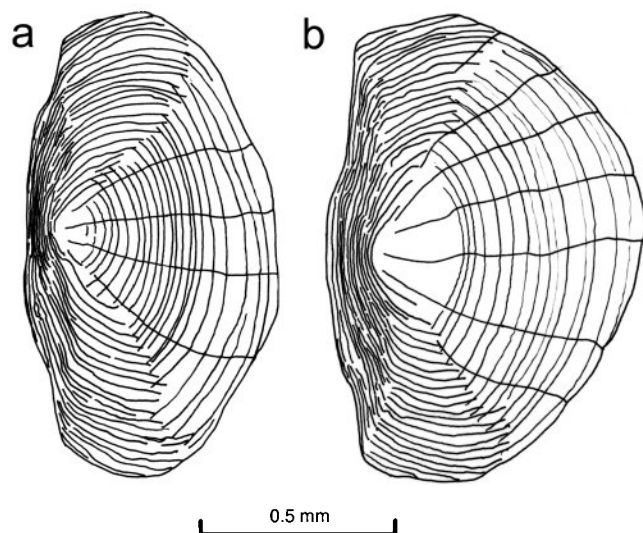


Fig. 3. Scale from mid-body slightly anterior to dorsal-fin origin: a, *Celestichthys margaritatus*, 18.5 mm; b, *Microrasbora rubescens*, 22.8 mm. Scales of “*Microrasbora*” *erythromicron* are similar to those of *C. margaritatus*.

of the dorsal, anal, caudal, and pelvic fins also indicate relationship of *Celestichthys* to Danioinae. Rasborinae, another Asian cyprinid group with numerous small and colourful species, almost invariably have anal fins with iii5 rays. Annandale (1918), in originally describing *Microrasbora*, referred some small colourful Southeast Asian rasborines to the genus. They are now in the genus *Boraras* Kottelat & Vidthayanon, 1993 (Conway, 2005).

The most relevant species of *Microrasbora* for comparison with *Celestichthys* (and also with “*M.*” *erythromicron*) is *M. rubescens* (the type species of *Microrasbora*). Several other small danioin species are currently referred to *Microrasbora* (Kottelat & Witte, 1999). These are *M. gatesi* Herre, 1939 (type locality Hlegu, Irrawaddy basin, lower Myanmar); *M. kubotai* Kottelat & Witte, 1999 (type locality in Ranong Province, Thailand); and *M. nana* Kottelat & Witte, 1999 (type locality Sittang basin, Myanmar). None are very colourful and all differ significantly from *Celestichthys*. All have abdominal vertebral counts substantially fewer than post-abdominal: 13+16=29 in *M. kubotai* (Kottelat & Witte, 1999: 51; 12–13+17–18=29–31 in *M. nana* (op cit.: 52); and *M. gatesi* 13+18=31 (radiograph of holotype, online Primary Types Imagebase, California Academy of Sciences). All apparently have 10/9 principal caudal fin rays (not verified in *M. gatesi*).

The most diminutive rasborines retain the generalized cyprinid count of 10/9 principal caudal fin rays but they tend to reduce the number of pharyngeal tooth rows from three to only one, whereas *Celestichthys* has reduced the number of principal caudal fin rays to 9/8 while retaining (like “*Microrasbora*” *erythromicron*) three rows of pharyngeal teeth. Asian cyprinids with fewer than 10/9 principal caudal fin rays are all diminutive species of Danioinae. In addition to *C. margaritatus*, they include “*Microrasbora*” *erythromicron* with 9/9; *Danionella translucida* and *D.*

mirifica with 9/9 (Roberts, 1986; Britz, 2003); and *Paedocypris* spp. with 8/8 (7/7 branched caudal fin rays) (Kottelat et al., 2005). Like some of the smallest rasborines, *Danionella* and *Paedocypris* have only a single row of pharyngeal teeth.

It is unclear whether the mandible of *C. margaritatus* has a danioin notch. This feature, supposedly diagnostic of Danioinae, was reported as present in “*M.*” *erythromicron* by Kottelat & Witte (1999: 50) but absent in this taxon and in *M. rubescens* by Fang (2003: 723). It might be present but reduced in these taxa and in *Celestichthys* due to the shortening of their jaws. By rotating the dentary it is possible to observe a small but definite concavity near the symphysis that might be the danioin notch, at least in *Celestichthys* and “*M.*” *erythromicron*. These both have a short but stout latero-posteriorly-directed bony process on the side of the mandible just behind the notch or where the notch supposedly would be. This feature, also perhaps diagnostic of danioins, may be termed the “danioin mandibular knob”. It apparently is better developed in males than in females. The premaxillary bone including its ascending process is more slender or elongate something missing here in *Celestichthys* than in *M. rubescens*. In the drawing it appears that the distal end of the maxillary is much more expanded in *Celestichthys* than in *M. rubescens*. This is partly true, but is also due to the expanded part of the maxillary in the latter species being oriented so that it is not viewed in the drawing. In both fishes the expanded end of the maxillary is embedded in a mobile cartilaginous element (not depicted) connected to the coronoid process of the lower jaw.

Microrasbora rubescens reportedly has 15+17=32 vertebrae (Roberts, 1986: 239) (“*M.*” *erythromicron* was reported with 16+16=32 vertebrae in the same paper). The similarity of their vertebral counts might indicate relationship among the three taxa but could also be due to convergence, since the three species are all very small and have vertebral counts near the lower limit for the family Cyprinidae. The following vertebral counts, similar but slightly lower than those in *Celestichthys*, were obtained from preserved aquarium specimens of “*M.*” *erythromicron* (ZRC 46264): 29(5), 30(20), and 31(1). The abdominal+post-abdominal vertebral counts for these specimens are 14+15=29(5); 13+17=30(1); 14+16=30(15), 15+15=30(4); and 14+17=31(1). The predominance of counts of 14+16=30, representing 57.6% of the 26 specimens radiographed, is notable. In *C. margaritatus* the most frequently observed count of 15+16=32 represents 32.5% of the 40 specimens radiographed. The difference in the two predominant counts is that the count for “*M.*” *erythromicron* has one less abdominal vertebrae than the count for *Celestichthys*.

Bold pelvic colouration does occur in other danioins, e.g. *Danio* (formerly *Brachydanio*) *rerio* but boldly-coloured pectoral fins are highly exceptional in Cyprinidae; pectoral fins of *Microrasbora rubescens* and “*Microrasbora*” *erythromicron* are colourless. Adult males and females are sexually dimorphic in ways unusual in Cyprinidae. Males have somewhat larger dorsal, anal, caudal and pelvic fins, as

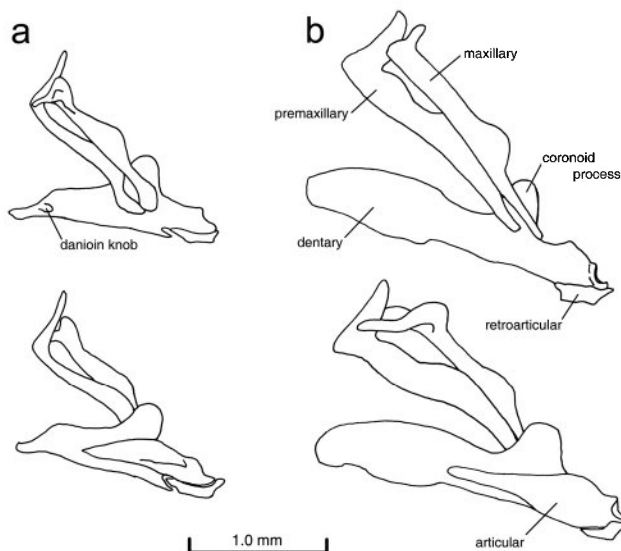


Fig. 4. Jaws in lateral (above) and medial (below) view: a, *Celestichthys margaritatus*, 18.5 mm (male); b, *Microrasbora rubescens*, 22.8 mm (female?). The jaws of “*Microrasbora*” *erythromicron* resemble those of *C. margaritatus*.

well as a distinctly deeper caudal peduncle. Colouration of the females is almost as beautiful as in males, but they have colourless pectoral and pelvic fins; their dorsal, anal and caudal fins, are similarly but less boldly coloured than those of the males. The spots on the flanks of females tend to be somewhat smaller and more numerous. No other cyprinid has body and fin colour like this new species. Colouration of "*Microrasbora*" *erythromicron*, which might be its closest relative, is also striking but quite different. One striking difference is that "*M.*" *erythromicron* has a small round black midpeduncular spot over the caudal fin base, and no black bars on the caudal fin. *Celestichthys* does not have a midpeduncular spot. On the other hand, "*M.*" *erythromicron* sometimes has a solid red slash across the proximal part of the anal fin, similar to the slash of red on the anal fin of *Celestichthys*. The basic background colour on the side of "*M.*" *erythromicron*, a darkish blue, is also similar to that in *Celestichthys*. In "*M.*" *erythromicron*, as in *Celestichthys*, females have similar colouration but are less colourful than males.

Cellular basis of colouration in Danioinae including *Celestichthys* and its nearest relatives

The often bright and highly-contrasting colours in species of the cyprinid subfamily Danioinae is determined by differential distribution of several types of pigmented cells, including melanophores, xanthophores, erythrophores and iridophores (Parichy & Johnson, 2001; Quigley et al., 2004, 2005). These cells originate in the neural crest, the neurectodermal embryonic structure that gives rise to the central nervous system. For background information on the neural crest see Hörstadius (1950), Gans & Northcutt (1983) and Hall (1999).

The different kinds of coloured cells or chromatophores and their relationship to colour patterns have been described in *Danio rerio*, *D. albolineatus*, *D. choprae*, *D. nigrofasciatus* and various hybrids between them (Parichy & Johnson, 2001; Quigley et al., 2004, 2005). Such studies, of interest in understanding how colour patterns evolve, may also provide insight into the phylogenetic history of Danioinae.

The following observations concern colouration of *Microrasbora rubescens*, "*Microrasbora*" *erythromicron* and *Celestichthys margaritatus*. They are based on colour photographs or on fresh specimens immediately after preservation in formalin. The differences in the colouration of these taxa, great as they are, can be described in terms of differential numbers and distribution of chromatophores and their interactions with iridophores. The chromatophores other than iridophores can be readily-observed with a dissecting microscope.

Wild *M. rubescens* are colourful: "in life the sides and ventral surface of the head, the caudal, anal, and sometimes the dorsal, fins are orange-scarlet in adults of both sexes. The whole body of the breeding male is suffused with the same colour. A dark mid-lateral streak extending forwards from the base of the tail to the level of the anterior border of the dorsal fin or further is often conspicuous. Sometimes it expands into a well-

defined spot at the base of the caudal" (Annandale, 1918: 50-51). The relatively pallid live specimens of *M. rubescens* reported on here (aquarium fish supposedly from Inle Lake) are silvery overall, with a thin red axial streak on the midline posteriorly, and with faint red colouration on the opercle and body. The fins are largely colourless or without an evident colour pattern. The overall colouration of these fish resembles that of a much larger species, *D. albolineatus*, and also *D. aff. albolineatus* described and illustrated by Quigley et al. (2004); perhaps these *M. rubescens* are conspecific with their *D. aff. albofasciatus*.

Examination of fish freshly fixed in formalin reveals only two kinds of pigment-bearing cells on the body and fins, melanophores and erythrophores. Given the extensive silvery sheen on the fish, iridophores presumably are present although they were not individually observed. Melanophores predominate on the dorsal, anal and caudal fins, and on the dorsal half of the body. They are particularly numerous on the dorsal half of the body, where they are closely packed in a broad arc paralleling the posterior (free) margin of the scales. Hence, as in so many fishes, the distribution of melanophores reflects the pattern of squamation, at least on the dorsal half of the body. In the dorsal, anal and caudal fins, melanophores occur mostly along the fins rays or on the fin membrane immediately behind or below each fin ray. Erythrophores occur interspersed among the melanophores, but are fewer in number and less widely distributed, on the dorsal half of the body, and by themselves or among the very few melanophores on the ventral half of the body. Unlike the melanophores, they are generally distributed, never clustered parallel to the scale margins. They also extend onto the caudal and anal fins, where their distribution is complementary to that of the melanophores. The melanophores on the caudal and anal fins occur mainly on the inter-radial membranes immediately adjacent to the rays, while the erythrophores occur mainly over the middle of the rays. Erythrophores are particularly abundant on the scale rows overlying the bases of the caudal and anal fins. While they extend the full length of the caudal fin, on the anal fin they extend only a very short distance beyond the anal fin scale sheath. The information available on colouration of live *M. rubescens* may be summarized as follows. Overall colouration of the body and fins tends to be patternless and uniform, either predominantly silvery or reddish. The chromatophores involved are melanophores, erythrophores and presumably iridophores.

In *Celestichthys*, there again seem to be two main kinds of chromatophores, melanophores and erythrophores. These are more numerous, somewhat larger, and therefore much more densely packed on many parts of the body and fins than in *M. rubescens*. The large bluish area on the sides has numerous closely packed melanophores. A few of these lie superficially to the scales, apparently in the epidermis, but the great majority are below the scales, presumably in the dermis. The dark areas on the sides of the flanks (bluish in life) are devoid of erythrophores. The round white spots have almost no chromatophores except for a very small number of melanophores, as well as somewhat more numerous erythrophores very low on the body near the reddened ventral

surface. Since they are often highly reflective, they presumably do have iridophores. The dorsal, anal and caudal fins in both sexes, and the pelvic and, to a less extent the, pectoral fins of the males, have broad red areas in which the only chromatophores are exceedingly numerous and very tightly packed erythrophores. There are also black areas in which the only chromatophores are melanophores, which are not so numerous or densely packed. Rather than being continuously distributed, they tend to be most numerous on the inter-radial membranes adjacent to the fin rays. The separation of erythrophore- and melanophore-dominated areas on the fins is nearly total. Erythrophore-dominated areas also occur on the body, particularly on the ventral surface in those individuals with reddish colouration ventrally.

In addition to melanophores and erythrophores, *Celestichthys* has smaller numbers of what appear to be pigment cells with white pigment or leucophores. These apparently are not iridophores because they do not gleam; viewed with transmitted light they are opaque. These are of about the same size as the melanophores and erythrophores. They are limited to areas dominated by erythrophores on the dorsal fin and on the upper and lower lobe of the caudal fin. Although the anal fin has similar red and black slashes of colour, it lacks leucophores. They are present in males and females, but are more abundant in the males.

Colouration of live “*M.*” *erythromicron* (Fig. 5) differs radically from that of *M. rubescens* and *C. margaritatus*. This is one reason for not placing this distinctive species in *Celestichthys*. A round black or dark spot due to a concentration of melanophores straddles the distal part of the middle of the hypural fan and the insertions of the middle caudal fin rays. Similar peduncular spots occur in many rasborines, in small adults of many species of *Systemus* and other small barbines and in small juveniles of large species of barbines, but are otherwise unknown in Danioinae. The peduncular spot of “*M.*” *erythromicron* perhaps is unique in being highlighted by a surrounding pale area or halo; whether this is due to iridophores or leucophores or to a combination of the two cell types is unknown. The alternating bluish and reddish vertical bars on the sides of the body also are quite unlike the plain reddish colouration of *M. rubescens* or the pearly white spots on a blue background on the sides of *C. margaritatus*.

Yet “*M.*” *erythromicron* and *C. margaritatus* perhaps do share one or two colour features indicative of a close relationship between them. While the pectoral fin of “*M.*” *erythromicron* is colourless or only very faintly coloured, the basal half of its pelvic and anal fins have a broad red swaths of solid red colour due to dense concentrations of erythrophores in the absence of any other chromatophores, as in red areas on these fins in *C. margaritatus*. Fainter red colouration due to erythrophores is present on the basal half of the dorsal and caudal fins. The gill covers, opaque silvery in *M. rubescens*, are transparent in “*M.*” *erythromicron* and *C. margaritatus*, while the gills showing through them are purplish red.

Ecology

The pond that is the type locality of the celestial pearl danio (Fig. 5) and the other ponds in which it has been found are in a rapidly developing area with the natural vegetation largely cleared away, extensive road construction and housing estates. The ponds are small and shallow, at most perhaps 30 cm deep, and maintained by seepage or springs. The water is clear unless roiled with recorded water temperatures of 22–24°C in January 2007. The present terrestrial vegetation is open grassland. The ponds have luxuriant submerged aquatic vegetation mainly of one or two species similar to *Elodea* or *Anacharis* (Hydrocharitaceae). Only three other fish species have been collected in the ponds: a *Microrashora*, similar to and perhaps the same species as *M. rubescens*; a small species of the nemacheiline loach genus *Yunnanilus*; and a piscivorous snakehead, *Channa harbourbutleri* (Annandale, 1918).

The number of ponds and their distribution is not known. Collectively they may be numerous and occupy a wide area, but individually they are apparently quite small and shallow, since fish collectors reportedly fish out one pond after another. Depending on the number and extent of the ponds and their accessibility, such intensive collecting and the ecological disturbance accompanying it could constitute a serious threat to *Celestichthys margaritatus* and *Yunnanilus* sp. unless they are more widely-distributed than known at this time. It may well be that both species have reproductive biology such that their numbers can be rapidly recuperated when they reinvade ponds that temporarily dry up and then refill. They might not adjust so readily to simultaneous exploitation of all of the ponds they inhabit.

Behaviour

Unlike most other danioins including *Devario*, *Danio* and *Microrashora* that quickly and constantly swim in open water often in response to each other’s movements, *Celestichthys margaritatus* have individually deliberate swimming movements. Typically staying stationary in shelter or focusing on food, they tend to hover obliquely, slightly upwards or downwards. When in motion they exhibit a darting



Fig. 5. Type locality and typical habitat of *Celestichthys margaritatus* near Hopong, Myanmar, Photo: Kamphol Udomritthiruj, Jan.2007.

movement, whether to food items or to exhibit aggression towards conspecifics, often “flaring” or rapidly erecting all of the fins at males competing for territory or food. Fin nipping of smaller males by larger or “alpha” males is commonly observed in small aquaria. When not breeding, both sexes will mix, with perhaps a slight tendency for individuals to associate more closely with others of their own gender. In all or most of these respects behaviour of “*Microrasbora*” *erythromicron* is similar to that of *C. margaritatus* than to that of other danioins.

Breeding in captivity

Aquarists associated with the Bolton Aquarium in Bolton, Lancashire, England have succeeded in breeding *Celestichthys margaritatus* (Clarke, 2006; Peter Liptrot and Paul Dixon, pers. comm.).

The following account of reproductive behaviour, received from Peter Liptrot on 6 January 2006 (pers. comm.), is quoted nearly verbatim: *Celestichthys margaritatus* do not appear to be true broadcast scatterers, but neither are they depositors such as *Trigonostigma* or *Sawbwa*. In our aquaria they spawn within either natural plants such as ‘Java Moss’ [provisional scientific identification *Taxiphyllum barbieri* (Card. & Coppey, 1991) (Iwatsuki, 1982); see Tan et al., 2005] or artificial media (woollen spawning ‘mops’). Ripe females have a spot of dark pigment at the vent, and when selecting fish for controlled spawning we have used this characteristic with some success for indicating readiness to breed. Although we have noticed chasing occurring, whereby 1–3 males will pursue a ripe female over distances of up to 45 cm, this hasn’t been seen to result in actual spawning. This takes place when individual males take up a display posture within 2–3 cm of the chosen site. Remaining stationary with its head down, body slightly curved, fins extended and quivering, the male waits for a female to approach. Once this occurs a very short chase ensues and the pair then push their way into the spawning substrate. If this has been observed by other males,

they will follow the pair in, but due to problems of observation within the spawning media it is unclear whether they are trying to fertilize some of the deposited eggs or feeding on them. The maximum egg production from a single female has been around 30 eggs per reproductive episode. The delay before a female is ready to spawn again has not been determined. The eggs take three to four days to hatch at around 76°F (24.4°C), and the larvae another four days to be fully free-swimming (we consider this to be unusually long for such a small cyprinid). During the first three days post-hatching, the larvae have significant dark pigmentation and adhere to solid surfaces by means of one or more adhesive structures on the head. At this stage they are very difficult to observe if there is any detritus in the aquarium. From the fourth day onwards the dark larval pigmentation is lost and they become free-swimming, and can be seen to start exogenous feeding on cultured micro-organisms. From here on husbandry is simple: the fish grow quickly and are undemanding. At around eight to ten weeks body shape changes noticeably to resemble that of the adults. Adult colour pattern starts showing at about 12 weeks.

Danioin colour patterns

There is a substantial and rapidly growing literature on developmental biology, genetics and phylogenetic implications of danioin colour patterns (Parichy & Johnson, 2001; Quigley et al., 2004, 2005). Most of the material reported upon in this article comes directly or indirectly from the aquarium trade, so is without locality data. This is unfortunate, as it means essential information is lacking and that the fish have not had access to their natural food for a more or less extended period. In any event, these studies show how closely-related species could have very different colouration. Thus, for example, it is theoretically possible for “*M.*” *erythromicron* and *C. margaritatus* to be very closely related despite the superficially great difference in their colouration. Although their overall colouration differs radically, it consists of colour traits occurring more or less widely in other danioins. Thus, the boldly striped fins of *Celestichthys* resemble those of *Danio rerio*, while the iridophore spots on its flank are similar to those in *D. dangila*.

Generic placement of “*M.*” *erythromicron*

Generic placement of “*M.*” *erythromicron* is momentarily left unresolved pending comparative studies of osteology and/or DNA to resolve relationships. Based on the evidence now available it seems likely that the species eventually will be placed in *Celestichthys*. This step is not taken here for the following reason. The highly distinctive species “*M.*” *erythromicron* described from Inle Lake and known only from there, presumably is endemic to the lake. Its closest-known relative also described from Inle Lake and for long thought to be endemic to it, supposedly is *M. rubescens*. It seems that reasonable hypothesis is that “*M.*” *erythromicron* evolved in Inle from ancestral stock similar to that of the present *M. rubescens*. *Celestichthys margaritatus* just as clearly evolved outside the lake from ancestral stock that might also have been similar to *M. rubescens*. This highly tentative conclusion



Fig. 6. “*Microrasbora*” *erythromicron*: Above, male, not preserved; below, female, not preserved. Images: Kamphol Udomritthiruj

is supported by the discovery of *M. cf. rubescens* living sympatrically in the small ponds.

If the two species did indeed evolve independently from the same or different species, placing them in *Celestichthys* would make the genus polyphyletic. This situation could change in the not unlikely event that additional *Celestichthys*-like species of Danioninae be recognized living outside of Inle Lake.

Captive breeding

Captive breeding of *Celestichthys margaritatus* could ease the pressure from commercial fish collectors on wild stocks and might be the only way for aquarists to have a continued supply of the species.

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