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## The identity of *Pethia punctata*, a senior synonym of *P. muvattupuzhaensis* (Teleostei: Cyprinidae)

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### Abstract

Francis Day described *Pethia punctata* from Cochin, on the Malabar (south western) coast of India. Although, the species is now recovered from its synonymy with *P. ticto*, an accurate diagnosis and description have been lacking. A redescription of *P. punctata* based on external morphology, osteology and genetics is provided, which revealed that *P. muvattupuzhaensis*, described from Muvattupuzha River, Ernakulam District, Kerala, India, is its junior synonym. *Pethia punctata* can be diagnosed from other known species in the genus by a combination of characters including lateral line complete, with 23–25 pored scales; 8 predorsal scales;  $\frac{1}{2}4/1\frac{1}{3}\frac{1}{2}$  scales in transverse line; dorsal fin originating almost opposite to, or slightly before pelvic-fin origin; gill rakers 7 on first ceratobranchial; 4+26 total vertebrae; a small black humeral spot covering anterior half of the fourth scale of the row below the lateral-line row; two minute dark spots below the humeral spot; a prominent spot on the caudal peduncle, surrounded by a golden hoop covering scales 19–21 of the lateral-line row; and dorsal fin with 2–3 longitudinal rows of black spots, third row occupying only anterior portion of the fin.

**Key words:** Kerala, *Puntius*, small barb, taxonomy, Western Ghats

### Introduction

*Pethia punctata* was described as *Puntius punctatus* by Day (1865, p. 302) from Cochin, on the Malabar coast of India. While earlier authors considered *P. punctata* as a synonym or a subspecies of *P. ticto* (Hora *et al.*, 1939; Jayaram 1991; Talwar & Jhingran 1991; Rema Devi 1992), others more recently have treated *P. punctata* as a valid species (Rema Devi *et al.* 1996; Menon *et al.* 2000; Beevi & Ramachandran 2005; Pethiyagoda *et al.* 2012; Katwate *et al.* 2014a, b). However, the recognition of *P. punctata* as a valid species has not until now been accompanied by a detailed description and diagnosis so as to facilitate definitive identification.

Beevi & Ramachandran (2005) described *Puntius muvattupuzhaensis* (now *Pethia muvattupuzhaensis*) as a small, elongate barb from the Muvattupuzha River, Ernakulam District, Kerala, India. The current distribution of the species is known to encompass the lower and middle reaches of the Muvattupuzha River, including the Ithipuzha and Murinjapuzha tributaries, and the lower reaches of Periyar River in Kerala (Beevi & Ramachandran 2005; 2009; Zeena & Beevi 2011). Except for these records, the species has not been documented in the scientific literature. Examination of the type material of *P. muvattupuzhaensis* deposited at the Southern Regional Center of the Zoological Survey of India (SRC-ZSI), Calicut, India, revealed several discrepancies with the original description, while the species showed remarkable similarity to *Pethia punctata*.

Here we provide morphometric, meristic, genetic and osteological evidence to characterize *P. punctata* based on topotypic material. Further, based on the examination of the type material and genetic analysis of fresh topotypic specimens we show that *P. muvattupuzhaensis* is a junior synonym of *P. punctata*.

## Material and methods

**Study sites and sampling.** Topotypes of *P. muvattupuzhaensis* were collected from Muvattupuzha River, Ooramana, Ernakulam District, Kerala, India (9°57'56"N, 76°31'17"E, 19 m a.s.l.). Putative topotypes of *P. punctata* were collected from near Cochin, Kerala. The specimens were fixed in 10% formalin and transferred to 70% ethanol for storage. The type series of *P. muvattupuzhaensis*, comprising of the holotype and four paratypes, available in the fish collection of SRC-ZSI, Calicut, India, were examined.

**External morphology, morphometry and osteology.** Measurements were taken point-to-point to the nearest 0.1 mm using digital calipers. Subunits of the body are presented as percent of standard length (SL), and subunits of the head as percent of head length (HL). Lateral-line pored scales were counted and the same scale row followed up to the base of the caudal fin for the lateral-scale count. Methods for taking counts and measurements follow Kullander (2008) and Pethiyagoda *et al.* (2012). The transverse scale-row count is taken from dorsal-fin origin, forwards and down to the lateral-line scale row, and then backwards and down to the pelvic-fin origin (Fig. 1). Values in parentheses after a count represent the frequency of that count. Topotypes of both, *P. muvattupuzhaensis* (BNHS FWF 114) and *P. punctata* (BNHS FWF 112) were cleared and stained for osteology following the procedure described by Potthoff (1984). Osteological nomenclature follows Conway (2011) and Mattox *et al.* (2013).

**Voucher specimens and museum abbreviations.** Voucher specimens are deposited in the museum collections of the Bombay Natural History Society (BNHS), Mumbai; the Wildlife Information Liaison Development (WILD) Society, Coimbatore; and the Western Regional Center of the Zoological Survey of India (ZSI-WRC), Pune. Other specimens examined are in Southern Regional Center of the Zoological Survey of India (ZSI/WGRC), Calicut, India; the Natural History Museum (BMNH), London, and the Museum of Comparative Zoology (MCZ), Harvard University.

**Statistical analysis.** Statistical analysis of the morphometric data was performed on size-adjusted measurements. The null hypothesis that the data conform to a multivariate normal distribution was tested using Doornik & Hansen (2008) omnibus. MANOVA was performed to understand whether the two nominal species form significantly different clusters (Huberty & Olejnik 2006). Pillay's trace statistic was performed to find the significant difference between the clusters (Harris 2001). Statistical analysis was performed in PAST (Hammer *et al.* 2001).

**Phylogenetic analysis.** Male and female specimens from the topotypic collection of *P. muvattupuzhaensis* and *P. punctata* were used for genetic analysis. Gills were harvested from three fresh specimens (WILD-14-PIS-110, BNSH FWF 120 and BNHS FWF 113) and preserved in absolute ethanol. DNA extraction, PCR amplification for cytochrome b (cytb) gene sequences and sequencing protocols follow Katwate *et al.* (2013). Sequences were analyzed by BLAST tool (Altschul *et al.* 1990). Sequences generated as part of the study are deposited in GenBank (KM364557–59). For phylogenetic analysis, we used the cytb gene sequence database from Katwate *et al.* (2014a). GenBank accession numbers, localities and the voucher numbers for the sequences used in the study are provided in Appendix A. Gene sequences were aligned using MUSCLE (Edgar 2004) and a molecular phylogenetic analysis was performed using the freeware MEGA 6 (Tamura *et al.* 2013). Best fit model for nucleotide substitution was selected from 24 models using MEGA 6 (Tamura *et al.* 2013) based on minimum Bayesian Information Criterion (BIC) value (Schwarz 1978; Nei & Kumar 2000). Pairwise distance between cytb sequences of *P. muvattupuzhaensis* and *P. punctata* were estimated based on the best nucleotide substitution model. Maximum likelihood analysis was performed to understand the relationship between various taxa. Reliability of the phylogenetic tree was estimated using bootstrap values run for 1000 iterations.

## Results and discussion

### *Pethia punctata* (Day, 1865)

*Puntius punctatus* Day, 1865

*Barbus punctatus* (Day, 1865): Day (1873)

*Barbus (Puntius) ticto* (non Hamilton, 1822): Hora *et al.* (1939)

*Puntius ticto* (non Hamilton, 1822): Jayaram (1991), Talwar & Jhingran (1991)

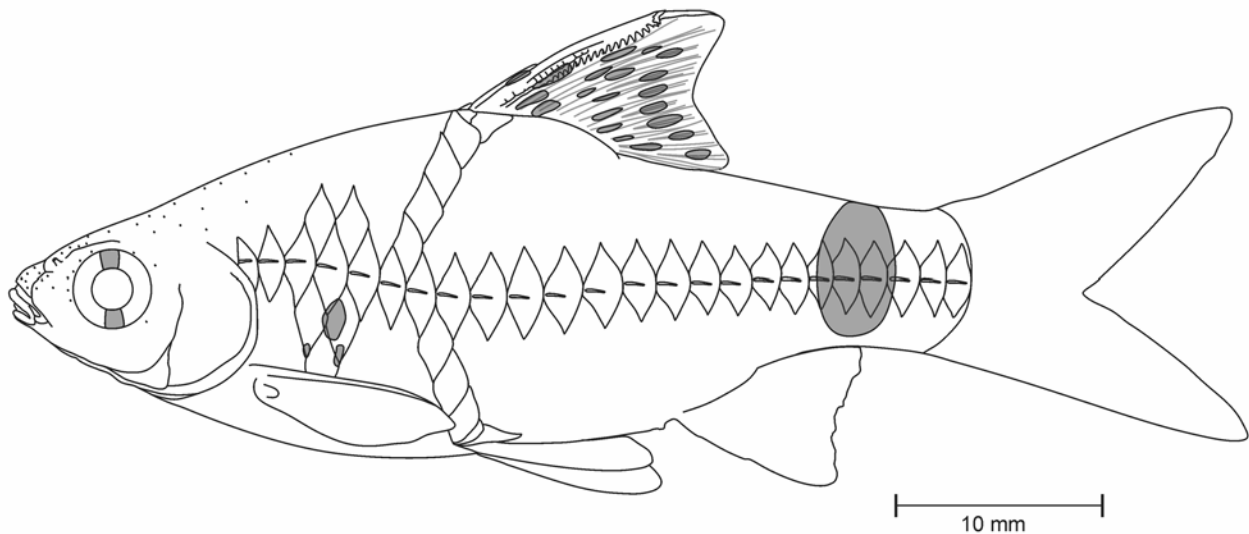
*Puntius ticto punctatus* Day, 1865: Rema Devi (1992)

*Puntius muvattupuzhaensis* Beevi & Ramachandran, 2005

*Pethia muvattupuzhaensis* (Beevi & Ramachandran, 2005): Pethiyagoda *et al.* (2012)

**Material examined:** Day's material (syntype?), 1 ex., MCZ 4303, Canara (Cannanore, on the Malabar Coast, Kerala, India), coll. F. Day (photograph examined); Day's material (syntype?), 1 ex., BMNH 1889.2.1.755, Wayanad, Kerala, India, coll. F. Day (photograph examined); 7 ex., BNHS FWF 107–113, collected from Vembanad Lake, Kerala, India (9°54'35"N, 76°20'34"E, 1–2 m a.s.l.), by Unmesh Katwate and Fabin Baby on 29.v.2014; 4 ex., WILD-14-PIS-111 to 114, collected from Edathua in the rivers flowing into the Vembanad Lake, Kerala, India (9°54'35"N, 76°20'34"E, 1–2 m a.s.l.), by Unmesh Katwate and Fabin Baby on 29.v.2014; 3 ex., ZSI-WRC-P/4092, collected from Edathua in the rivers flowing into the Vembanad Lake, Kerala, India (9°54'35"N, 76°20'34"E, 1–2 m a.s.l.), by Unmesh Katwate and Fabin Baby on 29.v.2014; 3 ex., CRG-SAC-2010.05.01-03, collected from near Cochin/Ernakulam, Kerala, by F. Baby on 18.v.2010; 6 ex. Other material: BNHS FWF 86–90, 92, Bandiwade, Gad River, Sindhudurga District, Maharashtra, collected on 15.ix.2013 by U. Katwate and S. Rane; 1 ex. BNHS FWF 91, collected from Terekhol River at Madkhol, Maharashtra, by U. Katwate, M. Paingankar and N. Dahanukar on 9.viii.2013.

**Diagnosis.** *Pethia punctata* can be distinguished from all other species of *Pethia* by the following combination of characters: barbels absent, lateral line complete, with 23–25 pored scales; predorsal scales 8; scales in transverse line  $\frac{1}{2}4/1/3\frac{1}{2}$ ; dorsal fin originating almost opposite to, or slightly before pelvic-fin origin; gill rakers 7 on first ceratobranchial; 4+26 total vertebrae including posterior most compound centrum; banding pattern includes a small black humeral spot covering anterior half of the fourth scale of the row below the lateral-line row; two minute dark spots below the humeral spot; a prominent spot on the caudal peduncle surrounded by a golden hoop covering scales 19–21 on the lateral-line row; and dorsal fin with 2–3 longitudinal rows of black spots, third row occupying only anterior portion of dorsal fin, extending also to 2 supernumerary and last unbranched fin rays.



**FIGURE 1.** *Pethia punctata*, topotype, BNHS FWF 109, male, 36.0 mm SL, showing distribution of prominent body pigmentation and banding pattern and lateral and transverse scale rows.

**Description.** Photograph of topotype and Day's material (possible syntypes) provided in Figs. 2A, B, C, D. Morphometric and meristic data provided in Table 1.

Body short, deep; compressed laterally; predorsal contour almost straight or slightly convex, rising gradually to dorsal-fin origin, thereafter sloping down towards hypural notch. Ventral profile convex up to posterior end of anal-fin base, sloping gradually towards hypural notch. Caudal peduncle broad, short, its length 1.3–1.6 times its depth.

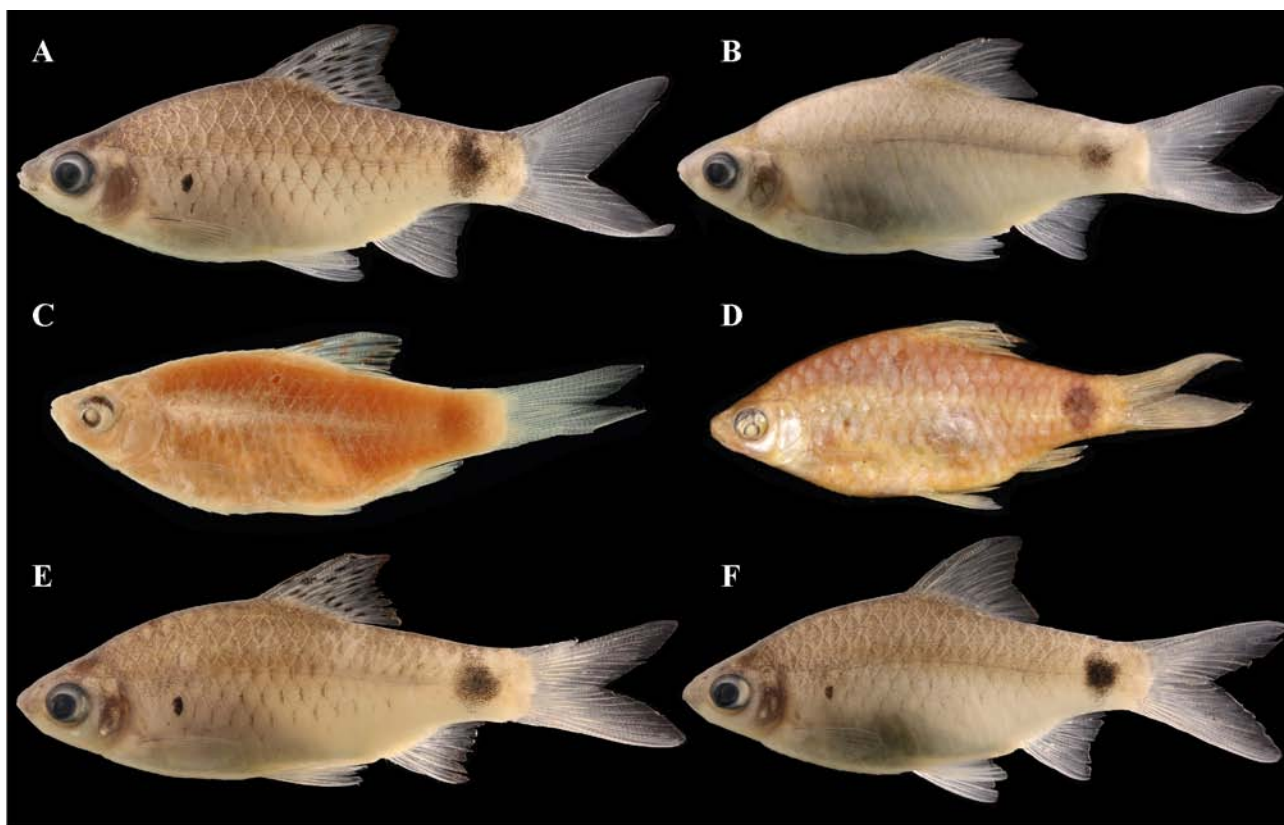
Head small, laterally compressed. Snout round, pointed, smooth, shorter than eye diameter, with a distinct lateral fold and fleshy overhanging upper lip. Eyes large, dorso-laterally positioned, closer to snout tip than margin of operculum, diameter 1.2–1.4 times interorbital width. Mouth small, subterminal, ventrally 'U' shaped, angle of

gape not reaching to vertical from anterior margin of eye. Upper and lower lips thick, fleshy, lower lip posterior groove not interrupted. Barbels absent.

Dorsal fin originating opposite or slightly anterior to pelvic-fin origin, closer to tip of snout than to base of caudal peduncle, its distal margin concave, height greater than head length. Dorsal fin with 3 unbranched (including 2 supernumerary rays) and 8 branched rays, last unbranched ray strong, osseous, densely serrated posteriorly, with 12 (1), 14 (6), 16 (2), 17 (3) or 18 (2) serrae on its distal half, 2 (10) or 3 (4) serrae on its apical half. Pectoral fin with one unbranched and 9 (2) or 10 (12) branched rays, its tip rounded, reaching one or two scales anterior to pelvic-fin origin or sometimes marginally reaching pelvic-fin origin. Pelvic fin with one unbranched and 7 branched rays, its tip rounded, not reaching vent when adpressed. Anal fin with 3 unbranched (including 2 supernumerary rays) and 5 branched rays, its distal margin concave with rounded corners. Caudal fin forked, lobes making more than half of fin length, tips pointed. Principal branched caudal-fin rays dorsally 9 (14), ventrally 8 (14); procurrent rays dorsally 7 (14), ventrally 6 (14).

Lateral line complete with 23 (11), 24 (2) or 25 (1) pored scales in lateral series, which curves gently downwards to base of pelvic fin, then running almost straight to caudal-fin base. Scales in transverse line  $\frac{1}{2}$ 4/1 $\frac{1}{2}$ , predorsal scales 8, prepelvic scales 9 (13) or 10 (1), preanal scales 13 (8) or 14 (6), circumpeduncular scales 12. Pelvic axillary scale present, reaching to  $\frac{1}{4}$ th of adpressed pelvic-fin length.

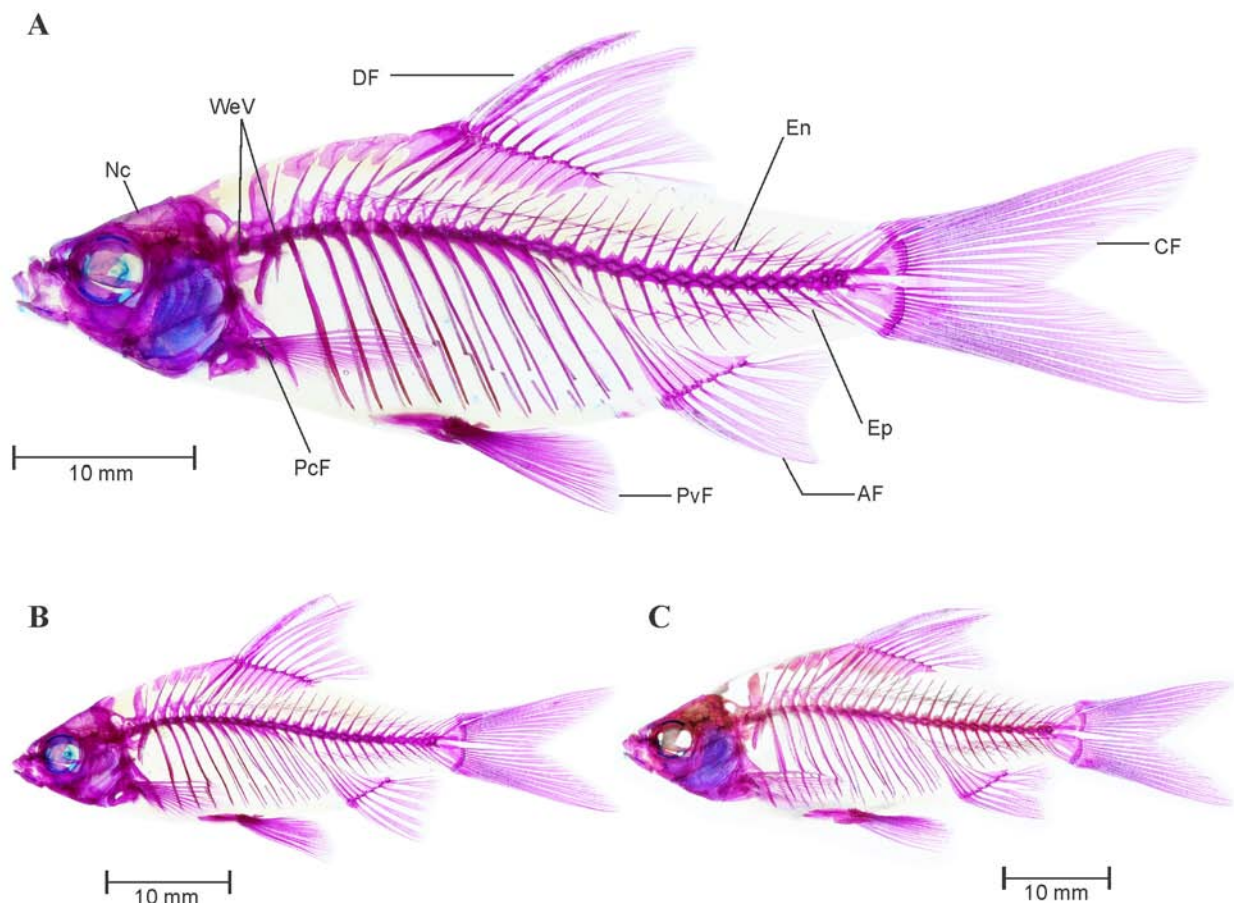
**Osteology.** Complete lateral view of the cleared and stained specimens of *P. punctata* (topotypic male and female) are provided in Figure 3A and C, respectively.



**FIGURE 2.** (A) *Pethia punctata*, topotype, male, BNHS FWF 109, 47.2 mm SL; (B) *Pethia punctata*, topotype, female, ZSI-WRC-accession numbers awaited, 53.7 mm SL; (C) Day's material, MCZ 4303, © President and Fellows of Harvard College, Museum of Comparative Zoology, Harvard University; (D) BMNH 1889.2.1.755 coll. by F. Day; (E) *Pethia muvattupuzhaensis*, topotype, male, BNHS FWF 117, 39.5 mm SL; (F) *Pethia muvattupuzhaensis*, topotype, female, BNHS FWF 119, 41.4 mm SL.

**Neurocranium:** Dorsal, lateral and ventral view of neurocranium provided in Figure 4A–C. In general appearance it is broad and wide at otic region, narrow and slopes dorsally towards ethmoid region. Ethmoid region composed of by mesethmoid, prethmoid, ethmoid, kinethmoid (Figure 4A), vomer and large winged lateral ethmoid. Ethmoid region slopes down anteroventrally, meet triangular mesethmoid, which placed in between lateral wings of ethmoid portion. Lateral ethmoid contributes large portion in ethmoid complex, clearly visible,

lateral processes well developed, tapers and slopes down anterolaterally, covers anterior most portion of orbital section. Prethmoid closely attached with anterior most tips of vomer. Nasals are in form of small bony plate, anterior down to the lateral ethmoid. Frontals large and contributes as largest anterior portion of neurocranium, with clear supraorbital lateral-line canal running along its lateral margin. Parietals large, with a sensory canal running on lateroposterior side forms distal margin of neurocranium. Orbitosphenoid large, distinct, attached dorsally with fontanel and ventrally with dorsovertical flap of parasphenoid. Pterosphenoid pierced, anteriorly attached with distal margin of orbitosphenoid. Parasphenoid long elongated with dorsovertical flap, attaches anteriorly with vomer and cartilaginous trabecula communis, runs posteriorly till otic region. Prootic with large auditory foramen seems to be the largest bone of the ventral surface of cranium, attached posterolaterally with anterior most part of the subtemporal fossa. Autopterotic forms posterolateral corner of the neurocranium, ventrally articulates with hyomandibular. On the ventral side of cranium, autopterotic forms the posterolateral wall of the deep subtemporal fossa. Supraoccipital attaches with the parietal on the anterior side, dorsally concave, spine well developed but does not supersedes weberian complex. Exoccipital large, well ossified, forms posterior wall of the otic capsule and the posterodorsal part of the otic bulla. Basioccipital large covers ventral portion of the otic bulla, and the articulation between the neurocranium and the first vertebra, it is a posteroventral most point of the occipital region. Basioccipital process well developed, elongated with round concave masticatory plate on the anterior side and it reaches half of weberian complex ventrally.

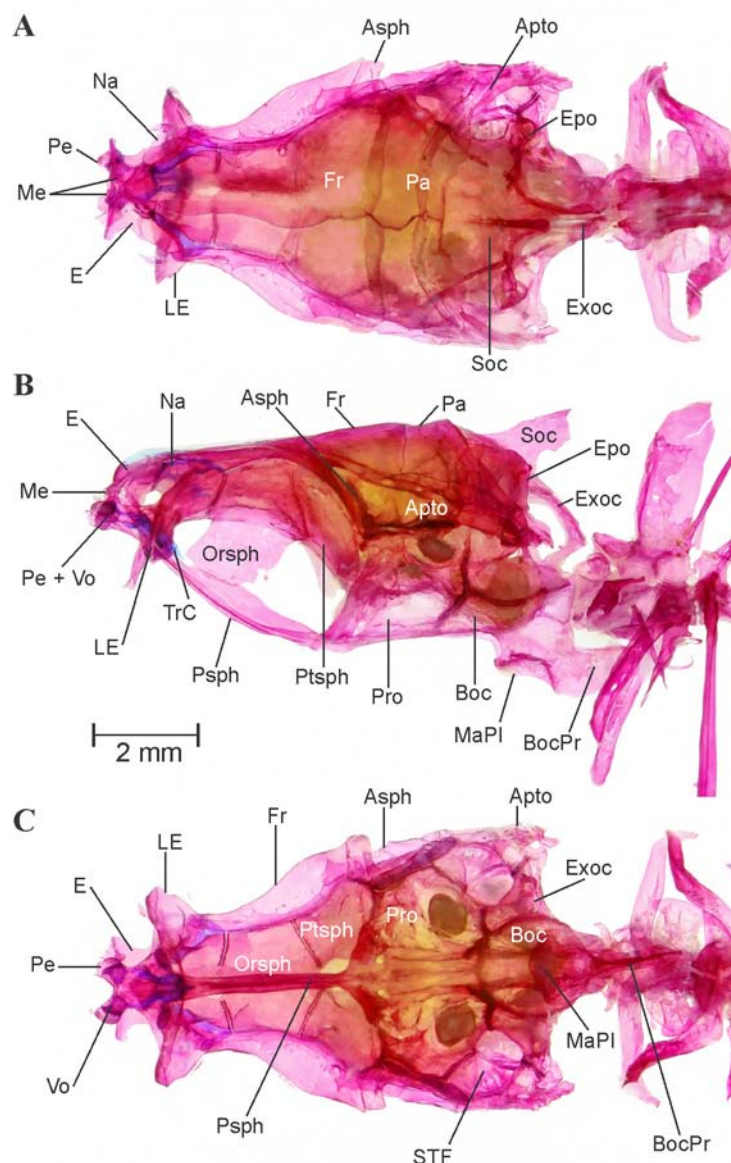


**FIGURE 3.** Lateral view of entire skeleton. (A) *Pethia punctata*, topotype, male, BNHS FWF 112, 56.1 mm SL; (B) *Pethia muvattupuzhaensis*, topotype, male, BNHS FWF 114, 40.7 mm SL; (C) *Pethia punctata*, topotype, female, BNHS FWF 111, 48.5 mm SL. AF, anal fin; CF, caudal fin; DF, dorsal fin; En, epineural intermusculars; Ep, epipleural intermusculars; Nc, neurocranium; PcF, pectoral fin; PvF, pelvic fin; WeV, Weberian vertebrae.

**Hyopalatine arch and opercular series:** See Figure 5A. The dorsal edge of the hyomandibular articulates with the autosphenotic, the autopterotic, and the prootic cranium bones and unites with the opercle in anterodorsal portion. Laterally, the hyomandibular overlaps with the posterodorsal margin of the preopercle. Opercle overlaps with interopercle and subopercle anteroventrally. The quadrate well ossified, zone of cartilage, a remnant of the

palatoquadrate cartilage is less visible. Its large posteroventral process extends laterally and overlaps anterior most point of the preopercle. On the anterior side, articular condyle of the quadrate articulates strongly with the anguloarticular. The metapterygoid is well ossified, ventrally attaches with the quadrate on anterior, preopercle and hyomandibular on posterior and overlaps laterally with symplectic. Endopterygoid and ectopterygoid well ossified like metapterygoid and overlaps on dorsal and anterior face of quadrate.

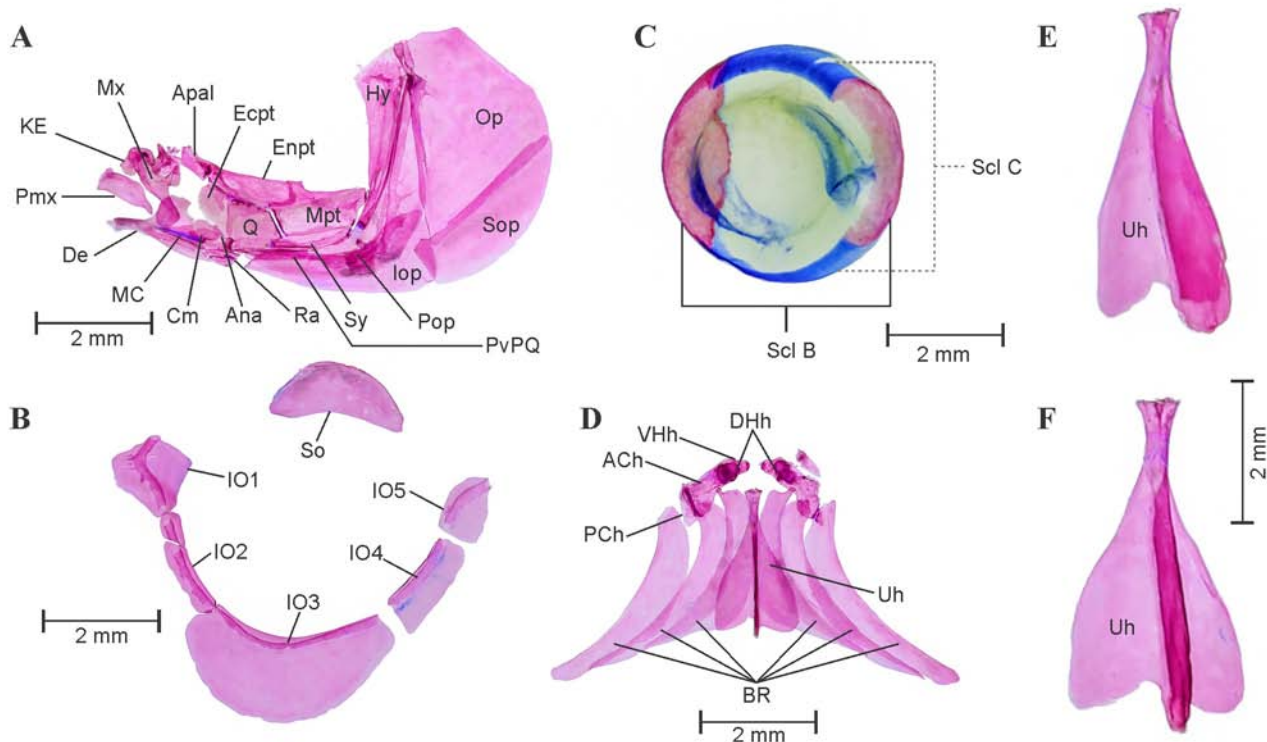
Upper jaw constitutes of premaxilla and maxilla, relatively large and well ossified. Premaxilla proportionately smaller than maxilla curved anteriorly and free of any joints with maxilla and dentary, while only attached with strong ligaments. Premaxilla articulates with the dentary on posteroventral surface. The lower jaw comprises Meckel's cartilage, dentary, anguloarticular and the coronomeckelian. Dentary articulates with maxilla on dorsolateral side, broad on posterior edge and pointed towards anterior edge. Coronomeckelian bone presents on dorsal surface of remnant of rod shape Meckel's cartilage. Kinethmoid a small bone contributes to ethmoid complex present in association with maxillae and premaxilla, bound with small ligaments.



**FIGURE 4.** *Pethia punctata*, topotype, female, BNHS FWF 111, 48.5 mm SL; neurocranium, dorsal (A), lateral (B) and ventral view (C). Apto, autopterotic; Asph, autosphenotic; Boc, basioccipital; BocPr, basioccipital process; E, ethmoid; Exoc, exoccipital; Epo, epioccipital; Fr, frontal; LE, lateral ethmoid; MaPI, masticatory plate of the basioccipital; Me, mesethmoid; N, nasal; Orsph, orbitosphenoid; Pa, parietal; Pe, prethmoid; Pro, prootic; Psph, parasphenoid; Ptsph, pterosphenoid; Soc, supraoccipital; STF, subtemporal fossa; TrC, trabecula communis; Vo, vomer.

**TABLE 1.** Morphometric data of *Pethia punctata*, topotypic material (n = 14; BNHS FWF 107–113, WILD-14-PIS-111 to 114, ZSI-WRC-P/4092) and *Pethia muvattupuzhaensis* type series and topotypic material (n = 19; type series: Holotype, F. 12241, ZSI / WGFRS, CLT; Paratypes, 2 ex., (uncatalogued specimens), ZSI / WGFRS, CLT; topotypic material: BNHS FWF 114 to 120, WILD-14-PIS-106 to 110, ZSI-WRC-P/4091).

Species	<i>Pethia punctata</i>		<i>Pethia muvattupuzhaensis</i>	
	Mean (s.d.)	Range	Mean (s.d.)	Range
<b>Morphometric</b>				
Total Length (mm)	63.7 (4.2)	58.6–71.7	54.0 (3.3)	48.3–59.9
Standard Length (SL, mm)	49.9 (3.4)	44.8–56.1	41.7 (2.9)	37.3–46.6
%SL				
Head length (HL)	27.0 (1.4)	25.1–29.9	26.7 (1.1)	25.1–28.8
Body depth	36.0 (1.2)	34.7–38.7	37.3 (2.0)	34.2–42.3
Body width at dorsal-fin origin	16.2 (1.5)	12.5–17.9	15.3 (2.6)	9.6–17.9
Body width at anal-fin origin	11.9 (1.7)	7.8–13.9	10.4 (1.8)	6.6–12.3
Pre-dorsal distance	48.8 (1.7)	46.1–51.4	49.1 (1.6)	46.3–51.9
Dorsal to hypural distance	54.2 (1.8)	50.3–56.5	54.4 (1.6)	51.0–57.3
Prepelvic distance	48.1 (1.8)	44.6–51.2	48.2 (1.8)	45.0–52.7
Preanal distance	70.8 (2.0)	67.0–74.7	71.5 (1.9)	68.8–75.4
Prepectoral distance	26.0 (1.5)	23.6–29.2	25.7 (1.2)	24.2–29.1
Dorsal-fin length	28.5 (1.6)	25.8–32.5	28.0 (1.5)	24.7–31.1
Dorsal-fin spine length	22.2 (1.7)	19.0–25.2	22.0 (1.5)	19.4–24.2
Length of dorsal-fin base	17.0 (1.4)	15.1–19.1	17.2 (1.3)	15.4–20.3
Pectoral-fin length	20.4 (1.0)	18.8–22.6	20.3 (1.7)	18.0–23.1
Anal-fin depth	17.4 (0.9)	15.7–18.9	16.6 (1.2)	14.5–19.5
Caudal-peduncle length	19.4 (1.5)	17.3–22.1	18.7 (1.5)	15.7–21.5
Caudal-peduncle depth	14.1 (0.4)	13.3–14.9	14.0 (0.7)	13.1–15.3
%HL				
Snout length	27.3 (2.5)	22.3–32.5	25.2 (2.5)	21.3–28.9
Eye diameter	30.4 (2.3)	27.6–33.6	33.4 (3.0)	28.5–38.2
Inter-orbital width	38.1 (2.3)	34.6–42.1	39.9 (2.6)	34.8–44.1
Head depth	77.0 (3.9)	69.6–81.2	77.3 (3.3)	72.6–83.0
Head width	54.5 (3.1)	47.8–59.9	54.3 (2.1)	50.5–60.0
<b>Meristics</b>				
Lateral-line scale		23–25		23–25
Number of lateral-line pores		23–25		23–25
Dorsal-fin spine serrae		12–18		11–18
Transverse scale rows		½4/1/3½		½4/1/3½
Predorsal scale		8		8
Prepelvic scale		9–10		9–10
Preanal scale		13–14		13–14
Circumpeduncular scales		12		12
Dorsal-fin rays		iii 8		iii 8
Pectoral-fin rays		i 9–10		i 9–10
Pelvic-fin rays		i 7		i 7
Anal-fin rays		iii 5		iii 5
Caudal-fin rays (procurrent)		7+6		7+6
Caudal-fin rays (principal)		9+8		9+8



**FIGURE 5.** *Pethia punctata*, topotype, female, BNHS FWF 111, 48.5 mm SL; (A) hyopalatine arch and preopercle, lateral view; (B) infraorbital series, lateral view; (C) scleral cartilage and bone; (D) hyoid and urohyal, dorsal view; (E-F) urohyal bone. ACh, anterior ceratohyal; Ana, anguloarticular; Apal, autopalatine; BR, branchiostegal ray; Cm, coronomeckelian; De, dentary; DHh, dorsal hypohyal; Ecpt, ectopterygoid; Enpt, endopterygoid; Hy, hyomandibular; IO1-5, infraorbital 1-5; Iop, interopercle; KE, kinethmoid; MC, Meckel's cartilage; Mpt, metapterygoid; Mx, maxilla; Op, opercle; Pmx, premaxilla; PCh, posterior ceratohyal; Pop, preopercle; PvPQ, posteroventral process of quadrate; Q, quadrate; Ra, retroarticular; Scl B, scleral bone; Scl C, scleral cartilage; So, supraorbital; Sop, subopercle; Sy, symplectic; Uh, urohyal; VHH, ventral hypohyal.

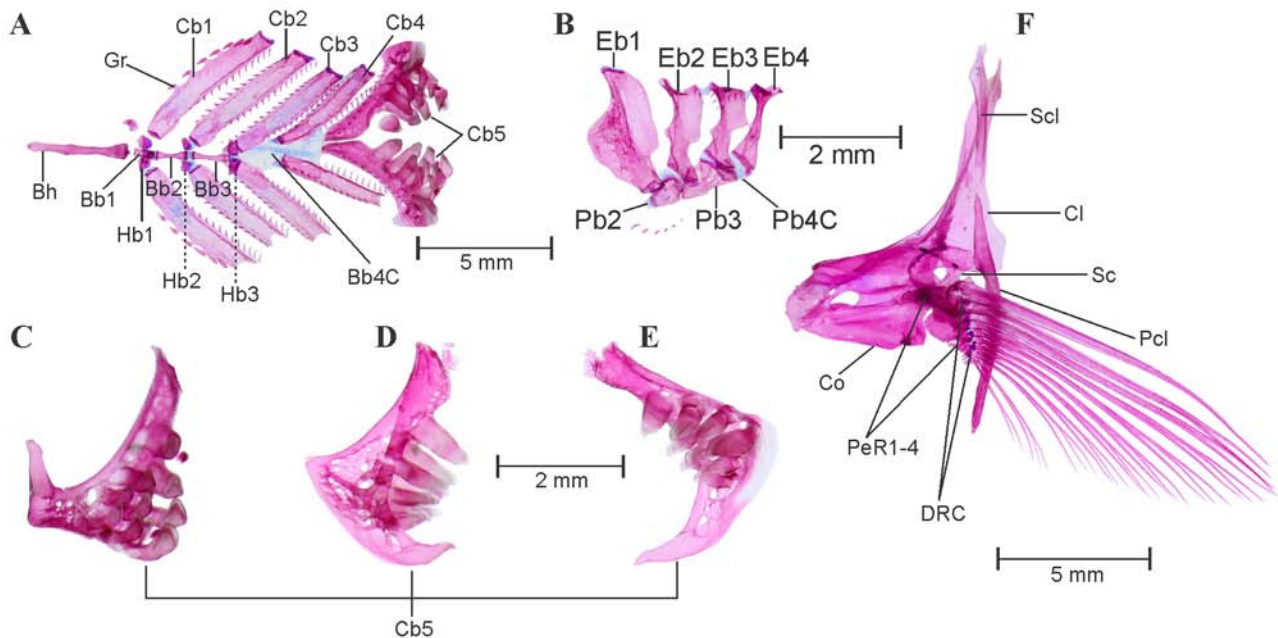
**Infraorbital series and scleral skeleton:** See Figure 5B and C. Five infraorbital bones present, bearing laterosensory canal which encircles the orbital margin. Infraorbital 1 is well developed rhomboidal and wider than infraorbital 2. Infraorbital 2 with laterosensory canal appears like a thin tube, encircles orbital margin. Infraorbital 3 large among all and overlaps preopercle. Infraorbital 4 and 5 are also well developed but less wider than infraorbital 1 and 3. Supraorbital large and well ossified, covers eye dorsally. Infraorbital 6 absent. Scleral bones well ossified, covers proximal and distal perimeter of eye sclera in lateral view, dorsal and ventral perimeter of eye is cartilaginous (Figure , 5C).

**Hyoid arch:** See Figure 5D–F. Anterior ceratohyal distinct, broad posteriorly, narrows down anteriorly. It is attached anteriorly with dorsal hypohyal and ventral hypohyal and posteriorly with posterior ceratohyal. Dorsal and ventral hypohyal are small and well ossified with no cartilaginous remnant. Large urohyal, with three well spread lateral flanges, attached anteriorly to ventral hypohyal with ligaments. Three well developed thin branchiostegal rays in each half of hyoid arch.

**Branchial arches:** See Figure 6A–E. Three well ossified cylindrical basibranchial, connected by congregated cartilage; first one is smaller than second and third. Three pairs of small, round, ossified hypobranchials, covered with cartilage, attaches to the respective basibranchial and ceratobranchial. First basibranchial attaches to the basihyal by ligaments; we did not identify a cartilaginous connection between basihyal and first basibranchial. Fourth and fifth ceratobranchial connects with basibranchial 3 by strong ligaments and very weak basibranchial 4 cartilage remnants. Well developed five pairs of ceratobranchials present. Ceratobranchial 1 with 7 gill rakers on anterior side and 14 on posterior. Ceratobranchial 2 with 14-15 gill rakers along their anterior margins and 17–18 on posterior. Ceratobranchial 3 with 14 gill rakers on anterior margin and 16 on posterior margin. Ceratobranchial 4 with 11 gill rakers on anterior margin and 15 on posterior margin. Ceratobranchial 5 well ossified, toothed having



13 gill rakers on anterior margin. Proximal half of ceratobranchial 5 marked with 3 rows of well ossified conical teeth. Anterior most teeth row comprises 2, middle 3 and posterior most comprises 4 conical and one small isolated tooth (Figure 6C–E). Upper gill arch constitutes by four pairs of well developed epibranchials. Epibranchial 1 broad and wing shaped while epibranchial 2, 3 and 4 are narrow bodied. We fail to locate pharyngobranchial 1, pharyngobranchial 2 and 3 are well developed, pharyngobranchial 4 cartilage very less developed and minute.

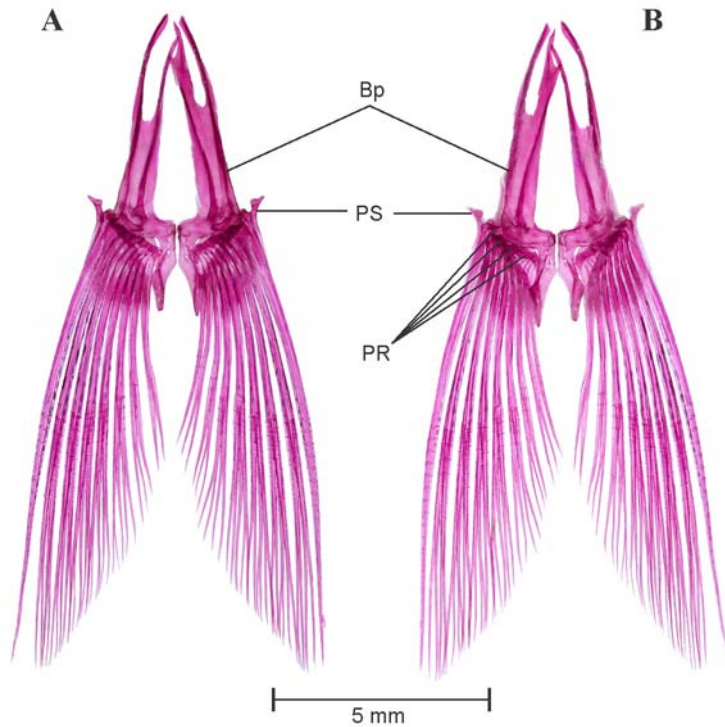


**FIGURE 6.** *Pethia punctata*, topotype, female, BNHS FWF 111, 48.5 mm SL; (A) ventral gill arches, dorsal view; (B) dorsal gill arches, ventral view; (C–E) 5<sup>th</sup> ceratobranchial teeth; (F) pectoral girdle, lateral view. Bb1–3, basibranchial 1–3; Bb4C, basibranchial 4 cartilage; Bh, basihyal; Cb1–5, ceratobranchial 1–5; Cl, cleithrum; Co, coracoid; DRC, distal radial cartilage; Eb1–4, epibranchial 1–4; Hb1–3, hypobranchial 1–3; Pb2–3, pharyngobranchial 2–3; Pb4C, pharyngobranchial 4 cartilage; Pcl, postcleithrum; PeR1–4, pectoral radial 1–4; Sc, scapula; Scl, supracleithrum.

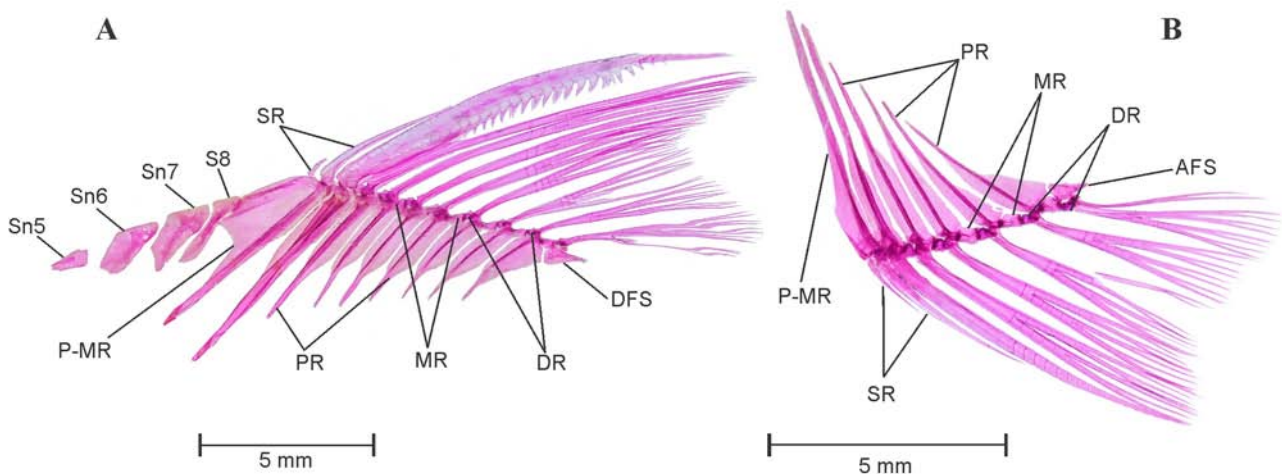
**Pectoral Girdle:** See Figure 6F. Pectoral girdle position most anteriorly and does not overlap weberian apparatus laterally; articulate with posterolateral surface of neurocranium through vertically aligned supracleithrum and cleithrum. Supracleithrum small, laterally overlaps with cleithrum by articulating more than 3/4<sup>th</sup> area. Cleithrum large, narrow and pointed dorsally, articulates with postcleithrum lateroventrally. Postcleithrum single, elongated and well developed supports pectoral girdle posteriorly. Coracoid large, rectangular, articulates posteriorly with scapula and pectoral radials, dorsally overlaps with cleithrum. Four large well ossified pectoral radials present. Six distal radials less ossified, with three medial elements predominantly cartilaginous. Nine distal radial present, first three distal radial appear to be well ossified whereas remaining six are cartilaginous and abbreviated as distal radial cartilage, supports one unbranched and 10 branched (i.9.i) pectoral fin rays.

**Pelvic girdle:** See Figure 7A–B. Pelvic girdle consists of a pair of thin, flat and elongated basipterygium, each supporting one unbranched and eight branched fin rays (i.7.i). On the anterior side basipterygium bears two spine-like processes, external and internal. External process slightly elongated than internal. Five well ossified pelvic radials present, pelvic splint is well developed and laterally associated with first unbranched pelvic fin ray.

**Dorsal fin:** See Figure 8A. Dorsal fin constitutes twelve dorsal fin rays (iii.8.i) supported by a series of pterygiophores placed between neural spines of vertebrae V8/V9–V14/V15. First three pterygiophores constitutes a large proximal–middle radial, articulates with two unbranched supernumerary and one serrated last unbranched dorsal fin ray, distal radial absent. Posterior to proximal–middle radial first two pterygiophores articulates with respective branched fin rays by having only distal radial, middle radials untraceable in this articulation. Middle radial appears in articulation with distal radial and dorsal base of pterygiophore on third proximal radial. All middle and distal radials are well ossified. Last tenth branched fin ray found to be closely united with ninth fin ray, whereas articulated separately with last pterygiophore, dorsal fin stay. Four free supraneurals, Sn5–8 present on anterior side of dorsal fin.



**FIGURE 7.** *Pethia punctata*, topotype, female, BNHS FWF 111, 48.5 mm SL; pelvic girdle, (A) ventral view, (B) dorsal view. Bp, basipterygium; PS, pelvic splint; PR, pelvic radial.

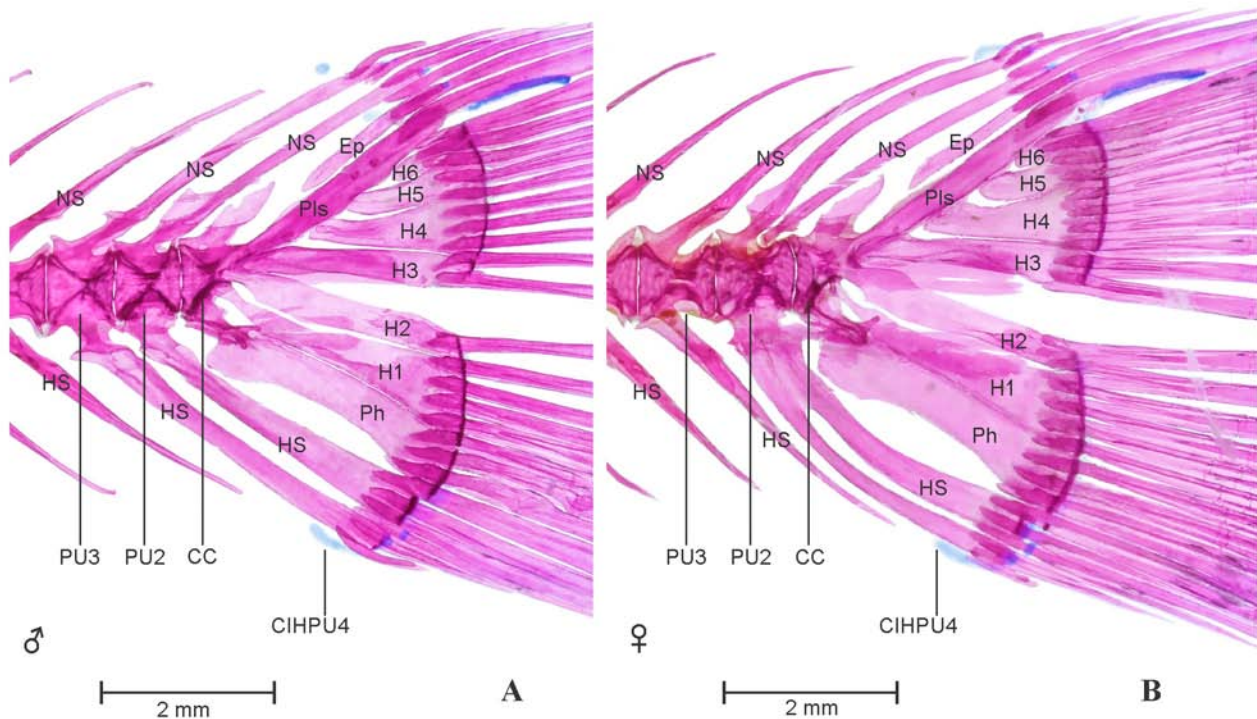


**FIGURE 8.** *Pethia punctata*, topotype, female, BNHS FWF 111, 48.5 mm SL; (A) dorsal fin, lateral view, (B) anal fin, lateral view. AFS, anal fin stay; DFS, dorsal fin stay; DR, distal radial; MR, middle radial; P-MR, proximal-middle radial; PR, proximal radial; Sn5-8, supraneural 5-8.

**Anal fin:** See Figure 8B. Anal fin constitutes 9 fin rays (iii.5.i) supported by a series of anal pterygiophores placed between hemal spines of vertebrae V18/V19–V20/V21. First three pterygiophores constitutes long, elongated proximal-middle radial, articulates with two unbranched supernumerary and last unbranched anal fin ray, distal radial present with no traces of middle radial. Middle radial appears in articulation with distal radial and dorsal base of pterygiophore on first proximal radial. All middle and distal radials are well ossified. Last sixth branched fin ray found to be closely united with fifth anal fin ray, whereas articulated separately with last pterygiophore, anal fin stay.

**Caudal fin:** See Figure 9A–B. Seven dorsal procurrent caudal-fin rays and six ventral procurrent caudal-fin

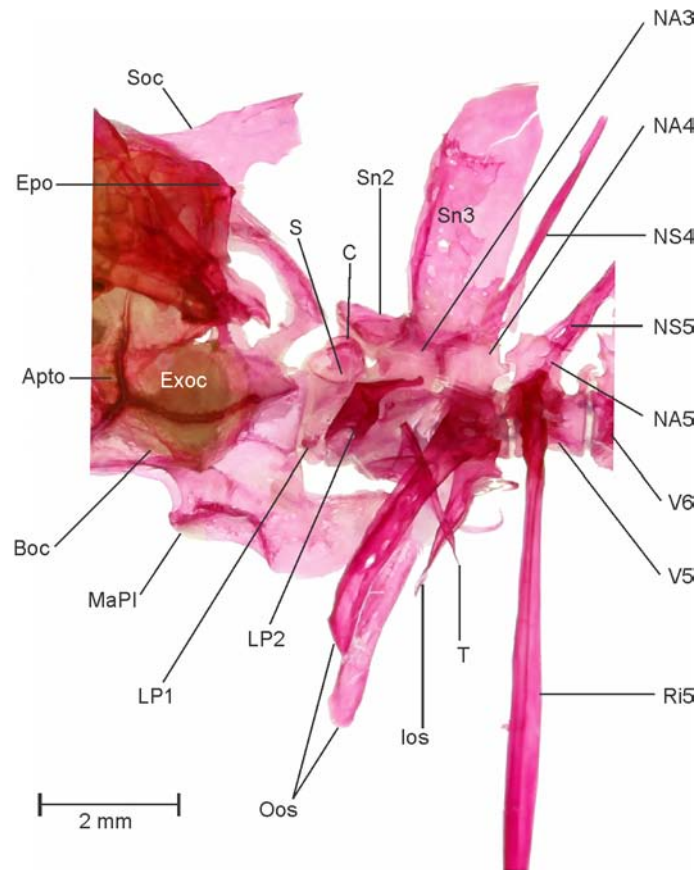
rays. Nine dorsal and eight ventral principal caudal fin rays present. Principal caudal fin rays supported by neural and hemal spines of third preural centrum, epural, pleurostyle, six hypurals and a parhypural. A single inter-hemal spine cartilage of preural centrum 4 present, placed anterior to ventral tip of hemal spine of preural centrum 3 in male and anterior to ventral tip of first hemal spine of preural centrum 2 in female. Single epural runs laterally with anterior margin of pleurostyle on dorsal half, distal tip of pleurostyle cartilaginous. Parhypural and hypural 1-6 well developed, broad and ossified. Sexual dimorphism in caudal fin skeleton is distinct in *P. punctata*. In male, pleural centra 2 supports single neural and hemal spine, pleural centra 3 supports well developed neural and hemal spine which support peduncle arch. In female, pleural centra 2 represents pair of neural and hemal spines, supports peduncle arch. Neural and hemal spines of third pleural centra less developed and short in female specimen as compare to male. Free uroneural absent



**FIGURE 9.** Caudal fin. (A) *Pethia punctata*, topotype, male, BNHS FWF 112, 56.1 mm SL; (B) *Pethia punctata*, topotype, female, BNHS FWF 111, 48.5 mm SL. CC, compound centrum; CIHPU4, inter-hemal spine cartilage of preural centrum 4; Ep, epural; H1-6, hypurals 1-6; HS, hemal spine; NS, neural spine; Ph, parhypural; Pls, pleurostyle; PU2-3, preural centra 2-3.

**Weberian apparatus:** See Figure 10. A complex structure made up by first four vertebral centra and their associated elements, covers the anterodorsal portion of swim bladder. The first centrum relatively small but distinct, possesses lateral process 1 (Lp1). Second centra equivalent in size of first, possesses large lateral process of second vertebra (Lp2). Lp2 is more developed and distinct than LP1 and grown laterally in wing shaped processes. Third and fourth centrum is much larger and each equivalent to size of first two. Scaphium and claustrum small, rounded and well ossified, present on dorsal in association with first centra. Tripus well developed, triangular grows posteriorly and overlaps inner arm of the os suspensorium, associated with third vertebral centrum. Outer arms of the os suspensorium much grown, long and reaches behind the postcleithrum bone of pectoral girdle. Neural arch 3 and neural arch 4 well developed and ossified. Supraneural 3 seems to be a second largest bone of weberian apparatus after outer arm of the os suspensorium. Supraneural 2, 3 and neural spine 4 is associated with neural arch 3 and 4. Supraneural 4 absent.

**Intermuscular bone:** See Figure 3A and C. Two series of intermuscular bones, epineural and epipleural. Total 23 epineural and 10 epipleural intermuscular bones present; first epineural appears before neural spine of seventh vertebra whereas first epipleural appears before hemal spine of sixteenth vertebra. All epipleural and epineural bifurcated at tip and becomes wider in caudal peduncle portion.



**FIGURE 10.** *Pethia punctata*, topotype, female, BNHS FWF 111, 48.5 mm SL; weberian apparatus, lateral view. Apto, autopterotic; Boc, basioccipital; C, claustrum; Epo, epioccipital; Exoc, exoccipital; Ios, inner arm of the os suspensorium; Lp1, lateral process of the first vertebral centrum; Lp2, lateral process of the second vertebral centrum; MaPl, masticatory plate of the basioccipital process; Na, neural arch; Ns, neural spine; Oos, outer arm of the os suspensorium; Ri, rib; S, scaphium; Sn, supra-neural; Soc, supraoccipital; T, tripus; V, vertebral centrum. Numbers after abbreviations refer to the vertebral centrum to which the structure is associated.

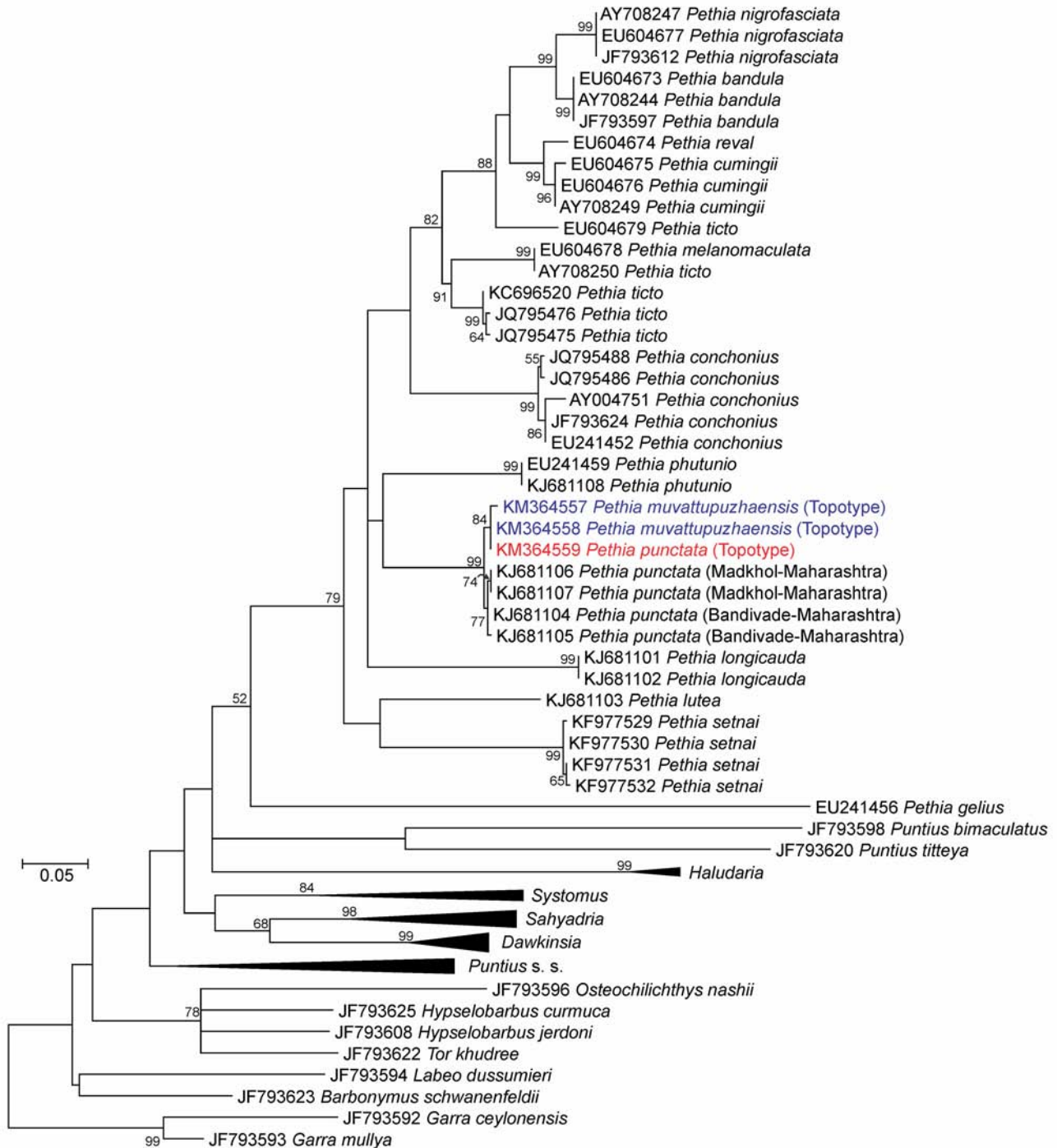
**Vertebral column:** See Figure 3A and C. 4+4 predorsal vertebrae; 4+26 total vertebrae including posterior most compound centrum, with 4+14 abdominal and 12 caudal vertebrae. Vertebral centra much elongated than deep. Neural pre and postzygopsis appears on all centra posterior to weberian complex. Hemal postzygopsis appear on all caudal vertebrae posterior to 15<sup>th</sup> centra. First hemal prezygopsis appears on 20<sup>th</sup> centra. Total 11 ribs are associated with 5<sup>th</sup> to 15<sup>th</sup> vertebrae.

**Coloration in life.** (Image 8a-d in Katwate *et al.* 2014b). Body yellow. Position of humeral and caudal spots same as described for preserved specimens, spot on caudal peduncle surrounded by a golden hoop covering scales 19–21 of lateral-line row, saddling caudal peduncle dorsally. Dorsal fin with 2–3 longitudinal rows of intense black spots, third one occupying only anterior portion of dorsal, yellow in breeding male, red and devoid of black spots in female. Pectoral, pelvic and anal fins yellow. Caudal fin yellow. Sclera bright yellow in breeding males, iridescent silver in females and immature males.

**Coloration in preservative.** For general appearance see Figure 2A–D and for illustration see Figure 10; body above lateral-line scale row brown; snout, head, dorsum dark brown; lower lip, cheek, opercular region below inferior border of eye cream; ventral region uniformly cream white. Body with prominent intense black humeral spot covering anterior half of fourth scale on scale row below lateral-line row; two minute black spots below humeral spot. A prominent spot on caudal peduncle covering 19<sup>th</sup>–21<sup>st</sup> scales of lateral-line row, saddling caudal peduncle dorsally. Dorsal fin with 2–3 longitudinal rows of dark black spots, third one occupy only front portion of dorsal and also extends on 2 supernumerary and last unbranched fin rays (Figures 1, 2A, 2C), in breeding and mature males, dorsal fin plain usually without any color bands in female (Figure 2B). Anal and caudal fins hyaline,

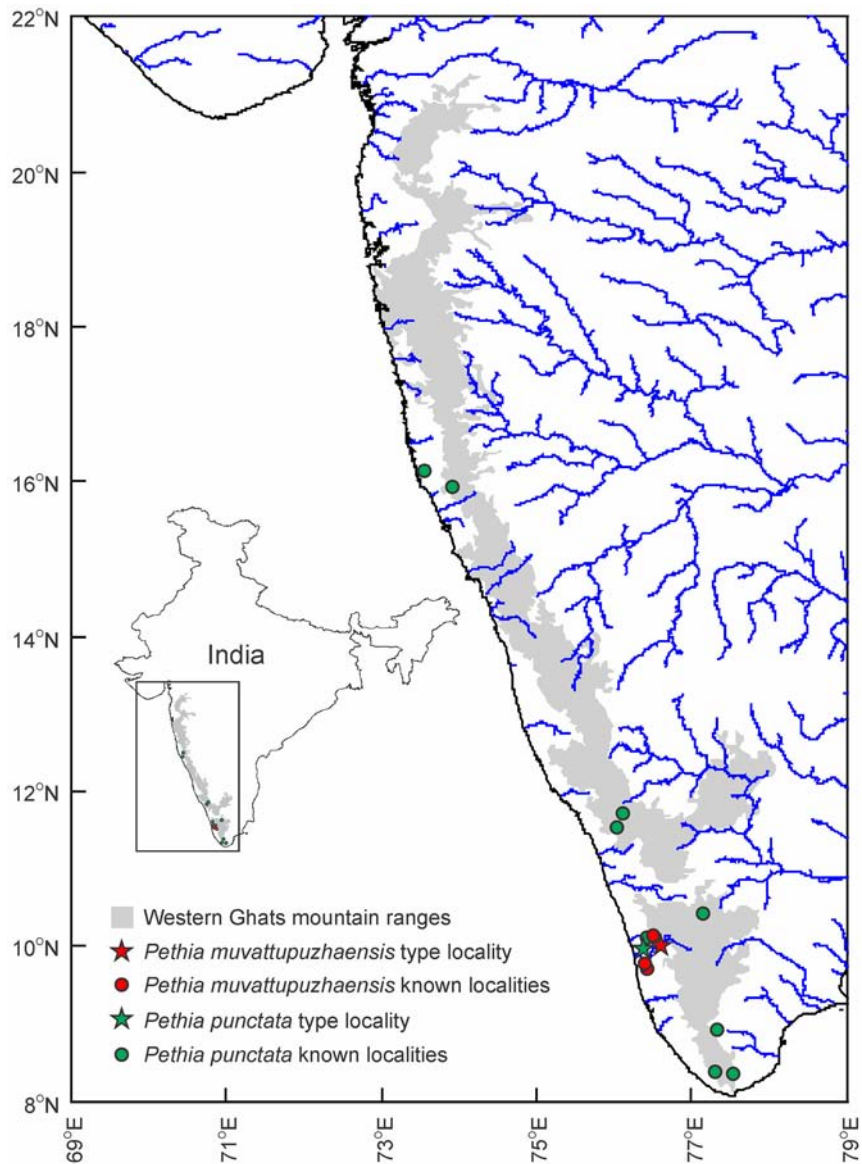
without any color bands or spots. Pectoral and pelvic fins lightly pigmented with melanophores. Base of scales studded with black melanophores.

**Phylogenetic position.** Phylogenetically, *P. punctata* from Cochin, Kerala (putative topotype), and Maharashtra (populations studied in Katwate *et al.* 2014b) formed a monophyletic group nested within genus *Pethia* (Figure 11).



**FIGURE 11.** Maximum likelihood tree for *Pethia* species based on cytochrome b sequences. Nucleotide substitution model used was Tamura & Nei (1993) with invariant sites and gamma distribution (TN93+G+I, BIC = 16921.42, lnL = -7579.84, I = 0.42, G = 0.79). Values along the nodes are percent bootstraps for 1000 iterations. *Garra* species are used as outgroup.

**Distribution.** *Pethia punctata* is a widely distributed species in the Western Ghats of India, mainly confined to west-flowing rivers, from 8°–16°N latitudes (Figure 12).



**FIGURE 12.** Distribution map of *Pethia muvattupuzhaensis* and *P. punctata*.

**Remarks.** While describing the fishes of “Cochin, on the Malabar Coast of India”, Day (1865) described *P. punctata* with evident and distinguishable prominent characters. Although Day (1865) did not designate a type for *P. punctata* in the description, there are surviving syntypes (Figure 1C, D) with collection localities. For the redescription, we use Day’s material as syntypes, with additional topotypes collected from the area encompassing the erstwhile state of Cochin, the type locality.

Day (1865), used the following combination of characters to distinguish *P. punctata*: no cirri; third dorsal spine strongly serrated posteriorly; lateral line first curves gently downwards, and from opposite base of ventral fin passes direct to centre of caudal fin; a black diffused spot on the twentieth and twenty-first scales of the lateral line; the anterior half of the fourth scale from the operculum, of the row next below the lateral line, deep black, and also a portion of the scale above and beneath it; fins yellowish; dorsal spotted with melanophores, in two longitudinal rows, with a third anteriorly, between the other two. Day’s (1865) lateral-line scale count of 27 for *P. punctata*, is apparently in error, for his syntypes and our topotypes possess only 23–25 lateral-line scales.

Species found in the Western Ghats region such as *P. lutea* Katwate, Katwate, Raghavan, Paingankar & Dahanukar, 2014; *P. narayani* (Hora, 1937) and *P. setnai* (Chhapgar & Sane, 1992) are close congeners of *P. punctata* in having complete lateral line. However, *P. punctata* differs from its Western Ghats congeners by having following combination of characters: greater number of lateral-line scales (23–25 vs. 19–22 in *P. lutea*, 22 in *P.*

*narayani*, 19–21 in *P. setnai*); dorsal fin with 2–3 longitudinal rows of intense black spots, third row occupying only anterior portion of fin (vs. plain and without any markings in *P. lutea*, *P. narayani* and *P. setnai*); two spots on body, one on humeral region, another on caudal peduncles (vs. three dark bands on body in *P. narayani* and *P. setnai*); and prominent intense-black humeral spot covering anterior half of fourth scale on scale row below lateral-line row (vs. covering third and fourth lateral line scale, extending to one scale up and down in *P. lutea*). *Pethia punctata* also differs from *P. narayani* by having more osseous and serrated last unbranched dorsal-fin ray (vs. last unbranched dorsal fin ray feeble and without serrations). *Pethia punctata* also differs from other Indian *Pethia* having a complete lateral line with a combination of characters that includes the presence of intense-black humeral spot covering the anterior half of the fourth scale on scale row below the lateral-line row (vs. absent in *P. expletiforis* and inconspicuous on the scale row below third and fourth scales of lateral-line row in *P. rutila*); 23–25 lateral-line scales (vs. 21–22 scales in *P. rutila*);  $\frac{1}{2}$ 4/1/3 $\frac{1}{2}$  scales in transverse line on body (vs.  $\frac{1}{2}$ 5/1/5 $\frac{1}{2}$  in *P. stoliczкана*) (Dishma & Vishwanath, 2013; Laltilanhlua *et al.* 2014; Day 1871; Linthoingambi & Vishwanath, 2007). *Pethia punctata* differs from *P. ticto* by having a complete lateral line (vs. incomplete lateral line) (Hamilton, 1822). A more detailed diagnosis for separating *P. punctata* from *P. ticto* will appear in a forthcoming study clarifying the identity of *P. ticto* (Katwate *et al.*, in prep.).

The distribution, habitat and ecology of *P. punctata* has been discussed in detail in Katwate *et al.* (2014b). While comparing the type material and topotypic specimens of *P. muvattupuzhaensis*, we observed that the characters suggested by Beevi & Ramachandran (2005) to distinguish it from *P. punctata* are invalid: the two nominal species are indistinguishable in all respects. Further details on the synonymy of *P. muvattupuzhaensis* are discussed below.

**Synonymy of *Pethia muvattupuzhaensis*.** *Pethia muvattupuzhaensis* was described based on the holotype (Figure 13) and four paratypes (Beevi & Ramachandran 2005). However, out of the five type specimens of *P. muvattupuzhaensis* in SRC-ZSI, only three belong to the genus *Pethia*, while two represent another cyprinid genus (Figure 14). We therefore studied only the holotype and the two paratypes that are conspecific with it. Comparison of type series (Figure 13) and recent collection of topotypic specimens of *P. muvattupuzhaensis* (Figure 1E, F) and *P. punctata* (Figure 1A, B) revealed no significant morphological or meristic differences between the two nominal species.



**FIGURE 13.** *Pethia muvattupuzhaensis*, holotype, F. 12241, ZSI / WGFRS, CLT, 44.2 mm SL, collected from Muvattupuzha river, Ooramana, Ernakulam District, Kerala, India, by K.S. Jameela Beevi & A. Ramachandran on 21.xi.2001.

Beevi & Ramachandran (2005) distinguished *P. muvattupuzhaensis* from *P. punctata* based on two prominent characters, namely: fewer lateral transverse scales rows ( $2\frac{1}{2}$ –3 vs. 4) and dorsal fin devoid of rows of spots (vs. present). However, examination of the relevant type specimens of *P. muvattupuzhaensis* and fresh topotypic specimens shows that there are  $\frac{1}{2}$ 4 scales between the dorsal-fin origin and lateral-line scale row, and  $3\frac{1}{2}$  scales between lateral-line row and the pelvic-fin origin (Table 1). The transverse scale count of *P. muvattupuzhaensis* matches exactly with that of putative syntypes (MCZ 4303 and BMNH 1889.2.1.755, coll. F. Day) (Figure 4C, D) as well as topotypic specimens (Figure 4E, F) of *P. punctata* collected from near Cochin, Kerala, and additional specimens from other rivers draining the Western Ghats (Katwate *et al.* 2014). Further, Beevi & Ramachandran

(2005) mention  $2\frac{1}{2}$ –3 scales between the lateral-line row and the pelvic-fin origin. However, the illustration provided by them in the description as well as our own examination of the holotype (Figure 13) indicates the presence of  $3\frac{1}{2}$  scales, which is the same as that in *P. punctata* (Table 1).



**FIGURE 14.** Two paratypes of the *P. muvattupuzhaensis* within the type series, collected from Muvattupuzha river, Ooramana, Ernakulam District, Kerala, India, by K.S. Jameela Beevi & A. Ramachandran on 21.xi.2001, do not belong to the genus *Pethia* by having characters such as smooth (unserrated) last unbranched dorsal-fin ray and presence of rostral barbels.

Beevi & Ramachandran (2005) also distinguished *P. muvattupuzhaensis* from *P. punctata* by the absence of rows of dark spots on the dorsal fin. The specimens in the type series of *P. muvattupuzhaensis*, however, are female individuals. The rows of black spots on the dorsal fin of *P. punctata* are in fact a sexually dichromatic: only mature and breeding males possess these, whereas females are usually devoid of this character and have an unmarked dorsal fin (Katwate *et al.* 2014b). Recent collections of topotypes of *P. muvattupuzhaensis* (Figure 1E, F) and *P. punctata* (Figure 1A, B) support these characters. By including this sexually dichromatic character, male individuals of topotypic *P. muvattupuzhaensis* key out as *P. punctata* in the key provided by Beevi & Ramachandran (2005).

Beevi & Ramachandran (2005) also distinguished *P. muvattupuzhaensis* from *P. punctata* by morphometric characters such as a more elongated body, shallower body depth, and narrower caudal peduncle. A comparison of the morphometric and meristic values of the type series and topotypic specimens of *P. muvattupuzhaensis* with *P. punctata* (Table 1) do not reveal any such difference. Furthermore, we found that no significant osteological differences separate *P. muvattupuzhaensis* as distinct from *P. punctata* (see Figure 2 A–C).

Morphometric data was multivariate normal (Doornik & Hansen omnibus  $E_p = 33.54$ ,  $P = 0.7548$ ). There was no significant difference in the multivariate morphometry of the two species (MANOVA: Pillai's trace = 0.7494,  $F_{12,20} = 1.794$ ,  $P = 0.1498$ ). Genetically, topotypes of *P. muvattupuzhaensis* and *P. punctata*, as well as additional *P. punctata* populations from Maharashtra were not significantly different (Table 2, Figure 11), forming a monophyletic group supported by high bootstrap value (99%).

We conclude that the characters suggested by Beevi & Ramachandran (2005) to differentiate *P. muvattupuzhaensis* from *P. punctata* are invalid: the two “species” are indistinguishable in external morphology, osteology and in the gene fragments investigated. We consider *Pethia muvattupuzhaensis*, therefore, to be a junior synonym of *P. punctata*.



**TABLE 2.** Pairwise percent distances in cytochrome b (cytb) gene sequence (below the diagonal) and standard errors (above the diagonal) between the topotypic and widely distributed *Pethia punctata* and topotypic *P. muvattupuzhaensis* based on Tamura & Nei (1993) nucleotide substitution model with gamma distribution and 1000 bootstrap iterations.

Sr. No.	Species	1	2	3	4	5	6	7
1	KM364559 <i>Pethia punctata</i> (Topotype)		0.41	0.41	0.36	0.42	0.28	0.00
2	KJ681106 <i>P. punctata</i> (Madkhol, Maharashtra)	0.77		0.00	0.20	0.29	0.53	0.41
3	KJ681107 <i>P. punctata</i> (Madkhol, Maharashtra)	0.77	0.00		0.20	0.29	0.53	0.41
4	KJ681104 <i>P. punctata</i> (Bandivade, Maharashtra)	0.58	0.19	0.19		0.20	0.47	0.36
5	KJ681105 <i>P. punctata</i> (Bandivade, Maharashtra)	0.77	0.39	0.39	0.19		0.53	0.42
6	KM364557 <i>Pethia muvattupuzhaensis</i> (topotype)	0.39	1.17	1.17	0.97	1.17		0.28
7	KM364558 <i>P. muvattupuzhaensis</i> (topotype)	0.00	0.77	0.77	0.58	0.77	0.39	

**Material examined.** *Pethia muvattupuzhaensis* (n = 19): Holotype, F. 12241, ZSI / WGFRS, CLT, collected from Muvattupuzha river, Ooramana, Ernakulam District, Kerala, India, by K.S. Jameela Beevi & A. Ramachandran on 21.xi.2001; Paratypes, 2 ex., (uncatalogued specimens), ZSI / WGFRS, CLT, data same as holotype; 7 ex., BNHS FWF 114 to 120, collected from Muvattupuzha River, Ooramana, Ernakulam District, Kerala, India (9°57'56"N, 76°31'17"E, 19 m a.s.l.), by Unmesh Katwate and Fabin Baby on 31.v.2014; 5 ex., WILD-14-PIS-106 to 110, collected from Muvattupuzha River, Ooramana, Ernakulam District, Kerala, India (9°57'56"N, 76°31'17"E, 19 m a.s.l.), by Unmesh Katwate and Fabin Baby on 31.v.2014; 4 ex., ZSI-WRC-P/4091, collected from Muvattupuzha River, Ooramana, Ernakulam District, Kerala, India (9°57'56"N, 76°31'17"E, 19 m a.s.l.), by Unmesh Katwate and Fabin Baby on 31.v.2014.

*Pethia lutea* (n= 22): Holotype, BNHS FWF 71, collected from Bhira (18.441°N & 73.267°E, elevation 50m), Kundalika River, Raigad District, Maharashtra, India by Unmesh Katwate and Chetana Katwate on 23.xii.2012; Paratypes, 3 exs., BNHS FWF 72, 78 and 79, collected from Bhira (18.441°N & 73.267°E, 50 m), Kundalika River, Raigad District, Maharashtra, India by Unmesh Katwate and Chetana Katwate on 23.xii.2012; 1 ex., WILD-14-PIS-061, collected from Bhira (18.441°N & 73.267°E, 50m), Kundalika River, Raigad District, Maharashtra, India by Unmesh Katwate and Chetana Katwate on 23.xii.2012; 1 ex., ZSI-WRC-P/ 3686, collected from Bhira (18.441°N & 73.267°E, 50m), Kundalika River, Raigad District, Maharashtra, India by Unmesh Katwate and Chetana Katwate on 23.xii.2012; 3 exs., BNHS FWF 73, 80 and 81, collected from Karjat (18.922°N & 73.332°E, 48m), Ulhas River, Raigad District, Maharashtra, India by Neelesh Dahanukar and M. Paingankar on 23.vi.2012; 2 exs., BNHS FWF 74 and 82, collected from Mangaon (18.233°N & 73.256°E, 7 m), Kal River - tributary of Savitri River, Raigad District, Maharashtra, India by Unmesh Katwate and Chetana Katwate on 05.i.2013; 1 ex., BNHS FWF 75, collected from Mahad (18.091°N & 73.466°E, 16 m), Savitri River, Raigad District, Maharashtra, India by Unmesh Katwate, Chetana Katwate, Rajendra Pawar and Vishwas Shinde on 23.ix.2013; 1 ex., WILD-14-PIS-062, collected from Mahad (18.091°N & 73.466°E, 16 m), Savitri River, Raigad District, Maharashtra, India by Unmesh Katwate, Chetana Katwate, Rajendra Pawar and Vishwas Shinde on 23.ix.2013; 1 ex., ZSI-WRC-P/3687, collected from Mahad (18.091°N & 73.466°E, 16 m), Savitri River, Raigad District, Maharashtra, India by Unmesh Katwate, Chetana Katwate, Rajendra Pawar and Vishwas Shinde on 23.ix.2013; 1 ex., BNHS FWF 76, collected from Shivathar Ghal (18.148°N & 73.619°E, 145 m), Savitri River, Raigad District, Maharashtra, India by Unmesh Katwate, Chetana Katwate, Rajendra Pawar and Vishwas Shinde on 26.xi.2013; 1 ex., WILD-14-PIS-063, collected from Shivathar Ghal (18.148°N & 73.619°E, 145 m), Savitri River, Raigad District, Maharashtra, India by Unmesh Katwate, Chetana Katwate, Rajendra Pawar and Vishwas Shinde on 26.xi.2013; 1 ex., ZSI-WRC-P/3688, collected from Shivathar Ghal (18.148°N & 73.619°E, 145 m), Savitri River, Raigad District, Maharashtra, India by Unmesh Katwate, Chetana Katwate, Rajendra Pawar and Vishwas Shinde on 26.xi.2013; 2 exs., BNHS FWF 83 and 84, collected from Poladpur (17.983°N & 73.470°E, 34 m), Savitri River, Raigad District, Maharashtra, India by Unmesh Katwate and Chetana Katwate on 27.xi.2013; 2 exs., BNHS FWF 77 and 85, collected from Sangameshwar (17.187°N & 73.550°E, 12 m), Shastri River, Ratnagiri District, Maharashtra, India by Unmesh Katwate and Saurabh Rane on 16.ix.2013; 1 ex., WILD- 14-PIS- 064, collected from Sangameshwar (17.187°N & 73.550°E, 12 m), Shastri River, Ratnagiri District, Maharashtra, India by Unmesh Katwate and Saurabh Rane on 16.ix.2013.

*Pethia setnai* (n = 35): Holotype, ZSI-K FF2766, collected from Sanguem, Goa, by S. R. Sane on 1.iii.1985; Paratypes, 6 ex., ZSI-K FF2767, collected from Sanguem, Goa, by S. R. Sane on 1.iii.1985; 9 ex., BNHS FWF 53, 63 to 70, collected from Sanguem, Goa, by U. Katwate, M. Paingankar and N. Dahanukar on 10.viii.2013; 3 ex.; WILD-13-PIS-043 to 045, collected from Sanguem, Goa, by U. Katwate, M. Paingankar and N. Dahanukar on 10.viii.2013; 2 ex., ZSI-WRC-P/3567, collected from Sanguem, Goa, by U. Katwate, M. Paingankar and N. Dahanukar on 10.viii.2013; 9 ex., BNHS FWF 54 to 62, collected from Terekhol River at Madkhol, Maharashtra, by U. Katwate and N. Dahanukar on 12.vi.2013; 3 ex., WILD-13-PIS-046 to 48, collected from Terekhol River at Madkhol, Maharashtra, by U. Katwate and N. Dahanukar on 12.vi.2013; 2 ex., ZSI-WRC-P/3568, collected from Terekhol River at Madkhol, Maharashtra, by U. Katwate and N. Dahanukar on 12.vi.2013. Osteological details were obtained from Katwate et al. (2013).

*Pethia narayani* (n = 2): Syntypes, 2 ex., ZSI-K F12180/1, collected from Cauvery River, Coorg, by C.R.N. Rao (only photographs examined).

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## References

- Altschul, S.F., Gish, W., Miller, W., Myers, E.W. & Lipman, D.J. (1990) Basic local alignment search tool. *Journal of Molecular Biology*, 215, 403–410.  
[http://dx.doi.org/10.1016/S0022-2836\(05\)80360-2](http://dx.doi.org/10.1016/S0022-2836(05)80360-2)
- Beevi, K.S.J. & Ramachandran, A. (2009) Checklist of freshwater fishes collected from Ernakulam District, Kerala, India. *Journal of Threatened Taxa*, 1, 493–494.  
<http://dx.doi.org/10.11609/JoTT.o1559.493-4>
- Beevi, K.S.J. & Ramachandran, A. (2005) A new species of *Puntius* (Cyprinidae, Cyprininae) from Kerala, India. *Journal of the Bombay Natural History Society*, 102, 83–85.
- Conway, K.W. (2011) Osteology of the South Asian genus *Psilorhynchus* McClelland, 1839 Teleostei: Ostariophysi: Psilorhynchidae, with investigation of its phylogenetic relationships within the order Cypriniformes. *Zoological Journal of the Linnean Society*, 163, 150–154.  
<http://dx.doi.org/10.1111/j.1096-3642.2011.00698.x>
- Day, F. (1865) On the fishes of Cochin, on the Malabar Coast of India. *Proceedings of the Zoological Society of London*, 33, 286–318.  
<http://dx.doi.org/10.1111/j.1469-7998.1865.tb02337.x>
- Day, F. (1871) Monograph of Indian Cyprinidae. Part 2. *Journal and Proceedings of the Asiatic Society of Bengal*, 40, 277–336.
- Day, F. (1873) *Report on the fresh water fish and fisheries of India and Burma*. Office of the Superintendent of Government Printing, Calcutta. i-ccciv + 118 pp.
- Dishma, M. & Vishwanath, W. (2013) A new species of the genus *Pethia* from Mizoram, northeastern India (Teleostei: Cyprinidae). *Zootaxa*, 3736, 82–88.
- Doornik, J.A. & Hansen, H. (2008) An Omnibus Test for Univariate and Multivariate Normality. *Oxford Bulletin of Economics and Statistics*, 70, 927–939.

<http://dx.doi.org/10.1111/j.1468-0084.2008.00537.x>

- Edgar, R.C. (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, 32, 1792–1797.  
<http://dx.doi.org/10.1093/nar/gkh340>
- Hamilton, F. (1822) *An Account of the Fishes of River Ganges and its Branches*. George Ramsay and Co., London, vii+405 pp. [39 plates]
- Hammer, Ø., Harper, D.A.T. & Ryan, P.D. (2001) PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica*, 4, 1–9.
- Harris, R.J. (2001) *A primer for multivariate statistics*. Third Edition. Lawrence Erlbaum Associates Publishers, London.
- Hora, S.L., Misra, K.S. & Malik, G.M. (1939) A study of variations in *Barbus (Puntius) ticto* (Hamilton). *Records of the Indian Museum*, 41, 263–279.
- Huberty, C.J. & Olejnik, S. (2006) *Applied MANOVA and Discriminant Analysis*. 2nd Edition. John Wiley and Sons, N.J., 488 pp.
- Jayaram, K.C. (1991) Revision of the genus *Puntius* (Hamilton) from the Indian Region (Pisces: Cypriniformes, Cyprinidae, Cyprininae). *Records of the Zoological Survey of India, Occasional Paper*, 135, 1–178.
- Katwate, U., Paingankar, M.S., Raghavan, R. & Dahanukar, N. (2014a) *Pethia longicauda*, a new species of barb (Teleostei: Cyprinidae) from the northern Western Ghats, India. *Zootaxa*, 3846 (2), 235–248.  
<http://dx.doi.org/10.11646/zootaxa.3846.2.4>
- Katwate, U., Katwate, C., Raghavan, R., Paingankar, M.S. & Dahanukar, N. (2014b) *Pethia lutea*, a new species of barb (Teleostei: Cyprinidae) and new records of *P. punctata* from northern Western Ghats of India. *Journal of Threatened Taxa*, 6, 5797–5818.  
<http://dx.doi.org/10.11609/JoTT.o3929.5797-818>
- Katwate, U., Paingankar, M.S., Jadhav, S. & Dahanukar, N. (2013) Phylogenetic position and osteology of *Pethia setnai* (Chhapgar & Sane, 1992), an endemic barb (Teleostei: Cyprinidae) of the Western Ghats, India, with notes on its distribution and threats. *Journal of Threatened Taxa*, 5, 5214–5227.  
<http://dx.doi.org/10.11609/JoTT.o3857.5214-27>
- Katwate, U., Raghavan, R. & Dahanukar, N. (in prep.) The identity of Hamilton's Ticto Barb *Pethia ticto* (Teleostei: Cyprinidae) with genetic and osteological details.
- Kullander, S.O. (2008) Five new species of *Puntius* from Myanmar (Teleostei: Cyprinidae). *Ichthyological Exploration of Freshwaters*, 19, 59–84.
- Lalramliana, Knight, J.D.M. & Lalramliana, Z. (2014) *Pethia rutila* (Teleostei: Cyprinidae), a new species from Mizoram, northeast India. *Zootaxa*, 3827 (3), 366–374.  
<http://dx.doi.org/10.11646/zootaxa.3827.3.6>
- Linthoingambi, I. & Vishwanath, W. (2007) Two new fish species of the genus *Puntius* Hamilton (Cyprinidae) from Manipur, India, with notes on *P. ticto* (Hamilton) and *P. stoliczkanus* (Day). *Zootaxa*, 1450, 45–56.
- Mattox, G.M.T., Britz, R., Toledo-Piza, M. & Marinho, M.M.F. (2013) *Cyanogaster noctivaga*, a remarkable new genus and species of miniature fish from the Rio Negro, Amazon basin (Ostariophysi: Characidae). *Ichthyological Exploration of Freshwaters*, 23, 297–318.
- Menon, A.G.K., Rema, Devi, K. & Vishwanath, W. (2000) A new species of *Puntius* (Cyprinidae: Cyprininae) from Manipur, India. *Journal of the Bombay Natural History Society*, 97, 263–268.
- Nei, M. & Kumar, S. (2000) *Molecular Evolution and Phylogenetics*. Oxford University Press, New York, 333 pp.
- Pethiyagoda, R., Meegaskumbura, M. & Maduwage, K. (2012) A synopsis of the South Asian fishes referred to *Puntius* (Pisces: Cyprinidae). *Ichthyological Exploration of Freshwaters*, 23, 69–95.
- Potthoff, T. (1984) Clearing and staining techniques. In: Moser, H.G., W.J. Richards, D.M. Cohen, M.P. Fahay, A.W. Kendall, Jr. & S.L. Richardson (eds.). *Ontogeny and Systematics of Fishes. No. 1*. American Society for Ichthyology and Herpetology, Special Publication, pp. 35–37. [760 pp]
- Rema Devi, K. (1992) Fishes of Kalakad Wildlife Sanctuary, Tirunelveli District, Tamil Nadu, India, with a redescription of *Horallabiosa joshuai* Silas. *Records of the Zoological Survey of India*, 92, 193–209.
- Rema Devi, K., Indra, T.J. & Emiliyamma, K.G. (1996) On the fish collections from Kerala, deposited in Southern Regional Station, Zoological Survey of India by NRM Stockholm. *Records of the Zoological Survey of India*, 95, 129–146.
- Schwarz, G. (1978) Estimating the dimension of a model. *Annals of Statistics*, 6, 461–464.
- Talwar, P.K. & Jhingran, A.G. (1991) *Inland Fishes of India and Adjacent Countries*. Oxford-IBH Publishing Co. Pvt. Ltd., New Delhi, 1158pp.
- Tamura, K. & Nei, M. (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution*, 10, 512–526.
- Tamura, K., Stecher, G., Peterson, D., Filipowski, A. & Kumar, S. (2013) MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution*, 30, 2725–2729.  
<http://dx.doi.org/10.1093/molbev/mst197>
- Zeena, K.V. & Beevi, K.S.J. (2011). Fish diversity in Ithipuzha and Murinjapuzha, Kerala, India. *Journal of the Bombay Natural History Society*, 108, 98–102.

**APPENDIX A.** NCBI accession numbers, locations and voucher numbers for cytochrome b gene sequences used for this study.

Species	Location	Voucher	GenBank number
<i>Pethia punctata</i>	Ernakulam, Kerala, India	BNHS FWF 113	KM364559
<i>Pethia muvattupuzhaensis</i>	Ooramana, Kerala, India	WILD-14-PIS-110	KM364557
<i>Pethia muvattupuzhaensis</i>	Ooramana, Kerala, India	BNSH FWF 120	KM364558
<i>Pethia punctata</i>	Bandivade, Maharashtra, India	BNHS-FWF-89	KJ681104
<i>Pethia punctata</i>	Bandivade, Maharashtra, India	BNHS-FWF-90	KJ681105
<i>Pethia punctata</i>	Madkhol, Maharashtra, India	BNHS-FWF-91	KJ681106
<i>Pethia punctata</i>	Madkhol, Maharashtra, India	WILD-14-PIS-103	KJ681107
<i>Pethia longicauda</i>	Azara, Maharashtra, India	BNHS-FWF-100	KJ681101
<i>Pethia longicauda</i>	Azara, Maharashtra, India	WILD-14-PIS-075	KJ681102
<i>Pethia lutea</i>	Bhira, Maharashtra, India	BNHS-FWF-78	KJ681103
<i>Pethia setnai</i>	Madkhol, Maharashtra, India	BNHS-FWF-54	KF977531
<i>Pethia setnai</i>	Madkhol, Maharashtra, India	WILD-13-PIS-046	KF977532
<i>Pethia setnai</i>	Sanguem, Goa, India	BNHS-FWF-53	KF977529
<i>Pethia setnai</i>	Sanguem, Goa, India	WILD-13-PIS-043	KF977530
<i>Pethia conchoniis</i>	West Bengal, India	NBFR:PCS18	JQ795488
<i>Pethia conchoniis</i>	West Bengal, India	NBFR:PCS15	JQ795486
<i>Pethia conchoniis</i>	Aquarium collection	WHT8850_AQ4	JF793624
<i>Pethia conchoniis</i>	Aquarium trade	NRM 52524	EU241452
<i>Pethia conchoniis</i>	Aquarium trade	-	AY004751
<i>Pethia ticto</i>	Ruili, Yunnan, China	-	KC696520
<i>Pethia ticto</i>	Lucknow, Uttar Pradesh, India	NBFR:PTO13	JQ795476
<i>Pethia ticto</i>	Lucknow, Uttar Pradesh, India	NBFR:PTO12	JQ795475
<i>Pethia ticto</i>	Kandalama, Sri Lanka	-	AY708250
<i>Pethia ticto</i>	Boncron, India	WHT8815_6i	EU604679
<i>Pethia phutunio</i>	West Bengal, India	NRM 41712	EU241459
<i>Pethia phutunio</i>	Sambalpur, Odisha, India	BNHS-FWF-95	KJ681108
<i>Pethia melanomaculatus</i>	Kandalama, Sri Lanka	WHT8816_75	EU604678
<i>Pethia reval</i>	Kelani River, Sri Lanka	WHT8812_1	EU604674
<i>Pethia nigrofasciata</i>	Mawanana, Sri Lanka	WHT8838_64	JF793612
<i>Pethia nigrofasciata</i>	Galle, Sri Lanka	-	AY708247
<i>Pethia nigrofasciata</i>	Sri Lanka	WHT8811	EU604677
<i>Pethia bandula</i>	Sri Lanka	WHT8810	EU604673
<i>Pethia bandula</i>	Galapitamada, Sri Lanka	-	AY708244
<i>Pethia bandula</i>	Galapitamada, Sri Lanka	WHT8823_59	JF793597
<i>Pethia cumingii</i>	Sri Lanka	WHT8813	EU604675
<i>Pethia cumingii</i>	Bentota, Sri Lanka	WHT8814_46	EU604676
<i>Pethia cumingii</i>	Galle, Sri Lanka	-	AY708249
<i>Pethia gelius</i>	Aquarium trade	NRM 50829	EU241456
<i>Puntius bimaculatus</i>	Bentota, Sri Lanka	WHT8824_48	JF793598
<i>Puntius titteya</i>	Kalu River, Sri Lanka	WHT8846_11	JF793620
<i>Puntius thermalis</i>	Mawanana, Sri Lanka	WHT8826_54	JF793600

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APPENDIX A. (Continued)

Species	Location	Voucher	GenBank number
<i>Puntius chola</i>	Boncron, India	WHT8827_24i	JF793601
<i>Puntius sophore</i>	Boncron, India	WHT8845_217ai	JF793619
<i>Puntius cf layardii</i>	Gin River, Sri Lanka	WHT8829_69	JF793603
<i>Puntius kelumi</i>	Gin River, Sri Lanka	WHT8830_70	JF793604
<i>Puntius dorsalis</i>	Mamallapuram, Sri Lanka	WHT8831_35i	JF793605
<i>Puntius mahecola</i>	Kottayam, India	WHT8837_16i	JF793611
<i>Dawkinsia filamentosa</i>	Kottayam, India	WHT8833_27i	JF793607
<i>Dawkinsia filamentosa</i>	-	-	JX975487
<i>Dawkinsia singhala</i>	Menik, River, Sri Lanka	WHT8843_33	JF793617
<i>Dawkinsia srilankensis</i>	Pallegama, Sri Lanka	WHT8844_19	JF793618
<i>Dawkinsia tambraparniei</i>	-	-	JX975490
<i>Dawkinsia exclamatio</i>	-	-	JX975489
<i>Dawkinsia rohani</i>	-	-	JX975488
<i>Dawkinsia tambraparniei</i>	-	-	JX049981
<i>Dawkinsia arulius</i>	Aquarium trade	NRM 50830	EU241450
<i>Haludaria fasciata</i>	Chalaky, India	WHT8832_20i	JF793606
<i>Haludaria melanampyx</i>	Aquarium trade	NRM 50827	EU241458
<i>Haludaria fasciata</i>	Chengannur, India	-	AY708262
<i>Haludaria fasciata</i>	Pidavoor, Kerala, India	NBFGR:PFA6	JQ795453
<i>Sahyadria denisonii</i>	Sullya, Karnataka, India	CDR01	GQ247558
<i>Sahyadria denisonii</i>	Cherupuzha, Kerala, India	KGD01	GQ247559
<i>Sahyadria denisonii</i>	Iritty, Kerala, India	VLP02	JX470421
<i>Sahyadria denisonii</i>	Pullooranpara, Kerala, India	CLR02	JX470426
<i>Sahyadria chalakkudiensis</i>	Vettilapara, Kerala, India	CHD02	JX470424
<i>Sahyadria chalakkudiensis</i>	-	-	JX311437
<i>Sahyadria chalakkudiensis</i>	Koruthodu, Kerala, India	PMB11	JX481182
<i>Sahyadria denisonii</i>	Kadakkola, Kerala, India	ACL9	JX470431
<i>Systomus martenstyni</i>	Pallegama, Sri Lanka	WHT8835_21	JF793609
<i>Systomus sp WHT8836</i>	Elahera, Sri Lanka	WHT8826_76	JF793610
<i>Systomus sarana</i>	Boncron, India	WHT8842_21i	JF793616
<i>Systomus timbiri</i>	Menik River, Sri Lanka	WHT8840_35	JF793614
<i>Systomus pleurotaenia</i>	Gin River, Sri Lanka	WHT8839_12	JF793613
<i>Tor khudree</i>	Mawanana, Sri Lanka	WHT8848_85	JF793622
<i>Barbonymus schwanenfeldii</i>	Aquarium trade	WHT8849_AQ1	JF793623
<i>Hypselobarbus jerdoni</i>	Srirangapatam, India	WHT8834_28i	JF793608
<i>Hypselobarbus curmuca</i>	Chalaky, India	WHT8851_1i	JF793625
<i>Labeo dussumieri</i>	Elahera, Sri Lanka	WHT8820_80	JF793594
<i>Osteochilichthys nashii</i>	Chalaky, India	WHT8822_29i	JF793596
<i>Garra ceylonensis</i>	Homadola, Sri Lanka	WHT8818_9	JF793592
<i>Garra mullya</i>	Chalaky, India	WHT8819_31i	JF793593