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Sahyadria, a new genus of barbs (Teleostei: Cyprinidae) from Western Ghats of India

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Abstract: Redline Torpedo Barbs (Teleostei: Cyprinidae), comprising of two species, *Puntius denisonii* and *P. chalakkudiensis*, and six evolutionarily distinct lineages are endemic to the streams of the Western Ghats freshwater ecoregion in peninsular India. Based on molecular and osteological evidence, we demonstrate that these barbs comprise a distinct genus, for which we propose the name *Sahyadria*.

Keywords: Cypriniformes, freshwater fish, *Puntius denisonii, Puntius chalakkudiensis,* taxonomy.

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INTRODUCTION

The Redline Torpedo Barbs, presently placed under the polyphyletic genus *Puntius* Hamilton, 1822 (Teleostei: Cyprinidae), are represented by two species, *Puntius denisonii* (Day, 1865), its look alike *P. chalakkudiensis* Menon, Rema Devi & Thobias, 1999, (Images 1,2,3), and, six evolutionarily distinct lineages (John et al. 2013). Endemic to the rivers of the Western Ghats freshwater ecoregion in peninsular India, these barbs are extremely popular in the aquarium trade with more than 300,000 individuals collected from the wild and exported via airports in the last six years (Raghavan et al. 2013). Both *P. denisonii* and *P. chalakkudiensis* are also listed as 'Endangered' in the IUCN Red List of Threatened Species due to their restricted range, ongoing population decline, and deterioration of the quality of their habitats (Ali et al. 2011; Raghavan & Ali 2011).

In spite of this popularity and conservation significance, the taxonomy and systematics of these barbs, especially their generic allocation, has been rather uncertain. Since its description, *P. denisonii* has been placed under several genera including *Labeo* (Day, 1865 p.299), *Puntius* (Day, 1865 p.212; Jayaram, 1981, p.100), *Barbus* (Günther, 1868, p.146; Day, 1878, p.573; 1889, p.320) and *Hypselobarbus* (Rema Devi et al., 2005, p.1810). Very recently, Pethiyagoda et al. (2012) suggested that *P. denisonii* and *P. chalakkudiensis* warrant placement in a separate genus due to the strikingly different coloration and mouth shape compared to all other congeners.

Here, based on osteological and molecular evidence, we demonstrate that the Redline Torpedo Barbs comprise a distinct genus, for which we propose the name *Sahyadria.*

MATERIALS AND METHODS

Osteological descriptions are based on a cleared and stained specimen (CRG-SAC.2009.21.7) following the methods described in Potthoff (1984). Conway (2011) was followed for osteological nomenclature, and the results compared with published data of related genera (*Dawkinsia*, *Haludaria*, *Pethia*, *Puntius* and *Systomus*; see Pethiyagoda et al. 2012; Pethiyagoda 2013).

The DNA sequences (mitochondrial 16S rRNA and Cytochrome b gene/cytb) were downloaded from NCBI GenBank and used in conjunction with a dataset from an earlier study (Pethiyagoda et al. 2012). These were subsequently used to build the phylogenetic trees, check for monophyly and determine the generic status of these barbs. Sequences were aligned using MUSCLE (Edgar 2004). Protein coding gene (cytb) sequences were translated, aligned, and back-translated prior to the downstream analyses. Tree searches were carried out using maximum likelihood (ML) and Bayesian methodologies. Prior to the ML and Bayesian tree searches, the best-fit nucleotide substitution model was selected for the concatenated dataset using MrAIC (Nylander 2004). Maximum likelihood searches were carried out using Garli v2.0 (Zwickl 2006), ten runs of two replicates (10 × 2) each were run, and the best tree (with the best likelihood value), was selected. One hundred bootstrap replicates were carried out in Garli v2.0, and the bootstrap values were placed on the nodes of the best ML tree (determined earlier) using the sumtrees program from the Dendropy library (Sukumaran & Holder 2010). A Bayesian tree was built in MrBayes v 3.2.1 (Ronquist & Huelsenbeck 2003), and the analysis was performed for 4×10^5 generations sampling every 100^{th} tree. Split frequencies between two independent runs of the four chains were used to decide when to stop the analysis. The Bayesian posterior probabilities (pp) were summarized by building a majority rule consensus tree. The ML bootstrap values and the Bayesian pp's were mapped on the best ML tree recovered earlier. In a second approach, we used sequences from three previously published Cypriniformes phylogeny datasets (Ruber et al. 2007; Pethiyagoda et al. 2012; Dahanukar et al. 2013), and the sequences for the Redline Torpedo Barbs (mentioned above) to build an extended phylogeny to exactly discern the phylogenetic position of the genus within the family Cyprinidae. Maximum likelihood searches were carried out using PHYML (Guindon et al. 2010) and aLRT branch support (Anisimova & Gascuel 2006) values were mapped on the nodes of the phylogeny. The ML phylogeny was used to test for monophyly of the lineage of interest, using Rosenberg's *P* (Rosenberg 2007). The average pair wise tree distance among members of the focal species, and the average pairwise tree distance between the members of the focal species versus the members of the next closest clade were also calculated.

Voucher specimens referred to in this study are deposited in the museum of the Conservation Research Group at St. Albert's College (CRG-SAC), Kochi, India.

RESULTS

Sahyadria gen. nov.

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Type species: *Labeo denisonii* (Day, 1865).

Diagnosis: A genus of cyprinid fishes (Teleostei: Cyprinidae) differing from all South and Southeast Asian genera of Barbinae by the combination of characters and character states including: adult size ranging from 85–190 mm SL; one pair of maxillary barbels; dorsal fin with iii-iv unbranched and eight branched rays, where the last branched ray can be bifurcated right at the base giving appearance of the 9th branched ray; anal fin with ii-iii unbranched and five branched rays; last unbranched dorsal-fin ray weak, apically segmented, not serrated (Fig. 1c); lateral line complete, with 26–28 pored scales on the body; free uroneural absent (Fig. 1d); gill rakers simple, acuminate (not branched or laminate), in two rows with

Image 1. Syntypes of *Sahyadria denisonii* (a) BMNH 1864.7.9.6 (b) AMS B 7913 and (c) NMW 54059. (Photo credit: a - Natural History Museum, London/Rajeev Raghavan; b - Australian Museum/ Rohan Pethiyagoda; c - Natural History Museum, Vienna/Helmut Wellendorf)

Image 2. Topotypic material of *Sahyadria chalakkudiensis* (formalin preserved; CRG-SAC, Uncatalogued).

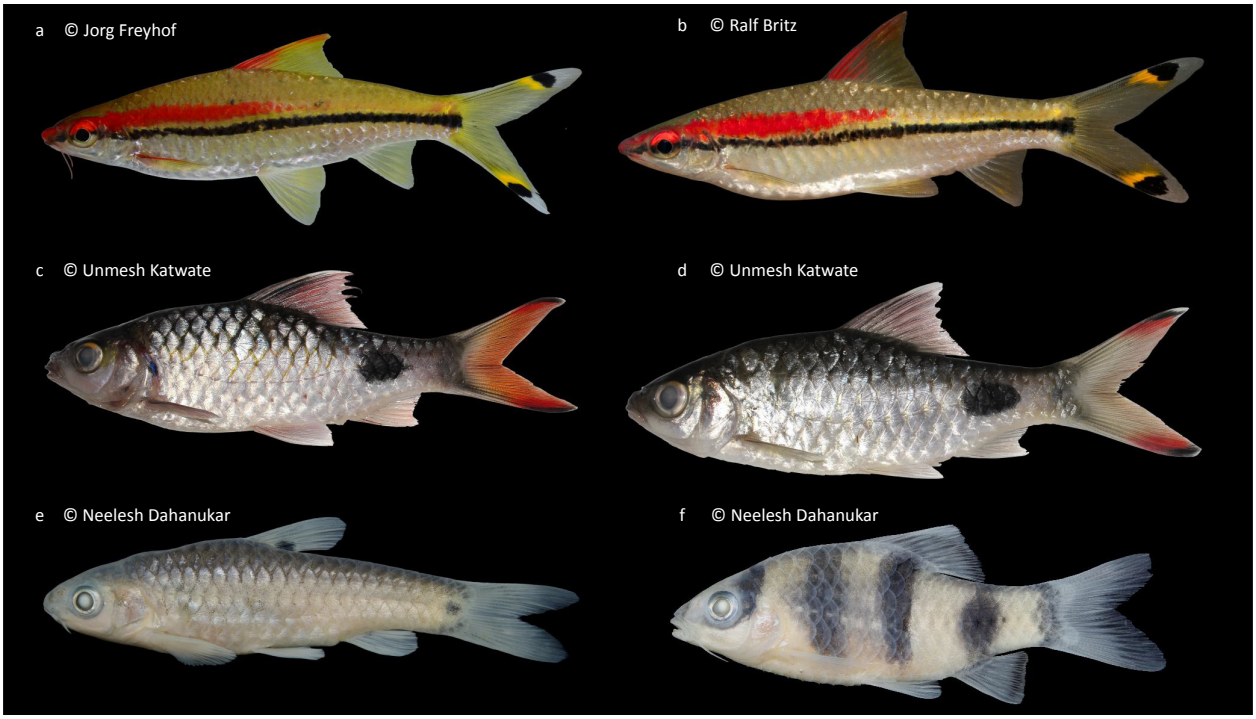

Image 3. *Sahyadria* and some related barbs. (a) *Sahyadria denisonii* (b) *Sahyadria denisonii* (c) *Dawkinsia* cf. *filamentosa* male, (d) *Dawkinsia* cf. *filamentosa* female, (e) *Puntius* cf. *bimaculatus* and (f) *Haludaria* cf. *fasciata*.

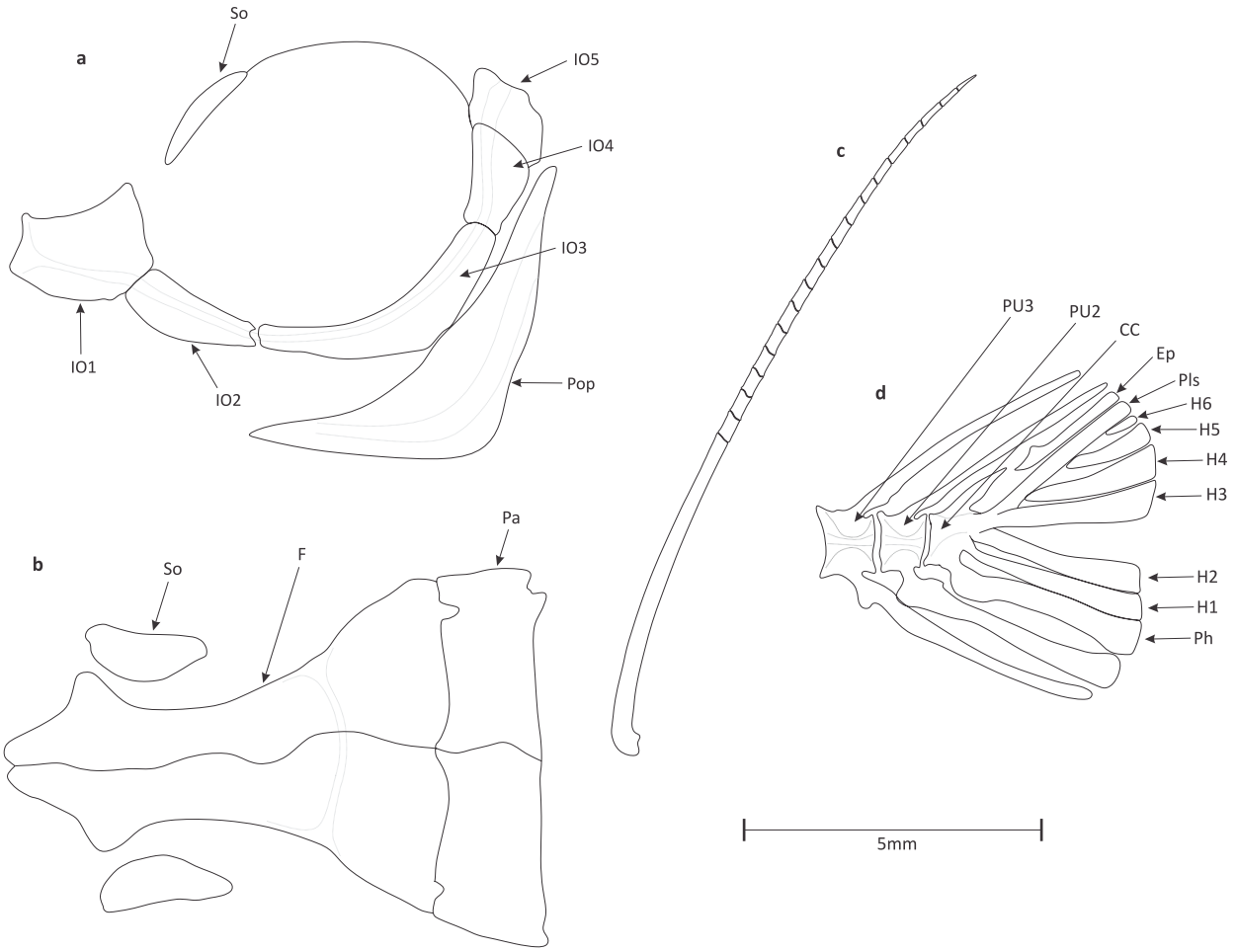

Figure 1. ***Sahyadria denisonii*****, (CRG-SAC 2009.21.7), 51.0mm SL. (a) Circumorbital series (IO1-5, infraorbitals; So, supraorbital; Pop, preopercle); (b) dorsal view of orbital region of cranium (F, frontal; Pa, parietal); (c) last unbranched dorsal-fin ray; and (d) caudal skeleton (CC, compound centrum; Ep, epural; H1-6, hypurals 1-6; 1-5; ; Ph, parhypural; Pls, pleurostyle; PU2, PU3, preural centra 2, 3). Note that the supraorbital sensory canal is not shown.**

12 and 18 rakers respectively; antrorse predorsal spinous ray absent; a post-epiphysial fontanelle absent (Fig. 1b); supraneurals five; infraorbital IO3 slender not overlapping preoperculum (Fig. 1a); pharyngeal teeth 5+3+2; 16 abdominal and 11 caudal vertebrae; and a distinct color pattern (Image 3a,b) with a wide blackish lateral stripe from snout to the base of caudal fin, black line along the lateral line, and scarlet stripe starting from snout until the mid body (varying by the species) above the black stripe. A yellow stripe present between the black and the scarlet stripes; starting from behind the operculum and ending at the hypural region. Caudal fin lobes with oblique black bands covering the posterior quarter towards the tip, and subterminal oblique yellow bands. Dorsal fin with or without a black blotch. In juveniles, a scarlet coloration covers half the height of anterior rays of the dorsal fin.

Phylogenetically, *Sahyadria* gen. nov. forms a monophyletic clade supported by high bootstrap value and Bayesian posterior probability (Fig. 2). The closest genus to *Sahyadria* is *Dawkinsia*, their separation also supported by high bootstrap value and Bayesian posterior probability. Further, in an extended analysis (Fig. 3) using three previously published datasets (Ruber et al. 2007; Pethiyagoda et al. 2012; Dahanukar et al. 2013), the phylogenetic position of the new genus *Sahyadria* is similar to the small dataset (Fig. 2), closest group being *Dawkinsia*. The test for monophyly, Rosenberg's *P*, the chance of obtaining monophyly stochastically, was not significant (Rosenberg's $P = 4.2x10^{-4}$). The intra-clade distance was 0.182 (*Sahyadria*) and inter-clade distance was 0.317 (*Sahyadria* vs. *Dawkinsia*).

Distribution: Genus *Sahyadria* is endemic to the Western Ghats of India, where they occur in 12 west flowing rivers between 9^0–12^0N latitudes.

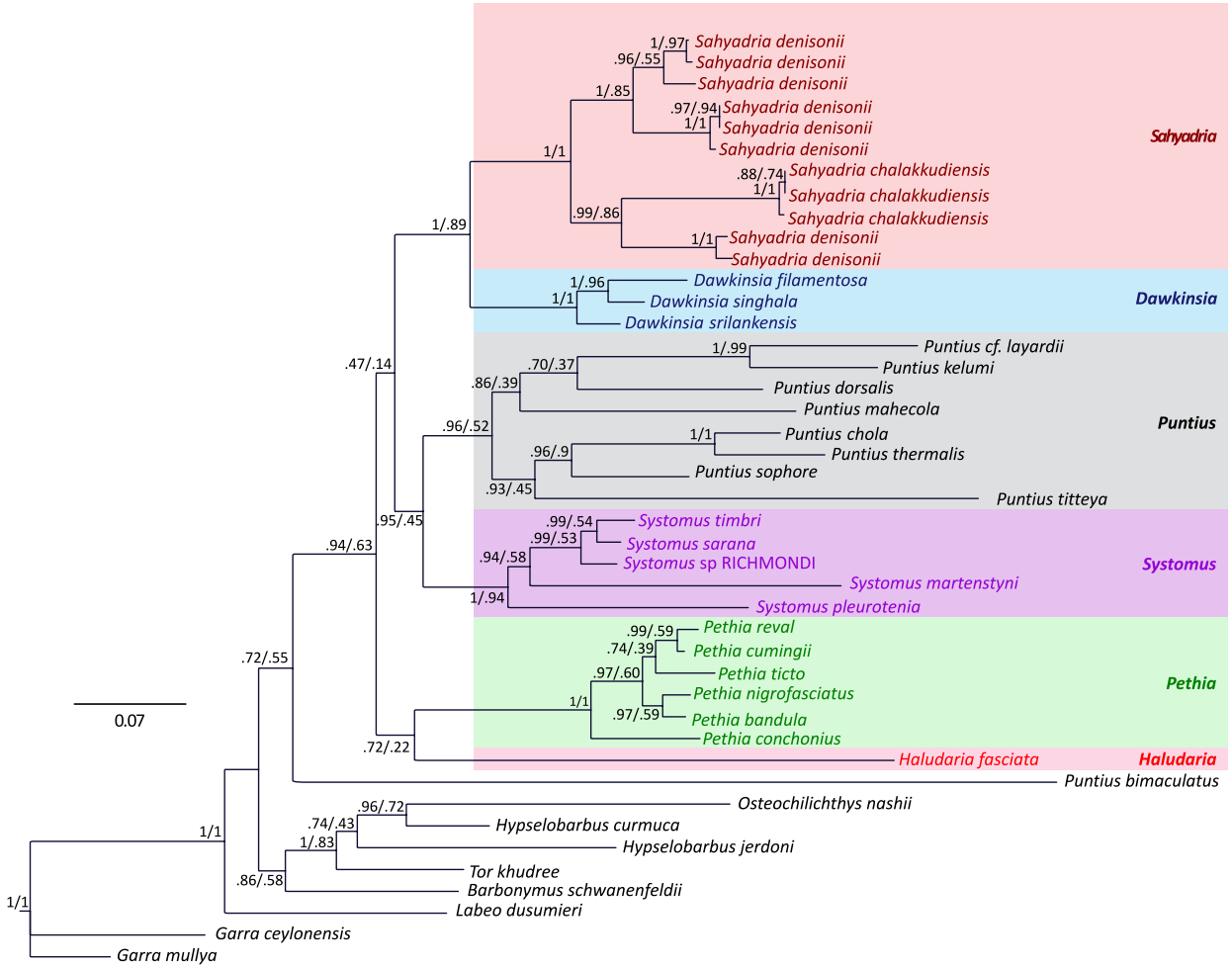

Figure 2. Phylogenetic tree based on concatenated mitochondrial cytochrome b (cytb) and 16s RNA gene sequences (accession numbers: see Pethiyagoda et al. 2012, John et al. 2013 and GQ247528 - GQ247532). Bayesian posteriorprobabilities/ML bootstrap values shown at nodes.

Etymology

The new genus is named after 'Sahyadri', noun, the vernacular name for the Western Ghats mountain ranges; gender feminine.

DISCUSSION

The genus *Sahyadria*, currently comprises of two species *S. denisonii* and *S. chalakkudiensis*, and six evolutionarily distinct lineages (John et al. 2013) all of which are endemic to the Western Ghats region. In their revision of South Asian fishes referred to as *Puntius*, Pethiyagoda et al. (2012) tentatively placed the Redline Torpedo Barbs under the genus *Puntius*. However, they mentioned that the two species have a "strikingly different coloration and mouth shape to all other congeners and are likely to warrant placement in a separate genus in the future".

Sahyadria can be differentiated from its closest sister taxa, *Dawkinsia* by slender frontal (vs. broader frontal), infraorbital IO3 larger than IO4 (vs. almost equal sized IO3 and IO4), IO4 short (vs. elongated), free uroneural absent (vs. present), presence of 16 abdominal and 11 caudal vertebrae (vs. 15 abdominal and 14–17 caudal vertebrae) and 26–28 lateral line scales (vs. 18–22). These two genera are also morphologically different (Image 3a,b,c,d) where *Sahyadria* has a pointed snout projecting beyond mouth, while *Dawkinsia* has a blunt snout and terminal mouth. The color pattern of the two genera is also distinctly different.

Sahyadria differs from the generic characters diagnosing *Puntius* in having broad and stout IO5 and IO4 (vs. large and slender), absence of post-epiphysial fontanelle (vs. present), absence of free uroneural (vs. present) and having 16 abdominal and 11 caudal vertebrae (vs. 12–14 abdominal and 14–16 caudal vertebrae). Additionally, from *Puntius bimaculatus*, which also lacks

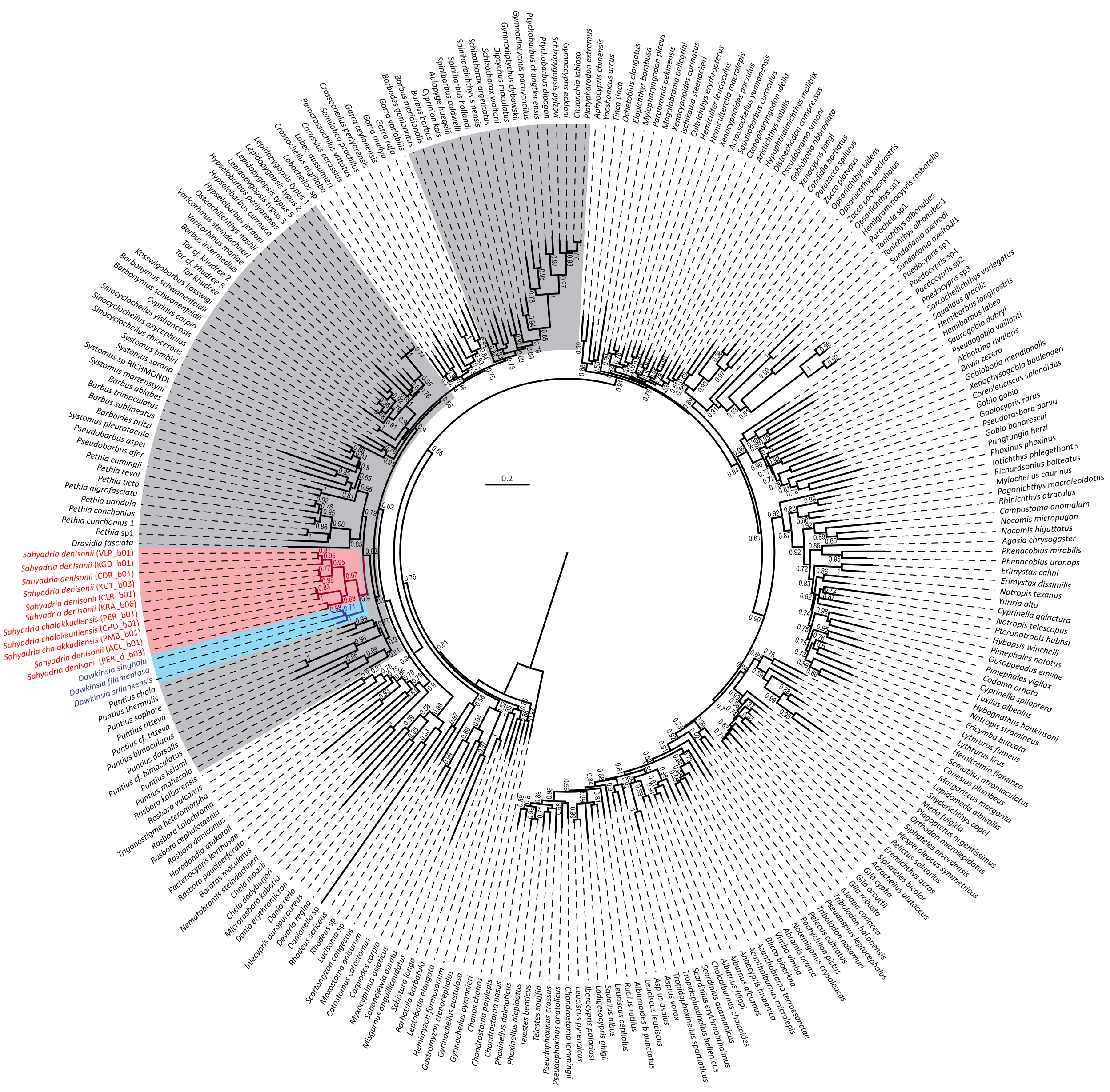

Figure 3. Exact phylogenetic position of *Sahyadria*. Genera currently considered in subfamily Barbinae are highlighted in grey and the clades of interest *Sahyadria* and *Dawkinsia* are highlighted in red and blue respectively.

the presence of post-epiphysial fontanelle, *Sahyadria* differs in pre-opercle non overlapping (vs. overlapping), frontal long and slender (vs. short and stout), presence of eight branched rays in the dorsal fin (vs. 7).

Sahyadria differs from *Haludaria* in pre-opercle non overlapping (vs. overlapping), elongated frontal (vs. short and stout), absence of rostral barbels (vs. presence). *Sahyadria* also substantially differs from *Haludaria* in the long and pointed head structure (Image 3a,b,e). Morphologically, *Sahyadria* has a long and slender caudal peduncle (vs. deep and short) and having a pointed snout projecting beyond mouth (vs. terminal mouth) (Image 3a,b,f). The color pattern in the two genera is also different.

Sahyadria can be differentiated from *Pethia* and *Systomus* based on the most prominent character of the last unbranched dorsal fin ray being non osseous and non serrated (vs. osseous and serrated). *Sahyadria* differs from *Pethia* in having 16 abdominal and 11 caudal vertebrae (vs. 11–13 abdominal and 13–16 caudal vertebrae) and 26-28 lateral line scales (vs. 19–24). *Sahyadria* also differs from *Systomus* in the absence of free uroneural (vs. presence), absence of rostral barbels (vs. presence) and 16 abdominal and 11 caudal vertebrae (vs. 14–15

abdominal and 17–19 caudal vertebrae).

The phylogenetic tree (Fig. 2) retrieves a monophyletic group comprising all the Redline Torpedo Barbs collected throughout its range. Except for the position of *Puntius bimaculatus,* our phylogeny resembles that of Pethiyagoda et al. (2012). An additional extended phylogeny with three previously published datasets (Ruber et al. 2007; Pethiyagoda et al. 2012; Dahanukar et al. 2013) in conjunction with the *Sahyadria* sequences revealed that its phylogenetic position was within Barbinae and that the closest genus was *Dawkinsia*. The Rosenberg's *P* value to test for monophyly (P-value <0.05) clearly showed that the clade (*Sahyadria*) was indeed distinct with clear separation from its sister group, the genus *Dawkinsia*. The tests for intra and inter-clade differentiation also pointed towards ample separation between the two groups and supported the reciprocal monophyly of both clades. Larger intra-clade distance values point towards higher diversity in the clade, and a higher inter-clade diversity shows that the two clades in comparison are increasingly distinct. The intra/inter ratio (0.57 in the case of *Sahyadria* vs. *Dawkinsia*) is another pointer towards the distinctness of the clades, where smaller values points towards smaller differentiation between the individuals of the focal clade than the differentiation between the two tested clades.

Our study thus clearly demonstrates the separation of Redline Torpedo Barbs from its congeners and its monophyly, thus warranting its placement into a new genus *Sahyadria*.

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