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Danio htamanthinus (Teleostei: Cyprinidae), a new species of miniature cyprinid fish from the Chindwin River in Myanmar

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Abstract

Danio htamanthinus, new species, is described from small streams in the vicinity of Htamanthi on the middle Chindwin River. It is most similar to *D. choprae* and *D. flagrans* from the Ayeyarwaddy River drainage, sharing the same elaborate colour pattern with dark vertical bars and a red interstripe along the posterior side, but is distinguished by the absence of a P+1 stripe and presence of a P stripe represented only by small spots. The uncorrected *p*-distance in the mitochondrial COI gene separates *D. htamanthinus* from *D. choprae* by 4.3% and from *D. flagrans* by 7.5%. The largest specimen is only 22.9 mm in standard length (male holotype), but the holotype and one other male, 19.5 mm SL, feature sex-specific pectoral-fin tubercles, and a female as small as 16.6 mm SL has ripening ova. In a phylogenetic analysis based on COI sequences, *D. htamanthinus* is sister taxon of *D. flagrans*+*D. choprae*, and those three species are sister group of *D. margaritatus*+*D.erythromicron.*

Key words: DNA barcode, freshwater, morphometrics, phylogeny, taxonomy

Introduction

The cyprinid fish genus *Danio* Hamilton, 1822, comprises 25 valid species in South and Southeast Asia (Kullander, 2015; Kullander & Britz, 2015). They are predominantly very small, schooling species found in small streams. Maximum known sizes range between 21.2 mm SL in *D. margaritatus* (Roberts, 2007) and nearly 90 mm SL in *D. dangila* (Hamilton, 1822) (Kullander, 2015). Phylogenetic relationships of *Danio* have been covered in several recent papers (e.g., Collins *et al.*, 2012; Fang; 2003; Fang *et al.* 2009; McCluskey *et al.*, 2015; Tang *et al*., 2010) and ornamental-fish aspects are covered by Cottle (2010).

The major concentration of species of *Danio* is in Myanmar, from where 16 valid species have been reported (Kullander, 2015; Kullander & Britz, 2015). Among them *Danio choprae* Hora, 1928, from near Myitkyina on the Ayeyarwaddy River in northern Myanmar, and *D. flagrans* Kullander, 2012, from tributaries to the Mali Hka River in Putao in northernmost Myanmar, are small species with known maximum size 30.7 and 32.1 mm SL, respectively. They possess a distinctive colour pattern consisting of dark vertical bars combined with striking red horizontal stripes (Kullander, 2012). Here we report one more species sharing a general colour pattern with *D. choprae* and *D. flagrans*. It was collected from small water bodies near Htamanthi on the middle Chindwin River.

Material and methods

Specimens are kept in the fish collections of the Academy of Natural Sciences of Drexel University (ANSP), Philadelphia, and the Swedish Museum of Natural History (NRM), Stockholm. Counts and measurements were made according to Fang (1997). Colour pattern terminology follows Fang (1998) and Kullander (2015). Horizontal dark stripes are identified by alphanumeric annotations: the P stripe is the dark stripe along the middle of the side, those above are numbered P+1, P+2, those below P-1, P-2, P-3; stripes on the anal fin are numbered with the middle one the A stripe, the proximal stripe A+1, and the distal stripe A-1. The term interstripe is used for pale

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areas between dark melanophore-rich stripes, in this paper without numbering. Fin-ray counts from pectoral, pelvic, dorsal, and anal fins were obtained directly from the specimens under a dissection microscope and with through-falling light. Vertebral counts were taken from X-radiographs made with a Philips MG-105 low voltage X-ray unit and Kodak X-Omat V plates, or as digital images with a Kevex 130kVP microfocus X-ray source with a Samsung/Rayence 17x17 inch DR panel. Abdominal vertebrae counts include the Weberian apparatus (assumed to contain four centra).

Statistics were calculated using SYSTAT v. 13 (Systat Software, 2009), except that the principal component analysis (PCA) of measurements was made using a separate procedure for component shearing, partialling out multivariate size residues from the second and further components as described by Humphries *et al*. (1981). The PCA was made with log-transformed measurement data to a tenth of a millimetre in a covariance matrix, and without rotation.

DNA was extracted from fin clips or muscle tissue using a Thermo Scientific KingFisher Duo (Thermo Fisher Scientific) fully automated liquid-handling instrument, with the Cell and Tissue DNA Kit (Thermo Fisher Scientific) and recommended protocol. PCR was performed using the primers and protocols of Ward *et al*. (2005). Sequencing of both strands of all fragments was carried out by Macrogen Europe (Amstelveen, The Netherlands). 654 basepairs from the 5′ end of the mitochondrial cytochrome oxidase subunit 1 (COI) gene (the standard DNA barcode region) were sequenced. Sequences were combined with relevant published sequences from GenBank, and selected sequences not deposited in GenBank were downloaded from the Barcode of Life Data Systems (BOLD, 2012). Sequences were analysed using the software Geneious R9 (Kearse *et al.*, 2012), except for the Bayesian phylogenetic analysis which was performed using MrBayes v3.2 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003). Bayesian phylogenetic analysis was performed with the model GTR + Γ + I, and the analysis was run for 4 million generations. Trees were sampled every 1000 generations, and the first 25% of pseudo-samples were discarded as “burn-in.” The average standard deviation of split frequencies was 0.004, the potential scale reduction factor 1.000, indicating that convergence had been reached. Convergence was further checked with the software Tracer (Rambaut & Drummond, 2007) version 1.4. The trace plot showed no trend, and effective sample size (ESS) was 6002.

New COI sequences produced for this paper are, with NRM voucher and GenBank accession numbers:

Danio choprae, NRM 51965, GenBank KX852477, Myanmar: Kamaing
Danio erythromicron (Annandale, 1918)*,* NRM 51833, GenBank KX852478, aquarium
Danio erythromicron, NRM 51963, GenBank KX852479, Myanmar: Inle Lake
Danio flagrans, NRM 41671, GenBank KX852480, Myanmar: Putao
Danio htamanthinus, NRM 42636, GenBank KX852481, Myanmar: Htamanthi
Danio htamanthinus, NRM 42621, GenBank KX852482, Myanmar: Htamanthi
Danio margaritatus, NRM 56722, GenBank KX852483, aquarium
Danio margaritatus, NRM 58611, GenBank KX852484, Myanmar: Baryin

Comparative material. *Danio* sp. cf. *htamanthinus,* ANSP 93955, 2, ca. 22 mm SL. Myanmar, Kachin State: Shinbwiyang [Shin Bway Yang], 26°41′41″N 96°12′31″E, J.H. Rehn, 1945. — *Danio choprae* and *D. flagrans* as listed in Kullander (2012), other species of *Danio* as listed in Kullander (2012), and references therein, Kullander (2015), and Kullander & Britz (2015). For the phylogenetic analysis complementary sequences of *D. choprae* and *D. flagrans* were downloaded from GenBank or BOLD (2012).

***Danio htamanthinus,* new species**

(Figs. 1 a–c)

Holotype. NRM 69502, adult male, 22.9 mm SL. Myanmar, Sagaing Division, stream outside Htamanthi village, 1 km W Chindwin River; 25°20′4″N 95°16′39″E. 1 Dec 2013. S.O. Kullander, M. Norén and B. Kajrup, field no. SOK-2013-029.

Paratypes. NRM 11997. 8, 17.1–22.1 mm SL; 42621, 1, not measured; NRM 69503, 10, 15.9–18.3 mm SL.

Same data as holotype. — NRM 11998. 3, 19.5–20.5 mm SL; 19, 15.0–20.5 mm SL; NRM 42613, 1, 16.8 mm SL; NRM 42636, 1 not measured; NRM 42638, 1, not measured; NRM 42639, 1, not measured. Myanmar: Sagaing Division, swamp and small stream 5 km W Htamanthi village; 25°19′44″N 95°14′26″E. 1 Dec 2013. S.O. Kullander, M. Norén and B. Kajrup, field no. SOK-2013-028.

Diagnosis. Similar to *Danio choprae* and *D. flagrans* in general colour pattern with several vertical bars on side of abdomen; red interstripe below P+2 stripe in life, and presence of well-developed tubercles on infraorbital ossicles. Distinguished from *D. choprae* and *D. flagrans* by absence of P+1 stripe (vs. presence) and P stripe expressed as row of spots posteriorly on side and caudal peduncle (vs. P stripe entire). Distinguished also from *D. flagrans* by deeper body (26.5% SL vs. 22.5–26.6% SL), shorter caudal peduncle (17.1–20.5% SL vs. 20.5–24.7% SL), and longer anal-fin base (19.0–21.8% SL vs.14.2–18.3% SL); from *D. choprae* by longer maxillary barbel (16.6–23.0% SL, vs. 9.3% SL, reaching to below pectoral-fin base adults vs. not reaching pectoral-fin base. Distinguished from all other species of *Danio* by characters in combination: rostral barbel present (absent in *D. erythromicron, D. margaritatus*, *D. nigrofasciatus*, *D. tinwini*, variable in *D. rerio*); mandibular barbel present (absent in *D. erythromicron* and *D. margaritatus*); lateral line absent (complete in *D. absconditus, D. annulosus, D. assamila, D. catenatus, D. concatenatus, D. dangila, D. feegradei,* and *D. meghalayensis*; abbreviated in *D. aesculapii, D. albolineatus, flagrans, D. jaintianensis, D. kerri, D. kyathit, D. quagga,* and *D. roseus*; abbreviated or absent in *D. choprae*), colour pattern consisting of vertical bars or spots anteriorly on side (vertical bars also posteriorly on side in *D. erythromicron*; light spots on dark ground in *D. annulosus, D. assamila, D. catenatus, D. concatenates, D. dangila, D. sysphigmatus,* and *D. margaritatus*; dark spots on light ground in *D. kyathit* and *D. tinwini*; horizontal stripes only in *D. albolineatus, D. jaintianensis*, *D. kerri, D. meghalayensis*, *D. nigrofasciatus*, *D. quagga*, *D. rerio,* and *D. roseus*; bars anteriorly, two horizontal rows of spots posteriorly in *D. aesculapii*); branched dorsal-fin rays 7½ (6½ in *D. aesculapii* and *D. tinwini*); circumpeduncular scale rows 10 (12 in *D. aesculapii, D. albolineatus*, *D. erythromicron, D. kerri;* 14 in *D. absconditus, D. annulosus, D. assamila, D. catenatus, D. concatenatus, D. dangila, D. feegradei,* and *D. meghalayensis*).

Description. General body features and pigmentation are illustrated in Figures 1 a–c. Proportional measurements are summarized in Table 1. Counts from up to 12 specimens (NRM 11997, NRM 11998, NRM 69502). Holotype count marked with asterisk.

Body compressed, elongate (body depth 26.5–32.0% SL), sexes isomorphic, except for males with tubercles on pectoral fin; two specimens dissected, 16.6 and 17.9 mm SL, are females with large ovaries with ripening ova. Head laterally compressed, slightly deeper than wide. Snout short, obtuse, shorter than eye diameter. Mouth terminal, oblique in profile, jaws about equal in anterior extension. Small projection at dentary symphysis fitting into depression in upper jaw. Maxilla reaching to slightly beyond vertical from anterior margin of orbit. Lower jaw ending anteriorly slightly below middle of eye. Dentary with anterior lateral lobe with pointed tubercles; laterally a distinct band of 2–3 dense rows of pointed tubercles; sparse pointed tubercles medially on dentary. Holotype with row of minute tubercles in single row on adocular margin of interorbitals 2 and 3. Rostral barbel long, reaching to posterior margin of orbit or slightly beyond; maxillary barbel long, reaching to or slightly beyond insertion of pectoral fin.

Many specimens with scales abraded so that accurate counts could not be made. Lateral line absent; about 28 (6), 29 (1), 30* (1), 31 (2) scales in a row representing expected lateral line course. Scales in a row along middle of side about 28*–30. Median predorsal scales 15 (2), 16* (4), 17 (4). Body lateral scale rows 7* (9) between dorsal-fin origin and pelvic-fin insertion. Circumpeduncular scale rows 10* (7). A row of scales along anal-fin base.

Dorsal-fin rays ii.7½* (12); anal-fin rays iii.11½ (2), iii.12* (8), iii.13½ (2); pectoral-fin rays i.10 (6), i.11* (5), i.12 (1); pelvic-fin rays i.6* (6), i.7 (6). Principal caudal-fin rays, 10+9 (6), 10+10* (1). Dorsal fin inserted at highest point of dorsum, at about midway distance from head to caudal-fin base, at vertical from anal-fin origin. Pectoral-fin insertion at about vertical through posterior margin of opercle; extending to pelvic-fin insertion. Pectoral-fin axial lobe well developed. Bands of minute tubercles present on lateral aspect of first two or three branched rays of pectoral fin in holotype and 19.5 mm specimen (NRM 11998); pectoral-fin tubercles absent in all other specimens. Pelvic-fin origin situated slightly anterior to middle of body, well anterior to dorsal-fin origin; pelvic fin reaching to or almost to anal-fin origin, usually slightly shorter. Pelvic axillary scale present. Caudal fin forked, lobes with rounded tips, lobes equal or upper lobe slightly longer than lower lobe; lower lobe slightly wider than upper lobe.

Vertebrae 15+18=33* (4), 16+17=33 (13), 16+18=34 (3); predorsal vertebrae 13* (3), 14 (7); vertebrae contained within caudal peduncle 7 (2), 8* (8). Pharyngeal bone with tooth formula 2,3,5-5,3,2.

TABLE 1. Morphometry of *Danio choprae, D. flagrans* (data from Kullander, 2012), and *D. htamanthinus* (NRM 11997, N=7; NRM 11998, N=2; NRM 69502, holotype). Measurements are in percent of standard length, except for standard length and total length (in mm). SD, standard deviation. Values separating species are highlighted in boldface. HT = holotype.

	Danio choprae					*Danio flagrans*					*Danio htamanthinus*					
	N	min	max	mean	SD	n	min	max	mean	SD	HT	n	min	max	mean	SD
Standard length (mm)	20	19.8	29.9	23.9	3.6	21	18.4	33.0	25.3	4.8	22.9	10	17.1	22.9	20.6	1.6
Total length (mm)	20	25.8	38.6	31.6	4.4	20	26.2	43.3	33.8	6.0	31.0	9	23.1	31.0	27.0	2.4
Body depth	20	**26.6**	**31.6**	28.6	1.4	21	**22.5**	**26.5**	24.9	0.9	29.7	10	26.5	32.0	29.8	1.5
Head length	20	23.3	27.3	24.5	0.9	21	23.5	26.8	25.1	1.0	24.5	10	23.5	26.1	24.7	0.9
Snout length	20	5.2	7.1	6.3	0.4	21	5.5	6.7	6.2	0.3	5.7	10	5.6	7.0	6.1	0.4
Head depth	20	17.2	19.8	18.6	0.7	21	16.6	19.6	18.1	0.7	17.0	10	17.0	19.8	18.6	1.0
Head width	20	13.3	15.2	14.2	0.6	21	12.6	15.5	13.9	0.5	13.5	10	13.5	15.3	14.2	0.6
Upper jaw length	20	8.6	10.6	9.4	0.6	21	8.7	10.9	10.0	0.5	10.0	10	9.0	10.8	9.9	0.6
Lower jaw length	20	9.9	13.1	11.3	0.8	21	11.1	13.0	12.0	0.4	11.4	10	11.0	12.7	11.8	0.5
Orbital diameter	20	8.0	9.9	9.0	0.5	21	7.7	9.8	8.7	0.5	9.2	10	8.6	9.9	9.2	0.5
Interorbital width	20	10.7	13.1	11.7	0.6	21	10.3	11.9	11.1	0.4	10.5	10	10.5	12.2	11.4	0.5
Caudal peduncle length	20	**16.1**	**19.1**	17.7	0.8	21	**20.5**	**24.7**	22.2	1.2	19.2	10	17.1	20.5	19.0	1.1
Caudal peduncle depth	20	11.8	15.1	13.6	0.9	21	11.4	13.3	12.2	0.5	13.1	10	11.7	13.3	12.5	0.6
Dorsal-fin base length	20	10.3	13.8	12.0	0.8	21	9.8	12.8	11.3	0.9	11.8	10	10.7	12.3	11.5	0.6
Anal-fin base length	20	**19.2**	**23.9**	21.3	1.3	21	**14.2**	**18.3**	16.2	1.1	21.8	10	19.0	21.8	20.3	1.1
Predorsal length	20	60.3	66.7	63.7	1.9	21	57.6	63.3	61.1	1.6	64.6	10	62.0	66.2	63.9	1.4
Preanal length	20	59.6	65.8	62.7	1.7	21	59.3	64.4	62.2	1.4	61.1	10	59.0	64.3	61.6	1.5
Prepelvic length	20	44.1	49.4	46.8	1.7	21	44.7	49.1	46.8	1.2	46.7	10	44.5	48.9	46.8	1.3
Pectoral-fin length	20	20.1	27.8	22.8	1.9	20	20.9	25.9	22.9	1.3	23.1	9	23.0	27.4	25.3	1.6
Pelvic-fin length	20	13.3	18.1	15.5	1.4	21	13.8	17.1	15.2	1.0	15.7	10	14.9	18.3	16.2	1.1
Rostral barbel length	20	**5.9**	**10.1**	8.2	1.1	21	**10.3**	**18.7**	14.7	3.1	9.2	10	**9.2**	**14.4**	11.7	1.5
Maxillary barbel length	20	**9.3**	**15.7**	13.2	1.5	21	14.2	23.1	18.4	2.7	16.6	10	**16.6**	**23.9**	19.9	2.2

FIGURE 1. *Danio htamanthinus*: **a**, holotype, NRM 69502, male, 22.9 mm SL; Myanmar: Sagaing Division, stream close to Htamanthi village; **b**, paratype, NRM 42613. 16.8 mm SL; Myanmar: Sagaing Division: 5 km W Htamanthi village; **c**, paratype, NRM 42613, 16.8 mm SL, live colours immediately after capture.

Colouration in preservative. Sexual dimorphism absent in colour pattern. Ground colour white to pale yellowish white. Dark markings brown or grey. Head dorsally pale brownish grey. Sides of head sparsely pigmented. Dorsum light brown with dark brown middorsal stripe anterior to dorsal fin; scale row adjacent to middorsal stripe conspicuously lighter. Next to it scales bearing narrow brown P+2 stripe from near gill cleft caudad. P+1 stripe absent. Sides anteriorly diffusely pigmented; from about distal part of pectoral fin caudad a number of dark vertical bars, extending vertically from level of hypothetical P+1 stripe level to about level of pectoral-fin base. Number of distinct bars (excluding spots) in measurement series of specimens 17.1–22.9 mm SL, 6 (3), 7* (5), 8 (2); bars gradually shorter caudally, becoming a row of more or less distinct small dark spots representing P stripe, and terminating with small spot at middle of base of caudal fin. Pectoral and pelvic fins hyaline. Dorsal fin hyaline with dark brown or blackish grey D stripe from middle of anterior margin obliquely across rays to tips of posterior rays; beyond that stripe hyaline. Anal fin basally grey, margined by black A stripe from middle of anterior margin caudad across rays to tip of posterior rays; distal to that stripe hyaline. Caudal fin hyaline or slightly pigmented; dark grey or black stripe along upper rays, dorsal margin white; ventral margin white.

Colour in life. A specimen photographed alive (Fig. 1c) with yellow margins to caudal fin lobes, anterior rays of dorsal fin, and yellow stripe distal to black A stripe. Red pigment patches on eye and dorsally on gill cover. Brilliant red interstripe stripe from caudal-fin base rostrad to anterior side, continued very narrow to gill cleft; red colour reaching fainter ventrally between black bars and blotches on caudal peduncle.

DNA. The COI sequences of two specimens of *D. htamanthinus* are identical, and differ from that of *D. choprae* and *D. flagrans* by 4.3% and 7.5% uncorrected *p*-distance. *Danio choprae* and *D. flagrans* differ by 7.0%.

Etymology. The specific name *htamanthinus* is an adjective formed on the name of the village near which the type series was collected.

Geographical distribution and habitat. *Danio htamanthinus* is so far only known from two small streams near Htamanthi on the right bank of the Chindwin River (Fig. 2) and probably also in Shin Bway Yang further upstream in the Chindwin river basin. The type locality (Fig. 3) was a small, shallow stream with clear water, margined by low vegetation, coming out of the forest and crossing a dirt road under a bridge. Associated species were *Paracanthocobitis* cf. *rubidipinnis* (Nemacheilidae), *Channa gachua* (Channidae), *Pethia meingangbii* and *P. thelys* (Cyprinidae), and undetermined species of *Badis* and *Dario* (Badidae), *Danio* and *Esomus* (Cyprinidae). A second locality near Htamanthi was a flooded cleared area with stagnant clear water over grasses and a small trickle of stream feeding it (Fig. 4). Associated species here were *Danio* cf. *quagga, Puntius chola*, *Pethia meingangbii, Rasbora ornata* (Cyprinidae), and undetermined species of *Amblyceps* (Amblycipitidae) and *Dario*.

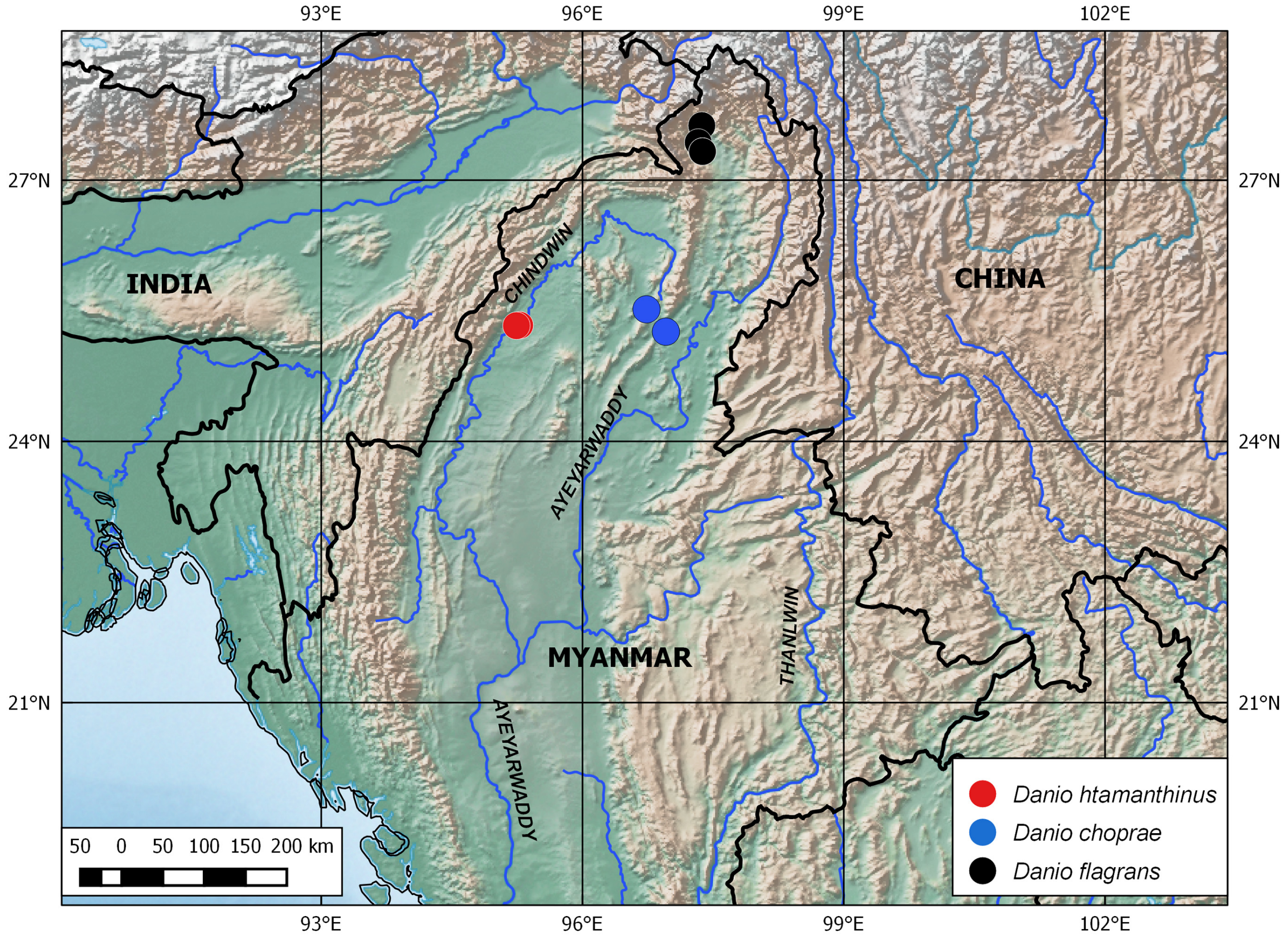

FIGURE 2. Map of northern part of Myanmar showing collecting localities of *Danio choprae, D. flagrans,* and *D. htamanthinus*.

FIGURE 3. Myanmar, Sagaing Division, small stream near Htamanthi, type locality of *Danio htamanthinus.*

FIGURE 4. Myanmar, Sagaing Division, small, flooded stream near Htamanthi, habitat of *Danio htamanthinus*, 1 December 2013.

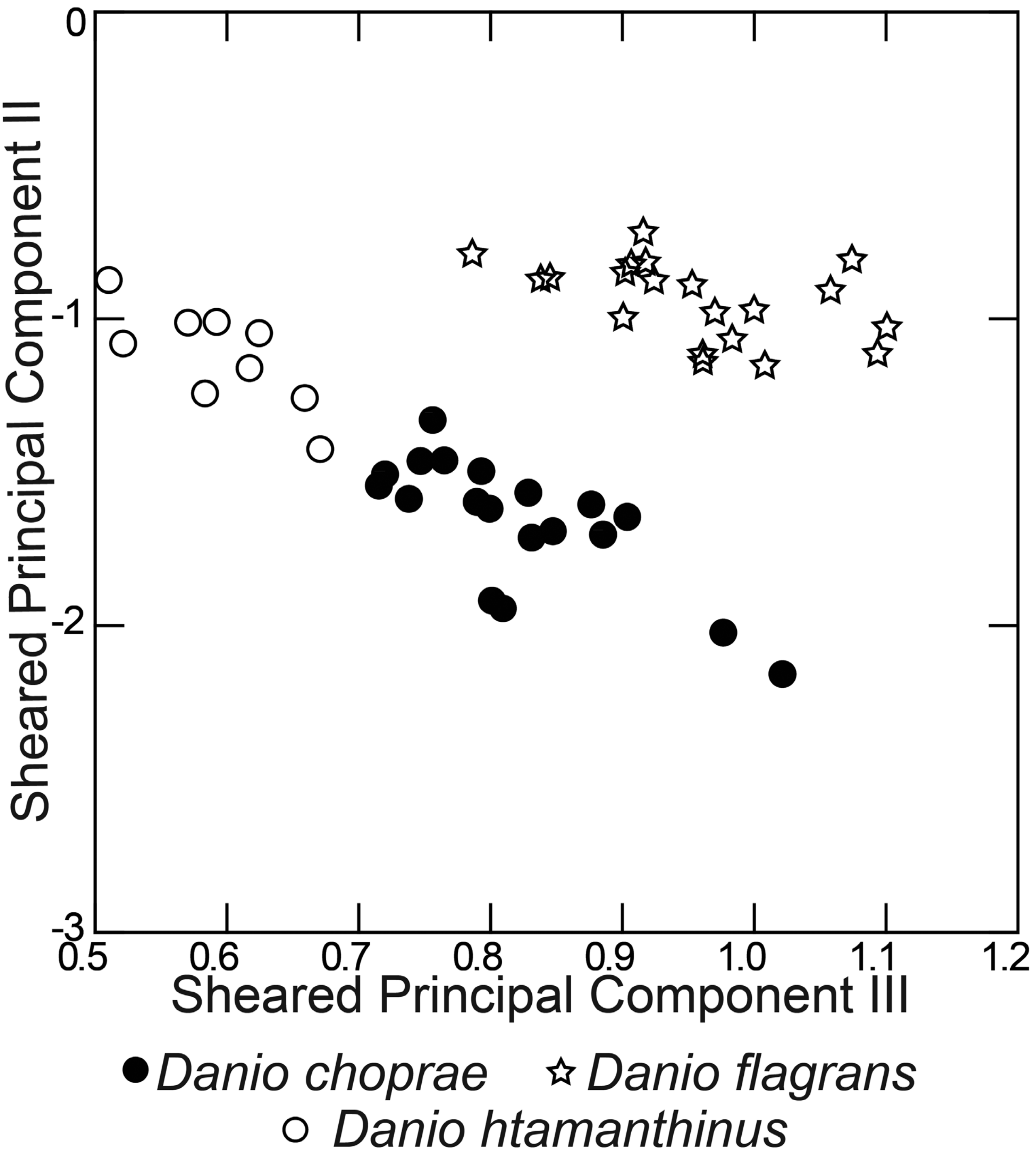

FIGURE 5. Principal Component Analysis of pooled morphometric data from *Danio choprae* (n=20), *Danio flagrans* (n=20) and *D. htamanthinus* (n=9). Plot of scores of Principal Component II (mainly variation in barbel lengths) on Component III (mainly lengths of maxillary barbel, anal-fin base and caudal peduncle).

Comparative morphometry. Proportional measurements of *D. htamanthinus* are similar to those of *D. choprae*, and they differ in the same way from *D. flagrans* in anal-fin base length and caudal peduncle length, but *D. choprae* stands out with shorter barbels than *D. htamanthinus* and *D. flagrans* (Table 1). Size variation within species samples is small, and significant allometries could not be detected. Although the examined specimens of *D. htamanthinus* are smaller than those of *D. flagrans* and *D. choprae*, allometries may thus not influence the ratios significantly, and comparison of ratios may be useful for identification. The Principal Component Analysis (Fig. 4; Table 2) also shows separation mainly on barbel length, but also detects the short anal fin and long caudal peduncle in *D. flagrans,* compared with the other two species.

TABLE 2. Component loadings from Principal Component Analysis of morphometric data from *Danio choprae* (N=20), *D. flagrans* (N=20), and *D. htamanthinus* (N=9); data from *D. choprae* and *D. flagrans* from Kullander (2010); the three highest loadings for each component are highlighted in **boldface**.

	PC I	PC II	Sheared PC II	PC III	Sheared PC III	PC IV	Sheared PCIV
Standard length	0.208	-0.092	-0.069	0.101	0.115	-0.009	-0.011
Body depth	0.171	-0.223	-0.202	-0.244	-0.234	0.272	0.271
Head length	0.192	-0.058	-0.037	0.189	0.203	0.093	0.091
Snout length	0.203	-0.093	-0.071	0.139	0.153	0.041	0.039
Head depth	0.192	-0.116	-0.095	0.076	0.089	0.12	0.118
Head width	0.189	-0.107	-0.086	0.110	0.123	0.166	0.164
Upper jaw length	0.226	-0.012	0.012	0.050	0.065	0.082	0.080
Lower jaw length	0.202	0.006	0.027	0.121	0.135	0.122	0.119
Orbital diameter	0.156	-0.078	-0.060	0.010	0.020	0.020	0.018
Interorbital width	0.187	-0.128	-0.107	0.052	0.065	0.157	0.155
Caudal peduncle length	0.222	0.148	0.170	**0.513**	**0.530**	-0.172	-0.175
Caudal peduncle depth	0.213	-0.255	-0.230	0.043	0.057	-0.193	-0.195
Dorsal-fin base length	0.221	-0.168	-0.143	-0.134	-0.120	**-0.580**	**-0.582**
Anal-fin base length	0.162	**-0.376**	**-0.355**	**-0.473**	**-0.465**	-0.191	-0.193
Predorsal length	0.212	-0.132	-0.108	-0.015	-0.002	0.130	0.128
Preanal length	0.215	-0.108	-0.084	0.134	0.149	0.142	0.140
Prepelvic length	0.207	-0.095	-0.072	0.136	0.150	0.196	0.194
Pectoral-fin length	0.168	-0.014	0.004	-0.095	-0.084	0.238	0.236
Pelvic-fin length	0.200	-0.108	-0.085	-0.041	-0.028	**-0.375**	**-0.377**
Rostral barbel length	0.406	**0.639**	**0.677**	-0.033	-0.007	**-0.240**	**-0.244**
Maxillary barbel length	0.299	**0.408**	**0.436**	**-0.532**	**-0.515**	0.238	0.234
Eigenvalue	0.6398	0.1385	N/A	0.0199	N/A	0.0073	N/A
Cumulative variance	76.8%	93.4%	N/A	95.8%	N/A	96.7%	N/A

Phylogenetic relationships. The close relationship of *D. choprae, D. flagrans, D. margaritatus* and *D. erythromicron* has already been indicated (Collins *et al.*, 2012; Fang *et al.* 2009; McCluskey *et al.*, 2015; Tang *et al.*, 2010), although we here present the first analysis of all species together. A Bayesian analysis of relationships based on the mitochondrial COI gene and with *Danio dangila* and *D. rerio* (Hamilton, 1822) as outgroup (Fig. 6) confirms the sister-species relationship of *D. margaritatus* and *D. erythromicron* from the Thanlwin drainage near Lake Inle; and recovers *D. choprae, D. flagrans* and *D. htamanthinus* as sister group of the Thanlwin species. *Danio htamanthinus* shows as sister species of the pair *D. choprae* and *D. flagrans*, which is unexpected given that *D. choprae* and *D. htamanthinus* are more similar in colour pattern and shape. On the other hand, the probability for the latter grouping is low. Inclusion of more data may show that the clade *D. choprae+D. flagrans+D. htamanthinus* represents a trichotomy, or that the similarities between *D. choprae* and *D. htamanthinus* are plesiomorphies.

Discussion

Danio htamanthinus has nearly the same colour pattern as *D. choprae* with several distinct vertical bars on the side. This colour pattern is almost the same as in *D. flagrans*, in which species, however, the vertical bars tend to be broader and more irregular. In life, all three species have a bright red or orange stripe from the middle of the side to the base of the caudal fin. The similarities in colour pattern are indicative of a close relationship, supported by

DNA analysis (Fig. 6). *Danio choprae* occurs in the upper Ayeyarwaddy River drainage, with type locality near Kamaing, close to Myitkyina (Kullander, 2012), whereas *D. flagrans* is known only from near Putao, in the Mali Hka drainage, a tributary of the upper Ayeyarwaddy River (Kullander 2012). The relatively scattered localities are due to limitations of access to other areas in the Irrawaddy drainage, and the distribution of each species may be expected to be greater.

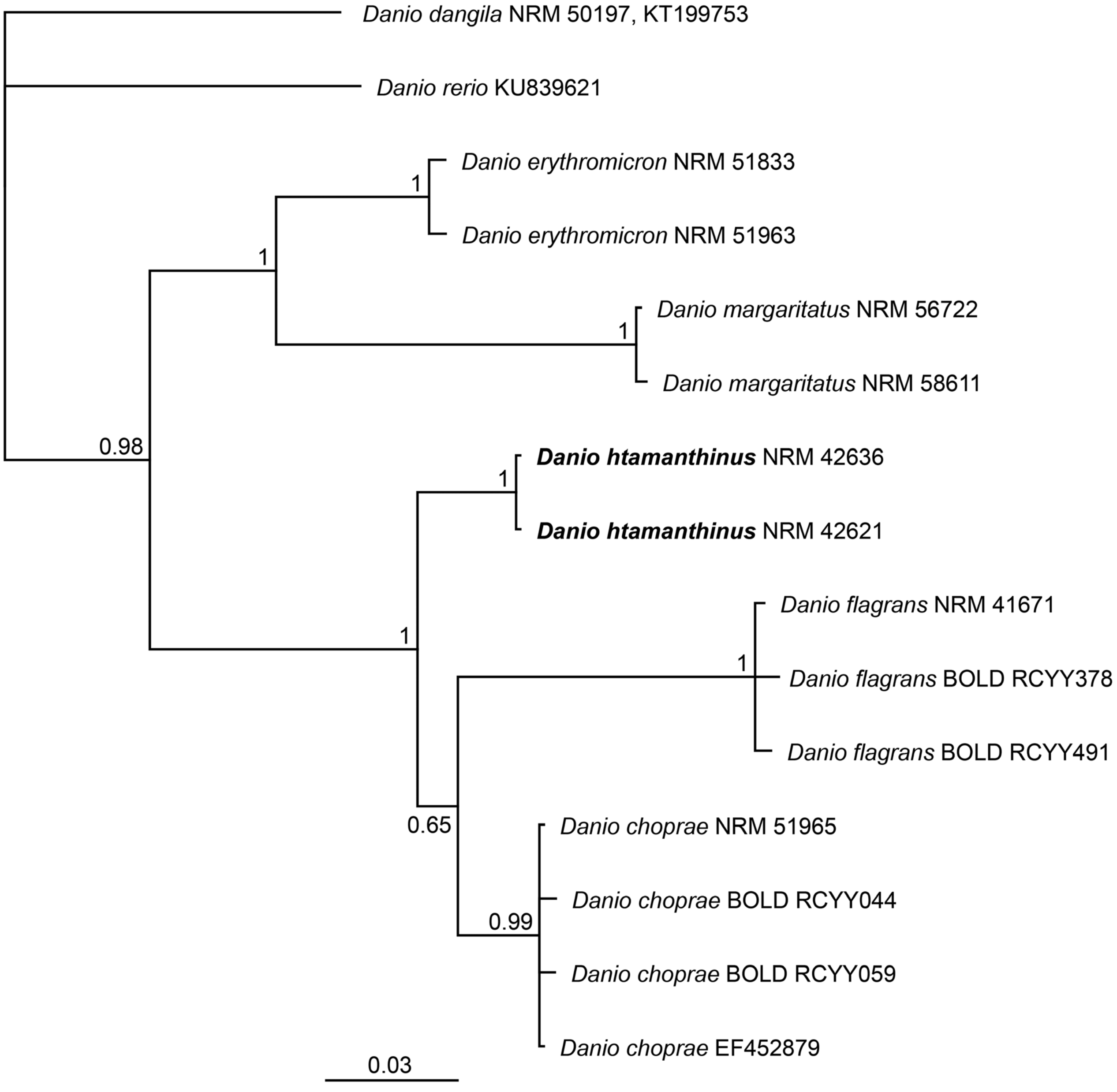

FIGURE 6. Phylogram of relationships from a Bayesian phylogenetic analysis of *D. htamanthinus* and related species, based on a 655 bp fragment of the mitochondrial COI gene. Internode values are Bayesian posterior probabilities. Sequences are tagged with Swedish Museum of Natural History voucher catalogue numbers (prefix NRM), GenBank sequence accession numbers (prefixed KT, KU, EF), or Barcode of Life identifier (prefix BOLD).

Danio flagrans was diagnosed from *D. choprae* as having a proportionally longer caudal peduncle, more vertebrae contained within the caudal peduncle (usually 9–10 vs. usually 7) and shorter anal-fin base with fewer anal fin rays (9.5–10.5, rarely 11.5, vs. 11.5–13.5). *Danio htamanthinus* shares caudal-peduncle and anal-fin base proportions with *D. choprae,* including a lower number of caudal-peduncle vertebrae (7–8), and higher number of anal fin rays (11.5–13.5). The proportional differences may have some correlation with habitat, *D. flagrans* occurring in faster running hillstream waters than the other two species which have been collected in both lotic and lentic habitats, as exemplified by the localities of *D. htamanthinus.* Habitat observations of the three species are, however, few.

Kottelat (2013) split *Danio* into the genera *Brachydanio* Weber & de Beaufort, 1916, *Celestichthys* Roberts, 2007, and *Danio*. *Danio choprae, D. erythromicron, D. flagrans,* and *D. margaritatus* were referred to *Celesticthys. Danio,* in the wide sense, however, is a monophyletic group, and in the absence of a phylogenetic analysis including all species, *Danio* in the wide sense is the only demonstrated monophyletic clade to account for all species referred to *Danio* in the sense of Fang (2003) and subsequent interrelationship analyses.

The distribution of *D. choprae, D. flagrans* and *D. htamanthinus* is clearly allopatric, but vast areas of Myanmar have not yet been subjected to ichthyological inventory. *Danio htamanthinus* is probably more widespread in the Chindwin basin. Two specimens of *Danio*, ANSP 93955, about 22 mm SL, from Shin Bway Yang in the upper Chindwin basin are in a poor state of preservation, discoloured and very soft. Fin and scale counts are difficult to make out without further damage to the specimens, and measurements are approximate. Nevertheless they most likely represent *D. htamanthinus.* Both have remnants of vertical bars on the side as in *D. htamanthinus* and *D. choprae*. Both have short caudal peduncle (15.8–16.9% SL) and long anal-fin base (20.3–23.3% SL), similar to *D. choprae* and *D. htamanthinus,* separating them from *D. flagrans*. In one of the specimens the barbels are long as in *D. htamanthinus*, but in the other specimen they are shorter, although the possibility that they have been broken cannot be excluded. The P stripe is represented by spots on the caudal peduncle, but the melanic colour pattern is strongly faded and the remnants may be misleading. The Shin Bway Yang specimens probably represent *D. htamanthinus*, but because of the poor state of preservation, they are excluded from the type series.

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