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# *Tanichthys kuehnei,* new species, from Central Vietnam (Cypriniformes: Cyprinidae)

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*Tanichthys kuehnei*, new species, is described from a stream in the Bach Ma Mountains in Hue Province in Central Vietnam. The new species differs from its congeners by having more branched rays in anal fin  $(9^{1}/_{2} \text{ vs. } 7-8^{1}/_{2} \text{ in } T.$  *micagemmae* and  $8^{1}/_{2}$  in *T. albonubes* and *T. thacbaensis*). Morphological and genetic characters suggest it to be closer related to *T. micagemmae*, the only other species of *Tanichthys* known from Central Vietnam. *Tanichthys kuehnei* differs from *T. micagemmae* by having a white anal-fin margin (vs. red).

#### Introduction

Cyprinid fishes of the genus *Tanichthys* are small (maximum 33 mm SL) but colourful and wellliked by ornamental fish hobbyists. The genus is characterised by confluent narial openings that are not separated by a skin wall and by males bearing cornified tubercles on the snout posterior to premaxilla (Freyhof & Herder, 2001). At present, three species are recognised: T. albonubes Lin, 1932 from southeast China (Guangxi, Guangdong and Hainan provinces) and northeast Vietnam (Quang Ninh Province), T. thacbaensis Nguyen & Ngo, 2001 from northwest Vietnam (Yen Bai Province) and T. micagemmae Freyhof & Herder, 2001 from Central Vietnam (Quang Tri and Quang Binh provinces) (Freyhof & Herder, 2001; Kottelat, 2001; Nguyen & Ngo, 2001; Yi et al., 2004; Chan & Chen, 2009; Li & Li, 2011; Luo et al., 2015). Fishes of the

genus *Tanichthys* inhabit moderately large to very small streams, with populations frequently being restricted to very small geographic areas and are often found only very locally (Freyhof & Herder, 2001), leading to strong isolation effects between the populations. This isolation was demonstrated in a genetic study by Luo et al. (2015) where each of six analysed wild populations of *T. albonubes* had own genetic signatures. The local occurrence in small streams also causes that members of *Tanichthys* are easily overlooked and therefore the existence of further, yet unknown populations can be predicted.

In 2009, an undescribed species of *Tanichthys* was exported from Central Vietnam for the ornamental fish trade (Bohlen, 2010; Seidel, 2010), reportedly from a stream in the Bach Ma Mountains in Vietnam. In the following years, several expeditions were undertaken by professional

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**Fig. 1.** *Tanichthys kuehnei*, live specimens. Top: male, about 25 mm SL. Bottom: female, about 22 mm SL. Vietnam: stream Rach Hoi Da (Photos by J. Kühne).

aquarium fish collectors to search for this species. Ichthyologists and naturalists searched in the proposed collecting stream as well as in adjacent streams, but the species remained undetected until 2017, when it was rediscovered. The same stream had been searched for this species in the years before, but in former years it had not been found, although suited habitat is found only in a short stretch of the stream. In the same year the species entered the ornamental fish trade for the second time.

The present paper formally describes this new species, based on specimens collected in 2017.

#### Material and methods

Measurements and counts follow Fang (1997). All measurements were made point-to-point with dial callipers to the next 0.1 mm and all material was measured by the same person within a 14 days time span. Osteological characters were studied from micro X-ray pictures taken from most of the analysed material. Information about coloration of wild-collected specimens of *T. albonubes* was taken from the pictures in Yi et al. (2004), Chan & Chen (2009) and Li & Li (2011). Museum acronyms are: IAPG, Institute of Animal Physiology and Genetics, Liběchov; NRM, Swedish Museum of



Fig. 2. Tanichthys kuehnei, top, ZFMK Ich-105342, holotype, male, 25.2 mm SL; bottom, ZRC 59656, paratype, female, 23.4 mm SL. Vietnam: stream Rach Hoi Da.

Natural History, Stockholm; RIA1, Research Institute for Aquaculture No 1, Ministry of Agriculture and Rural Development, Hanoi; SJB, collection of first author, USNM, National Museum of Natural History, Smithsonian Institution, Washington, D.C.; ZFMK, Zoological Research Institute and Museum Alexander Koenig, Bonn; ZRC, Lee Kong Chian Natural History Museum, Singapore.

A sample of muscle was taken from one paratype of *T. kuehnei* (IAPG A11237) and two specimens imported in 2009 (IAPG A4113 and IAPG A 4114) for sequencing of the mitochondrial cytochrome *b* gene. Comparative material included six sequences of *T. albonubes* from six wild populations in China (from Genbank, after Luo et al., 2015), seven specimens of *T. albonubes* from ornamental fish trade, and 12 sequences of *T. micagemmae* from Genbank and two specimens of *T. micagemmae* from ornamental fish trade.

Isolation of DNA, cytochrome b primers, PCR and sequencing protocols followed Bohlen et al. (2016). For phylogeny reconstruction we used neighbour joining (NJ), maximum parsimony (MP) and maximum likelihood (ML) analyses implemented in PAUP\* 4.0b10 (Swofford, 2003) and Bayesian inference (BI) as implemented in MrBayes 3.1.2 (Ronquist & Hueselbeck, 2003) and BEAST 2.4.8 (Bouckaert et al., 2014). The evolutionary model of nucleotide substitution was selected with use of model test (Lanfear et al., 2012) implemented in MEGA v. 7.0 (Kumar et al., 2007). The choice of a model followed the Bayesian information criterion. As outgroup we used a sequence of *Rhodeus ocellatus* (GenBank accession number AB769519).

NJ analysis was based on uncorrected p-distances. The starting trees for MP and ML analyses were obtained via random stepwise addition of taxa and reconstructions were carried out with heuristic searches. ML analysis was performed under the selected model (HKY+G). For all three types of analyses, statistical supports were assessed via 1000 non-parametric bootstrap replicates. BI in MrBayes was performed in two parallel runs, each of 10 million generations with six Metropolis Coupled Markov Chains Monte Carlo (MCMCMC) of default heating conditions. Sampling frequency was set to every 100 generations. The dataset was partitioned into codon positions, partitions unlinked and parameters were set according to selected best-fit models (K2+G, HKY and TN93+G+I for 1st, 2nd and 3rd codon position, respectively). The results were checked in Tracer v 1.6 (Rambaut et al., 2014) to see the effective sampling size (ESS) of the parameters. Likelihood scores of the sampled trees were inspected for stationarity by plotting them against the generation times. First 10 % of trees were discarded as burnin and the remaining trees were used to build a 50 % majority rule consensus trees. For the analyses in BEAST the partitions (codon positions) were also unlinked and assigned the estimated evolutionary models. As priors the Yule process of speciation and relaxed lognormal molecular clock were selected. The MCMC analyses was set to 10 million generations with sampling of every 1000 generations. Inspection of result in Tracer 1.6 (Rambaut et al., 2014) showed that ESSs for all parameters were sufficient (>200). A maximum clade credibility tree was built in TreeAnnotator v.1.8.0 (Rambaut & Drummond, 2010) after discarding the first 10 % of trees. A haplotype network was constructed employing the statistical parsimony (Templeton et al., 1992) implemented in TCS 1.21 software (Clement et al., 2002).

## Tanichthys kuehnei, new species (Figs. 1-2)

**Holotype.** ZFMK Ich-105342, 25.2 mm SL; Vietnam: Hue Province: stream Rach Hoi Da running from the Bach Ma Mountains into the Lang Co lagoon; 16°12'40" N 108°04'23" E; J. Kühne, 17 Aug 2017.

**Paratypes.** ZFMK Ich-105343, Ich-105344, Ich-105345, and Ich-105346, 4, 23.1–26.7 mm SL; ZRC 59656, 5, 23.4–25.2 mm SL; and IAPG A11237, 1, 21.9 mm SL; collected with the holotype.

**Non-types.** IAPG A4193–4195, 3, 28.1–30.6 mm SL; Vietnam: no details known; Saigon Aquarium, 2009.

Diagnosis. Tanichthys kuehnei is distinguished from the other species of the genus by having  $9^{1/2}$ branched rays in anal fin (vs. 7–8<sup>1</sup>/<sub>2</sub> in T. micagemmae and  $8\frac{1}{2}$  in T. albonubes and T. thacbaensis). It is further distinguished from *T. albonubes* and T. thacbaensis by having a black longitudinal stripe along the lateral midline and located on scale rows 5 and 6 below dorsal-fin origin (vs. dorsal to lateral midline, and located on scale rows 4 and 5 below dorsal-fin origin). Tanichthys *kuehnei* is distinguished from *T. albonubes* and *T. thacbaensis* by having a black midlateral stripe of similar width as the white lateral stripe immediately above (vs. less than half of its width). It differs from *T. albonubes* by having the ventral half below the black midlateral stripe white (vs. dark

brown). Besides the different number of anal-fin rays it differs from *T. micagemmae* by having the margin of the anal fin white (vs. red).

**Description.** For general appearance see Figures 1-2 and Table 1 for morphometric data of holotype and nine paratypes. Small (largest known size 30.6 mm SL), relatively elongated species (body depth at pelvic-fin origin 3.9-4.6 times in SL; depth of caudal peduncle 2.2-2.9 times in its length), moderately compressed. Dorsal profile of head convex between snout and level of anterior eye margin, then straight until nape. Back between nape and dorsal-fin origin straight, decreasing along base of dorsal fin and afterwards tapering slightly until caudal-fin base. Ventral profile of snout convex, then straight until line through posterior margin of pupil. Ventral profile of body convex until anal-fin origin, straight along anal-fin base, afterwards nearly horizontal and straight until caudal-fin base. Head 3.8-4.3 times in SL. Eye large, 2.7-3.1 times in lateral head length, no barbels, nostrils confluent, anterior half of joint opening with a low rim.

Dorsal fin with 2 unbranched and  $6\frac{1}{2}$  or  $7\frac{1}{2}$  branched rays; vertical through origin of dorsal fin slightly closer to anal-fin origin than to pelvic-fin base. Caudal fin with 14–16 branched rays; forked (length of central rays about 1.4–1.6 times in length of upper lobe). Anal fin with 3 unbranched and  $9\frac{1}{2}$  branched rays, pelvic fin with 7 rays, reaching anus which is located in front of anal-fin origin. Pectoral fin with 10–11 rays, not reaching pelvic-fin origin. Four + 28–29 vertebrae; no lateral line, 30–32 scales in lateral midline, last 2 located on basal part of caudal fin, 8 or 9 scales between dorsal-fin origin and pelvic-fin origin.

**Sexual dimorphism.** Males with cornified tubercles on the snout posterior to premaxilla and longer anal-fin rays. Males with more intense red colour in anal and pelvic fins. Morphometric differences include height of caudal peduncle (9.8–11.7 % SL in females and 11.9–13.5 % in males), length of anal-fin base (10.0–11.2 % SL in females, 12.7–14.5 % in males) and length of pelvic fin (females 8.3–10.8 % SL, males 12.4–13.9 %), but these characters should be revised with more material.

**Coloration.** Preserved specimens with brown dorsal head and back, flank crème-white with numerous small black spots. A black midlateral stripe running from shortly behind eye, almost reaching caudal-fin base, its maximum width at level of dorsal and anal-fin base, decreasing cranially and caudally; maximum width slightly less than diameter of pupil. A white stripe along upper margin of black stripe from posterior margin of operculum to caudal-fin base, its width similar to width of black stripe and about equal over whole length. A conspicuous black dot on caudal-fin base, slightly larger than pupil, covering bases of 7-9 innermost fin rays. Margin of joint narial openings black; a black dot or area below the eye present in most specimens. Black stripe in distal part of dorsal, anal, pelvic and caudal fins, parallel to margin and close, but reaching margin only in central caudal fin. Margin of anal and pelvic fins white in males, grey in females, margin of caudal and pelvic fins grey.

In live specimens, dorsal side of head and body light brown, belly white, posterior part of body below black stripe grey. Black midlateral stripe with blue iridescent shine, white midlateral stripe white to slight orange. Central area of caudal fin light red, in both sexes, pelvic and anal fin red in males, light red in females. Intensity and extent of red colour variable, often disappearing under aquarium condition.

Genetic characters. Sequences of the complete mitochondrial cytochrome b gene have been deposited to Genbank under the accession numbers MG952920 (paratype of T. kuehnei IAPG A11237) and MH918657-MH918664. All analyses recovered T. kuehnei as a monophylum and placed T. albonubes as sister to a lineage containing T. micagemmae plus T. kuehnei. A sister relationship between T. kuehnei and T. micagemmae was recovered by NJ, MP and BI in BEAST, while ML and BI in MrBayes suggested that T. kuehnei stems out of *T. micagemmae*. It would mean that an ancestor of T. kuehnei became isolated within the T. micagemmae distribution and due to the bottleneck effect and genetic drift underwent an accelerated evolution leading to morphologically identifiable species. Anyway, T. kuehnei is genetically different from T. micagemmae; the haplotype network generated in TCS shows a difference of 13 bp between T. kuehnei and T. micagemmae.

**Distribution.** Until now known only from the type locality in the eastern Bach Ma mountains in the lower stretch of a stream coming from the forests of the proposed Bac Hai Van Nature Reserve.

	holotype and paratypes $(n=9)$		
-	mean	SD	range
Standard length (mm)			23.1-26.7
In percent of standard length			
Lateral head length (HL)	24.8	1.1	23.2-26.5
Pre-dorsal length	56.3	1.0	54.6-57.6
Pre-pelvic length	48.0	1.1	45.6-49.4
Pre-pectoral length	24.7	1.8	20.7-26.2
Pre-anal length	60.8	1.8	56.4-62.5
Head depth at eye	14.9	0.5	14.2-15.8
Body depth at pelvic-fin origin	24.1	1.2	22.0-25.8
Depth of caudal peduncle	12.1	1.0	9.8-13.5
Length of caudal peduncle	28.1	1.1	26.4-30.1
Maximum head width	12.9	0.5	12.0-13.5
Body width at dorsal-fin origin	11.6	0.6	10.8-12.7
Length of dorsal-fin base	9.5	1.2	7.3-11.5
Length of anal-fin base	12.7	1.6	10.0-14.5
Length of pelvic-fin base	12.0	1.7	8.3-13.9
Length of pectoral-fin base	17.9	1.2	15.7-19.6
In percent of head length			
Eye diameter	35.7	1.9	32-38
Interorbital width	36.2	2.8	32-41
Snout length	24.5	1.3	23-27

**Table 1.** Morphometric data of holotype and eight paratypes of *Tanichthys kuehnei* (ZFMK Ich-105342, ZFMK Ich-105343, ZRC 59656). Mean and standard deviation (SD) include the holotype.



Fig. 3. Type locality of Tanichthys kuehnei; Vietnam: stream Rach Hoi Da (Photo by J. Kühne).



Fig. 4. Group of *Tanichthys kuehnei* in the wild at the type locality. Vietnam: stream Rach Hoi Da (Photo by J. Kühne).

**Notes on biology:** The species has been found in 2009 and in 2017 easily and in numbers large enough to allow export in the ornamental fish trade, but has not been found in the years between despite intense searches in nearly every year by ornamental fish collectors, ichthyologists or naturalists. It appears as if population size undergoes drastic changes or that the population is generally living higher in the stream and only in certain years expands downstream.

Time of sampling was in the peak of the dry season. The inhabited stream cascaded down a steep slope, then changed into a 1-2 km long stretch with moderate flow at the foot of the hill before entering the brackish lagoon Lang Co. Tanichthys kuehnei was observed in the upper portion of the moderate stretch in small basins washed out by the stream, which split into several side arms. Basins were usually not more than 40-60 cm deep, water was very clear with pH around 7 and very low conductivity, bottom was sandy with rocks, and the only submerged vegetation was filamentous algae. Tanichthys kuehnei formed loose groups in all water levels at the inflow of water into the basin. Other fish species observed were Oryzias pectoralis, Macropodus spechti, Barbodes semifasciolata, Cobitis laoensis, Glossogobius sp. and Oreochromis niloticus.

Collected specimens were kept for a short period in aquarium, where they spawned, indicating that they were in reproductive period when being collected.

**Etymology.** Named after Jens Kühne (Mahachai Tours, Nakhon Si Thammarat, Thailand), in recognition of his efforts to locate the new species in the field. A noun in genitive case.

**Remarks.** The new species is easily identified as member of the genus *Tanichthys* by the presence of cornified tubercles on the snout of males, the confluent nostrils and the presence of a black and a white stripe along the flank. *Tanichthys thacbaensis* was described by Nguyen & Ngo (2001) on the base of two specimens collected in Thac Ba in 1962. Shortly after collection, the Thac Ba reservoir was completed and inundated the sampling locality. Since the type specimens of *T. thacbaensis* are lost (A. T. Bùi, pers. comm.) and no further specimens have ever been found, all that is available is the original description with a drawing, hampering the comparison with other species. Since the text of the description is in Vietnamese language



**Fig. 5.** Map with known distribution area of the four species of *Tanichthys*.

and not easy to translate, we provide an English translation of the description here (see below). However, *T. thacbaensis* is a large species of up to 30 mm SL, has 8½ branched rays in the anal fin and the black lateral stripe is located in the upper half of the body, making it similar to *T. albonubes*. As mentioned above, *T. kuehnei* differs from *T. thacbaensis* by having 9½ branched rays and the black stripe along (vs. dorsal to) lateral midline. *Tanichthys micagemmae* is the only known species besides *T. kuehnei* to occur in Central Vietnam.

The phylogenetic analysis (Fig. 6) shows *T. kuehnei* to be closer related to *T. micagemmae* than to *T. albonubes*, a result that is supported by the fact that these two species occur in Central Vietnam (about 100 km air line between closest known populations), while *T. albonubes* is found much further north. Moreover, *T. kuehnei* and *T. micagemmae* share some morphological character states that are different in *T. albonubes* (e.g. position and thickness of the black midlateral stripe and light colour of ventral half of body).

**Comparative material.** *Tanichthys albonubes.* IAPG A11216-11221, 6, 27.0-29.8 mm SL; Saigon Aquarium, collected in the wild in Vietnam. – NRM 10403, 3, 17.3-19.8 mm SL; China: Guangdong Province: White Cloud Mountain. – SJB 6335-6338, 4, 33.4-35.4 mm SL and SJB 6346-6375, 30, 15.7-25.4 mm SL; aquarium stock. – USNM 94886, paratypes, 2, 15.5-19.3 mm SL;



**Fig. 6.** Phylogenetic tree of cytochrome *b* dataset of *Tanichthys*. The depicted tree results from the NJ analyses, the statistical supports at the nodes represent bootstrap values of NJ, ML and MP analyses generated in PAUP and posterior probabilities of BI conducted in MrBayes and BEAST (NJ/MP/ML/BI-MrBayes/BI-BEAST). New sequences are marked by asterisks; the remaining ones have been downloaded from GenBank. Paratype of *T. kue-hnei* indicated by bold accession number.

China: Guangdong Province: White Cloud Mountain (photographs and X-ray).

*Tanichthys micagemmae.* IAPG uncatalogued, 5, 23.2–23.8 mm SL; aquarium stock. – ZFMK 39098, holotype, 18.8 mm SL; Vietnam: Quang Binh Province: stream Bau Dung. – ZFMK 39099–39118, paratypes, 20, 13.4–23.1 mm SL; same data as holotype.

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**Fig. 7.** Haplotype network of the cytochrome *b* gene of *Tanichthys* with focus on the relationships between *T. mica-gemmae* and *T. kuehnei*. Data for the more distantly related *T. albonubes* are simplified for better visualisation. Paratype of *T. kuehnei* indicated by bold accession number.

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### Appendix

English translation of the original description of *Tanichthys thakbaensis* from Nguyen & Ngo, 2001 in telegraphic style (translated by H. N. Thang):

'1. Names:

a. Official name: *Tanichthys thacbaensis* Hảo & Vân nov. sp.

c. Nomenclature type: *Tanichthys thacbaensis* Hảo & Vân nov. sp.

d. Vietnamese name: Cá Diếc nhằng

2. Description:

2 specimens: total length 36–39 mm; standard length 27–30 mm; collected from Thac Ba lake, Yen Bai province, April 1962.

a. Description:

Round scales quite large, cover whole body. No lateral line. Abdomen edge from pelvic fin to anal fin bold. Anus closely in front of anal fin.

b. Colour: Body colour dotted light green; light yellow at the belly. Prior parts of mouth and eyes have light

green lines which create a line through the eyes and gill cover, reaching to base of caudal fin, where is large spot; around this spot, colour is light yellow. Above vertical stripe is another parallel stripe which has light yellow colour. From origin of anal fin to origin of caudal fin another dark green stripe exists. There is a similar stripe at the top of the head which is through dorsal fin to the end point of caudal fin.

c. Comparison with other species: This species is different from *T. albonubes* Lin by having a higher body and longer head (standard length = 3.75-3.86 times body depth at dorsal-fin origin and 3.6-3.75 times lateral head length), longer mouth, smaller eyes and narrower distance between eyes (T = 3.7-4.0 snout length = 3.2-3.8 eye diameter = 2.5-2.7 interorbital distance). Gill rakers on first arch: 25. There are 2 black stripes along the fish body.

d. Standard sample: Holotype: total length = 39 mm, standard length = 30 mm, code: H.01.70.01.01 collected at Thac Ba Yen Binh – Yen Bai in April, 1962. Paratype 2 specimen, code: H.01.70.01.01, collected at the same location and time as holotype. Samples were deposited to specimen room of Research Institute of Aquaculture in Bac Ninh province, Viet Nam.

3. Distribution:

a. inside Viet Nam: Fish distributed mainly at the North mountain of Viet Nam, collected at the lake of Yen Bai province, Viet Nam.

b. worldwide: no records.

4. Biology and ecology: Not known.

5. Value:

a. In science: species was described at the first time in Viet Nam; endemic species at Thac Ba lake, Yen Bai province.

b. In economy: Small size fish, distribute at rice field and lake, small population. No recorded economic value. However, beautiful color of the fish may support it as ornamental fish in the market.

c. Resources situation: Rare and not popular in the nature.'

D=3.6-7; A=3.8; P=1.11; V=1.6. Gill rakers on first arch: 25. Pharyngeal teeth: 4.5-5.4; Scales in lateral count: 30-32; Scales in vertical count: 8; Scales before dorsal fin: 15; Scales around caudal peduncle: 12. Standard length = 3.75-3.86 times body depth at origin of dorsal fin = 3.6-3.75 times lateral head length = 1.1-1.8 times pre-dorsal length = 2.4-2.6 times post-dorsal length. Lateral head length = 3.7-4.0 times snout length = 3.2-3.8times eve diameter = 2.5-2.7 times interorbital distance; interorbital distance = 1.2-1.5 times eye diameter. Long and compressed body. No keel between pelvic fin and anal fin. Blunt mouth. Upper jaw a little longer than lower jaw, end of mouth not reaching level of anterior margin of eye. No barbels. The post-gutter of the lip is interrupted at the middle part. Narrow cheek. Large eyes. Distance between eyes quite large. Gill rakers small and thick. Pharyngeal teeth small, pointed; head of teeth curved. Origin of dorsal fin behind pelvic fin. Base of dorsal fin ends before origin of anal fin. Fin without hard spine, only soft rays. Pectoral fin reaching pelvic fin. Pelvic fin not reaching anal fin. Caudal fin strongly emarginated with two equal parts.