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# Two new species of shovel-jaw carp *Onychostoma* (Teleostei: Cyprinidae) from southern Vietnam

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## Abstract

Two new species of large shovel-jaw carps in the genus *Onychostoma* are described from the upper Krong No and middle Dong Nai drainages of the Langbiang Plateau in southern Vietnam. These new species are known from streams in montane mixed pine and evergreen forests between 140 and 1112 m. Their populations are isolated in the headwaters of the upper Sre Pok River of the Mekong basin and in the middle of the Dong Nai basin. Both species are differentiated from their congeners by a combination of the following characters: transverse mouth opening width greater than head width, 14–17 predorsal scales, caudal-peduncle length 3.9-4.2 times in SL, no barbels in adults and juveniles, a strong serrated last simple ray of the dorsal fin, and small eye diameter (20.3-21.5% HL). *Onychostoma krongnoensis* sp. nov. is differentiated from *Onychostoma dongnaiensis* sp. nov. by body depth (4.0 vs. 3.2 times in SL), predorsal scale number (14-17 vs. 14-15), dorsal-fin length (4.5 vs. 4.2 times in SL), caudal-peduncle length (3.9 vs. 4.2 times in SL), colour in life (dark vs. bright), and by mitochondrial DNA (0.2% sequence divergence). Molecular evidence indicates that both species are members of *Onychostoma* and are distinct from all congeners sampled (uncorrected sequence divergences at the 16S rRNA gene of >2.0\% for all *Onychostoma* for which homologous 16S rRNA sequences are available).

Key words: Langbiang Plateau, Onychostoma krongnoensis sp. nov., Onychostoma dongnaiensis sp. nov., southeast Asia, Vietnam

#### Introduction

The first shovel-jaw cyprinid species, *Barbus gerlachi* Peter 1880, now assigned to the genus *Onychostoma* Günther 1896, was described from specimens collected in the Xi Jiang drainage in Wuzhou, Guangxi, southern China. *Onychostoma* is characterized by the lower lip being restricted only to the sides of the lower jaw, which bears a sharp cornified sheath on the cutting edge (Chen 1989, Shan *et al.* 2000). There are 20 valid species distributed in hill-streams in east and southeast Asia (Shan *et al.* 2000; Kottelat 2001a, b; Nguyen & Ngo 2001; Xin *et al.* 2009; Jang-Liaw & Chen 2013). Chen (1989) and Xin *et al.* (2009) divided members of *Onychostoma* into three groups based on mouth width, mouth-opening shape, and postlabial groove length. The narrow mouth group has a horse-shoe-shaped mouth opening with its width less than the corresponding head width and a long postlabial groove; this mouth type occurs in *Onychostoma barbatum* (Lin 1931), *O. elongatum* (Pellegrin and Chevey 1934), *O. lini* (Wu 1939), and *O. uniforme* (Mai 1978).

The moderate mouth group has an almost transverse mouth opening with its width equal to the corresponding head width and a short labial groove; this mouth type occurs in *O. angustistomata* (Fang 1940), *O. breve* (Wu and Chen in Wu *et al.*1977), *O. daduense* (Ding 1994), *O. fangi* Kottelat 2000, *O. fusiforme* Kottelat 1998, *O. macrolepis* (Bleeker 1871), *O. meridionale* Kottelat 1998, *O. ovale* Pellegrin and Chevey 1936, *O. rarum* (Lin 1933), *O. simum* (Sauvage and Dabry de Thiersant 1874), and *O. virgulatum* Xin, Zhang and Cao 2009. The wide mouth group has a transverse mouth opening with its width greater than the corresponding head width and a very short labial groove; this mouth type occurs in *O. alticorpus* (Oshima 1920), *O. barbatulum* (Lin 1931), *O. gerlachi* (Peters 1880), *O. leptura* (Boulenger 1900), and *O. minnanensis* Jang-Liaw and Chen 2013 (Xin *et al.* 2009).

During field expeditions in the Langbiang Plateau at high-elevations in the Krong No drainage, a catchment of the Sre Pok River, a tributary of the Mekong River, and at low-elevations in the middle Dong Nai drainage, from 2011 to 2014, we collected specimens described here as two new, large *Onychostoma* species on the basis of morphological and molecular characteristics.

### Materials and methods

We recorded morphological data from specimens fixed in 10% formalin and stored in 70% ethanol. Specimens were deposited at the University of Science, Ho Chi Minh City, Vietnam (UNS); Zoological Reference Collection of the Lee Kong Chian Natural History Museum, National University of Singapore (ZRC), and National Museum of Nature and Science, Japan (NSMT). Morphometric data were taken to the nearest 0.1 mm with digital calipers. Methods of measurements, counts, and terminology of the mouth structure followed Kottelat and Freyhof (2007). Abbreviations are as followed: SL – standard length; HL – head length; HW – head width. Sex was determined by internal gonadal inspection. We obtained comparative morphological data from museum specimens of *Onychostoma* and photographs of these species in life, preserved and from the literature: *O. gerlachi* (Bănărescu 1971a; Taki 1975; Nguyen 2001; Kottelat 2001a; Lothongkham & Musikasinthorn 20-05), *O. leptura* (Bănărescu 1971a; Nichols & Pope 1927; Chevey & Lemasson 1937; Nguyen 2001), *O. meridionale* (Kottelat 1998), *O. fusiforme* (Kottelat 1998) (Appendix) and other *Onychostoma* spp. (Xin *et al.* 2009; Jang-Liaw & Chen 2013). Due to the undiagnosed diversity within the genus, where available we relied on examination of topotypic material and/ or original species descriptions.

In addition to the Krong No and Dong Nai specimens, we included *O. meridionale* Kottelat 1998 in the molecular analysis due to its morphological similarity with the specimens from Krong No. Locality information and accession numbers for all sequences included in the analysis can be found in Table 3.

In order to support the generic placement of the new species, we obtained fin samples from the new species and compared them to species currently assigned to the genus *Onychostoma* (Wang *et al.* 2012). We analyzed ~1524 base pairs (bp) of mitochondrial DNA for the 16S ribosomal RNA gene from eight adults of the new species. DNA was extracted using DNeasy tissue extraction kits from Qiagen. We used two pairs of primers (16Sp1F-16Sp1R and 16Sp2F-16Sp2R) of Wang *et al.* (2012) to amplify the 16S rRNA gene. Standard PCR protocols were used, and PCR products were purified using ExoSap-IT (USB Corporation, OH, USA). Purified templates were sequenced directly by Macrogen (Seoul, Korea). Sequences were edited with Sequencher v. 4.10 (Gene Codes) and deposited in GenBank under accession numbers KJ754097–KJ754105. Homologous fragments of 16S for *Onychostoma* species were downloaded from GenBank so that types or topotypes of every species of *Onychostoma* reported from Vietnam, China, or Taiwan were included in the analysis (Table 1). These sequences were trimmed to match the length of the fragment obtained here. The data set was aligned using the Clustal option in MEGA v. 6.06 and refined by eye. Uncorrected pairwise sequence divergence was calculated using MEGA v. 6.06.

Maximum-likelihood phylogenetic analyses were performed in raxmlGUI v. 1.3 with GTR+I+ $\Gamma$  model, which was performed in jModelTest v. 2.1.4. One-hundred independent searches were performed, each starting with a random tree. The best likelihood from each of these runs was selected as our maximum-likelihood estimate, and statistical support for this topology was obtained by running 1000 bootstrap replicates in raxmlGUI v. 1.3. Phylogenetic trees were modified by TreeGraph v. 2.0.47. Branches receiving  $\geq$ 45% bootstrap support were considered to be well-supported for 16S rRNA (Li *et al.* 2008). The outgroup *Percocypris pingi* (GenBank Accession No. GQ406259) was chosen from the species closely related to the genus *Onychostoma* based on the cyprinid phylogeny of Wang *et al.* (2012). Molecular phylogenies were used primarily to support the placement of the newly collected specimens within the genus *Onychostoma*, rather than to resolve phylogenetic relationships within the group.

TABLE 1. Morphometric and meristic characters of Onychostoma krongnoensis sp. nov. Range and mean include the holotype.

	Holotype	Range	Mean±SD	Ν
Standard length (mm)	205	16.6–23.8	19.9±1.5	24
Morphometrics				
% SL				
Head length	18.5	17.8–20.6	19.1±0.7	24
Depth of body	24.4	23.4–28.2	25.3±1.0	24
Body width at dorsal-fin origin	11.7	10.4–13.9	12.2±0.9	24
Predorsal length	40.5	39.4-43.0	40.9±1.1	24
Prepectoral length	19.5	17.5–21.9	$19.0{\pm}1.0$	24
Prepelvic length	44.9	42.9-47.8	44.9±1.1	24
Preanal length	69.3	65.7–71.6	68.7±1.3	24
Distance between pectoral- and pelvic-fin origins	22.9	20.6-26.1	23.6±1.3	24
Distance between pelvic- and anal-fin origins	20.5	18.9–23.6	21.1±1.2	24
Depth of caudal peduncle	10.2	7.6–10.2	8.1±0.5	24
Length of caudal peduncle	25.4	23.8–26.4	25.0±0.7	24
Length of dorsal fin	22.0	19.9–24.1	$21.7{\pm}1.0$	24
Length of dorsal-fin base	16.6	13.6–16.7	15.5±0.9	24
Length of pectoral fin	17.1	16.2–18.8	$17.2 \pm 0.7$	24
Length of pelvic fin	18.0	15.3–18.9	$17.2 \pm 0.9$	24
Length of anal fin	19.5	15.1–24.4	18.3±2.4	24
Length of anal-fin base	8.8	6.4–9.0	8.2±0.6	24
% HL				
Head depth	84.21	68.4–89.5	82.0±4.4	24
Head depth at eye	71.1	61.5-71.6	66.9±2.9	24
Maximum head width	68.4	59.2-70.3	65.4±3.2	24
Snout length	34.2	31.3-48.9	36.3±3.6	24
Interorbital width	47.4	37.8-50.9	45.9±2.6	24
Eye diameter	21.1	17.8–21.9	20.1±1.2	24
Mouth width	47.4	40.0–50.0	44.8±2.4	24
Counts				
Lateral-line scales	48	47–50	$47.9{\pm}0.7$	24
Scales between lateral line and origin of dorsal fin	7	7–7	$7.0{\pm}0.0$	24
Scales between lateral line and origin of pelvic fin	5	5–5	$5.0{\pm}0.0$	24
Scales between lateral line and origin of anal fin	5	5–5	$5.0{\pm}0.0$	24
Circumpeduncular scales	16	15–16	16.0±0.2	24
Predorsal scales	17	14–17	15.4±0.8	24

#### Results

# Onychostoma krongnoensis sp. nov.

**Holotype:** UNS00805, adult female, 205 mm SL; upper Ea Krong No drainage: upper Mekong River in montane evergreen forest in Bidoup-Nui Ba National Park, Lam Dong Province, Vietnam (12°15'17.36" N, 108°40'29.15" E, 1077 m), 5 March 2013 by Hoang Duc Huy, Pham Manh Hung and Tran Trong Ngan (Fig. 1).



FIGURE 1. Type localities of Onychostoma krongnoensis (black star) and O. dongnaiensis (white star).

**Paratypes:** UNS00601 (adult female, 211 mm SL), UNS00881 (adult female, 198 mm SL), UNS00882 (adult female, 215 mm SL), UNS00883 (adult female, 198 mm SL), 12 March 2012, collected at same locality as holotype (12°14'57.47" N, 108°40'31.41" E, 1101 m); UNS00785 (adult female, 207 mm SL), UNS00799 (adult female, 208 mm SL), UNS00804 (adult female, 216 mm SL), UNS00806 (adult male, 192 mm SL), ZRC 54623 (adult female, 196 mm SL), 5 March 2013, collected at same locality as holotype (12°14'57.47" N, 108°40'31.41" E, 1101 m); UNS00810 (adult male, 185 mm SL), UNS00811 (adult male, 201 mm SL), UNS00812 (adult male, 179 mm SL), NSMT-P 121247 (adult male, 166 mm SL), 6 March 2013,

collected at same locality as holotype (12°15'5.25" N, 108°38'32.48" E, 1030 m); UNS00824 (adult male, 192 mm SL), UNS00825 (adult male, 214 mm SL), 7 March 2013, collected at same locality as holotype; UNS00819 (adult female, 206 mm SL), UNS00823 (adult male, 202 mm SL), UNS00832 (adult female, 238 mm SL), UNS00887 (adult female, 203 mm SL), 8 March 2013, collected at same locality as holotype (12°15'5.25" N, 108°38'32.48" E, 1025 m); NSMT-P 121248 (adult female, 202 mm SL), UNS00885 (adult male, 180 mm SL), ZRC 54624 (adult male, 177 mm SL), 23 June 2014, collected at same locality as holotype (12°16'23.68"N 108°26'30.17" E, 672 m).

**Etymology.** Specific epithet is in reference to the type locality in the upper Krong No drainage. Suggested common name: Krongno srang (English), Cá srang Krông Nô (Vietnamese). Srang is the vernacular name of this species of *Onychostoma* by the K'Ho people in the Langbiang Plateau.

**Diagnosis.** Onychostoma krongnoensis is distinguished from its congeners by a combination of (1) mouth wide (width 1.4–1.6 times in HW), (2) predorsal scales 14–17, (3) caudal peduncle slender and long (2.5–3.3 times longer than deep), (4) no barbels in adults and juveniles, (5) a strong serrated last simple ray of dorsal fin, (6) eye diameter small (4.6–5.6 times in HL), and (7) tip of snout with 2–3 irregular transverse rows of 13–46 small and large tubercles (Figs. 2a, b, c; 3a, b, c, d).



**FIGURE 2.** Lateral view of *Onychostoma krongnoensis* specimens (a) female holotype UNS00805 in life, (b) female holotype UNS00805 in preservative, (c) male paratype UNS00825 in preservative. Scale 10 mm.

**Description.** General appearance shown in Figure 2; meristic and morphometric data of 27 adult type specimens given in Table 1. Head longer than deep, dorsal profile strongly convex. Snout stout and rounded, longer than eye diameter. Interorbital area slightly convex. Mouth subterminal. Maxillary reaching vertical of anterior margin of orbit. Upper lips thick, upper region covered by rostral fold (rostral cap) and lower 1/4 mostly exposed. Tip of snout with 2–3 irregular transverse rows of 18–40 small and large tubercles; each one occupies a moderate depression in the dermis. Mouth width about 1.4–1.6 times in maximum HW; no barbels (Fig. 3d).

Body relatively elongate, moderately compressed; caudal peduncle slender and long, about 2.5–3.3 times longer than deep. Dorsal body profile convex, ventral profile rounded. Lateral line complete, 47–50 scales; 14–17 predorsal scales; 7/1/5 scales in transverse row anterior to pelvic-fin insertion.



FIGURE 3. Front view of head of *Onychostoma krongnoensis* (a) female holotype UNS00805, 205 mm SL, (b) immature paratype UNS00821, 123 mm SL, (c) male paratype UNS00825, 214 mm SL; *O. dongnaiensis* (e) female holotype UNS00851, 172 mm SL, (f) female paratype UNS00852, 196 mm SL, (g) male paratype ZRC 54625, 157 mm SL; *O. leptura* (i) female UNS00684, 186 mm SL. Ventral view of head of *O. krongnoensis*, wide mouth (d) male paratype UNS00825; *O. dongnaiensis*, wide mouth (h) female holotype UNS00851; *O. leptura*, wide mouth (j) female UNS00684; *O. meridionale*, moderate mouth (k) immature UNS00826, 108 mm SL; *O. uniforme*, narrow mouth (l) female UNS00689, 130 mm SL. Scale 10 mm.

Dorsal fin high with 4 simple and 8 branched rays; first simple ray as a tiny process, vestigial in a few specimens; last simple ray very strongly serrated; dorsal-fin origin inserted slightly in front of vertical with pelvic-fin origin; distal margin slightly concave. Pectoral fin pointed with 1 simple and 16 branched rays. Pelvic fin pointed, with 1 simple and 8 branched rays; axillary scale present. Anus immediately in front of anal fin. Anal fin with 3 simple and 5 branched rays; short (15.1–16.7% SL) and small in male; long (18.7–24.4% SL) and large in female (Fig. 2b, c). Breeding tubercles numerous and dense in anal-fin rays of males but few and sparse or absent in females (Fig. 2b, c). Caudal fin deeply forked with 9+8 principal rays, 8+7 being branched.

**Colour in life.** Head dark turquoise on back and around orbital, yellow on side, white on lower jaw. Body dark turquoise on back, yellow to pinkish orange on lateroventral surface, snowy white belly with longitudinal black stripe running along lateral line. Scales silver and more bluish between back and lateral line; scales bordered with black reticulate pattern, becoming dim on ventral, and black at scale bases. Fins hyaline with random black pigments on interradial membranes. Dorsal fin dark turquoise at origin, bold on rays, pinkish on distal margin. Pectoral fin and pelvic fin yellow pink, bolder at origin than on distal margin. Anal fin pinkish orange on rays and blackish on distal margin. Caudal fin near peduncle dark turquoise yellow, pinkish on tips.



**FIGURE 4.** High elevation main river at type locality of *Onychostoma krongnoensis*. Habitats altering from fast flowing pool (a) with bed of detritus and falling leaves, running to riffles (c) and swift current (b) over bedrock, large boulder and cobble substrates covered with periphyton (sites continuous); low elevation main river at type locality of *Onychostoma dongnaiensis* (d) running to riffles.

**Colour in preservative.** Similar to that of fresh condition except as noted below. Upper half of body including head dark grey. Lower half of body including head pale white except anterior half of body nearly greyish white. All turquoise, yellowish, silverly bluish, pinkish orange and snowy whitish colors lost (Fig. 2b, c).

**Comparisons.** In general, *O. krongnoensis* most resembles *O. leptura* and *O. gerlachi* when compared with congeners in the Mekong basin and Red river basin. *Onychostoma krongnoensis* differs from *O. leptura* in having scales in transverse row 7/1/5 vs. 7/1/4, predorsal scales 14–17 vs. 12–13, eye small with diameter 17.8–21.9 vs.

22.9–30.3% HL, snout tip covered with 13–43 vs. 2–6 tubercles. *Onychostoma krongnoensis* differs from *O. gerlachi* by having predorsal scales 14–17 vs. 12–15, caudal peduncle 25.0 vs. 21.2% SL, prepectoral length 17.5–21.9 vs. 21.4–26.5% SL, eye small with diameter 17.8–21.9 vs. 27.4–35% HL.

Onychostoma krongnoensis differs from O. meridionale in having mouth 1.4–1.6 vs. 2.2–2.3 times in maximum HW (Fig. 3d, k), last simple dorsal ray strongly serrated vs. not serrated posteriorly, eye diameter 4.6–5.6 vs. 3.7-4.5 times in HL, and scales in transverse row 7/1/5 vs. 6.5/1/6.5. Onychostoma krongnoensis differs from O. fusiforme in having mouth 1.4–1.6 vs. 2.0–2.2 times in maximum HW, eye diameter 4.6–5.6 vs. 3.7-4.4 times in HL, scales in transverse row 7/1/5 vs. 6.5/1/4-4.5.

Ecology. All specimens of the new species were found in the Ea Krong No drainage basin, consisting of montane mixed pine and evergreen forest between 596-1112 m (Fig. 4a, b, c). Onychostoma krongnoensis lives in clear water. Water conditions of 17-26 °C, pH 6.5-7.2, DO 80-88.6%, conductivity 1-10 µS.cm<sup>-1</sup>, and flow velocity 0.17–0.67 m/s were recorded. Individuals of O. krongnoensis were found in lotic habitats of main streams. When foraging, they preferred erosional zones such as runs and riffles with swift currents over periphyton covering bedrock, large boulders and cobble substrates. When shoaling, they preferred the depositional zones such as fast flowing pools with detritus and fallen leaves (habitats alternate in rapid succession as shown in Fig. 4a, b, c) (pers. obs.). This is similar to the habitat of O. gerlachi (Lothongkham & Musikasinthorn 2005). The shovel-jaw carp shoal with other cyprinids such as the white barb, *Poropuntius* sp., and mahseer, *Neolissochilus stracheyi*, in deep pools (approx. 2 m depth) or in streams shadowed by canopy (Fig. 4a). Sometimes they shoal to scrape the periphyton off bedrock, large boulders and cobbles under open areas (Fig. 4c). The shovel-jaw carp was observed flipping while foraging so that its belly and lateral body faces upwards. The new species shares its habitat with Poropuntius sp., Neolissochilus stracheyi, Annamia normani, Schistura spp., Glyptothorax sp. and Channa gachua. In lower reaches of their distribution in Ea Krong No, they share habitat with Poropuntius sp., Tor spp., Lobocheilus delacouri, Crossocheilus reticulatus, Garra fuliginosa, Garra cambodgiensis, Gyrinocheilus aymonieri, Annamia normani, Schistura spp., and Channa gachua. The shovel-jaw carp uses its lower jaw with a sharp cornified sheath on the cutting edge to scrape off and feed on algae. The stomachs of all specimens contained pinnate diatoms (Order Bacillariales) and filamentous algae (Spirogyra sp., Mougeotia sp., and Oedogonium sp.). During wet season surveys (July 2011 and May 2013), many juvenile fishes were observed.

**Conservation status.** Onychostoma krongnoensis is an indigenous fish of the Langbiang Plateau in Ea Krong No drainage. This species is one of the most important food fishes in the upper Ea Krong No River and plays an important role in local fish consumption. It has been long recognized by the local K'Ho minority people due to their preference for its bitter taste, reportedly due to their algae-filled guts. During the dry season when river water levels are low (December to April), local people harvest this species using cast nets. Harvesting in this manner is unlikely to affect *O. krongnoensis* populations.

The main river of the Ea Krong No river system is approximately 85 km long and runs inside Bidoup-Nui Ba National Park and Chu Yang Sin National Park, and *O. krongnoensis* populations are within protected areas. The ichthyofauna of high elevation forest streams is highly endemic (Allen *et al.* 2012), and the isolated *O. krongnoensis* populations are probably endemic to this high elevation (>500 m) drainage in the Langbiang Plateau. Given the available information, we suggest the species should be considered Data Deficient following IUCN's Red List categories (IUCN 2014, version 11).

#### Onychostoma dongnaiensis sp. nov.

**Holotype:** UNS00851, adult female, 172 mm SL; upper Da Teh main stream: middle Dong Nai drainage in montane evergreen forest in Lam Dong Province, Vietnam (11°36'13.80"N, 107°35'49.60"E, 172 m) 11 March 2014 by Pham Manh Hung and Hoang Duc Huy (Fig. 1).

**Paratypes:** NSMT-P 121249 (adult female, 155 mm SL), 11 March 2014, collected at same locality as holotype; UNS00852 (adult female, 196 mm SL), UNS00853 (adult female, 180 mm SL), UNS00854 (adult female, 166 mm SL), ZRC 54625 (adult male, 157 mm SL), 7 March 2014, collected at same locality as holotype; ZRC 54626 (adult female, 173 mm SL), 11 April 2014, collected at same locality as holotype (11°38'13.79"N, 107°37'26.14"E, 195 m).

Etymology. Specific epithet is in reference to the type locality of middle Dong Nai drainage. Suggested

common name: Dongnai srang (English), Cá srang Đồng Nai (Vietnamese). Srang is the vernacular name of this species of *Onychostoma* by the Ma people in the Da Teh catchment of the middle Dong Nai River, Lam Dong Province.

**Diagnosis.** *Onychostoma dongnaiensis* is distinguished from its congeners by a combination of (1) mouth wide (width 1.4–1.6 times in HW), (2) predorsal scales 14–15, (3) body depth high (3.1–3.4 times in SL), (4) eye diameter small (4.2–4.9 times in HL), (5) no barbels in adults and juveniles, (6) a strong serrated last simple ray of dorsal fin, and (7) dorsal-fin length high (3.8–4.5 times in SL) (Figs. 5a, b, c; 3e, f, g, h).



**FIGURE 5.** Lateral view of *Onychostoma dongnaiensis* specimens (a) female holotype UNS00851 in life, (b) female holotype UNS00851 in preservative, (c) male paratype ZRC 54625 in preservative. Scale 10 mm.

**Description.** General appearance shown in Figure 5; meristic and morphometric data of seven adult type specimens given in Table 2. Head longer than deep, dorsal profile strongly convex. Snout stout and rounded, longer than eye diameter in adults. Interorbital area slightly convex. Mouth subterminal. Maxillary reaching vertical of anterior margin of orbit. Upper lips thick, upper region covered by rostral fold (rostral cap) completely and exposed at corners. Epidermal tubercles on snout numerous but small to medium-sized and sparse in all type specimens except holotype, which has no tubercles (Fig. 3e). Mouth width about 1.4–1.6 times in maximum HW; no barbels (Fig. 3h).

	Holotype	Range	Mean±SD	Ν
Standard length (mm)	172	15.5–19.6	17.1±1.4	7
Morphometrics				
% SL				
Head length	19.8	19.4–21	20.3±0.7	7
Depth of body	29.2	29.2-32.7	30.8±1.1	7
Body width at dorsal-fin origin	16.0	12.7–16	14.5±1.3	7
Predorsal length	43.7	40.8-45.8	43.3±1.5	7
Prepectoral length	18.7	18.7–21.7	20.0±1.1	7
Prepelvic length	46.9	46.9–51.2	48.6±1.5	7
Preanal length	70.0	69.7–73.5	70.8±1.3	7
Distance between pectoral- and pelvic-fin origins	24.5	24.5-28.7	26.4±1.8	7
Distance between pelvic- and anal-fin origins	19.2	17.8–22.4	19.2±1.6	7
Depth of caudal peduncle	9.3	9.2–10	9.6±0.3	7
Length of caudal peduncle	23.6	22-24.3	23.2±0.7	7
Length of dorsal fin	23.3	22.3–26	23.5±1.2	7
Length of dorsal-fin base	16.3	15.6–17.6	16.7±0.8	7
Length of pectoral fin	18.1	17.8–19.4	18.3±0.6	7
Length of pelvic fin	19.2	17.4–19.2	$18.4{\pm}0.6$	7
Length of anal fin	21.0	17.8–21.7	20.6±1.3	7
Length of anal-fin base	8.7	7.7–11.1	9.2±1.3	7
% HL				
Head depth	88.2	71.4-89.5	81.1±6.6	7
Head depth at eye	67.6	57.1-70.3	64.3±5.0	7
Maximum head width	67.6	57.1-67.6	62.3±3.2	7
Snout length	35.3	33.3-36.8	34.7±1.3	7
Interorbital width	47.1	40.5-55.3	47.1±4.6	7
Eye diameter	20.6	20.3-23.8	21.7±1.5	7
Mouth width	41.8	33.3-44.7	40.5±3.6	7
Counts				
Lateral-line scales	47	44–47	46.1±1.2	7
Scales between lateral line and origin of dorsal fin	7	7	$7.0{\pm}0.0$	7
Scales between lateral line and origin of pelvic fin	5	5	$5.0{\pm}0.0$	7
Scales between lateral line and origin of anal fin	5	5	5.6±0.5	7
Circumpeduncular scales	16	16–16	16.0±0.0	7
Predorsal scales	15	14–15	14.7±0.5	7

TABLE 2. Morphometric and meristic characters of Onychostoma dongnaiensis sp. nov. Range and mean include the holotype.

Body high, moderately compressed; caudal peduncle slender, about 2.4–2.6 times longer than deep. Dorsal body profile convex, ventral profile rounded. Lateral line complete, 45–47 scales; 14–15 predorsal scales; 7/1/5 scales in transverse row anterior to pelvic-fin insertion.

Dorsal fin high with 4 simple and 8 branched rays; first simple ray as a tiny process, vestigial in a few specimens; last simple ray strongly serrated; dorsal-fin origin inserted slightly in front of vertical with pelvic-fin origin; distal margin anteriorly concave and posteriorly convex. Pectoral fin pointed with 1 simple and 16 branched rays. Pelvic fin pointed, with 1 simple and 8 branched rays; axillary scale present. Anus immediately in front of anal fin. Anal fin with 3 simple and 5 branched rays; short (17.8% SL) and small in male; long (20.0–21.7% SL) and large in female (Fig. 5b, c). Caudal fin deeply forked with 9+8 principal rays, 8+7 being branched.

**Colour in life.** Head dark turquoise on back, light turquoise around orbital and on side, white on opercula and lower jaw. Body light turquoise on back, silver white on lateroventral surface, snowy white belly with a longitudinal black stripe running along lateral line. Scales silverly bluish between back and lateral line; lateral-line scales and row of scales just above lateral line in anterior half of body pigmented at center of scale bases. Fins hyaline. Dorsal fin dark turquoise at origin, bold on rays; distal margin concave anteriorly and convex posteriorly; reddish at distal part. Pectoral fin and pelvic fin yellowish green and reddish at first rays and distal parts. Anal fin yellowish green on rays, pinkish distally and hyaline on distal margin. Caudal fin near peduncle dark turquoise, yellowish to red on tips.

**Colour in preservative.** Similar to that of fresh condition except as noted below. Upper half of body including head brown. Lower half of body including head pale ivory colour except anterior half of body darker. Pigments at scale bases on almost whole body outstanding. All turquoise, yellowish, silverly bluish, reddish and snowy whitish colors disappeared (Fig. 5b, c).

**Ecology.** All specimens of the new species were found in the Da Teh catchment of middle Dong Nai drainage in evergreen forest between 140–200 m (Fig. 4d). *Onychostoma dongnaiensis* lives in clear water but some specimens were collected in silty water. Water conditions of  $26.4-28.1^{\circ}$ C, pH 7.02–7.6, DO 82.7–91.4%, conductivity 12–25  $\mu$ S.cm<sup>-1</sup> and flow velocity 0.12–0.77 m/s were recorded. The species occurred in swift currents over bedrock, large boulders and cobble substrates covered with periphyton (Fig. 4d). This is similar to the habitat of *O. krongnoensis*. When feeding, *O. dongnaiensis* flips rocks and scrapes algae from them. *Onychostoma dongnaiensis* is found with *Mystacoleucus obtusirostris*, the bonylip barb *Osteochilus vittatus*, *Poropuntius deauratus*, *Channa gachua*, *Yasuhikotakia morleti*, and *Acantopsis dialuzona*.

**Conservation status.** The conservation status of this new species requires particular attention. It has been long recognized by the local Ma minority people. It is likely to be restricted to relatively small stretches of low-elevation forest streams of the middle Dong Nai drainage and hence particularly vulnerable to threatening processes such as siltation and overfishing. Siltation (from road construction, deforestation, agriculture etc.) which covers rocks and stones is a particular threat to this species as it covers the algae growing on the stones (Fig. 4d). This species feeds on the algae and animals living in the algae. Anthropogenic modification of stream morphology, logging, deforestation, agriculture and overfishing frequently occur in the Da Teh catchment. These activities impact the aquatic environment of this species so its populations are potentially threatened. Given the available information, we suggest the species should be considered Data Deficient following IUCN's Red List categories (IUCN 2014, version 11).

**Comparisons.** In general, *O. dongnaiensis* most closely resembles *O. leptura* and *O. gerlachi* when compared with congeners in the Mekong basin and Red river basin. From detailed measurements and comparisons of specimens of both species, *O. dongnaiensis* differs from *O. leptura* in having scales in transverse row 7/1/5 vs. 7/1/4, predorsal scales 14–15 vs. 12–13, eye small with diameter 20.3–23.8 vs. 22.9–30.3% HL and serrated last simple dorsal ray vs. smooth last simple dorsal ray. *Onychostoma dongnaiensis* differs from *O. gerlachi* by having predorsal scales 14–15 vs. 12–15, caudal peduncle 23.2 vs. 21.2% SL, prepectoral length 18.7–21.7 vs. 21.4–26.5% SL, eye small with diameter 20.3–23.8% vs. 27.4–35% HL. *Onychostoma dongnaiensis* differs from *O. meridionale* in having mouth 1.4–1.6 vs. 2.2–2.3 times in maximum HW (Fig. 3h, k), the last simple dorsal ray strongly serrated vs. not serrated posteriorly, eye diameter 4.2–4.9 vs. 3.7–4.5 times in HL, and scales in transverse row 7/1/5 vs. 6.5/1/6.5. *Onychostoma dongnaiensis* differs from *O. fusiforme* in having mouth 1.4–1.6 vs. 2.0–2.2 times in maximum HW, caudal peduncle 2.2–2.6 vs. 3.0–3.6 times longer than deep, eye diameter 4.2–4.9 vs. 3.7–4.4 times in HL, and scales in transverse row 7/1/5 vs. 6.5/1/4–4.5.

Mai *et al.* (1992) described one specimen as the name *Scaphidonichthys* sp. from Phu Lap, Tan Phu, Dong Nai province near the Dong Nai River at elevation (11°27'17.23" N, 107°29'4.40" E, 155 m). We consider this species conspecific with *O. dongnaiensis* based on examination of its morphometrics (Mai *et al.* 1992) similar to our *O. dongnaiensis* specimen (UNS00856) collected at the same locality.

**Morphological data.** Predorsal scales and eye diameter are considered reliable taxonomic characters, and to our knowledge, the short eye diameter (4.2–5.6 times in HL) in the two new species is extreme among all *Onychostoma* species from southeast Asia (Taki 1975, Kottelat 1998, Lothongkham & Musikasinthorn 2005).

*Onychostoma krongnoensis* is most similar to *O. dongnaiensis*, but differs most dramatically in colour in life. *Onychostoma krongnoensis* is dark turquoise on the back and yellow to pinkish orange on lateroventral surface (Fig. 2), and *O. dongnaiensis* is light turquoise on the back, and bluish silver-white on the lateroventral surface (Fig. 5). *Onychostoma krongnoensis* also has a slightly deeper body (3.5–4.3 vs. 3.1–3.4 times in SL) and a slightly shorter caudal-peduncle (3.8–4.2 vs. 4.1–4.5 times in SL). The number of epidermal tubercles on the snout also may vary in the two species with more on *O. krongnoensis*, although seasonal and ontogenetic variation has not been studied.

**Molecular data.** The newly collected specimens from the Krong No and Dong Nai rivers are embedded within a clade containing all Mekong *Onychostoma* species (Fig. 6). This clade receives 100% bootstrap support. *Onychostoma meridionale* was also embedded within this clade.



**FIGURE 6.** Maximum-likelihood tree based on 16S ribosomal RNA mitochondrial gene sequences for species of *Onychostoma* (Wang *et al.* 2012) and for the outgroup *Percocypris pingi*. Numbers on branches are ML bootstrap values (values  $\geq$  45% shown).

Our molecular phylogeny suggests that the Krong No and Dong Nai river specimens are most closely related to *O. gerlachi*, differing by 2.0% and 2.1%, respectively, to *O. gerlachi*, and >2.0% to all *Onychostoma* in the analysis. The Krong No and Dong Nai specimens are closely related to each other and differ by 0.2% sequence divergence, which is larger than the difference between *O. angustistomata* and *O. rarum* by 0.1% (Table 3). *Onychostoma meridionale* appears most closely related to *O. gerlachi*, but differs by 2.1% from this species and >2.0% from all other *Onychostoma* specimens in the analysis.

	1	2	3	4	5	9	7	8	6	10	11	12	13	14
1. O. alticorpus Taiwan KC791686														
2. O. angustistomata China HQ235714	0.045													
3. <i>Ò. barbatulum</i> Taiwan K C896762	0.063	0.052												
KJ754099	0.063	0.066	0.070											
5. U. etongatum China: Rong'an, Guangxi Zhuang Auto, Region GO406754	0.072	0.068	0.065	0.084										
6. O. gerlachi China: Jinghong, Yunnan Prov. DO&45867	0.067	0.064	0.076	0.021	0.089									
7. O. krongnoensis sp. nov. KJ754097	0.062	0.064	0.067	0.002	0.083	0.020								
8. <i>O. leptura</i> China: Xilin, Guangxi Zhuang Auto, Region GQ406257	0.060	0.058	0.063	0.030	0.080	0.033	0.028							
9. O. unu China: Youyang, Chongqing JQ343982	0.048	0.044	0.041	0.052	0.065	0.059	0.050	0.046						
10. O. macrolepis China: Taian, Shandong Prov. GOM6258	0.054	0.050	0.051	0.057	0.073	0.060	0.055	0.053	0.019					
11. O. meridionale	0.074	0.067	0.082	0.018	0.092	0.021	0.020	0.037	0.064	0.065				
12. <i>O. ovale</i> China: Tian'e, Guangxi Prov. JX074089	0.035	0.014	0.029	0.047	0.050	0.049	0.047	0.039	0.029	0.051	0.056			
13. <i>O. rarum</i> China HQ235715	0.044	0.001	0.051	0.064	0.068	0.063	0.063	0.056	0.043	0.050	0.066	0.012		
14. <i>O. simum</i> China: Hejiang, Sichuan Prov. D0845861	0.024	0.044	0.050	0.056	0.066	0.062	0.055	0.054	0.041	0.047	0.066	0.033	0.042	
15. Percocypris pingi China: Hejiang, Sichuan Prov. GOM6759	0.073	0.067	0.074	0.089	0.085	0.089	0.087	0.076	0.070	0.072	0.095	0.052	0.066	0.070

# Discussion

With two new species described here, a total of 22 species of *Onychostoma* are now recognised, with five species occurring in the Mekong drainage. *Onychostoma krongnoensis* inhabits the Ea Krong No river drainage of the Langbiang Plateau. These mountain rivers are perennial, shallow water bodies characterized by low temperature,

high turbulent current, and rocky substratum. As adaptions to strong water currents, *O. krongnoensis* has dense epidermal tubercles on the snout tip, a slender, streamlined body with an increased number of predorsal scales (mostly 17 scales), and a longer caudal peduncle compared to other species of *Onychostoma*.

Tubercles of cyprinids exhibit sexual dimorphism and ontogenetic variation in the size, shape and distribution (Wiley & Collette, 1970). In contrast to other species of *Onychostoma*, in *O. krongnoensis* the epidermis of the snout possesses 2–3 irregular transverse rows of 13–43 tubercles in juveniles and adults of both sexes (Fig. 3b). Variation in the surface of the snout in hill-stream fishes has been suggested to be an adaptation to life in torrential streams (Hoshiyar *et al.* 2013). The snout epidermis of *O. krongnoensis* is subject to frictional stress as it comes into contact with water current, thus supporting this hypothesis. In contrast, species that inhabit low elevation streams with slower velocity, such as *O. dongnaiensis*, *O. leptura*, and *O. uniforme*, have fewer smaller tubercles on the snout (Hoang *et al.* pers.obs.). Breeding tubercles also occur on the anal-fin rays of males of *O. krongnoensis* and other stream cyprinids (Witkowski & Rogowska 1991; Poncin *et al.* 2011).

Body shape affects movement in stream fishes (Chuang *et al.* 2006) and may be a useful tool for predicting habitat preferences (Stolbunov *et al.* 2011). For *O. krongnoensis*, the slender, streamlined body with a large number of predorsal scales (15–17) and long caudal peduncle may provide greater swimming ability and allow it to inhabit rapid rivers and streams at high elevations such as those in the Ea Krong No drainage. In contrast, *O. dongnaiensis* and *O. leptura* have a deeper body, fewer predorsal scales (12–15) and a short caudal peduncle, which may be more suitable features for slow, low elevation streams such as the middle of the Dong Nai and Red river drainages (Hoang *et al.* pers.obs.). Further studies on the capabilities and physiological mechanisms of *O. krongnoensis* that allow the species to live in such rapid streams would be interesting.

Pronounced sexual dimorphism in the anal-fin size has been observed for several species of *Onychostoma* (Hoang *et al.* pers. obs.), but has not been reported. This morphological character highlights the importance of making intraspecific comparisons separately for each sex. The functional role of the sexual dimorphism in *O. krongnoensis* and *O. dongnaiensis* is unknown.

**Species diversity of** *Onychostoma* **in Mekong basin.** *Onychostoma krongnoensis* and *O. dongnaiensis* are very similar to *O. gerlachi, O. fusiforme* and *O. meridionale,* and have probably been misidentified due to the similarities in their adult overall morphology and geographic distributions. Shan *et al.* (2000), Kottelat (2001, 2009), Lothongkham & Musikasinthorn (2005) and Kano *et al.* (2013) described *O. gerlachi* as a widespread species from the Pearl River, upper Red River to the Langcang River, Nam Ou, Se Banghiang River-Mekong basin, and the Nan River-Chao Phraya basin. Kottelat (1998), Xin *et al.* (2009) and Kano *et al.* (2013) recorded *O. fusiforme* as occurring from the Langcang River (Yunnan, China), Ing River, Kok River (Thailand) to Nam Theun River (Laos). Kottelat (1998, 2007, 2011), Kano *et al.* (2013) and our survey (2013) described and recorded *O. meridionale* from the Se Bangfai River, Sekong River (Laos), Sesan River (Cambodia), Sa Thay River-tributary of the Sesan River partly in the Vietnam Central Highlands to the Tonle Sap River (Cambodia). Even amongst geographically close river basins such as the Sekong River, Sesan River and Sre Pok River in the middle Mekong basin, there are distinct species of Onychostoma present: O. krongnoensis in the Srepok River, *O. meridionale* in the Sekong and Sesan rivers.

Onychostoma krongnoensis and O. dongnaiensis are the first records of the genus in the upper Srepok River and middle Dong Nai River on the Langbiang Plateau, southern Vietnam. Among species of the Mekong group, O. gerlachi occurs the furthest north. This species could be the ancestral lineage of the Mekong clade. Despite the lack of O. fusiforme in our molecular data, phylogenetic analysis and geographical distribution suggest that this lineage may have migrated towards the southern Mekong basin. The geological event that separated the Dong Nai dranage from the Mekong drainage would have isolated O. dongnaiensis from O. krongnoensis. Detailed and comprehensive surveys to gain a better understanding of geographical distributions, and molecular phylogenetic analysis for Onychostoma in the Mekong basin are essential.

#### **Comparative material examined**

Onychostoma gerlachi: Wuchow, Kwangsi (U.S.N.M. 94594 (2 spec.), F.M.N.H 47328).

Onychostoma gerlachi: Laos, Luang Prabang (IBRP 4167).

Onychostoma gerlachi: Thailand, Nan Province (RLIKU 21-35, RLIKU 95-97).

Onychostoma leptura: Vietnam, Yen Bai Province, Ngoi Thia-upper Red River (UNS00684).

Onychostoma leptura: Vietnam, Phu Tho Province, Ha Giang Province, Yen Bai Province (Coll. I. O. I. Nº 462).

*Onychostoma leptura*: Nodoa, Hainan Island (B.M.N.H. 1889. 11. 30: 21, Hainan Isl.; N.M.W. 10073, Hainan Isl.; A.M.N.H. 10681, Nodoa, Hainan Island).

Onychostoma leptura: northern Vietnam, Red River drainage, (M.N.H.N. 34-258, 34-259, 1937-14).

Onychostoma leptura: Vietnam, Lao Cai Province, Thanh Hoa Province (H. 01.73.01.01; H. 01.73.01.02).

*Onychostoma meridionale*: Laos, upper Xe Bangfai (ZRC 41783, CMK 12263), Vietnam, Kon Tum Province, Sa Thay River-upper Sesan River (UNS00826).

Onychostoma fusiforme: Laos, Nam Theun (ZRC 41782, CMK 12521, 12711).

Onychostoma uniforme: Vietnam, Yen Bai Province, Ngoi Thia-upper Red River (UNS00689).

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