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Taxonomic revision of the genus *Poropuntius* (Teleostei: Cyprinidae) in Peninsular Malaysia

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Abstract

Up to three nominal species of the cyprinid fish genus *Poropuntius* (i.e. *P. deauratus* [Valenciennes in Cuvier & Valenciennes 1842], *P. normani* [Smith 1931], and *P. smedleyi* [de Beaufort 1933]) have been reported to occur in Peninsular Malaysian freshwater ecosystems. However, low morphological differentiation among species of *Poropuntius* causes confusion and it is still unknown how many valid species of *Poropuntius* occur in this region. The goal of this study is to review the taxonomic status of *Poropuntius* in Peninsular Malaysia by using morphological and molecular characters. Principal Component Analysis (PCA) on a morphometric dataset including 281 specimens of *Poropuntius* from Peninsular Malaysia and *P. normani* from Thailand (type locality) failed to identify non-overlapping clusters within sampled specimens. A phylogenetic tree based on cytochrome oxidase subunit I (COI) showed intraspecific levels of genetic differentiation within *Poropuntius* of Peninsular Malaysia and the specimens of *P. normani* from Thailand form a monophyletic group. Our results strongly support the presence of only one species of *Poropuntius* in Peninsular Malaysia, *P. normani*. We demonstrate that *P. smedleyi* described from Johor, southern Peninsular Malaysia, is a junior synonym of *P. normani*. The previous reports of the presence of *P. deauratus* in Peninsular Malaysia are doubtful because this species was described from Vietnam where, in all evidence, it is endemic.

Key words: Cypriniformes, *Poropuntius normani*, Taxonomy, Morphology, Phylogeny

Introduction

The genus *Poropuntius* (Cyprinidae) was described by Smith (1931) to classify a new species collected from Chanthaburi of Thailand, *Poropuntius normani* Smith 1931. *Poropuntius* is distinguished from other cyprinid genera mainly by the following combination of characters: weakly ossified and serrated last simple dorsal fin ray, presence of two pairs of well-developed barbels, and large tubercles covering the tip of the snout and extending over the lacrimal bones (Rainboth 1996; Roberts 1998). Many species of *Poropuntius* are also characterized by bold black or dusky submarginal stripes on the upper and lower caudal fin lobes. Smith (1931) indicated that *Poropuntius* may superficially resemble the genus *Lissochilus* (currently a synonym of *Neolissochilus*), but the latter could be distinguished by the occurrence of tubercles on the cheek and a weakly ossified last simple dorsal-fin ray with a smooth posterior edge. *Poropuntius* also resembles *Puntius*, but the tuberculation on the snout and the occurrence of a horny sheath on the lower lips separate the two genera.

As currently understood, *Poropuntius* has a large geographic distribution from southern China (Yunnan Province) to Myanmar, Thailand, Indochina, the Malay Peninsula, and Sumatra (Roberts 1998; Kottelat 2013) with the Mekong drainage the likely diversity center for the genus. Thirty-four nominal species have been listed by

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Kottelat (2013) from Southeast Asian freshwater habitats and these handsomely colored fish, reaching 10–20 cm in standard length, are usually found in foothill streams with clear water and swift currents (Roberts 1998).

In Peninsular Malaysia, specimens of *Poropuntius* are commonly known as "*ikan tengas daun*" or "*ikan daun*" (Mohsin & Ambak 1983). They occur in streams with good water quality (Zakaria-Ismail & Fatimah 2002), and the distribution of the genus is well-documented (Ng & Tan 1999; Shah *et al.* 2009; Hashim *et al.* 2012; Miyazaki *et al.* 2013). However, few works have addressed the taxonomy and systematics of *Poropuntius* (with the notable exceptions of Rainboth [1996] and Roberts [1998]) and it is unclear how many valid species occur in this region.

Up to three nominal species of *Poropuntius* have been reported in Peninsular Malaysia: *Poropuntius deauratus* (Valenciennes in Cuvier & Valenciennes 1842) (type locality: Vietnam [= "Cochichina"]), *P. normani* (Smith 1931) (type locality: Chanthaburi, Thailand) and *P. smedleyi* (de Beaufort 1933) (type locality: Johor, southern Malay Peninsula, Malaysia) (Ismail 1989; Ng & Tan 1999; Shah *et al.* 2009; Hashim *et al.* 2012; Kano *et al.* 2013; Miyazaki *et al.* 2013).

Poropuntius deauratus was described from Vietnam and the presence of this species in Peninsular Malaysia would represent a significant range extension (Mohsin & Ambak 1983; Ng & Tan 1999; Shah *et al.* 2009; Kano *et al.* 2013). *Poropuntius smedleyi* is morphologically very similar to *P. normani* from Thailand and their reciprocal identification is challenging (Roberts 1998). The only character that distinguishes *P. smedleyi* and *P. normani* is the number of circumpeduncular scales (12 in *P. smedleyi* vs. 14 in *P. normani*) (Smith 1931; de Beaufort 1933). However, our preliminary observation on samples from Peninsular Malaysia found that samples from Pahang River possess 13 circumpeduncular scales, an intermediate character between *P. smedleyi* and *P. normani*. This observation has raised a question about the validity of the statuses of species of *Poropuntius* in Peninsular Malaysia, particularly whether specimens in the region are con or heterospecific.

The main objective of this paper is to investigate the morphological and molecular variations of *Poropuntius* in Peninsular Malaysia to clarify the species diversity in the region.

Materials and methods

Morphological data. Most of the specimens of *Poropuntius* used in this study were newly collected from freshwater habitats throughout Peninsular Malaysia and Thailand (Fig. 1). Preserved specimens are stored in the Zoological Museum of the University of Malaya, Kuala Lumpur (UMKL), and the Ichthyological Collections of Department of Biological Sciences, University Malaysia Terengganu (UMT). For comparative purposes, some Malaysian and other Southeast Asian materials deposited in the Zoological Reference Collection of the Lee Kong Chian Natural History Museum of Singapore (ZRC), National Museum of Natural History, Washington D.C., United States of America (USNM), Muséum National d'Histoire Naturelle, Paris, France (MNHN), and Ubon Ratchathani University, Thailand were also examined. The abbreviation of institutions that hold the vouchers for the GenBank data used here are as follows: KIZ and KIZZLP for Kunming Institute of Zoology, Chinese Academy of Science, China; ihb for Freshwater Fish Museum at the Institute of Hydrobiology, Chinese Academy of Science, China; and SLM-PS(SL) for repository store at the Freshwater Fisheries Research Division of Jelebu, Malaysia.

A total of 281 specimens of *Poropuntius* were examined from 87 localities of the 20 major drainages in Peninsular Malaysia and some localities of Thailand (including the type locality of *P. smedleyi* and *P. normani*) (Fig. 1). The fishes were identified according to Smith (1931), de Beaufort (1933) and Roberts (1998). Preliminary morphological observations indicate that the specimens of *Poropuntius* (281 from Peninsular Malaysia and 9 from Thailand) are similar, with all of them having heavy tuberculation on the snout, a yellowish caudal fin with dark upper and lower margins, the last simple dorsal-fin ray moderately long with its posterior edge strongly serrated, and circumpeduncular scale rows ranging from 12 to 14. Following the species description by Smith (1931) and de Beaufort (1933), fish with circumpeduncular scale rows 12 and 14 were identified as *P. smedleyi* and *P. normani*, respectively, and the fish with 13 circumpeduncular scale rows were labelled as *Poropuntius* cf. *normani*.

A total of 33 measurements and 21 counts were collected following the methods of Hubbs & Lagler (1964) and Rainboth (1996). The measurements were taken to the nearest 0.1 mm with digital calipers on the left side of the fish. Measurements of the body parts were recorded as proportions of standard length (SL), and measurements of the head were recorded as proportions of head length (HL). All examined specimens had a full series of morphometric and meristic data for morphological analysis.

TABLE 1. GenBank accession numbers and associated information for samples of *Poropuntius* and *Neolissochilus soroides* included in the phylogenetic analysis.

Species	GenBank accession	Voucher	Locality	Source
P. normani Chanthaburi 1	MH550536	UMKL 12287-1	Thailand, Chanthaburi, Kao Sabap, Pliew Waterfall	Present study
P. normani Chanthaburi 2	MH550535	UMKL 12287-2	Thailand, Chanthaburi, Kao Sabap, Pliew Waterfall	Present study
P. normani Chanthaburi 3	MH550534	UMKL 12287-3	Thailand, Chanthaburi, Kao Sabap, Pliew Waterfall	Present study
P. smedleyi Johor 1	MH550529	UMKL 12178-1	Malaysia, Johor, Kota Tinggi Waterfall	Present study
P. smedleyi Endau 1	MH550533	UMKL 12001-1	Malaysia, Johor, Selai, Pandan river	Present study
P. smedleyi Endau 2	MH550532	UMKL 12003-1	Malaysia, Johor, Selai, Tempaang river	Present study
P. smedleyi Muar 1	MH550524	UMKL 12103-1	Malaysia, Negeri Sembilan, Kuala Pilah, Langkap river	Present study
P. smedleyi Muar 2	MH550523	UMKL 12103-2	Malaysia, Negeri Sembilan, Kuala Pilah, Langkap river	Present study
P. normani Klang 1	MH550526	UMKL 12179-1	Malaysia, Selangor, Gombak, Gombak river	Present study
P. normani Klang 2	MH550525	UMKL 12179-2	Malaysia, Selangor, Gombak, Gombak river	Present study
P. normani Bernam 1	MH550538	UMKL 12168-1	Malaysia, Perak, Kampung Behrang Hulu	Present study
P. normani Bernam 2	MH550537	UMKL 12168-2	Malaysia, Perak, Kampung Behrang Hulu	Present study
P. normani Perak 1	MH550520	UMKL 12172-1	Malaysia, Perak, Tapah, Woh river	Present study
P. normani Perak 2	MH550519	UMKL 12172-2	Malaysia, Perak, Tapah, Woh river	Present study
P. normani Golok 1	MH550531	UMKL 12180-1	Malaysia, Kelantan, Tanah Merah, Jedok river	Present study
P. normani Golok 2	MH550530	UMKL 12180-2	Malaysia, Kelantan, Tanah Merah, Jedok river	Present study
P. normani Kelantan 1	MH550528	UMKL 12051-1	Malaysia, Kelantan, Gunung Stong, Kenarong river	Present study
P. normani Kelantan2	MH550527	UMKL 12051-2	Malaysia, Kelantan, Gunung Stong, Kenarong river	Present study
P. normani Terengganu 1	MH550518	UMKL 12148-1	Malaysia, Terengganu, Sekayu Waterfall	Present study
P. normani Terengganu 2	MH550517	UMKL 12148-2	Malaysia, Terengganu, Sekayu Waterfall	Present study
P. cf. *normani* Pahang 1	MH550522	UMKL 12081-1	Malaysia, Pahang, Jerantut, Kiol river	Present study
P. cf. *normani* Pahang 2	MH550521	UMKL 12081-2	Malaysia, Pahang, Jerantut, Kiol river	Present study
P. normani	KY319981	Unavailable	Laos, Bolaven Plateau	GenBank
P. normani	KY319982	Unavailable	Laos, Bolaven Plateau	GenBank
P. smedleyi	JF781242	SLM-PS(SL)-11	Malaysia, Selangor, Kepong	Song *et al.* 2013
P. smedleyi	JF781238	SLM-PS(SL)-02	Malaysia, Selangor, Kepong	Song *et al.* 2013
P. normani	JX066757	Unavailable	Laos, Boli Kham Xai	Yang *et al.* 2012
P. smedleyi	KT001012	Unavailable	Malaysia	GenBank
P. smedleyi	KT001011	Unavailable	Malaysia	GenBank
P. smedleyi	KT001060	Unavailable	Malaysia	GenBank
P. smedleyi	KT001013	Unavailable	Malaysia	GenBank
P. opisthopterus	KM610796	ihb201306234	China, Yunnan, Nujiang river	Chen *et al.* 2015
P. opisthopterus	KM610793	ihb201306228	China, Yunnan, Nujiang river	Chen *et al.* 2015
P. opisthopterus	KJ994616	KIZ0348	China, Yunnan, Longling	Zheng *et al.* 2016
P. huangchuchieni	KJ994656	KIZZLP20120096	China, Yunnan, Menglun	Zheng *et al.* 2016
P. laoensis	KP263423	Unavailable	Thailand, Phayao, Doi Phu Nang, Than Sawan	GenBank
P. laoensis	KP263422	Unavailable	Thailand, Phayao, Doi Phu Nang, Than Sawan	GenBank
Neolissochilus soroides	MH550539	UMKL 12241-1	Malaysia, Selangor, Kuala Kubu, Baru, Gumut river	Present study

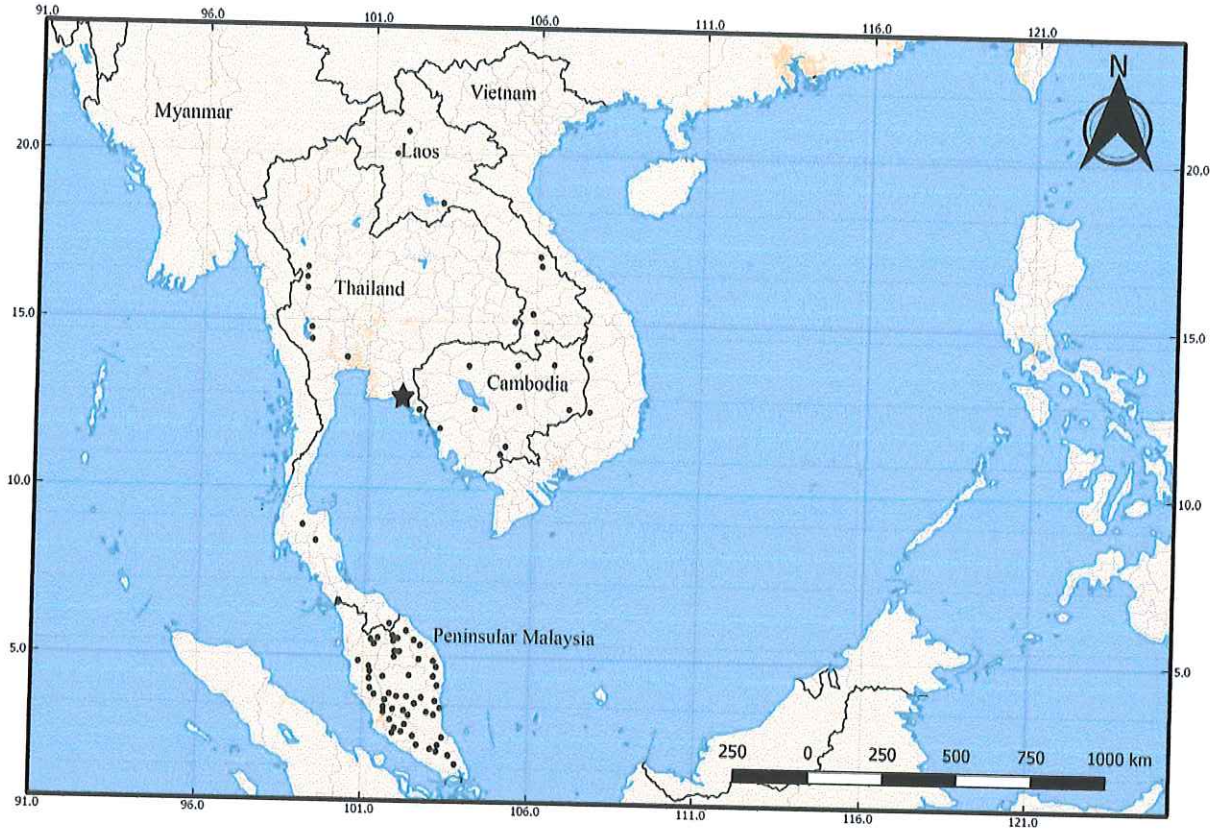

FIGURE 1. Geographic distribution of known localities of *Poropuntius normani* in Peninsular Malaysia and several localities in Southeast Asia (black dots), and the type locality of *P. normani* in Pliew Waterfall, Kao Sabap, Chanthaburi, Thailand (black star). Map made with QGIS Development Team (2018) (Accessed on 11 January 2018).

A sheared principal component analysis (PCA) in which the covariance matrix was factored (Humphries *et al.* 1981; Bookstein *et al.* 1985) was used to examine the variation in morphometric data. Specimens were coded based on species according to morphological keys and the sheared second (PC2) and third (PC3) principal components of the data which represent the shape factors independent of size, were plotted with 95% confidence intervals. The sheared PCA was performed on the log-transformed measurements using the statistical program PAST3 (Hammer *et al.* 2001).

Molecular. Phylogenetic relationships were reconstructed using the cytochrome c oxidase I (COI) gene for 22 individuals from several populations of *Poropuntius* spp. (Table 1) for which material could be obtained. The pectoral-fin clips of the voucher specimens were stored in 95% ethanol for DNA extraction. The voucher specimens were subsequently fixed in 10% formalin and preserved in 70% ethanol and given catalogue numbers before being deposited in the Zoological Museum of Universiti Malaya (UMKL). Voucher specimen information is shown in Table 1.

Genomic DNA was extracted using a DNA extraction kit (G-spin™Total from iNtRON Biotechnology, Inc., Scongnam, South Korea). The COI gene region was amplified and sequenced using the primers FishF1: 5' TCA ACC AAC CAC AAA GAC ATT GGC AC3' and FishR1: 5' TAG ACT TCT GGG TGG CCA AAG AAT CA3' (Ward *et al.* 2005). Each sample for the polymerase chain reaction (PCR) consisted of a 50 µl reaction containing 20 µl of sterile water, 2 µl of genomic DNA, 25 µl of Master Mix (NEXpro™, Korea), and 1.5 µl of 10 mM of each primer. The PCR cycling conditions followed Ward *et al.* (2005). PCR reactions were performed using an Applied Biosystems Veriti 96-Well Thermal Cycler (Applied Biosystems, Inc., Foster City, CA, USA). Sanger sequencing was done by MyTACG Bioscience Enterprise, Malaysia. All sequences were edited and aligned using MEGA v7.0 (Kumar *et al.* 2015).

A Neighbor-Joining (NJ) phylogenetic tree was plotted using MEGA7 v7.0 (Kumar *et al.* 2015). The NJ bootstrap values were estimated using 1000 replicates with Kimura's two-parameter model of substitution (K2P distance) (Kimura 1980). A Maximum Likelihood (ML) analysis was performed with an online server Smart Model Selection in PhyML (Guindon *et al.* 2010). An automatic model selection was implemented based on the Akaike information criterion (AIC) (Lefort *et al.* 2017). The best-fit model was the HKY85 +G model with a fixed invariable site and a gamma shape parameter of 0.099 estimated by the program. For comparison, COI sequences of *Poropuntius* spp. from GenBank (*P. normani*, *P. smedleyi*, *P. opisthopterus*, *P. laoensis*, and *P. huangchuchieni*) were included in the dataset. *Neolissochilus soroides* was used as the out-group to root the tree. GenBank accession numbers for all sequences are presented in Table 1.

Results

Morphology. Result of the sheared PCA of the morphometric data indicate that size accounted for 96.2% of the observed variance. The sheared PC2 and PC3 accounted for 0.85% and 0.48% of the observed variance, respectively. Prenostril length (0.55) and caudal-peduncle depth (-0.31) had the highest loadings on sheared PC2. Meanwhile, anal-fin base length (0.55) and the distance of the pelvic-fin base origin to the anal-fin base origin (-0.43) had the highest loading on sheared PC3.

The scatterplot clusters with 95% confidence interval ellipses for *P. normani* from Thailand (Chanthaburi, Trat, Ubon-Ratchathani, Bangkok, and Narathiwat) and specimens identified as *P. smedleyi, P. normani*, and *P.* cf. *normani* from Peninsular Malaysia were compared to test for allopatric morphological divergence. The PCA of the morphometric data showed substantial overlap in the examined characters and failed to form clear distinguishable clusters when PC2 was plotted against PC3 (Fig. 2) indicating low structured variation in body shape among the populations studied and failing to separate populations or putative species. Thus, the PCA result are consistent with the presence of only one species of *Poropuntius* across Peninsular Malaysia which is morphologically similar to *P. normani* from Thailand.

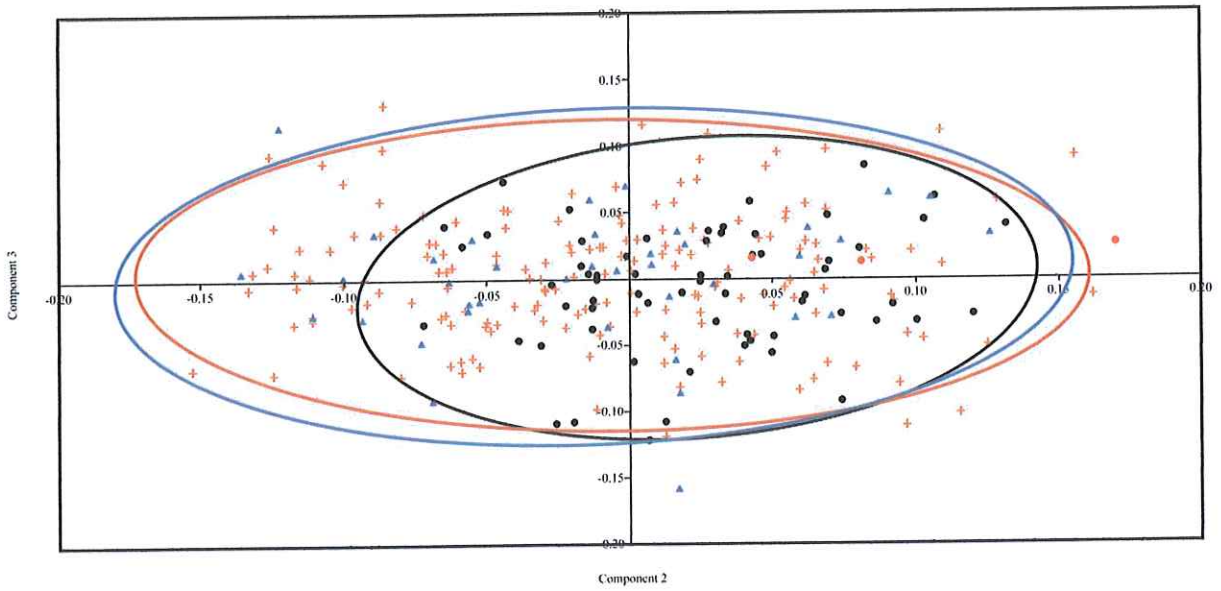

FIGURE 2. Scatterplots with 95% confidence interval ellipses of the sheared second principal component (PC2) and sheared third principal component (PC3) of morphometric data on *Poropuntius* from Peninsular Malaysia and Thailand. *Poropuntius smedleyi* (black dots), *P. normani* (red +, and red dot indicates specimens from type locality) and *P.* cf. *normani* (blue triangle).

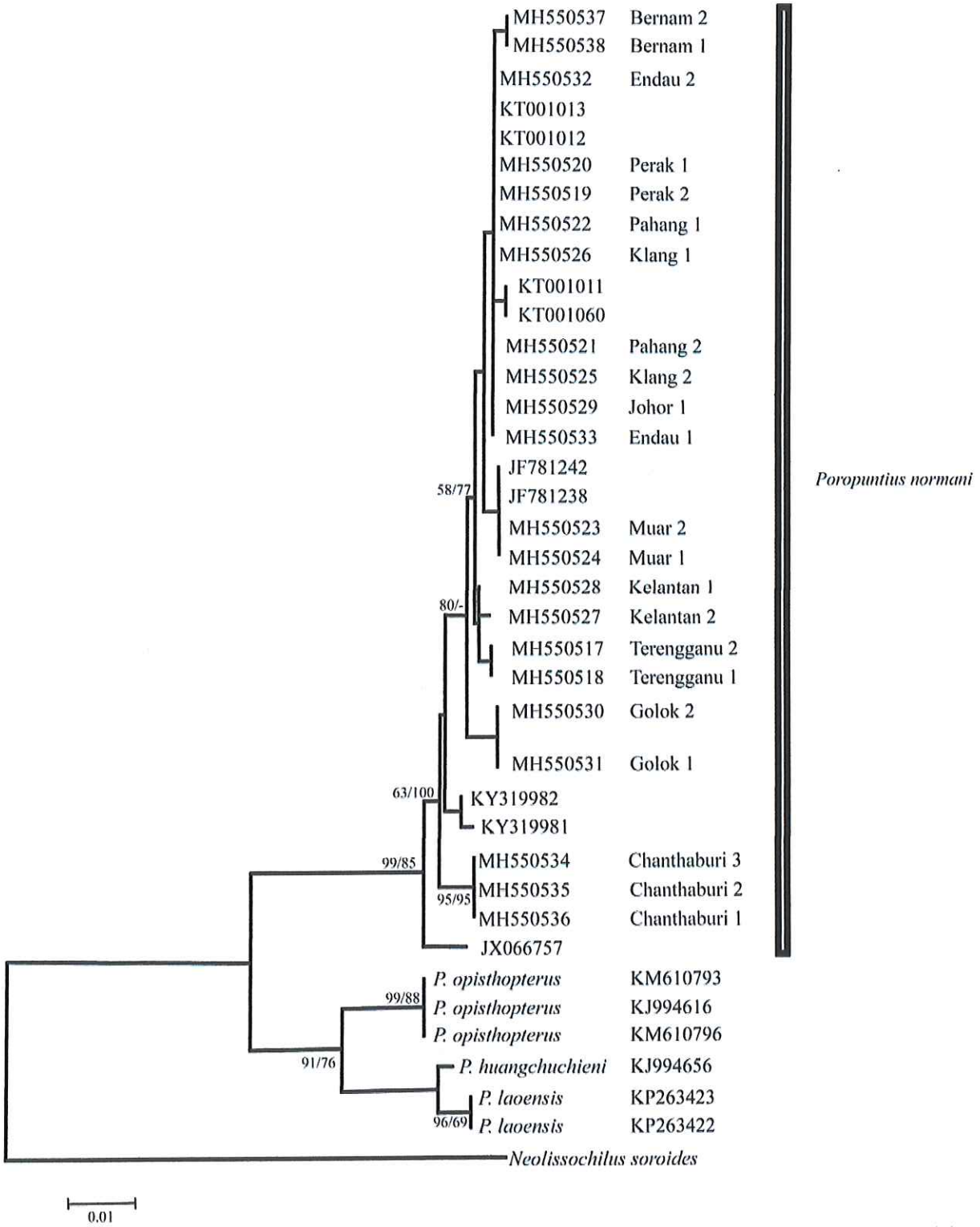

FIGURE 3. Neighbor-joining phylogenetic tree of *Poropuntius* based on COI sequences. Bootstrap values (neighbor-joining [NJ]/maximum likelihood [ML]) are shown on the nodes.

Molecular. The aligned COI sequences consisted of 575 characters; of these, 51 were parsimony informative, 52 were variable but parsimony uninformative, and 472 were constant. NJ and ML analyses produced phylogenetic trees with the same topology but with different bootstrap support values (Fig. 3). Only the NJ tree is presented for the sequences of COI. The phylogenetic tree based on the COI sequences revealed two main lineages (Fig. 3). The

first lineage consisted of all specimens of *Poropuntius* from Peninsular Malaysia, along with specimens of *P. normani* from Thailand and Laos, with 99% bootstrap support, whereas the second lineage consisted of specimens of *P. opisthopterus*, *P. laoensis*, and *P. huangchuchieni* with 91% bootstrap support. *Poropuntius normani* is distantly separated from *P. opisthopterus*, *P. huangchuchieni*, and *P. laoensis* by 5.39–6.09%, 5.74–6.61%, and 6.09–6.78%, respectively (Table 2). Genetic variability was observed within the first lineage, suggesting some levels of population structure across Malaysia, Thailand, and Laos. However, low genetic differentiation distances (0.00–1.91%) within this lineage preclude the recognition of more than one species among the studied populations. All specimens of *Poropuntius* from Peninsular Malaysia, including specimens diagnosable as *P. smedleyi* and *P.* cf. *normani* are indeed conspecific with *P. normani* from Thailand and Laos. This result is supported by both morphological and molecular data presented herein.

TABLE 2. Inter- and intra-specific uncorrected p genetic distance (%) of *Poropuntius* spp.

	1	2	3	4
1. *P. normani*	0.00—1.91			
2. *P. opisthopterus*	5.39—6.09	0.00		
3. *P. huangchuchieni*	5.74—6.61	3.13	—	
4. *P. laoensis*	6.09—6.78	2.78	0.70	0.00

Discussion

Several authors (Mohsin & Ambak 1983; Ng & Tan 1999; Shah *et al.* 2009; Kano *et al.* 2013) have identified specimens of *Poropuntius* in Peninsular Malaysia as *Poropuntius deauratus*. This may be due to the lack of taxonomic works available at that time which caused the authors to misidentify specimens as *P. deauratus* as their appearance is much similar to the Vietnamese *P. deauratus*. The general resemblance of *P. deauratus* and *P. normani* also caused Roberts (1998) to treat *P. normani* as a synonym of *P. deauratus*. Later, Kottelat (2000) re-examined the specimens of *P. deauratus* from Vietnam and found that this species can be distinguished from *P. normani* based on its general appearance, caudal-fin coloration (very pale yellow with dusky upper and lower margins vs. yellowish with dark upper and lower margin in *P. normani*), morphology of the last simple dorsal-fin ray (more slender in *P. deauratus* compared to *P. normani*), and denticulation type of the last simple dorsal-fin ray (weak or almost smooth vs. strongly serrated in *P. normani*). The observation by Kottelat (2000) is congruent with the observation of Sauvage (1881) who examined the holotype (MNHN 0000-2727) of *Barbus deauratus* (now *Poropuntius deauratus*) and also noticed the weak denticulation of the last simple dorsal-fin ray. Kottelat (2000) also reported that *P. deauratus* is restricted to central Vietnam and the reports on its occurrence outside this area, including Peninsular Malaysia, are doubtful.

De Beaufort (1933) noted that *P. smedleyi* (from Kota Tinggi, Johor) differs from *P. normani* by the following characters: 12 circumpeduncular scale rows (vs. 14 in *P. normani*) and convex interorbital space (vs. flat in *P. normani*). However, our extensive morphological examination of specimens of *Poropuntius* across Peninsular Malaysia did not find any distinctive characters to differentiate these species, with the interorbital shapes varying from flat to convex and circumpeduncular scale counts ranging from 12 to 14 (Table 5). This variation of characters may be due to ecophenotypic factors as demonstrated in other cyprinid fishes such as *Neolissochilus* (Khaironizam *et al.* 2015) and *Tor* (Walton *et al.* 2017). A similar pattern was hypothesized in populations of *Lobocheilos rhabdoura*, with specimens inhabiting fast-flowing sections of streams having slenderer bodies compared to specimens from populations inhabiting slow-moving habitats (Ciccotto & Page 2016).

Additional analyses using morphometric and molecular data (Figs. 2 and 3), confirm the presence of only one species of *Poropuntius* in Peninsular Malaysia. Moreover, all specimens of *Poropuntius* from Peninsular Malaysia and those from the type locality of *P. normani* (holotype, USNM 90297—Fig. 4A) are morphologically and genetically similar, supporting the conclusion that all specimens examined are conspecific. Article no. 23 Principal of Priority of the International Code of Zoological Nomenclature (ICZN 1999) is applicable here as the oldest available name, *Poropuntius normani* (Smith 1931), is retained and *Poropuntius smedleyi* (de Beaufort 1933) is a synonym of *P. normani*.

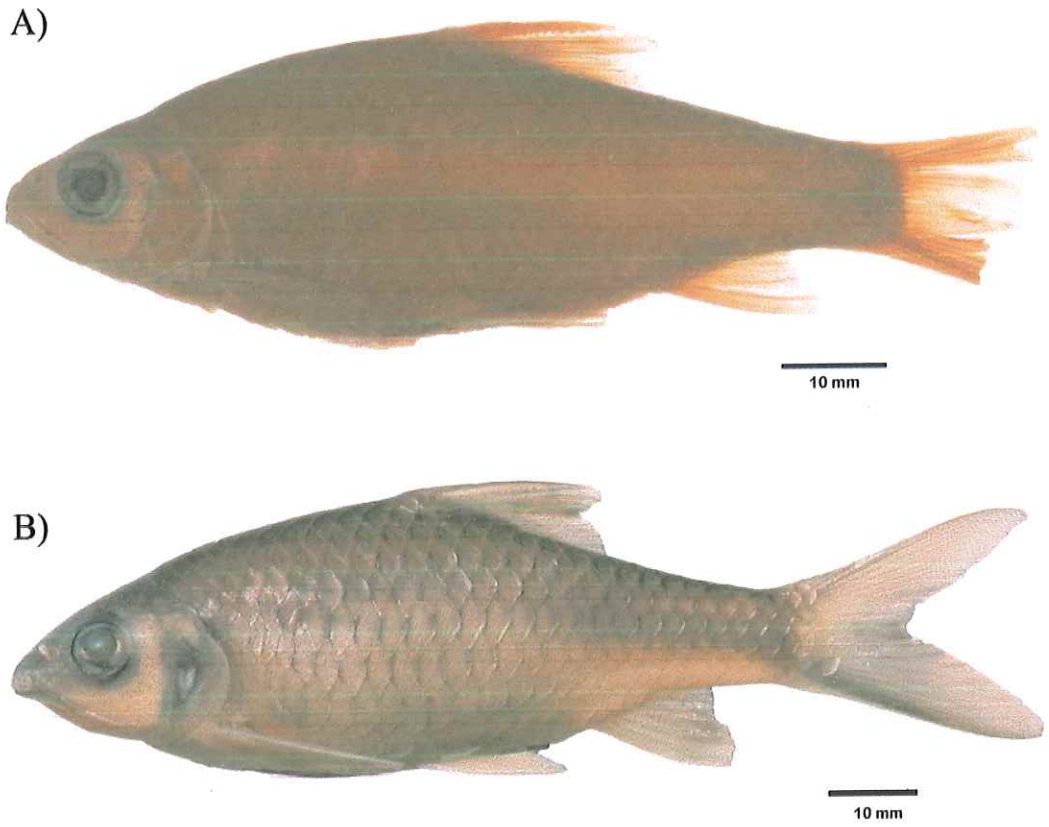

FIGURE 4. *Poropuntius normani*; A) Holotype, USNM 90297, Chanthaburi, Thailand. Photo by Sandra J. Raredon, National Museum of Natural History, Smithsonian Institution; B) UMKL 12006, 109.85 mm SL, Gunung Ledang, Johor, Malaysia.

Poropuntius

Poropuntius Smith 1931: 14 (type species by monotypy *Poropuntius normani* Smith 1931).

Description. Body moderately elongated and compressed. Snout bluntly rounded; tubercles irregularly arranged on tip of snout to lacrimal bones and visible with bare eyes. Several mature males in breeding condition have been observed having extensive tuberculation on the head, posterior half of body, and anal fin (Fig. 5). Mouth subterminal, lower jaw shape varies from rounded to truncated, lower jaw always subequal to upper jaw. Most species have yellowish horny sheath on lower lip, two pairs of well-developed barbels (rostral and maxillary); few species have small maxillary barbels; one species has no barbels. Dorsal-fin rays iv, 7–8 with last simple ray ossified and serrated posteriorly. Pectoral-fin rays i, 12–16, with first ray longest; pelvic-fin rays i, 8 (rarely 7), with first ray being longest; anal-fin rays iii, 5, pointed with third ray longest. Most species have bold black or dusky submarginal stripes on upper and lower caudal-fin lobes; stripe in lower lobe tends to be bolder than in the upper one. See Roberts (1998) for detailed description.

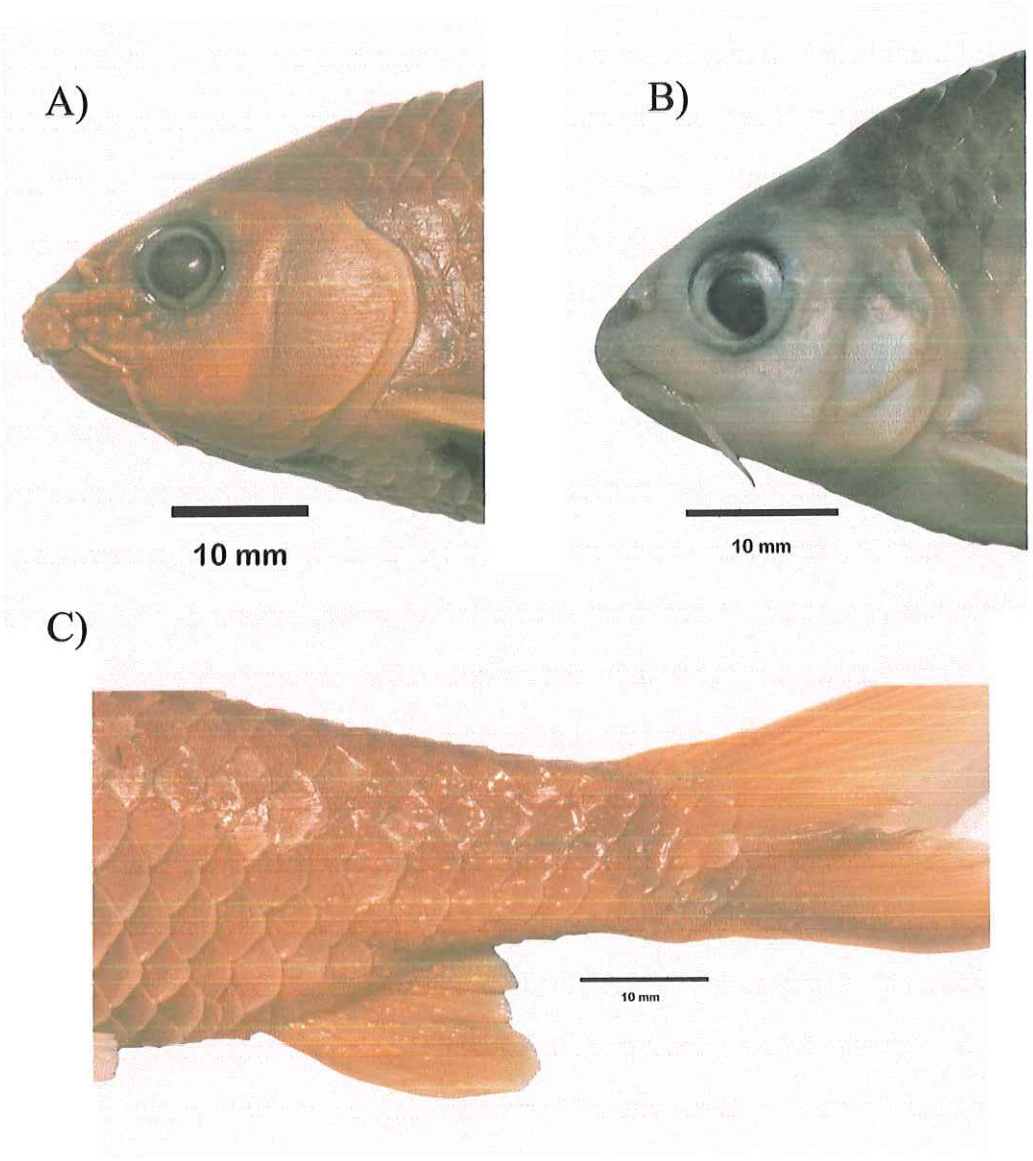

FIGURE 5. A) Large tubercles on snout in reproductive males (UMKL 12234); B) Small tubercles on snout in females (UMKL 12290); and C) heavily tuberculation on posterior half of the body, anal fin and caudal fin in reproductive males (UMKL 12234) of *Poropuntius normani*.

Poropuntius normani **Smith 1931**
(Fig. 4B)

Poropuntius normani Smith 1931: 15 (type locality Pliew waterfall, Kao Sabap, near Chanthaburi, southeastern Thailand).
Lissochilus smedleyi de Beaufort 1933: 34 (type locality Johor, southern Malay Peninsula).
Acrossocheilus deauratus: Menon 1954: 25; Bishop 1973; Mohsin & Ambak 1983: 216.
Poropuntius deauratus: Lim, Kottelat, & Ng 1990: 315; Lim, Ng, & Kottelat 1990: 38; Ng & Tan 1999: 38; Shah *et al.* 2009: 20; Hashim *et al.* 2012: 8; Sah *et al.* 2012: 64.
Poropuntius smedleyi: Ismail & Fatimah 2005; Roberts & Khaironizam 2008: 56; Hashim *et al.* 2012: 8; Ismail *et al.* 2013: 65; Amirrudin & Ismail 2014: 66.
Poropuntius normani: Miyazaki *et al.* 2013: 9.

Diagnosis. *Poropuntius normani* is distinguished from all other species of *Poropuntius* except for *P. deauratus* and *P. laoensis* by having a bright lemon yellow caudal fin, with bold submarginal dark stripes on the upper and lower lobes. *Poropuntius normani* is distinguished from *P. deauratus* by having its last simple dorsal-fin ray robust and strongly serrated along its posterior margin (vs. slender and very weakly serrated). *Poropuntius normani* can be distinguished from *P. laoensis* by having 22 to 31 (mode = 26) pored lateral-line scales (vs. 32 to 36 [mode = 33] for *P. laoensis*).

Description. Morphometric and meristic data of 281 specimens of *P. normani* given in Tables 3 and 4. Body moderately deep; dorsal profile rising gently from tip of snout to origin of dorsal fin, sloping gently ventrally from origin of dorsal fin to end of caudal peduncle. Body depth approximately 3.1 times in SL; caudal peduncle slender and moderately long, about 1.5–1.8 times longer than deep. Dorsal-fin origin above or posterior to vertical through pelvic-fin insertion. Distance between pelvic-fin and pectoral-fin insertions slightly greater than distance between pelvic-fin insertion and anal-fin origin. Pelvic fin shorter than pectoral fin length, about 1.3 times in HL.

Head moderately compressed, snout slightly pointed, longer than eye diameter. Prenostril length about 4.8 times in HL. Tip of snout with 2–3 irregular transverse rows of tubercles. Mouth subterminal, lower lips always covered with yellowish horny sheath and post-labial groove always interrupted. Rostral and maxillary barbels almost equal in length, both longer than eye diameter.

Dorsal-fin rays iv, 8 and last branched ray always split at base; anal-fin rays iii, 5 last branched ray always split to base; pelvic-fin rays i, 7–8; pectoral-fin rays i, 12–15. Dorsal-fin height lower than dorsal-fin depressed length. First simple dorsal-fin ray short, always embedded into the skin. Last simple ray ossified and serrated posteriorly, bearing 16–25 denticles. Dorsal-fin base length longer than anal-fin base length. First and second simple anal-fin rays short, always attached to third and longest simple rays with distal margin concave when fin erect. Caudal fin forked with convex distal margin of each lobe. Upper and lower lobes nearly equal in length.

TABLE 3. Meristic data of *Poropuntius normani*. Data includes specimens from the type locality in Thailand and Peninsular Malaysia.

Characters	All (n = 281)
Dorsal-fin spines and rays	iv, 8
Anal-fin spines and rays	iii, 5
Pectoral-fin spines and rays	i, 12–15 (mode = 13)
Pelvic-fin spines and rays	i, 8
Perforated lateral-line scales	22–31 (mode = 26)
Perforated scales in lateral line on hypural plates	2–5 (mode = 3)
Transverse scales rows above perforated lateral-line	5–7 (mode = 6)
Transverse scales rows below perforated lateral-line	3–4 (mode = 3)
Circumferential scale rows	18–24 (mode = 22)
Circumpeduncular scale rows	11–15 (mode = 14)
Predorsal scales	9–13 (mode = 10)
Scales from end of dorsal-fin base to origin of caudal-fin base	8–14 (mode = 11)
Scales at dorsal-fin base	5–8 (mode = 6)
Scales from isthmus to pelvic-fin base	13–18 (mode = 14)
Scales from end of pelvic-fin base to origin of anal-fin base	6–8 (mode = 7)
Scales from end of anal-fin base to origin of caudal-fin base	5–7 (mode = 6)
Scales at anal-fin base	5–7 (mode = 5)
Total gill rakers	8–15 (mode = 10)
Gill rakers on upper arm	2–4 (mode = 3)
Gill rakers on lower arm	5–10 (mode = 7)
Serration number on last simple dorsal-fin ray	16–24 (mode = 22)

TABLE 4. Range, mean, and standard deviation (SD) of the morphometric data of *Poropuntius normani.* Data for all specimens includes specimens from the type locality in Thailand and Peninsular Malaysia.

Characters	All (n = 281)	
	Range	(Mean ± SD)
Standard length (mm)	35.0–188.2	
% of standard length		
Body depth	27.8–36.9	(31.9 ± 1.8)
Head length	22.0–31.0	(26.3 ± 1.4)
Caudal peduncle length	14.1–22.4	(17.9 ± 1.4)
Caudal peduncle depth	7.6–14.5	(11.9 ± 0.9)
Dorsal-fin base length	13.2–17.9	(15.8 ± 0.9)
Dorsal-fin depressed length	21.0–30.5	(26.6 ± 1.7)
Dorsal-fin height	17.7–29.3	(24.3 ± 2.0)
Anal-fin base length	7.2–12.9	(10.0 ± 0.9)
Anal-fin depressed length	16.0–24.7	(20.5 ± 1.3)
Anal-fin height	14.9–23.6	(20.0 ± 1.4)
Pectoral length	20.1–27.2	(23.6 ± 1.3)
Pelvic length	17.0–23.3	(20.4 ± 1.2)
Predorsal length	49.1–59.6	(53.3 ± 1.6)
Posdorsal length	43.0–61.9	(50.0 ± 2.1)
Prepectoral length	20.9–31.4	(26.0 ± 1.6)
Postpectoral length	70.8–86.5	(76.7± 2.2)
Preanal length	71.3–81.2	(75.6 ± 2.1)
Postanal length	21.0–30.5	(25.9 ± 1.6)
Prepelvic length	41.7–56.7	(51.5 ± 1.8)
Postpelvic length	45.8–57.7	(51.3 ± 2.0)
Dorsal-fin base to pectoral-fin base	32.9–42.6	(37.9 ± 1.5)
Dorsal-fin base to anal-fin base	32.4–42.1	(37.3 ± 1.6)
Pelvic-fin base to anal-fin base	21.7–37.2	(25.9 ± 1.8)
Pectoral-fin base to pelvic-fin base	23.5–32.7	(27.1 ± 1.3)
Pectoral-fin base to anal-fin base	25.5–58.0	(52.0 ± 2.9)
% of head length		
Head depth	68.8–89.0	(78.8 ± 2.9)
Head width	54.1–71.7	(61.6 ± 3.3)
Snout length	20.0–38.5	(34.0 ± 2.2)
Prenostril length	14.1–27.3	(21.0 ± 2.0)
Orbital length	21.0–38.4	(29.7 ± 2.7)
Preoccipital length	73.1–92.1	(81.0 ± 3.2)
Preopercle length	68.1–81.0	(75.1 ± 2.0)

Entire body covered by moderately large scales. Number of pored scales on lateral-line 22–31; pored scales on caudal fin 2–5; predorsal scales 9–13; 5–7/1/3–4 scales in transverse row origin of dorsal-fin to anterior anal-fin insertion; circumferential scales rows 18–24; circumpeduncular scales rows 12–14. Total gill rakers on first gill arch 8–15.

Coloration. In live specimens, dorsum and upper half of side of body varies from silvery to light greenish coloration. Lower half of side and ventrum silvery-white. Pectoral, pelvic, and anal fins vary from yellowish to

some darkening on first simple rays, other unbranched rays hyaline. Caudal fin bright lemon yellow, with submarginal black stripes on upper and lower lobes. Iris silvery blue. Preserved specimens silvery to yellowish-brown on body and fins.

Ecological notes. *Poropuntius normani* is collected in benthic habitats of small to large rivers, typically in streams with swift currents and good water quality (Zakaria-Ismail & Fatimah 2002). In Peninsular Malaysia, this species is frequently syntopic with *Neolissochilus soroides*.

Distribution. Currently, *P. normani* is known from the Mekong basin in Laos, Cambodia, Thailand, and Vietnam and in the Mae Khlong basin in Thailand. In Malay Peninsula, *P. normani* is found from the headwaters of the Tapi River basin in Surat Thani and Nakhorn Srithammarat southward into the Narathiwat province to the southern tip of the Peninsular Malaysia (Fig. 1).

Remarks. Usually in *P. normani*, the size of tubercles on the tips and sides of the snout are larger and more prominent in males (Figs. 5A and B). Sometimes during the breeding season, (from November to January), which coincides with the wet season in Peninsular Malaysia, the males show extensive breeding tuberculation on the snout, dorsum of the head, posterior half of the body, and the anal fin (Fig. 5C). This character is also observed in other species of *Poropuntius* (Roberts 1998).

TABLE 5. Number of circumpeduncular scales rows in *Poropuntius normani* from Thailand and Peninsular Malaysia.

Country	Drainages	No. of circumpeduncular scales		
		12	13	14
Thailand (n = 9)	Trat			2
	Chanthaburi*			3
	Mekong (Ubon-Ratchathani)			4
Peninsular Malaysia (n = 272)	Johor	12	3	
	Endau	34	1	
	Batu Pahat	7	3	
	Muar	1	1	3
	Linggi	2	3	
	Klang		2	18
	Bernam			5
	Perak		1	28
	Perlis			10
	Golok			13
	Kelantan		1	11
	Terengganu		1	9
	Besut			5
	Kemaman			5
	Setiu			5
	Dungun	1	6	3
	Pahang	11	5	52
	Kuantan	1	5	4

Note: Asterisk (*) mark indicates specimens collected from similar drainage of type locality.

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Material examined

Poropuntius normani. **Peninsular Malaysia**: UMKL 12001, 11 ex., 66.1–113.1 mm SL; Johor: Selai: Sg. Pandan, a tributary of Sg. Selai; coll. A.H. Muhammad Rasul, 16 Feb. 2016. UMKL 12006, 13 ex., 60.3–126.2 mm SL; Johor: Taman Negara Johor, Gunung Ledang; coll. A.H. Muhammad Rasul, 18 Feb. 2016. UMKL 12007, 9 ex., 45.3–76.8 mm SL; Johor: Bekok: Hutan Lipur Sg. Bantang; coll. A.H. Muhammad Rasul, 18 Feb. 2016 UMKL 12178, 16 ex., 61.6–113.9 mm SL; Johor: Kota Tinggi: Sg. Pelepah, a tributary of Kota Tinggi Waterfall; coll. A.H. Muhammad Rasul, 8 Dec. 2015. UMKL 12003, 5 ex., 53.0–139.3 mm SL; Johor: Selai: Sg. Tempaang, a tributary of Sg. Selai; coll. A.H. Muhammad Rasul, 16 Feb. 2016. UMKL 5061, 1 ex., 74.2 mm SL; Johor: Selai: Sg. Selai near base camp; coll. M. Zakaria-Ismail, 16 Apr. 2002. UMKL 12005, 9 ex., 43.0–52.1 mm SL; Johor: Selai: Takah Tinggi; coll. A.H. Muhammad Rasul, 16 Feb. 2016. ZRC 6662–6666, 5 ex., 28.8–69.5 mm SL; Johor: Kota Tinggi; Johor River drainage; Lombong waterfall; coll. J.R. Hendrickson, 17 Feb. 1962. ZRC 600, 4 ex., 59.7–73.5 mm SL; Johor: Kota Tinggi; Johor River drainage; Kota Tinggi waterfall; coll. M.W.F. Tweedie, Mar. 1938. ZRC 16716–16720, 5 ex., 35.1–61.6 mm SL; Johor: Kota Tinggi; coll. C.F. Lim, 28 Sept. 1977. UMKL 12116, 20 ex., 43.8–89.6 mm SL; Negeri Sembilan: Hutan Lipur Lenggeng; coll. A.H. Muhammad Rasul, 13 Oct. 2016. UMKL 12110, 12 ex., 46.2–75.1 mm SL; Selangor: Hutan Lipur Sg. Tekala; coll. A.H. Muhammad Rasul, 13 Oct. 2016. UMKL 12181, 5 ex., 80.3–108.3 mm SL; Selangor: Batu 19, Jln. Gombak-Bentong lama; coll. A.H. Muhammad Rasul, 5 Dec. 2015. UMKL 12172, 10 ex., 73.9–123.7 mm SL; Perak: Tapah: Sg. Woh; coll. A.H. Muhammad Rasul, 26 May 2016. UMKL 12175, 9 ex., 33.4–98.5 mm SL; Perak: Bidor: Sg. Bidor, near Kg. Poh; coll. A.H. Muhammad Rasul, 26 May 2016. UMKL 12009, 1 ex., 134.6 mm SL; Perak: Sg. Anak Kuok, Royal Belum; coll. A.H. Muhammad Rasul, 3 Feb. 2016. UMKL 12011, 5 ex., 66.9–96.4 mm SL; Perak: Sg. Ta-Ng, Royal Belum; coll. Linda, 3 Feb. 2016. UMKL 12045, 10 ex., 75.1–101.4 mm SL; Kelantan: Sg. Kenarong, near Gunung Stong; coll. A.H. Muhammad Rasul, 10 Jan. 2016. UMKL 12180, 13 ex., 42.9–71.7 mm SL; Kelantan: Tanah Merah: Sg. Jedok; coll. A.H. Muhammad Rasul, 21 Nov. 2015. UMKL 12062, 9 ex., 42.5–78.2 mm SL; Kelantan: Jeli: Sg. Bawang, a tributary of Sg. Golok; coll. A.H. Muhammad Rasul, 25 Mar. 2016. UMKL 12148, 12 ex., 33.6–124.0 mm SL; Terengganu: Kuala Berang: Hutan Lipur Sekayu; coll. A.H. Muhammad Rasul, 28 Sept. 2016. UMKL 12081, 3 ex., 86.9–111.6 mm SL; Pahang: Jerantut: Ulu Sg. Kiol; coll. A.H. Muhammad Rasul, 27 Apr. 2016. UMKL 12080, 3 ex., 52.6–69.1 mm SL; Pahang: Maran: Hutan Lipur Lubuk Yu; coll. A.H. Muhammad Rasul, 27 Apr. 2016. UMKL 12084, 3 ex., 61.7–74.5 mm SL; Pahang: Jerantut: Hutan Lipur Lata Meraung; coll. A.H. Muhammad Rasul, 27 Apr. 2016. UMKL 12083, 5 ex., 36.4–90.8 mm SL; Pahang: Gambang: Air terjun Sg. Pandan; coll. A.H. Muhammad Rasul, 28 Apr. 2016. UMKL 12076, 7 ex., 36.6–93.2 mm SL; Pahang: Raub: Lata Jarum; coll. A.H. Muhammad Rasul, 26 Apr. 2016. UMKL 12082, 3 ex., 41.0–68.6 mm SL; Pahang: Karak: Sg. Perdak; coll. A.H. Muhammad Rasul, 29 Apr. 2016. UMKL 12077, 5 ex., 78.1–107.1 mm SL; Pahang: Raub: Lata Lembik; coll. A.H. Muhammad Rasul, 26 Apr. 2016. UMKL 12234, 7 ex., 95.7–122.8 mm SL; Pahang: Pahang River drainage; Sungai Bertam, a tributary of Sungai Jelai; coll. Zakaria-Ismail, 20 Aug. 1990. UMKL 12236, 19 ex., 34.4–107.1 mm SL; Pahang: Pahang River drainage; Sungai Lemoi, a tributary of Sungai Bertam; coll. A. H. Muhammad Rasul, 16 Mar. 2017. UMKL 12165, 13 ex., 47.4–74.8 mm SL; Perlis: Taman Negeri Wang Kelian; coll. A.H. Muhammad Rasul, 27 July 2016. Uncataloged specimens deposited in Universiti Malaysia Terengganu 1, 21 ex., 76.1–159.1 Pahang: Cameron Highland; Pahang River drainage; Sungai Telom, a tributary of Sungai Bertam; coll. Amirrudin, 22 May 2007. Uncataloged specimens deposited in Universiti Malaysia Terengganu 2, 5 ex., 153.7–188.0 mm of SL; Terengganu: Hulu Dungun, Dungun River drainage, Sg. Dungun, Station 14; coll. Amirrudin, 8 June 2004.

Thailand: USNM 90297 (holotype), 1 ex., 136.5 mm SL, Thailand: Chanthaburi, Kao Sabap; H.M. Smith, 1931; UMKL 12287, 3 ex., 92.8–109.8 mm SL; Thailand: Chanthaburi, Kao Sabap, Pliew waterfall; coll. A.H. Muhammad Rasul & K. Sontaya, 22 July 2017. ZRC 40847, Thailand: Trat province, Nam Tok Saphan Hin

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Poropuntius laoensis. Uncataloged collection of Ubon Ratchathani University 17103, 1 exp., 123.6 mm SL.

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