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Taxonomic Status of *Fowlea tytleri* (Blyth, 1863) from the Andaman Islands

GUNTHER KÖHLER[1], NI LAR THAN[2] AND PANUPONG THAMMACHOTI[3*]

[1]*Senckenberg Forschungsinstitut und Naturmuseum, Senckenberganlage 25, 60325 Frankfurt a.M., GERMANY*
[2]*East Yangon University, Thanlyin, Yangon, MYANMAR*
[3]*Department of Biology, Faculty of Science, Chulalongkorn University; Bangkok 10330, THAILAND*
[*]Corresponding author. Panupong Thammachoti (panupong.th@chula.ac.th)
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ABSTRACT.– The analysis of mtDNA data (16S ribosomal RNA) shows that the *Fowlea* (formerly *Xenochrophis*) of the Andaman islands, formerly referred to as *Fowlea* (or *Xenochrophis*) *tytleri* are conspecific with *F. piscator* (Schneider, 1799). Specimens from the Andaman islands form a mixed clade together with individuals from the mainland and differ from the latter by genetic distances of 0.2–0.5% (except for 1.7% from a single mainland specimen that also differs from other mainland samples of *F. piscator* by 1.4–1.5% genetic distance). The genetic distances between individuals of *F. piscator* and its supposed sister taxon *F. flavipunctatus* vary from 4.8 to 7.3%. In pholidosis, *F. tytleri* and *F. piscator* are essentially identical. Based on the evidence from mtDNA and morphological data (the latter taken from the literature), we formally place *Fowlea tytleri* (Blyth, 1863) in the synonymy of *F. piscator* (Schneider, 1799).

KEY WORDS: Biodiversity studies, mtDNA, reptiles, Southeast Asia, *Xenochrophis*

INTRODUCTION

The keelback water snakes of the genera *Fowlea* and *Xenochrophis* have a complex taxonomic history. For decades, these snakes were traditionally grouped in a single genus, *Xenochrophis*, but most recently split into two genera (Purkayastha *et al.* 2018). The species *X. bellulus* (Stoliczka, 1871), *X. cerasogaster* (Cantor, 1839), *X. maculatus* (Edeling, 1864), *X. trianguligerus* (Boie, 1827), and *X. vittatus* (Linnaeus, 1758) remained in *Xenochrophis* whereas the following species were transferred to the genus *Fowlea*: *F. asperrimus* (Boulenger, 1891), *F. flavipunctatus* (Hallowell, 1860), *F. melanzostus* (Gravenhorst, 1807), *F. piscator* (Schneider, 1799), *F. punctulatus* (Günther, 1858), *F. sanctijohannis* (Boulenger, 1890), *F. schnurrenbergeri* Kramer, 1977, and *F. tytleri* (Blyth, 1863). In 1863, Blyth described the new species *Tropidonotus tytleri* from "Andaman" (= Andaman Islands, India). This taxon has been treated as a synonym of *X. melanzostus* (or *X. piscator melanzostus*) (e.g., Smith 1943; Biswas 1984; Whitaker and Captain 2004) until it was resurrected from this synonymy and recognized as a full species by Vogel and David (2006), and later confirmed as such by Vogel and David (2012).

During field work in Myanmar and Thailand we collected individuals of keelback water snakes at several places and thus became interested in the geographic variation of this group of snakes. In particular, we aimed to study the genetic and morphological differentiation of *F. flavipunctatus* and *F. piscator* and to document the areas of sympatric occurrence. In the course of analyzing the geographic variation of the sampled snakes we added sequences of additional species of this group of snakes from Genbank. Here we report on the lack of genetic differentiation in mtDNA between specimens from the Andaman Islands (currently recognized as *F. tytleri*) and *F. piscator* from several Asian mainland localities.

MATERIALS AND METHODS

Specimens examined for this study were collected by GK, NLT, and PT (see Appendix 1 for specimens examined). Specimens labeled with GK field numbers were deposited in the collections of Senckenberg Forschungsmuseum Frankfurt (SMF), in the collection of the Chulalongkorn University, Museum of Natural History (CUMZ), Bangkok, or at East Yangon University (EYU), Thanlyin, Myanmar. Data of variation in external morphology in the nominal species *Fowlea tytleri* and *F. piscator* were taken from Vogel and David (2012).

Prior to preservation of collected specimens in the field, we took color photographs of each individual in life. We euthanized the snakes with a pericardial injection of T61. We cut tissue samples from the tongue or lateral body wall and preserved these in 98% non-denatured ethanol for DNA extraction. The tissue samples were deposited in the collection of SMF and CUMZ. Specimens were then preserved by injecting a solution of 5 mL absolute (i.e., 36%) formalin in 1 L of 96% ethanol into the body cavity, and stored in 70% ethanol. Coordinates and elevation were recorded using Garmin GPS receivers with built-in altimeters. All coordinates were recorded in decimal degrees, WGS 1984 datum.

In evaluating species' boundaries within and among populations, we followed the evolutionary species concept (Simpson 1951; Wiley 1978). As lines of evidence for species delimitation, we applied a phenotypic criterion (external morphology) and the genetic distinctness of a mitochondrial genetic marker.

We extracted DNA following the protocol of (Ivanova, Waard and Hebert 2006). To eliminate potential PCR-inhibiting contaminants, the tissue samples were incubated for 14 hours at 4°C in 200 µL low PBS buffer (20 µL PBS in 180µL of water) before overnight digestion with the vertebrate lysis buffer at 56°C. After extraction, DNA was eluted in 50 µL TE buffer. Fragments of the mitochondrial 16S rRNA (16S) were amplified in an Eppendorf Mastercycler® Pro using the following protocol: initial denaturation for 2 min at 94°C; followed by 40 cycles with denaturation for 35 s at 94°C, hybridization for 35 s at 48.5°C, and elongation for 60 s at 72°C; final elongation for 10 min at 72°C. The reaction mix for each sample contained 1 µL DNA template, 14 µL water, 2.5 µL PCR-buffer, 1 µL 25 mM $MgCl_2$, 4 µL 2.5 mM dNTPs (Invitrogen), 0.5 µL (containing 2.5 units) Taq Polymerase (PeqLab), and 1 µL of the primer (16S: forward: L2510, 5'–CGCCTGTTTAT CAAAAACAT–3'; reverse: H3056, 5'–CCGG TCTGAACTCAGATCACGT–3'; from Eurofins MWG Operon). DNA extraction, PCR, and sequencing for the samples from Myanmar were done at SMF, for those from Thailand were done at Chulalongkorn University, Bangkok. We generated 12 new sequences for this study (see Appendix 2). Additionally, we downloaded relevant 16S sequences from GenBank (Appendix 2).

We aligned the sequences with MUSCLE (Edgar 2004) using the default settings in Geneious 6.1.2. (Kearse *et al.* 2012). For software applications, sequence data formatting was converted using the online server Alter (Glez-Peña *et al.* 2010). The best substitution model for each gene was identified using PartitionFinder2 (Lanfear *et al.* 2017), with linked branch lengths via PhyML 3.0 (Guindon *et al.* 2010). Model selection used the corrected (for finite sample size) Akaike Information Criterion (AICc) (Burnham and Anderson 2002). *Oligodon fasciolatus* was used as outgroup (Pyron, Burbrink and Wiens 2013).

Bayesian Inference analyses (BI) used MrBayes 3.2 (Ronquist *et al.* 2012) with five runs with eight chains. The first 25% of trees

were discarded as burn-in. MCMC runs used an initial set of 1,000,000 generations with sampling every 500 generations, and adding 500,000 generations until chains reached convergence. Convergence was considered achieved when the standard deviation of split frequencies was 0.015 or less. Additionally, convergence was diagnosed by PSRF (Potential Scale Reduction Factor), which should approach 1.0 as runs converge (Gelman and Rubin 1992). We used the IQTree webserver (Trifinopoulos *et al*. 2016) to run a Maximum Likelihood (ML) analysis using 10,000 ultrafast Bootstrap approximation (UFBoot) replicates with 10,000 maximum iterations and minimum correlation coefficient of 0.99 (Minh, Nguyen and Haeseler 2013), plus 10,000 replicates of Shimodaira-Hasegawa approximate likelihood ratio (SH-aLRT), which proved to be accurate with a high statistical power (Guindon *et al.* 2010). We used FigTree 1.3.1 (http://tree.bio.ed.ac.uk/software/figtree/) for viewing the trees. We estimated evolutionary genetic divergence, computing uncorrected pairwise distances with MEGA 7.0.26 (Kumar, Stecher and Tamura 2016) to assess the degree of intra- and interspecific differences, using a Bootstrap estimation method of 10,000 replications.

Abbreviations used are IFL (infralabials), SPL (supralabials), and TL (total length).

RESULTS

Specimens labeled *Fowlea tytleri* from the Andaman islands form a mixed clade together with individuals of *F. piscator* from the mainland (Fig. 1) and differ from the latter by genetic distances of 0.2–0.5% (except for 1.7% from a single mainland specimen that also differs from other mainland samples of *F. piscator* by 1.4–1.5% genetic distance) (Table 1). The genetic distances between individuals of *F. piscator* and its supposed sister taxon *F. flavipunctatus* vary from 4.8 to 7.3%. However, in our trees, the Genbank samples KC347376 (labeled *F. asperrimus*) and KX277271 (labeled *F. piscator*) are placed between the clades of *F. piscator*/*tytleri* and *F. flavipunctatus*. The taxonomic relevance of this peculiar finding cannot be evaluated here any further since we do not have access to the voucher specimens of these two samples.

In pholidosis, *F. tytleri* and *F. piscator* are essentially identical (Table 2). Based on the evidence from mtDNA and morphological data, we formally place *Fowlea tytleri* (Blyth, 1863) in the synonymy of *F. piscator* (Schneider, 1799).

DISCUSSION

Given the close genetic similarity between the keelback water snakes from the Andaman Islands and those from the Asian mainland suggests a recent colonization of the islands or repeated colonization events from the mainland over a longer period of time. In their phylogenetic study of various species of keelback water snakes based on the three mtDNA markers ND4, 12S, and cytB, (Purkayastha *et al.* 2018) also found that *F. tytleri* is grouped with a sample of *F. piscator* from near the type locality of the latter taxon with a mean genetic distance of only 2.5% in ND4.

Vogel and David (2006:241) stated that "the population of the Andaman Islands belongs to a distinct species, for which the combination *Xenochrophis tytleri* is available". However, four of the five supposedly diagnostic characters they present, refer to details in color pattern, and their 5th character compares the number of ventral scales with another species, *F. melanzostus*. Neither Vogel and David (2006) nor Vogel and David (2012) present any pholidotic or morphometric character that would separate *F. tytleri* from *F. piscator*. The island populations are reported to differ from the mainland populations in some details of color

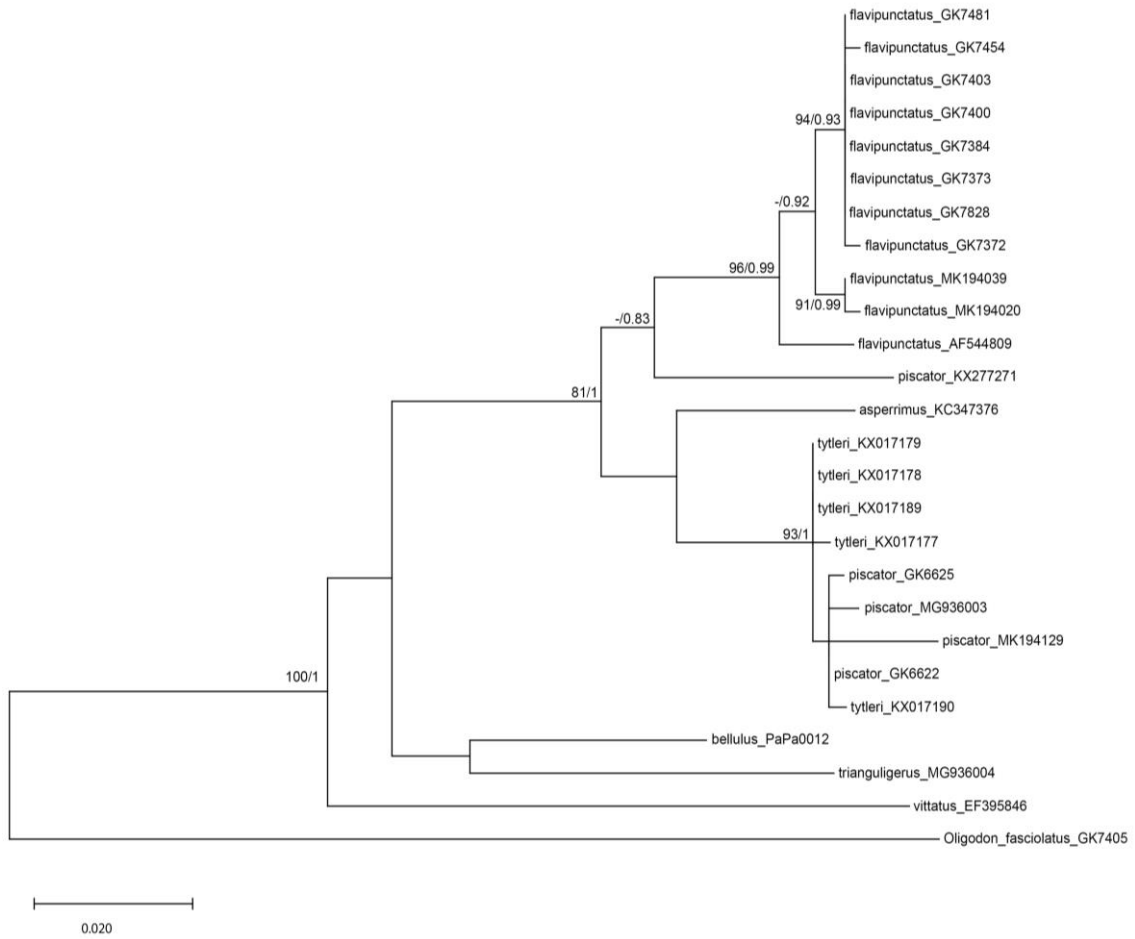

FIGURE 1. Phylogenetic tree of keelback water snakes of the genera *Fowlea* and *Xenochrophis* from a Maximum-Likelihood analysis of the mitochondrial marker 16S. Numbers at nodes are bootstrap values (left) and Bayesian posterior probabilities (right), but only for nodes with bootstrap values higher than 75. A scale bar of genetic distance is indicated. The tree is rooted using *Oligodon fasciolatus*.

pattern. However, mainland *F. piscator* are also known to show considerable variation in color pattern (see e.g., photos in Khan 2002, Schleich and Kästle 2002, Vogel and David 2012).

Mohan, Swamy and Shanker (2018) analyzed the genetic variation of the keelback water snakes on the Andaman archipelago, and found very low genetic variation (i.e., <1% between studied population based on the mtDNA marker ND4). They further concluded that salt waters between close-by islands are weak dispersion barriers. The Andaman archipelago is a continental group of islands in the Bay of Bengal, about 300 km south-southwest of the western tip of Myanmar. Geological data suggest that these islands have repeatedly been connected to the Asian mainland during the last glacial maxima with periods of low sea level (Bandopadhyay and Carter 2017).

TABLE 1. Mean genetic distances (in %) of *Xenochrophis* and *Fowlea* taxa included in the 16S dataset. See text for details

	flavipunctatus	***piscator***	***bellulus***	***tytleri***	***trianguligerus***	***asperrimus***
flavipunctatus						
piscator	5.2%					
bellulus	6.3%	5.9%				
tytleri	6.0%	1.6%	6.2%			
trianguligerus	6.9%	7.4%	5.4%	7.1%		
asperrimus	5.4%	3.8%	5.4%	3.8%	6.9%	
vittatus	8.4%	10.0%	7.1%	10.2%	7.6%	8.3%

TABLE 2. Variation in selected characters of external morphology (scalation and morphometrics) in the nominal species *Fowlea tytleri* and *F. piscator*. Data were taken from the respective species accounts and Table 3 in Vogel and David (2012). Table 3 in Vogel and David (2012) reports sample sizes as *Fowlea tytleri* (n=11) and *F. piscator* (n=130).

Characters	***F. piscator***	***F. tytleri***
ventrals males	128–143	131–138
ventrals females	136–154	140–145
subcaudals males	79–96	79–86
subcaudals females	68–88	76–77
dorsal scale rows	19–19–17 (rarely 21–19–17)	19–19–17
cloacal plate	divided	divided
loreals	1	1
preoculars	1	1
postoculars	3 or 4	3, rarely 4
anterior temporals	2, rarely 1, 3 or 4	2, rarely 1
posterior temporals	2, rarely 1 or 3	2, rarely 3
supralabials	9, rarely 8 or 10	9
SPL contact eye	4 and 5, rarely only 4th or only 5th	4 and 5
infralabials	10, rarely 9 or 11	10, rarely 9
IFL contact chin shields	5, rarely 6	5 first
tail length/TL males	28.3–33.1%	30.8–32.4%
tail length /TL females	24.7–30.0%	27.0–27.9%

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APPENDIX 1. Specimens Examined

Fowlea piscator—**Myanmar:** Bago: Bago Yoma: 18.89740: 95.90525: 430: GK-7116; Bago, near airport (17.32926, 96.44005), 50 m: GK-6625; Yangon: Yae Kyaw village, Kha Yan Township (16.95404, 96.48498), 10 m: GK-6622; East Yangon University (16.74030, 96.28518), 65 m: GK-7151, 7173.

Fowlea flavipunctatus—**Thailand:** Roi Et: near Ban Sa At Na Di (16.16382, 104.11223), 160 m: GK-7372-73, 7384, 7394, 7400-01, 7403, 7418, 7429; near Ban Sa At Na Di (16.16666, 104.11851), 150 m: GK-7454; near Ban Sa At Na Di (16.16166, 104.11279), 150 m: GK-7481; near Ban Sa At Na Di (16.16765, 104.11749), 155 m: GK-7828; Nakhon Phanom: Ban Kan Luang (16.96000, 104.41766), 175 m: GK-7904.

APPENDIX 2. GenBank Accession numbers of genetic sequences (16S) used

Species	GenBank No.	voucher
Fowlea asperrimus	KC347376	RS-J
Fowlea flavipunctatus	AF544809	not given
Fowlea flavipunctatus	MK194020	CHS260
Fowlea flavipunctatus	MK194039	CHS306
Fowlea flavipunctatus	MZ672173	GK7372
Fowlea flavipunctatus	MZ672169	GK7373
Fowlea flavipunctatus	MZ672168	GK-7384
Fowlea flavipunctatus	MZ672167	GK-7400
Fowlea flavipunctatus	MZ672166	GK-7403
Fowlea flavipunctatus	MZ672165	GK7454
Fowlea flavipunctatus	MZ672164	GK7481
Fowlea flavipunctatus	MZ672172	GK7828
Fowlea piscator	KX277271	not given
Fowlea piscator	MG936003	USNM 587046
Fowlea piscator	MK194129	CHS659
Fowlea piscator	MZ672171	GK-6622
Fowlea piscator	MZ672170	GK-6625
Fowlea tytleri	KX017177	1615
Fowlea tytleri	KX017178	1625
Fowlea tytleri	KX017179	1627
Fowlea tytleri	KX017189	1677
Fowlea tytleri	KX017190	1679
Oligodon fasciolatus	MZ672178	GK7405
Xenochrophis bellulus	MZ672174	PaPa-0012
Xenochrophis trianguligerus	MG936004	USNM 587045
Xenochrophis vittatus	EF395846	FMNH 257460