

Letter to the editor

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Phylogenetic placement and diet of homalopsid snake *Miralia alternans* (Ruess, 1833)

DEAR EDITOR,

Miralia alternans (Ruess, 1833) is distributed in Borneo, Sumatra, and Java in Southeast Asia. The species is morphologically similar to *Raclinia indica*, a monotypic genus known from Peninsular Malaysia. Here, we collected a juvenile specimen of *M. alternans* from Borneo, and report on its coloration in life and first prey item recovered from the species. We also explored the phylogenetic position of the genus using molecular phylogenetic analysis of mitochondrial DNA (mtDNA) gene cytochrome *b* (*cyt b*) and nuclear gene prolactin receptor (*PRLR*), confirming *M. alternans* as sister to *R. indica*, with the two genera exhibiting relatively high genetic divergence in *cyt b* (13.0%–13.1%).

Mud snakes (Homalopsidae), which include 56 species in 29 genera, are primarily distributed in Southeast Asia (Uetz et al., 2022). *Enhydris* Sonnini & Latreille, 1802, the formerly largest genus in the family, was divided into 15 genera by Murphy & Voris (2014). While several recent studies have inferred the phylogenetic relationships within the family, the positions of certain genera were not investigated, as half of the genera are only known from museum specimens (Bernstein et al., 2021). The monotypic genus *Miralia* Gray, 1842 was previously considered a synonym of *Enhydris* (e.g., Gyi, 1970), but later resurrected by Murphy & Voris (2014). Several studies have identified morphological similarities between *Miralia alternans* and *Raclinia indica*, suggesting they may be closely related (Gyi, 1970). However, molecular analysis has not yet been conducted due to the lack of *M. alternans* tissues (Murphy, 2007; Quah et al., 2018). In 2010, we collected a juvenile *M. alternans* snake in Kuching, Sarawak, Malaysian Borneo, with tissue from this specimen used to clarify its phylogenetic position.

The *M. alternans* specimen was collected during a field

survey at the Matang Wildlife Centre, Kuching, Sarawak, Malaysia, in August 2010. Tissue samples were taken for genetic analyses and deposited with the voucher specimen in the Sarawak Research Collection, Sarawak Forest Department (SRC). In addition to the new specimen, we examined the morphological characters of previously deposited specimens in the Museum Zoologicum Bogoriense (MZB), Sarawak Museum (SM), and Zoological Reference Collection of the Lee Kong Chian Natural History Museum at the National University of Singapore (ZRC). Scale terminology and measurements followed Murphy & Voris (2014) and Quah et al. (2018).

For molecular analyses, DNA was extracted and fragments of the mitochondrial gene *cyt b* (1 053 bp) and nuclear gene *PRLR* (573 bp) were amplified by polymerase chain reaction (PCR). The PCR products were sequenced with PCR primers and BigDye v3.1 using Sanger sequencing methods, and the obtained sequences were deposited in GenBank under accession numbers LC667473 (for *cyt b*) and LC667474 (for *PRLR*). In addition to the newly sequenced data for *M. alternans*, we used Homalopsidae sequencing data from Bernstein et al. (2021) to identify the phylogenetic position of *M. alternans* among other homalopsid genera (Supplementary Table S1). Maximum-likelihood (ML) and Bayesian inference (BI) methods were used to conduct phylogenetic analyses. Uncorrected *P*-distances for the *cyt b* region among sequences were calculated. Details are provided in the Supplementary Materials and Methods.

The specimen (SRC 00064) was collected on 30 August 2010 in lowland primary forest at the Matang Wildlife Centre, Kuching, Sarawak, Malaysia. The specimen was found at 1930h under leaf litter, close to a small, shallow, sandy-

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bottomed stream (1–2 m wide). To date, only three specimens have been reported from Borneo (Gyi, 1970; Murphy, 2007; Murphy & Voris 2014). Thus, the new specimen represents the fourth record of the species from Borneo.

Scale counts of *M. alternans* showed variation in supralabials 7–8, with 3rd–4th, 4th, or 4th–5th bordering orbit, supralabial formula 2-2-3, 3-1-3, 3-2-2, 3-1-4, or 3-2-3, 1st or 2nd supralabials contacting nasal cleft, infralabials 8–11, preoculars 0–1 (when no preocular prefrontals touching

anterior side of orbit), postoculars 1–2, mid dorsal scale rows 19–20, ventrals 127–164 in males and 120–152 in females, and subcaudals 28–39 in males and 23–36 in females (Supplementary Tables S2, S3). In life, dorsum glossy dark purple brown with narrow, transverse orange bands about one dorsal scale in length; first band on occiput lighter in color than other bands, about three dorsal scales in length; venter glossy dark purple brown, with yellow white bands about two ventrals in length, most interrupted by dark areas on midline

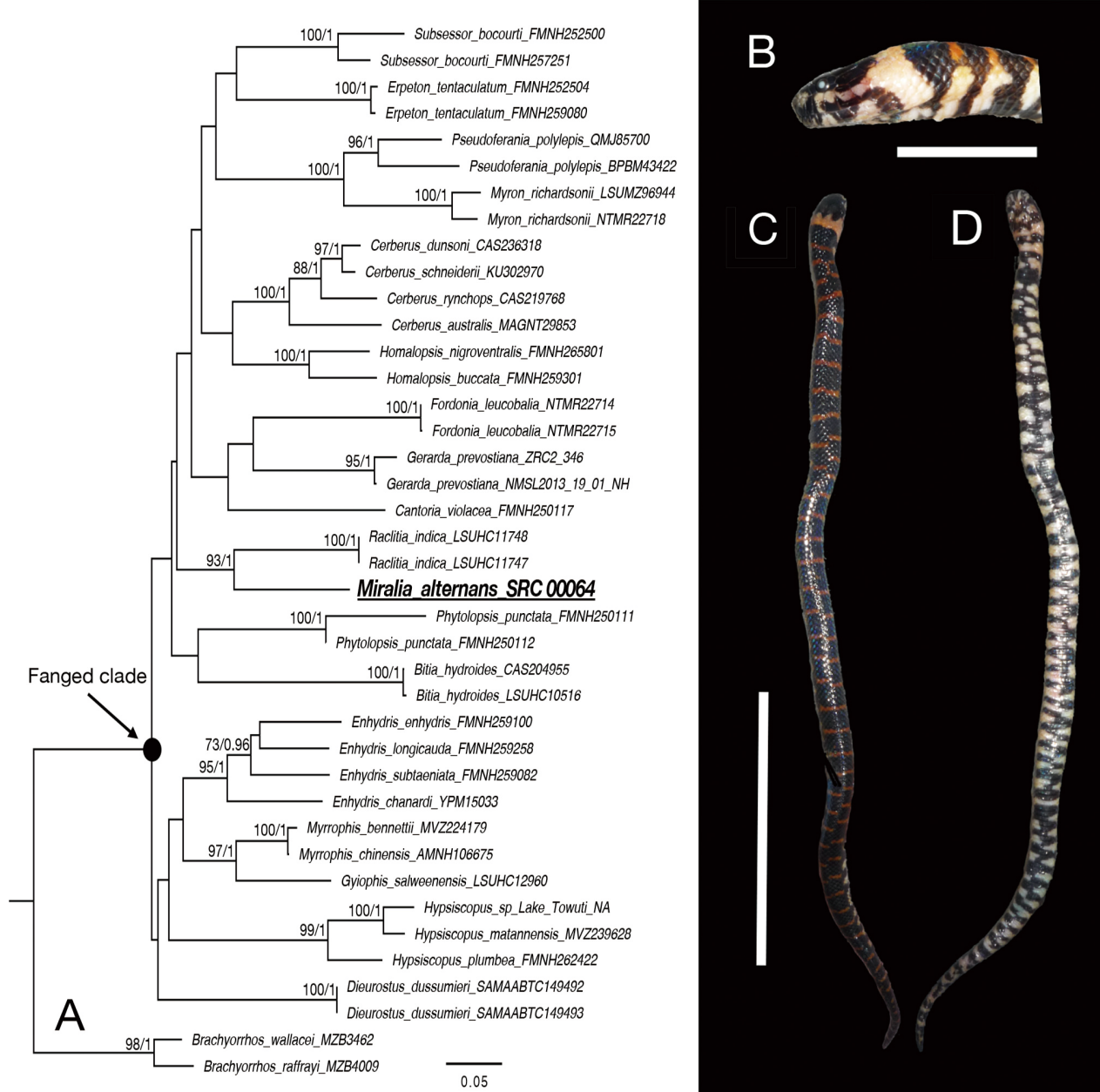


Figure 1 Phylogenetic relationships of Homalopsidae and *Miralia alternans* specimen

A: Maximum-likelihood tree based on mitochondrial gene *cyt b* and nuclear gene *PRLR* for samples of Homalopsidae. Numbers above branches represent bootstrap supports (not shown if below 70%) for ML (left) and posterior probabilities (not shown if below 0.95) for BI (right). B–D: Lateral profile of head (B), dorsal (C), and ventral (D) aspects of *Miralia alternans* specimen (SRC 00064). Scale bar: 10 mm (B) and 50 mm (C, D). Photos by K. Nishikawa.

(Figure 1B, C, D). Color pattern was generally similar in all preserved specimens, but dorsal transverse bands were less clear and first band on occiput was shorter in adult specimens than in juvenile specimens.

The phylogenetic tree topologies using ML and BI were identical, except that the basal lineage relationships were not strongly supported in both analyses. Therefore, we only present the ML tree (Figure 1A). Analysis indicated that *M. alternans* was nested within the fanged clade of Homalopsidae, although clade monophyly was not well supported. The sister relationship between *M. alternans* and *R. indica* was strongly supported, with an uncorrected *P*-distance of 13.0%–13.1% in *cyt b* between the two species (Supplementary Table S4).

One examined specimen (MZB Ophi. 4877) was fixed as it was in the process of regurgitating an Asian swamp eel (*Monopterus cf. albus*) (Supplementary Figure S1).

The present study revealed huge morphological variation within *M. alternans* (Supplementary Tables S2, S3), indicating that the species may include multiple cryptic species. We only used samples from Sarawak and Java for morphological examination and from Sarawak for molecular analysis. Thus, taxonomic assessment of the species using wider morphological and phylogenetic sampling is needed.

Phylogenetic analysis indicated and confirmed a sister relationship between *M. alternans* and *R. indica*, as inferred in previous studies based on morphology (Gyi, 1970; Murphy et al., 2011). Although *M. alternans* and *R. indica* are morphologically similar, their genetic distance based on the mitochondrial *cyt b* gene was relatively high (13.0%–13.1%). Maximum interspecific genetic distances in *cyt b* within a genus are about 9%–12% and minimum intergeneric genetic distances within a family are about 10%–14% (Supplementary Table S4), comparable to the genetic divergence between *M. alternans* and *R. indica*.

Gray (Gray, 1849) suggested the possibility that *M. alternans* may be a variety of *R. indica*. Although the two species are morphologically similar, the number of ventrals generally differs (120–164 in *M. alternans* vs. 152–175 in *R. indica*), and the two species are deeply genetically divergent. Thus, we confirmed distinct species status for both species based on molecular and morphological differences.

However, the generic status of the two species may be reconsidered, as we could not find morphological differences between *Miralia* and *Raclitia* comparable to those of different genera. Both these genera were described in Gray (1842), and thus *Miralia* could be treated as a junior synonym of *Raclitia* or vice versa. To determine their generic treatment, additional morphological data, such as maxillary teeth and hemipenial morphology, as well as genetic material, especially topotypic *M. alternans* from Java, are crucial.

Murphy et al. (2011) suggested that *M. alternans* and *R. indica* may be fossorial and vermivorous. We found *Monopterus cf. albus* (Supplementary Figure S1) as a food item of *M. alternans* for the first time, and one specimen (SRC 00064) was collected very close to a stream. Therefore, we can infer that *M. alternans* is at least partly aquatic and feeds

on fish. However, more information on its natural history is required to reveal the dietary and behavioral habits of this rare species.

SCIENTIFIC FIELD SURVEY PERMISSION INFORMATION

Field surveys and specimen collection were approved by the State Government of Sarawak (Research Permit No. NPW.907.4.2(III)-68).

SUPPLEMENTARY DATA

Supplementary data to this article can be found online.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

I.F., T.K., and K.E. conceived and designed the study. I.F. performed the experiments, measured the specimens, and wrote the manuscript. M.M., K.N., and K.E. revised the manuscript. M.Y.H. arranged the field survey. M.Y.H., M.M., K.N., and K.E. collected the specimens in the field. All authors read and approved the final version of the manuscript.

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