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 *Raclitia 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 *Raclitia 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-
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
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.

**ACKNOWLEDGEMENTS**

The State Government of Sarawak kindly permitted us to conduct the project and the RDID provided facilities for conducting research and molecular experiments, including sequencing. We are grateful to R. ak S. Pungga, P. ak Meleng, and T. Itokka for their support in obtaining permission to conduct research. We thank A. Hamidy (MZB) and K.O. Chan (ZRC) for kindly providing access to specimens deposited in the collections of their respective institutions. We thank H. Ota for valuable comments and suggestions for identification.

Ibuki Fukuyama1,2, Tomonori Kodama2, Koshiro Eto3, Masafumi Matsui1, Mohamad Yazid Hossman4, Kanto Nishikawai,6

1 Graduate School of Human and Environmental Studies, Kyoto University, Yoshida-Nihomatsu, Sakyo, Kyoto 606-8501, Japan
2 Graduate School of Science, Kyoto University, Kitashirakawa-Oiwake, Sakyo, Kyoto 606-8502, Japan
3 Kitakyushu Museum of Natural History &Human History, Higashidaga 2-4-1, Yahatahigashi, Kitakyushu, Fukuoka 805-0071, Japan
4 Research, Development, and Innovation Division, Forest Department Sarawak, Kuching, Sarawak 93250, Malaysia
5 Graduate School of Global Environmental Studies, Kyoto University, Yoshida-Honnachi, Sakyo, Kyoto 606-8501, Japan
6 Corresponding author, E-mail: kawashibi@gmail.com

**REFERENCES**

Bernstein JM, Murphy JC, Voris HK, Brown RM, Ruane S. 2021. Phylogenetics of mud snakes (*Squamata: Serpentes: Homalopsidae*): a
paradox of both undescribed diversity and taxonomic inflation. *Molecular Phylogenetics and Evolution*, 160: 107–109.

Gray JE. 1842. Monographic synopsis of the water snakes, or the family Hydridae. *Zoological Miscellany*, 2: 59–68.

Gray JE. 1849. Catalogue of the Specimens of Snakes in the Collection of the British Museum. London: Edward Newman.

Gyi KK. 1970. A Revision of Colubrid Snakes of the Subfamily Homalopsinae. Lawrence: University of Kansas, 47–223.

Murphy JC. 2007. Homalopsid Snakes, Evolution in the Mud. Malabar: Krieger Publishing Company.

Murphy JC, Mumpuni, Sanders KL. 2011. First molecular evidence for the phylogenetic placement of the enigmatic snake genus *Brachyorrhos* (Serpentes: Caenophidia). *Molecular Phylogenetics and Evolution*, 61(3): 953–957.

Murphy JC, Voris HK. 2014. A checklist and key to the homalopsid snakes (Reptilia, Squamata, Serpentes), with the description of new genera. *Fieldiana Life and Earth Sciences*, (8): 1–43.

Quah ESH, Wood PL Jr, Lee Grismer L, Sah SAM. 2018. On the taxonomy and phylogeny of the rare Selangor Mud Snake (*Racilitha indica*) Gray (Serpentes, Homalopsidae) from Peninsular Malaysia. *Zootaxa*, 4514(1): 53–64.

Uetz P, Freed P, Aguilar R, Hošek J. 2022 [2022-04-11]. The Reptile Database. http://www.reptile-database.org.