Volume 30 (October 2020), 234-237

Herpetological Journal

SHORT NOTE

https://doi.org/10.33256/hj30.4.234237

Published by the British Herpetological Society

Contributions to *Lycodon zawi*, a little-known colubrid snake (Reptilia: Serpentes: Colubridae)

Lal Biakzuala[1], Vanlal Hrima[3], Michael Vanlalchhuana[2], Andrew Vanlallawma[2], Mathipi Vabeiryureilai[1], Lal Muansanga[1], Sarathbabu Subbarayan[2], Nachimuthu Senthil Kumar[2] & Hmar Tlawmte Lalremsanga[1]

[1]Developmental Biology and Herpetology Laboratory, Department of Zoology, Mizoram University, Tanhril 796004, India

[2]Department of Biotechnology, Mizoram University, Tanhril 796004, India

[3]Biodiversity and Nature Conservation Network, Aizawl 796001, Mizoram, India

Since the original description of *Lycodon zawi* almost two decades ago from Myanmar and Northeast India, little is known on the systematics, distributional range as well as the natural history of the species. Hence, this paper attempts to contribute updated information to enhance the genetic data, morphology, distributional records, and natural history on its feeding and the hitherto unknown breeding habit of this species from Mizoram State, Northeast India.

Keywords: Diet, eggs, morphology, phylogeny, Zaw's wolf snake

The colubrid snake *Lycodon zawi* Slowinski, Pawar, Win, Thin, Gyi, Oo & Tun, 2001 commonly known as Zaw's wolf snake was described from Myanmar and north-east India on the basis of 11 individuals (Slowinski et al., 2001). A combination of characters such as the presence of preocular scale, loreal scale not in contact with internasal, anal scale divided, poorly developed white cross-bands on a brownish-black dorsum, and without a well-developed band on the neck (nape) differentiate *L. zawi* (Fig. 1A) from its congeners (Slowinski et al., 2001). It is a nocturnal, oviparous species known to feed mainly on skinks and geckos (Slowinski et al., 2001; Whitaker & Captain, 2004). The known distributional records in India include the States of Assam (Garbhanga Reserved Forest and Tinkopani Reserve Forest), Mizoram (Ngengpui Wildlife Sanctuary, Keifang and Pachhunga University College Campus), Meghalaya (Nongkhyllem Wildlife Sanctuary, Balpakram Tiger Reserve) (Slowinski et al., 2001; Dutta et al., 2013), Tripura (Vanghmun, Jampui hills) (Majumder, 2018), and West Bengal (Ghosh et al., 2017). Outside India, it has been recorded from northern Myanmar (Sagaing Division and Rakhine State) (Slowinski et al., 2001) and Bangladesh (Lawachara National Park) (Reza, 2010). It is currently listed as Least Concern in IUCN Red List of threatened species (Wogan & Vogel, 2012). Given the paucity of data on the morphology, genetic characters, natural history and distributional range after the original description of the species, herein we provide additional data to fill the gaps in the aforementioned status.

During this study (2007–2019), we collected 17 specimens comprised mainly of dead on road (DOR) and individuals killed by local people in Mizoram State. We document *L. zawi* from 24 localities covering eight Districts of Mizoram State. Those are represented by 11 localities in Aizawl District, four in Lunglei District, three in Mamit District, two in Serchhip District, and one locality each in Lawngtai, Kolasib, Hnahthial and Champhai Districts (200–1216 m a.s.l). Details of collection data are presented in Supplementary Materials. Two live specimens we collected (MZMU 1049 and MZMU 1061) and these were anaesthetised using 250mg/kg of 0.7 % sodium bicarbonate buffered MS-222 (Tricaine Methanesulfonate) solution by intracoelomic injection, and then euthanised using a second intracoelomic injection of 0.1 ml unbuffered 50 % (v/v) MS-222 solution (see Conroy et al., 2009). Then, catalogued, fixed in 10 % buffered formalin and later transferred to 70 % ethanol in the Reptile Section, Departmental Museum of Zoology, Mizoram University (MZMU). The scalation terminology of Campbell & Lamar (2004) was used. The number of ventral scale (Ve) was counted according to Dowling (1951). Measurements were taken with a slide-caliper (Mitutoyo, 505–671) to the nearest 0.1 mm except snout-vent length (SVL) and tail length (TaL), which were measured with a ruler to the nearest 1 mm. The terminology for hemipenis by Dowling & Savage (1960) was followed. The relative tail length (RTaL) was calculated as tail length/total length (TaL/TL). Tissue samples collected from dead specimens were stored in –20 ˚C at 90 % ethanol in the facility of Developmental Biology and Herpetology Laboratory, Mizoram University, India.

We extracted DNA for a single individual of the specimen (MZMU 1379) using a DNeasy Blood and Tissue Kit (Qiagen™, Valencia, California, USA) following the standard protocol provided within the kit. PCR amplification on fragments of the mitochondrial cytochrome c oxidase I (*cox1*) gene was performed using

Correspondence: Hmar Tlawmte Lalremsanga (htlrsa@yahoo.co.in)

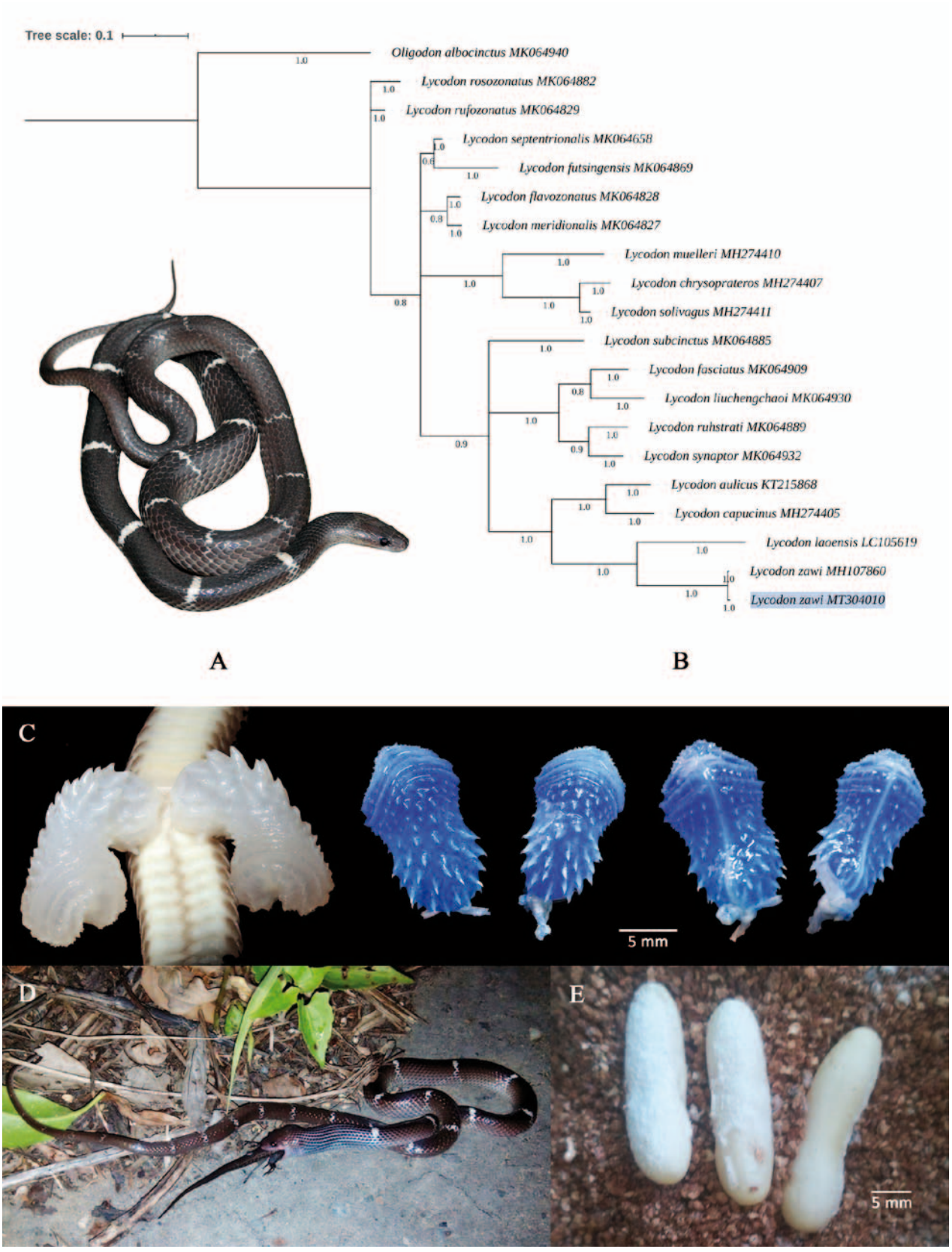

Figure 1. **(A)** Specimen of adult female *L. zawi* (MZMU 1061) in life, captured from Mission Veng, Mizoram, India. **(B)** Bayesian phylogeny based on partial *cox1* for Lycodon with other congeners. The sample generated in this study is highlighted in blue. **(C)** Everted hemipenis of *L. zawi* (MZMU 1049) (left), processed hemipenis (MZMU 1058) in asalcal view (middle two), and salcus view (right two). **(D)** *Lycodon zawi* swallowing *Eutropis macularia* in Mizoram, NE India. **(E)** Eggs of *L. zawi* just after dusting with antifungal powder, and later deposited to Departmental Museum of Zoology, Mizoram University (voucher number MZMU E1).

LCO1490 and HCO2198 primers (Folmer et al., 1994). The generated partial *cox1* sequence was deposited in the GenBank repository (accession number MT304010). In our phylogenetic analyses, we included 18 publicly available sequences of *Lycodon* species recovered from GenBank including one *Oligodon albocinctus* sequence (MK064940), which was used as an outgroup. We aligned our sequential data and GenBank database sequences together by using ClustalW (Thompson et al., 1994), and the Kimura 2 (K2P) genetic distances (Kimura, 1980) were calculated using MEGA X (Kumar et al., 2018). MrModeltest 2.3 (Nylander, 2004) was used to calculate the best fit model for Bayesian inference (BI) phylogeny. The BI phylogeny was constructed in MrBayes 3.2.5 by selecting nst=6 and rates=invgamma for the GTR+I+G model. The MCMC (one cold and three hot chains) was run for 20,000,000 generations sampling every 1000 generations, and burn-in was set to 25 %. The analysis was terminated when the standard deviation of split frequencies was less than 0.01 (Ronquist & Huelsenbeck, 2003).

We confirmed the identification of the species based on morphology (Slowinski et al., 2001; Purkayastha, 2013) and the *cox1* data (Fig. 1B). The morphometrics and pholidosis of 17 specimens we collected in this study are summarised: In males (n=8), SVL 260–514 mm, TaL 57–110 mm, RTaL 0.149–0.207 (mean=0.191), Ve 183–205, Subcaudals (Sc) 59–68. In females (n=9), SVL 169–517 mm, TaL 41–140 mm, RTaL 0.142–0.215 (mean=0.189), Ve 192–213, Sc 43–70. In sex pooled, dorsal scale rows 17:17:15, supralabials 8–9, suplalabials touching eye 3rd–5th (rarely 4th–5th), infralabials 8–10, temporals 2+3, loreal single, anal shield divided, 12–20 white transverse band on body and 3–6 poorly developed bands on the tail in five specimens. Hemipenes reaching up to 8–10 Sc, unforked, proximal half of organs with large spines followed by spinules. Ridges found at distal end of the asulcal surface extending around the sides toward the sulcus. A single sulcus spermaticus terminated in an expanded trough at the tip which agree well with the original hemipenis description by Slowinski et al. (2001) (Fig. 1C). Moreover, the specimens collected from Mission Veng and Sawleng, Mizoram, India represent the largest known female (MZMU 1061, total length 650 mm) and second largest male (MZMU 1,049, total length 599 mm) specimen for the species (see Slowinski et al., 2001; Whitaker & Captain, 2004; Dutta et al., 2013; Purkayastha, 2013), respectively.

The BLAST of the generated partial *cox1* gene matched *L. zawi* collected from Aizawl, Mizoram, India (MH107860), with a 99.76 % identity, 0 % gaps and an e-value of 0.0. From the studied mitochondrial *cox1* dataset (422 bp) of the genus *Lycodon*, a total of 143 variable sites were diagnosed with 15.5 % of mean K2P genetic distance with each other, ranging from 0.2 % to 20.8 %. The BI analysis of *L. zawi* of our specimen showed a well-supported monophyletic clade with the conspecific sequence obtained from the GenBank database (MH107860) with 0.2 % intraspecific genetic distance. Minimum and maximum interspecific genetic distances were diagnosed with *L. laoensis* (14.6 %) and *L. subcinctus* (20.8 %), respectively. Genetic distances of 16.5 %, 18.01 % and 18.07 % were also diagnosed with its congeneric species sympatric in the region *L. aulicus*, *L. septentrionalis* and *L. fasciatus*, respectively. The genetic relationship of *L. zawi* and *L. laoensis* in the present single locus tree (*cox1*) contradicts with the earlier work that used multiple genes (*c-mos*, *cytb* and *nd4*) to derive well-supported trees (Lei et al., 2014). However, we suggested the discordance between our single locus tree and the multilocus trees of *L. zawi* (Lei et al., 2014) may be due to any of the conditions such as model misspecification, due to the result of different evolutionary processes, inherent to the finite amount of data or error in sampling process (see Goodman et al., 1979; Pamilo & Nei, 1988; Takahata, 1989; Maddison, 1997; Page & Charleston, 1997; Mallo & Posada, 2016).

During a night survey on 11 August 2016, at ca. 22:30, a single *L. zawi* was observed preying on a skink, *Eutropis macularia* in the wild at Mizoram University Campus, India (23°44'15.69" N, 92°39'44.15" E, 780 m/asl., Fig. 1D) which was not mentioned specifically by earlier workers where Slowinski et al. (2001) reported that the food consists of small skinks: *Sphenomorphus maculatus* and geckos (*Hemidactylus frenatus* and *H. garnoti*). On 30 June 2019, at 15:00, a gravid female was captured from a bunch of grasses in a flower garden near the Guest House of Mizoram University, India. The female laid three eggs on 23 July 2019 (Fig. 1E), and the female was released back to the wild after taking the necessary morphological data. Eggs were soft, whitish, with a leathery texture, elongated ellipsoid, and measured 30.3 x 8.6 mm, 30.7 x 8.9 mm, and 30.9 x 8.6 mm. The eggs were incubated in a plastic container with vermiculite bedding and the eggs were occasionally dusted with clotrimazole, an antifungal powder (1 % w/w). None of the eggs hatched because of fungal infection or due to error in the optimal incubation conditions; or the eggs were not fertilised at all, hence data on the incubation period, temperature and humidity requirements, neonate size and other reproductive habits are not available at this time. All the specimens were encountered during monsoon season mostly at night (April to late September during 2013–2019). From our present work, we show that the species is not uncommon in the State and that is a species that has an apparent preference for riparian forests, where most specimens were found. Individuals were mostly active at night, and this nocturnal activity is congruent with the observations of Slowinski et al. (2001). *Lycodon zawi* were often encountered crossing tarmac roads as well as near and inside human habitations, possibly in search of prey.

Our work confirms the species identity and contributes further molecular data to a global database (GenBank Accession number: MT304010; specimen voucher MZMU 1379). It also bestowed new ranges of Ve 183–205 vs. 179 –197 in male (Slowinski et al., 2001; Reza, 2010) and 192–213 vs. 204–207 in female (Slowinski et al., 2001; Dutta et al., 2013), and new range of Sc in female i.e 43–70 vs. 49 (Dutta et al., 2013). Also, expands the known elevational range from 100–750 m/asl. to the zone of 200–1216 m/asl. (see Whitaker & Captain, 2004; Reza, 2010; Das, 2012; Dutta et al., 2013; Majumder, 2018). In addition, the species is also likely to occur in Bhutan (Jigme Tshelthrim Wangyal, pers. comm.; Vishal Santra pers. comm.), which

would extend its distributional range further towards the north-west of the known range.

ACKNOWLEDGEMENTS

We are very thankful to the Chief Wildlife Warden of Environment, Forests and Climate change Department, for issuing a herpetofaunal collection permit within the State (No.A.33011/2/99-CWLW/22). This research was financially supported by DST-SERB, New Delhi. We are grateful to Vishal Santra for proofreading the manuscript. We acknowledge DBT, New Delhi for the Advanced State Level Biotech Hub, Mizoram. Heartfelt thanks to B. Lalruatfela, Romalsawma, Binoy Kumar Barman, Ht Decemson, and Lalrinsanga for their assistance in this study.

REFERENCES

Campbell, J. A. & Lamar, W. W. (2004). *The Venomous Reptiles of the Western Hemisphere. Vol. 2*. Ithaca: Comstock Pub. Associates.

Conroy, C. J., Papenfuss, T., Parker, J. & Hahn, N. E. (2009). Use of tricaine methanesulfonate (MS222) for euthanasia of reptiles. *Journal of the American Association for Laboratory Animal Science* 48, 28–32.

Das, I. (2012). *A Naturalist's Guide to the Snakes of South-east Asia* (Malaysia, Singapore, Thailand, Myanmar, Borneo, Sumatra, Java and Bali). Oxford: John Beaufoy Publishing.

Dowling, H. G. (1951). A proposed standard system of counting ventrals in snakes. *British Journal of Herpetology* 1, 97–99.

Dowling, H. G. & Savage, J. M. (1960). A guide to the snake hemipenis: a survey of basic structure and systematic characteristics. *Zoologica* 45, 17.

Dutta, D., Sengupta, S., Das, A. K. & Das, A. (2013). New distribution of records of *Lycodon zawi* (Serpentes: Colubridae) from Northeast India. *Herpetology Notes* 6, 263–265.

Folmer, O., Black, M., Hoeh, W., Lutz, R., & Vrijenhoek, R. (1994). DNA primers for amplifcation of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* 3, 294–299.

Ghosh, A., Mukherjee, N. & Rahut, B. (2017). Geographic Distribution: *Lycodon zawi* (Zaw's Wolf Snake). *Herpetological Review* 48, 129.

Goodman, M., Czelusniak, J., Moore, G. W., Romero-Herrera, A. E. & Matsuda, G. (1979). Fitting the gene lineage into its species lineage, a parsimony strategy illustrated by cladograms constructed from globin sequences. *Systematic Biology* 28, 132–163.

Kimura, M. (1980). A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* 16, 111–120.

Kumar, S., Stecher, G., Li, M., Knyaz, C. & Tamura, K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution* 35, 1547–1549.

Lei, J., Sun, X., Jiang, K., Vogel, G., Booth, D. T. & Ding, L. (2014). Multilocus phylogeny of *Lycodon* and the taxonomic revision of *Oligodon multizonatum*. *Asian Herpetological Research* 5, 26–37.

Letunic, I. & Bork, P. (2019). Interactive Tree Of Life (iTOL) v4: recent updates and new developments. *Nucleic Acids Research* 47, W256–W259.

Maddison, W. P. (1997). Gene trees in species trees. *Systematic Biology* 46, 523–536.

Majumder, J. (2018). Record of *Lycodon zawi* (Squamata: Colubridae) in Tripura and its range extension in northeast India: an Indo-Burma biodiversity hotspots of the world. *Biodiversity International Journal* 2, 303–304.

Mallo, D. & Posada, D. (2016). Multilocus inference of species trees and DNA barcoding. *Philosophical Transactions of the Royal Society B: Biological Sciences* 371, 20150335.

Nylander, J. (2004). Mr.Modeltest v2, program distributed by the author. Uppsala: Evolutionary Biology Centre, Uppsala University, Uppsala.

Page, R. D. & Charleston, M. A. (1997). From gene to organismal phylogeny: reconciled trees and the gene tree/species tree problem. *Molecular Phylogenetics and Evolution* 7, 231–240.

Pamilo, P. & Nei, M. (1988). Relationships between gene trees and species trees. *Molecular Biology and Evolution* 5, 568–583.

Purkayastha, J. (2013). *An Amateur's Guide to Reptiles of Assam*. Assam: EBH Publishers (India).

Reza, A. H. M. A. (2010). Colubrid snake *Lycodon zawi* (Serpentes: Colubridae) from Lawachara national park in Bangladesh. *Russian Journal of Herpetology* 17, 75–77.

Ronquist, F. & Huelsenbeck, J. P. (2003). MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19, 1572–1574.

Slowinski, J. B., Pawar, S. S., Win, H., Thin, T., Gyi, S. W., Oo, S.L. & Tun, H. (2001). A new Lycodon (Serpentes: Colubridae) from northeast India and Myanmar (Burma). P*roceedings of the California Academy of Sciences* 52, 397–405.

Takahata, N. (1989). Gene genealogy in three related populations: consistency probability between gene and population trees. *Genetics* 122, 957–966.

Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* 22, 4673–4680.

Whitaker, R. & Captain A. (2004). *Snakes of India: The Field Guide*. Chennai: Draco Books.

Wogan, G. & Vogel, G. (2012). *Lycodon zawi*. The IUCN Red List of Threatened Species 2012:e.T176846A1449629. https://dx.doi.org/10.2305/IUCN.UK.20121.RLTS.T176846A1449629.en [accessed 23 April 2020].

Accepted: 5 August 2020

Please note that the Supplementary Materials are available via the Herpetological Journal website: https://thebhs.org/publications/the-herpetological-journal/volume-30-number4-october-2020