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DOI
Date submitted 26/1/2022
Date accepted 13/10/2022
Available online

Hamadryad Vol. 39 (1&2), pp. 96–103, 2022.

Contributions to the morphology and molecular phylogenetics of *Gonyosoma prasinum* (Blyth, 1854) (Reptilia: Squamata: Colubridae) from Mizoram, India

CITATION. Lalrinsanga[1], H.T. Lalremsanga[1], L. Ht. Decemson[1], V. Mathipi[1], L. Tanpuii[1], L. Muansanga[1], L. Biakzuala[1] (2022). Contributions to the morphology and molecular phylogenetics of *Gonyosoma prasinum* (Blyth, 1854) (Reptilia: Squamata: Colubridae) from Mizoram, India. *Hamadryad: 39, 96-103*.

KEYWORDS. Green Trinket Snake, morphometry, natural history, Northeast India, systematics

The Green Trinket Snake *Gonyosoma prasinum* (Colubridae) is arboreal (Smith 1943; Das 2012) and predominantly diurnal in habit (Whitaker & Captain 2008). Individuals of *G. prasinum* have a uniformly greenish dorsum (Blyth 1854; Smith 1943; Chan-Ard et al. 2015; Das 2012). Recent work indicates that the snake is distributed in India and Myanmar (David et al. 2022) and is listed as a species of 'Least Concern' (Wogan et al. 2021). Within India, they are found in the states of Arunachal Pradesh, Assam, Mizoram, Manipur, Nagaland, Meghalaya, and West Bengal. The snake is typically encountered in sub-montane or montane forests, near water in forest hills at elevations between 80–2650 m (Das 2012). Although distribution records are available, little else is known about the snake and no genetic data was hitherto available from India (see Blyth 1854; Smith 1943; Grossmann 2002; Whitaker & Captain 2008; Das 2012, 2018; Chan-Ard et al. 2015; Das & Das 2017). In this report, we provide morphological data of *G. prasinum* from Mizoram (Northeast India) and mitochondrial DNA sequences (*16s rRNA* and *Cyt b*). Additionally, we use the mitochondrial DNA to reconstruct a molecular phylogeny of the members of the genus *Gonyosoma*.

In this work, we documented the species from a total of 20 localities (Fig. 1), out of which the snake was known only from one locality (Mizoram University campus; Laltanpuia et al. 2008). A total of seven specimens (three males and four females) were collected from six different localities (Table 1). The morphol-

ogy of these snakes largely conforms to the descriptions available in published literature (e.g., Smith 1943; Grossmann 2002). However, many of our specimens differed from the morphology detailed in published literature. We found differences in the number of subcaudal scales in two of our specimens (MZMU2551 and MZMU2620) wherein, they had 113 and 116

Table 1. Morphological data of *Gonyosoma prasinum* specimens from Mizoram, India.

Museum vouchers	MZMU0024 Juvenile	MZMU920 Juvenile	MZMU2619	MZMU2630	Mean±SD N=4	MZMU2009	MZMU2551	MZMU2620	Mean±SD N=3
Date of collection	26/9/2008	5/9/2016	8/11/2021	16/11/2020		10/9/2020	22/7/2021	30/9/2021	
Locality	Mizoram University campus, Aizawl	Mizoram University campus, Aizawl	Tanhril, Aizawl	Samtlang, Aizawl		Maubuang, Aizawl	Murlen, Champhai	Durtlang North, Aizawl	
Sex	F	F	F	F	F	M	M	M	M
Snout-vent length	263	394	1050	635	585.50±345.88	609	525	588	574.00±43.72
Tail length	92	95	305	196	172.00±100.99	217	186	205	202.67±15.63
Eye-nostril	3.66	3.00	7.65	5.07	4.85±2.06	5.98	5.36	4.86	5.4±0.56
Eye diametre	4.30	2.80	5.88	3.80	4.20±1.28	4.63	4.39	3.64	4.22±0.52
Snout length	5.50	4.90	11.34	7.41	7.29±2.91	8.38	7.61	7.90	7.96±0.39
Snout width	4.27	2.90	6.78	6.25	5.05±1.79	5.23	5.73	5.15	5.37±0.31
Head length	15.03	15.50	33.51	23.90	21.99±8.70	24.86	22.42	22.95	23.41±1.28
Head width	8.50	8.10	11.30	12.27	10.04±2.06	13.51	10.56	11.66	11.91±1.49
Ventrals	196	202	207	199	201.00±4.69	196	195	199	196.67±2.08
Subcaudals	112	111	111	96	107.50±7.68	109	113	116	112.67±3.51
Dorsal scale rows	19:19:13	19:19:15	19:19:15	17:19:17		19:19:17	19:19:17	17:19:15	
Supralabials	9/9	9/9	9/9	9/9		9/9	9/9	9/9	
Supralabialstouching eye	4-6th/4-6th	4-6th/4-6th	4-6th/4-6th	4-6th/4-6th		4-6th/4-6th	4-6th/4-6th	4-6th/4-6th	
Infralabials	9/10	10/10	10/10	10/10		9/9	9/9	9/9	
Loreals	0/0	1/1	1/1	1/1		1/1	1/1	1/1	
Temprals	2+2/2+2	3+3/3+2	2+3/2+3	2+2/2+2		2+2/2+2	2+2/2+2	2+2/2+2	
Preoculars	1/1	1/1	1/1	1/1		1/1	1/1	1/1	
Postoculars	2/2	2/2	2/2	2/2		2/2	2/2	2/2	
Anal shield divided	No	No	No	No		No	No	No	

Table 2. Detailed information of 16S and Cytb sequences used in this study.

Species	Voucher	16S	Cytb	Location	Reference
Gonyosoma prasinum	MZMU2630	ON533525	ON548552	Mizoram, India	This study
G. prasinum	MZMU2009	OL442122	-	Mizoram, India	This study
G. prasinum	SEABRI2019120043	-	MZ322864	Htamanthi, Sagaing, Myanmar	Liu et al. 2021
G. prasinum	SEABRI2019120075	-	MZ322863	Htamanthi, Sagaing, Myanmar	Liu et al. 2021
G.* cf. *prasinum	CHS298	MK194035	MK201383	China	Li et al. 2020
G. boulengeri	CHS243	MK194009	MK201361	Hainan, China	Li et al. 2020
G. boulengeri	CHS242	MK194008	MK201360	Mengzi, Yunnan, China	Li et al. 2020
G. frenatum	CHS139	MK193938	MK201290	Huangshan, Anhui, China	Li et al. 2020
G. frenatum	CHS138	MK193937	MK201289	Huangshan, Anhui, China	Li et al. 2020
G. oxycephalum	ROM37622	KX694646	KX694870	Not specified	Alencar et al. 2016
G. oxycephalum	No voucher	-	AF471084	Not specified	Lawson et al. 2005
G. margaritatus	No voucher	-	KM870886	Not specified	Chen et al. 2014
G. jansenii	No voucher	-	DQ902113	Sulawesi	Burbrink & Lawson 2007
G. coeruleum	KIZ2019028	-	MZ322867	Mengla, Yunnan, China	Liu et al. 2021
G. coeruleum	KIZ2019025	-	MZ322870	Mengla, Yunnan, China	Liu et al. 2021
G. coeruleum	KIZ20200729	-	MZ322866	Zhenyuan, Yunnan, China	Liu et al. 2021
G. coeruleum	KIZ20200904	-	MZ322865	Menglian, Yunnan, China	Liu et al. 2021
Coelognathus radiatus	CHS556	MK194066	MK201411	China	Li et al. 2020

scales, respectively. The scale counts are slightly higher than the previously known upper limit of the subcaudal scale (111 scales; Smith 1943; Grossmann 2002; Whitaker & Captain 2008). One specimen (MZMU0024) lacked a loreal scale which was present in all other specimens observed and the presence of the loreal scale is a key diagnostic feature *fide* Smith (1943). Some of our specimens also had a variable number of dorsal scale rows (Table 1) and we consider this character as a variable trait rather than an anomaly. We also found that some of the specimens were marginally longer compared to published literature (MZMU2619, Fig. 2, 135 cm vs. 120 cm.; Whitaker & Captain 2008; Chan-Ard et al. 2015; Das 2012). In addition to the information provided by Smith (1943) we observed that the hemipenis of the examined male specimens extended up to the 7th–8th caudal plate; the calyces are elongated longer in the apical part (partially everted) than that of the basal part with spinous points; apical notch with tiny spines; the proximal plicate area is smooth and without spine; sulcus spermaticus single, prominent, and runs up to the tip (Fig. 3).

Table 3. Partitioning schemes and nucleotide evolutionary models selected for the Bayesian Inference (BI) phylogenetic analysis.

Partitions	Sites	Models
P I	16S, Cytb 3rd codon pos	GTR+G
P II	Cytb 1st codon pos	HKY+I
P III	Cytb 2nd codon pos	TIM+G

In our study, individuals of *G. prasinum* were encountered in terrestrial habitats (e.g., three individuals from the vicinity of a metalled road among bushes or shrubs, and one from the ground in a homestead garden) but they were occasionally seen in arboreal habitats as well. In captivity, we observed three individuals that were housed separately. One individual, (MZMU2009) preferred feeding on *Hemidactylus frenatus* than frogs, such as *Fejervarya multistriata, Minnervarya asmati, Microhyla berdmorei,* and *Sylvirana lacrima* when offered together. The other two individuals were not

Table 4. Uncorrected p-distance of Gonyosoma species estimated based on Cytb fragment. Sequence generated in this study is indicated by asterisk (*).

	Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	*Gonyosoma prasinum* MZMU2630*																
2	*Gonyosoma prasinum* SEABRI2019120043	0.022															
3	*Gonyosoma prasinum* SEABRI2019120075	0.022	0.000														
4	*Gonyosoma prasinum* CHS298	0.118	0.118	0.118													
5	*Gonyosoma boulengeri* CHS243	0.137	0.125	0.125	0.133												
6	*Gonyosoma boulengeri* CHS242	0.142	0.133	0.133	0.150	0.027											
7	*Gonyosoma frenatum* CHS139	0.135	0.123	0.123	0.140	0.079	0.072										
8	*Gonyosoma frenatum* CHS138	0.135	0.130	0.130	0.152	0.077	0.077	0.032									
9	*Gonyosoma oxycephalum* ROM37622	0.147	0.147	0.147	0.150	0.149	0.156	0.154	0.164								
10	*Gonyosoma oxycephalum*	0.149	0.149	0.149	0.150	0.149	0.156	0.154	0.164	0.007							
11	*Gonyophis margaritatus*	0.125	0.130	0.130	0.135	0.123	0.128	0.126	0.120	0.164	0.164						
12	*Gonyosoma jansenii*	0.161	0.157	0.157	0.150	0.154	0.152	0.152	0.159	0.067	0.070	0.168					
13	*Gonyosoma coeruleum* KIZ2019028	0.118	0.121	0.121	0.022	0.128	0.142	0.138	0.147	0.149	0.149	0.128	0.154				
14	*Gonyosoma coeruleum* KIZ2019025	0.123	0.126	0.126	0.029	0.135	0.145	0.142	0.150	0.157	0.157	0.135	0.161	0.012			
15	*Gonyosoma coeruleum* KIZ20200729	0.123	0.123	0.123	0.024	0.125	0.142	0.138	0.154	0.147	0.147	0.128	0.156	0.010	0.012		
16	*Gonyosoma coeruleum* KIZ20200904	0.121	0.121	0.121	0.024	0.126	0.140	0.133	0.149	0.150	0.150	0.135	0.150	0.014	0.015	0.010	
17	*Coelognathus radiatus* CHS556	0.178	0.174	0.174	0.198	0.183	0.186	0.181	0.179	0.197	0.197	0.190	0.202	0.193	0.195	0.195	0.191

seen to feed in captivity. During the winter months (November to January), the one individual we observed, was found immersed inside the provided water bowl, particularly when the terrarium air temperature fell below 20 °C; the head was held outside while the remaining body was coiled inside the water with temperature ranging between 21 °C and 22 °C. But, during the summer months (March and May), the other two individuals we had observed so far in captivity did not show this kind of behaviour, instead, they were seen to climb vegetation but would mostly coil on the ground and hide underneath rocks.

Specimens were euthanized using MS-222 following Conroy et al. (2009); then, fixed in 10% buffered formalin solution and subsequently stored in 70% ethanol. Liver tissues were dissected for DNA extraction and stored in 95% ethanol at -20 °C. We extracted genomic DNA from two specimens (MZMU2009 and MZMU2630) using the QIAamp DNA Mini Kit. The fragments of 16S rRNA (*16S*) and Cytochrome *b* (*Cyt b*) loci were amplified in a PCR reaction using published *16S* primers pairs L02510 (Palumbi1996) and H03063 (Rassmann 1997), and the primers (Snk) from Dubey et al. (2009) was utilized for *Cyt b* gene. Amplicons were subjected to Sanger's sequencing at Barcode BioScience Pvt Ltd., Bangalore, India. The raw sequences were checked for the quality score by Sequence Analyzer v2 and high-quality sequences were included in the analysis. Both datasets (*16S*

Table 5. Uncorrected p-distance of Gonyosoma species estimated based on 16S fragment. Sequences generated in this study are indicated by asterisk (*).

Species		1	2	3	4	5	6	7	8
1	*Gonyosoma prasinum* MZMU2630*								
2	*Gonyosoma prasinum* MZMU2009*	0.000							
3	*Gonyosoma boulengeri* CHS242	0.051	0.051						
4	*Gonyosoma* cf. *prasinum* CHS298	0.043	0.043	0.055					
5	*Gonyosoma boulengeri* CHS243	0.053	0.053	0.013	0.055				
6	*Gonyosoma frenatum* CHS139	0.055	0.055	0.017	0.060	0.019			
7	*Gonyosoma frenatum* CHS138	0.053	0.053	0.019	0.058	0.021	0.002		
8	*Gonyosoma oxycephalum* ROM37622	0.064	0.064	0.072	0.081	0.070	0.066	0.068	
9	*Coelognathus radiatus* CHS556	0.077	0.077	0.060	0.087	0.060	0.062	0.062	0.090

and *Cyt b* loci) contain the newly generated sequences and other sequences of *Gonyosoma* species along with one *Coelognathus radiatus* sequence (outgroup) retrieved from NCBI database (Table 2). We aligned sequences using the default parameters of MUSCLE (Edgar 2004), implemented in MEGA X (Kumar et al. 2018). Subsequently, the two aligned datasets were concatenated in SequenceMatrix (Vaidya et al. 2011) and partitioning (P) was done by gene and codon positions (pos). To reconstruct the Bayesian Inference (BI) phylogeny, the best partitioning schemes and models (Table 3) were

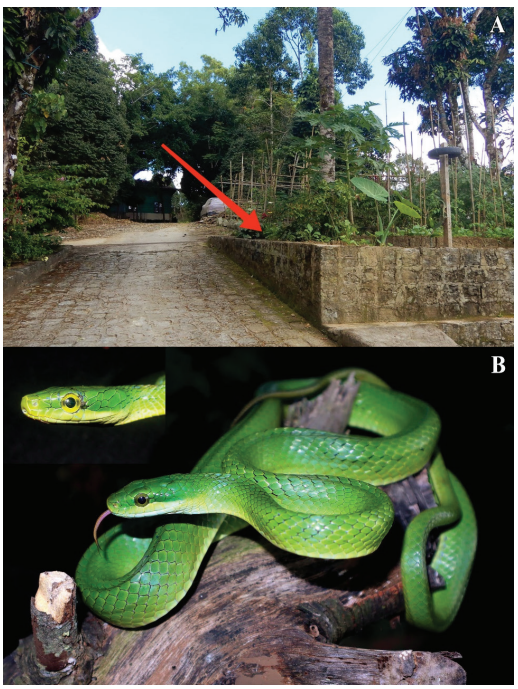

Figure 1. (A) Microhabitat of *Gonyosoma prasinum* (MZMU2619) at a homestead garden, Tanhril Vengpui, Mizoram, India. (B) Female of *G. prasinum* (MZMU2619) in life; inset showing the antero-lateral view of the head lacking a loreal scale (MZMU0024) in life.

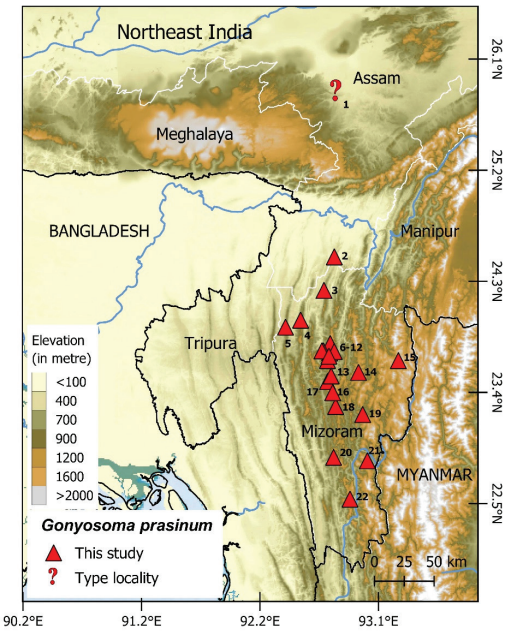

Figure 2. Map showing the imprecise type locality (question mark) and new distributional records (red triangles) of *Gonyosoma prasinum* from Mizoram, Northeast India: 1. Imprecise type locality (question mark was put at the approximate position of the unverified spotting in Assam by Liu et al. (2021)); 2. Kolasib; 3. North Sabual; 4. Tuidam; 5–11. Republic Vengthlang, Durtlang North, Tanhril, MZU campus, Berawtlang, Saikhamakawn, Samtlang; 12. Phulpui; 13. Tawizo; 14. Murlen National Park; 15. Maubuang; 16. Sialsuk; 17. Thenzawl; 18. Khawlailung; 19. Lunglei; 20. Muallianpui; 21. Lawngtlai.

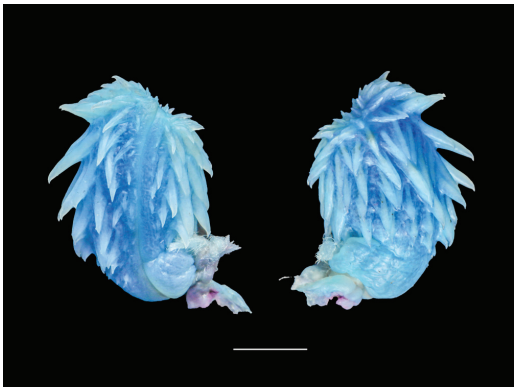

Figure 3. Hemipenis of MZMU2009, sulcal (left) and asulcal (right) views. Scale bar = 5 mm.

selected using PartitionFinder version 2 (Lanfear et al. 2017) based on the lowest Bayesian Information Criterion. Using the selected models, the BI phylogenetic analysis was subsequently performed by running four chains of Markov Chain Monte Carlo for 10 million generations by sampling every 10,000 generations in Mr.Bayes v3.2.5 (Ronquist et al. 2012). As a burn-in, the first 25% of the sampled trees were discarded, and Bayesian posterior probabilities (PP) were used to assess the nodal support. We visualized the BI tree in FigTree v1.4.4 (Rambaut 2019). In the *Cyt b* loci-based estimation of uncorrected p-distances, a genetic distance of 2.2% was detected between a specimen from Mizoram, India (MZMU2630) and those from Sangaing, Myanmar (SEABRI2019120043; SEABRI2019120075). A relatively high genetic distance (11.8%) was recovered between the *G.* cf. *prasinum* specimen from China (CHS298) and the other *G. prasinum* samples (India + Myanmar) (Table 4). Using the *16S* loci dataset, we found that the two specimens (MZMU2009; MZMU2630) from Mizoram, India were not genetically different from each other (genetic distance of 0.0%) but found a genetic distance of 4.3% between the Mizoram specimens and *G.* cf. *prasinum* from China (CHS298). The *16S* sequences of *G. prasinum* from Mizoram also depicted interspecific genetic divergence ranging between 5.1% (with *G. boulengeri*; CHS242) and 6.4% (with *G. oxycephalum*; ROM37622) (Table 5). Our BI phylogenetic relationship showed that populations from Mizoram are immediate sister to the population from Myanmar having strong nodal support values (PP=1.0), while the specimen of *G.* cf. *prasinum* from China (CHS298) is grouped with the type specimens of the recently described *G. coeruleum* (Fig. 4). In addition, we speculate that the specimen from China (CHS298) can be elevated

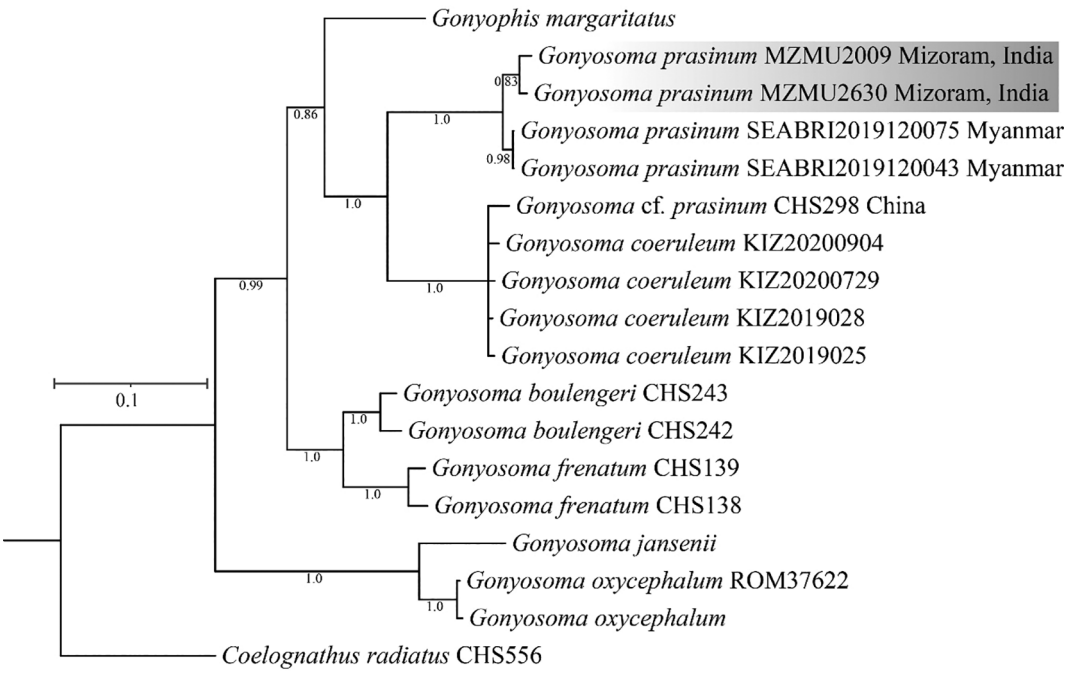

Figure 4. Bayesian inference phylogeny of Gonyosoma species inferred from concatenated sequences of 16S and Cytb. Bayesian posterior probability values are given at each node.

either as a new lineage or *G. coeruleum* by integrating morphological data.

The identity of *G. prasinum* was unresolved because of the lack of morphological and genetic data for comparison. Liu et al. (2021) pointed out that the original description of the species is insufficient for identifying the species. Moreover, the original description by Blyth (1854) does not specify the precise type locality and only states it as "Asám" (Blyth 1854) which comprised the whole of Northeast India during the colonial era. Our findings, where we provide detailed morphological measurements and genetic information of this species would bolster our understanding of this species across parts of Northeast India.

Acknowledgments

We are thankful to the Principal Chief Conservator of Forests (PCCF) and Chief Wildlife Warden, Aizawl, Government of Mizoram for the permit No.A.33011/2/99-CWLW/225 for herpetofaunal collection in Mizoram. We expressed sincere thanks to Amit Kumar Bal and Lalengzuala Tochhawng for field assistance. We acknowledge the following funding agencies: Defence Research Development Organisation (DRDO), Ministry of Defence, Government of India (No. DFTM/07/3606/NESTC/ABR/M/P-01); Department of Biotechnology (DBT), New Delhi (No. DBT-NER/AAB/64/2017); Department of Science and Technology-Science and Engineering Research Board (DST-SERB), New Delhi (DST No. EEQ/2021/000243). The author HTL is grateful to the International Herpetological Symposium, Inc. (IHS), USA; LB is also thankful to The Rufford Foundation, UK for the financial grant (Grant No. 36737-1).

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DOI
Date submitted 3/1/2022
Date accepted 21/6/2022
Available online