STUDY ON THE MORPHOLOGY, DISTRIBUTION AND PHYLOGENETIC STATUS OF THE GENUS *BUNGARUS* (REPTILIA: SERPENTES: ELAPIDAE) IN MIZORAM, INDIA

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STUDY ON THE MORPHOLOGY, DISTRIBUTION AND PHYLOGENETIC STATUS OF THE GENUS *BUNGARUS* (REPTILIA: SERPENTES: ELAPIDAE) IN MIZORAM, INDIA

BY

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Submitted in partial fulfillment of the requirements for the degree of

Master of Philosophy in Zoology of

Mizoram University, Aizawl.

CERTIFICATE

This is to certify that Study on the morphology, distribution and phylogenetic status of the genus *Bungarus* (Reptilia : Serpentes: Elapidae) in Mizoram, India written by Lalbiakzuala has been written under my supervision.

He has fulfilled all the required norms laid down within the M.Phil. regulations of Mizoram University. The dissertation is the result of his own investigation. Neither the dissertation as a whole nor part of it was ever submitted by any other University for any degree.

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DECLARATION

I, Lalbiakzuala, hereby declare that the subject matter of this dissertation is the record of work done by me, that the contents of this dissertation did not form basis of the award of any previous degree to me or to do the best of my knowledge to anybody else, and that the dissertation has not been submitted by me for any research degree in any other University/Institute.

This is being submitted to the Mizoram University for the degree of Master of Philosophy in Zoology.

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CHAPTER 1

INTRODUCTION

Snakes (Serpentes) are phenotypically diverse carnivorous reptiles (Secor and Diamond, 1998; Castoe et al., 2008, 2009). All over the world, the total number of snakes recorded so far is 3709 species (Uetz et al., 2019). India harbours a total of 304 species of snakes; subsequently, there have been a number of newly described species namely *Trimeresurus arunachalensis* (Captain et al., 2019), *Hebius lacrima* (Purkayastha and David, 2019), *Smithophis atemporalis* (Giri et al., 2019), as well as a new country records such as *Pareas margaritophorus* (Lalbiakzuala and Lalremsanga, 2019), etc. Thus, the number of snake species in India will probably be more than that reported in the recent most Checklist of Reptiles by Aengals et al. (2018). In northeast India, Ahmed et al. (2009) reported a total of 102 species of snakes, and in addition to the previous record of 52 snake species in Mizoram by Lalremsanga and Lalronunga (2017), there have been a recent new state report such as *Hebius venningi* (Lalbiakzuala and Lalremsanga, 2019), *Euprepriophis mandarinus* (Ashaharraza et al., 2019). It is very difficult to classify all snakes with conserved morphology even within the sublevels of Serpentes. The biodiversity of snake is decreasing globally due to hunting and trading for health foods, medicinal products, and pets. This is a serious issue, and in need of attention especially in the context of conservation biology, thus leading to an effort in producing a collection of an entire diversity of snakes alongside a modern, accurate taxonomy (Supikamolseni et al., 2015).

Snakes of the genus *Bungarus*, Daudin, 1803, commonly known as Kraits belong to the family Elapidae which are defined primarily by the presence of two permanently erect canaliculated front fangs, known as the proteroglyphous condition. They are medium to large-sized venomous elapid snakes which can reach up to 2 m

in length. They can be distinguished from other terrestrial elapids by a mid-dorsal row of enlarged, hexagonal scales (Smith, 1943). Other distinctive characters include vertebrae with laterally expanded prezygapophysial and postzygapophysial processes and usually high neural processes (Hoffstetter, 1939; Hoffstetter and Gasc, 1969). Their distinctive triangular appearance in cross-section is due to the high neural processes resulting in a raised mid-dorsal ridge. These characters indicate that *Bungarus* is monophyletic (Slowinski, 1994). A total of 16 species were recorded under the genus *Bungarus* (Abtin et al., 2014; Wallach et al., 2014), 15 species are restricted to the south and southeastern part of Asia (Slowinski, 1994; David and Ineich 1999; Kuch et al., 2005), and one in Iran (Abtin et al., 2014). According to IUCN Red List (2019), only two species of Kraits are listed as Vulnerable and the rest are either Least Concern or not listed. From northeast India, four species under the genus *Bungarus* are recorded (Smith, 1943) namely: *Bungarus fasciatus, Bungarus niger, Bungarus lividus* and *Bungarus bungaroides* (Fig 1– 4). From the state of Mizoram, *Bungarus niger* and *Bungarus fasciatus* were first reported by Laltanpuia et al. (2008), and Pawar and Birand (2001) respectively. Throughout their range, kraits are nocturnal and active predators. They are among the most dangerous and medically important venomous snakes (Warrell, 1999). Venoms of elapid snakes (King Cobras, Cobras, Mambas, Kraits, coral snakes, sea snakes and Australasian terrestrial venomous snakes) comprising of neurotoxins and myotoxins have proved to be valuable tools for studying neuromuscular transmission and muscle regeneration. Venoms of kraits, b-bungarotoxin (BTX) acts pre-synaptically, a-BTX and g-BTX antagonize binding of acetylcholine post-synaptically at peripheral neuromuscular junctions, and k-BTX blocks neuronal nicotinic receptors (Rowan,

2001; Nirthanan and Gwee, 2004; Doley and Kini, 2009). According to the WHO report (2019), in India alone it has been estimated that as many as 2.8 million people are bitten by snakes, and 46,900 people die from snakebite every year. A total of only six proven cases of *B. niger* envenomation have been published from Bangladesh (N=5; Fatal = 2) by Faiz et al. (2010), and Nepal (Fatal=1) by Pandey et al. (2016). It was confirmed that the bite of *B. niger* is also capable of fatal neuro-myotoxic envenoming, Faiz et al. (2010) suggested that its venom should be considered when antivenoms are designed. The bite of *B. fasciatus* is surprisingly rare, with a record of one man who died after 15 hours (Whitaker and Captain, 2008). However, the ophidian fauna of Mizoram is still poorly known, and in need of more research to have a clear cut information on the diversity, distributional localities as well as altitudinal ranges of the snake species found within this region. No matter how conventional taxonomic procedures for snakes which solely based on morphology are well established (Cox et al., 2012), they are time-consuming due to the fact that limited availability as well as differences between life stages, and sexes of the snake specimens is not uncommon. So, according to Supikamolseni et al. (2015), accurate identification of species needs modern techniques such as molecular approaches in addition to traditional taxonomic methods.

Fig 1: *Bungarus fasciatus* (Banded Krait) (MZMU 1421) from Keitum, Serchhip District, Mizoram (Photographed by Lalbiakzuala).

Fig 2: *Bungarus niger* (Greater Black Krait) from MZU Campus, Mizoram (Photographed by Lalbiakzuala).

Fig 3: *Bungarus lividus* (Lesser Black Krait) from Rajabhatkhawa, Alipurduar District, West Bengal (Photographed by Avrajjal Ghosh).

Fig 4: *Bungarus bungaroides* (Northeastern Hill Krait) from Sikkim (Photographed by Vishal Santra).

CHAPTER 2

REVIEW OF LITERATURE

Morphological characters

Snakes of the genus *Bungarus*, Daudin, 1803, have six conserved morphological characters in accordance to Slowinki (1994), such as the vertebral scales, postzygapophysial processes, choanal process of the palatine, subcaudals, demarcation between calyculate and spinose zones of hemipenis, and colour pattern. According to Smith (1943), the maxillary bones do not extend forward beyond the palatine, and the venom fangs are followed by two to four small teeth. The head is not distinct from the neck; head shields normal, loreal absent; eye moderate or small, with a round pupil. The scales are smooth, in 13–15 rows, strongly enlarged vertebral row, except in *Bungarus lividus*; tail is moderate; subcaudals single or some with paired. The hemipenis extends to the 6^{th}–9^{th} caudal plate; one-third or half of the hemipenis is calyculate, and the remainder spinose. The calyces near the tip of the organ are smallest and increase in size by following to the spinose area, and considerable variation within the species in the number and form of the spines is also noted. The total length of *B. fasciatius* was reported to be 2250 mm, clutch size of 4–14 eggs (22–38 mm long) with incubation period of 61 days, hatchlings size of 250–300 mm in length; and a total length of 1800 mm in *B. niger*, but currently with unstudied reproductive habits (Das and Das, 2012, 2017). However, there is scanty of literature regarding detailed comparative morphological study within and between the two species.

Distribution

The factors governing the elevational distributional pattern as well as the diversity pattern along the elevational gradient attained much attention (Rahbek, 1997; Rickart, 2001). Although mid elevation peak is known (Rahbek, 1997), decrease in the diversity with an increase in elevation is proposed as a general pattern (Brown, 1988; Stevens, 1992). According to the existing literatures (eg: Uetz et al., 2019; Das and Das, 2017, etc.), the distributional range of *B. fasciatus* includes Bangladesh, Myanmar, Cambodia, Southern China, India, Bhutan, Nepal, Indonesia, Laos, Malaysia, Singapore, Thailand, and Vietnam up to an elevation range of more than 2500 m asl., and known to feed on *Xenochrophis piscator* (Checkered Keelback), *Xenopeltis unicolor* (Sunbeam Snake), *Ptyas mucosa* (Dhaman), *Amphiesma stolatum* (Buff Striped Keelback), *Ptyas korros* (Indo-Chinese Rat Snake), *Boiga trigonata* (Common Cat Snake), *Daboia russelii* (Russel's Viper), *Enhydris enhydris* (Rainbow Water Snake), *Cylindrophis ruffus* (Red-tailed Pipe Snake), *Ovophis tonkinensis* (Tonkin Pit-Viper), carrion of *Cylindophis ruffus* (Red-tailed Pipe Snake), skinks, fish and eggs of snakes (Daniels, 2002). The distributional range of *B. niger* includes Eastern Himalayas of eastern and north-eastern India, Bangladesh, Bhutan, and Nepal. And are known to inhabit tropical evergreen and moist deciduous forests, plantations, grasslands and around human settlements, between elevations of 100 to 1500 m asl. Reported to feed on other snakes, but little is known about which species they preyed upon (Ahmed et al., 2009). In spite of the fact that Das (2018) recorded few localities for the species within Mizoram, a detail

survey or documentation is not available on the status of distribution pattern for the snake belonging to the genus *Bungarus* in the statewide.

Molecular characterization

Molecular identification techniques have showed to be an effective tool for identifying species (Purcell et al,2004; Teletchea et al., 2005; Jerome et al., 2003; Guha and Kashyap, 2005; Fajardo et al., 2006; Wong et al., 2004; Dubey et al., 2009; Dubey et al., 2011). The most commonly used gene as a marker for barcoding is the mitochondrial COI gene because it has been studied in many vertebrates and exhibits interspecific nucleotide divergence that is greater than its intraspecific nucleotide divergence (Chaves et al., 2008). Other mitochondrial genes, such as 16S rRNA and Cytb (Xia et al., 2012; Nicolas et al., 2012), have also been employed as barcodes with varying levels of success. DNA barcoding of snakes based on COI, 12S rRNA, and Cytb genes are well established in India, China, and the USA (Wong et al., 2004; Pook and McEwing, 2005; Dubey et al., 2011; Gaur et al., 2012). The generated sequences are compared with the available reference sequences in the DNA databases to obtain species identification (Dubey et al., 2011). However, no report or publication was available on the molecular characterization of snakes belonging to the genus *Bungarus* in Mizoram.

Review of literature reveals that there is scanty of literature regarding the comparative morphological study within and between the two species. In spite of the fact that few localities for the species has been recorded within Mizoram, a detail survey or documentation is not available on the status of distribution pattern for the snake belonging to the genus *Bungarus* in the statewide. No report or publication

was available on the molecular characterization of snakes belonging to the genus *Bungarus* from Mizoram. Moreover, there is limited number of DNA marker gene sequence from these species in databases, moreover the genetic diversity radiated by the two species is waiting to be unveiled.

CHAPTER 3

OBJECTIVES

Objectives

1. To survey and document diversity and distribution of species belonging to the genus *Bungarus* in Mizoram.
2. To study morphological and meristic variations among different species under the genus *Bungarus*.
3. To analyze the phylogenetic status of different species under the genus *Bungarus* using COI marker gene.

CHAPTER 4

MATERIALS AND METHODS

Collection of sample

To survey and document the diversity and distribution of species belonging to the genus *Bungarus* in Mizoram, field survey were conducted by utilizing Visual Encounter Survey (VES). This technique is easy to implement, inexpensive and efficient over diverse habitats (Manley et. al., 2004). Snake samples collected by hand, snake hook and tongs were euthanised by injection with chlorobutanol prior to fixation with formalin and were followed by storing in 70% ethanol. Muscle tissue samples taken from freshly roadkilled specimens collected in a field were preserved in 70% ethanol. Blood samples were drawn from the ventral tail vein using a 1 mL Insulin syringe and kept in K_3 EDTA Blood Vials. Geographical coordinates of localities were determined by Global Positioning System (GARMIN Montana 650). The collected snake samples were transfered in the Reptile Section, Departmental Museum of Zoology, Mizoram University (MZMU). The observations on the natural history were also catalogued and deposited as an image voucher in Lee Kong Chian Natural History Museum, National University of Singapore [ZRC (IMG)]. Information on their distribution data was also collected from private collections and photographs from different localities within the study area. Map is prepared using QGIS and the digital elevation model (DEM) is from SRTM (Shuttel Radar Topography Mission) of 30 metre spatial resolution downloaded from Open Topography (https://opentopography.org/).

Identification and morphological study

To study morphological and meristic variations among different species under this genus, different morphological characters which were useful to distinguish between species as well as those regarded as useful for further taxonomical studies were recorded. The following straight-line measurements were taken with a Mitutoya (series 505 – 671) dial caliper to the nearest 0.1 mm, except the snout-vent length (from the tip of snout to the posterior margin of anal plate) and tail lengths (from the posterior edge of anal plate to the tip of tail) which were measured to the nearest of 1 mm with a measuring tape: head length (distance between angle of jaw and snout-tip), maximal head width, maximal eye diameter. The scalation terminology of Campbell and Lamar, (2004) was used. The number of ventral scale was counted according to Dowling (1951). Dorsal scale rows were given as three values, where counted one head length behind the angle of the jaw, at mid body, and one head length before the cloaca. The sexes were determined by using a metal probe in a live specimen (Fig 5), whereas in a preserved specimen it was determined by dissecting to see the testis as well as the ventral side of the tail to find the retractor muscles which connect the distal end of hemipenis which is the characteristics of male (Fig 6). The tail-body ratio (TBR) was calculated by dividing the tail length (TaL) by the snout-vent length (SVL). The hemipenis was processed on freshly dead specimens by cutting the retractor muscles prior everting by injecting a preservative (4 % formalin or Glycerol mixed with Giemsa stain). The terminology for hemipenis by Dowling and Savage (1960) was followed.

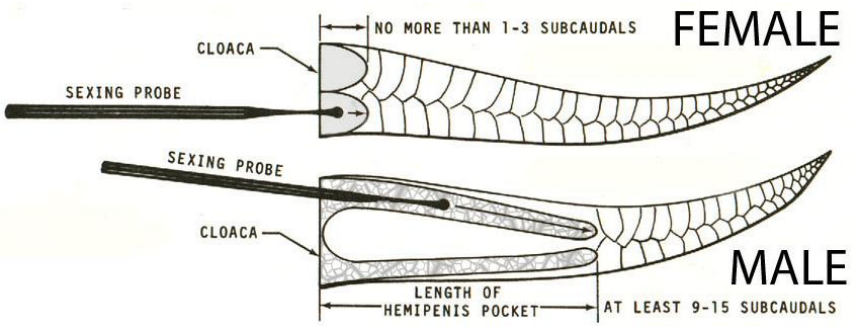

Fig 5: Sexing by using probe; figure from Laszlo (1975).

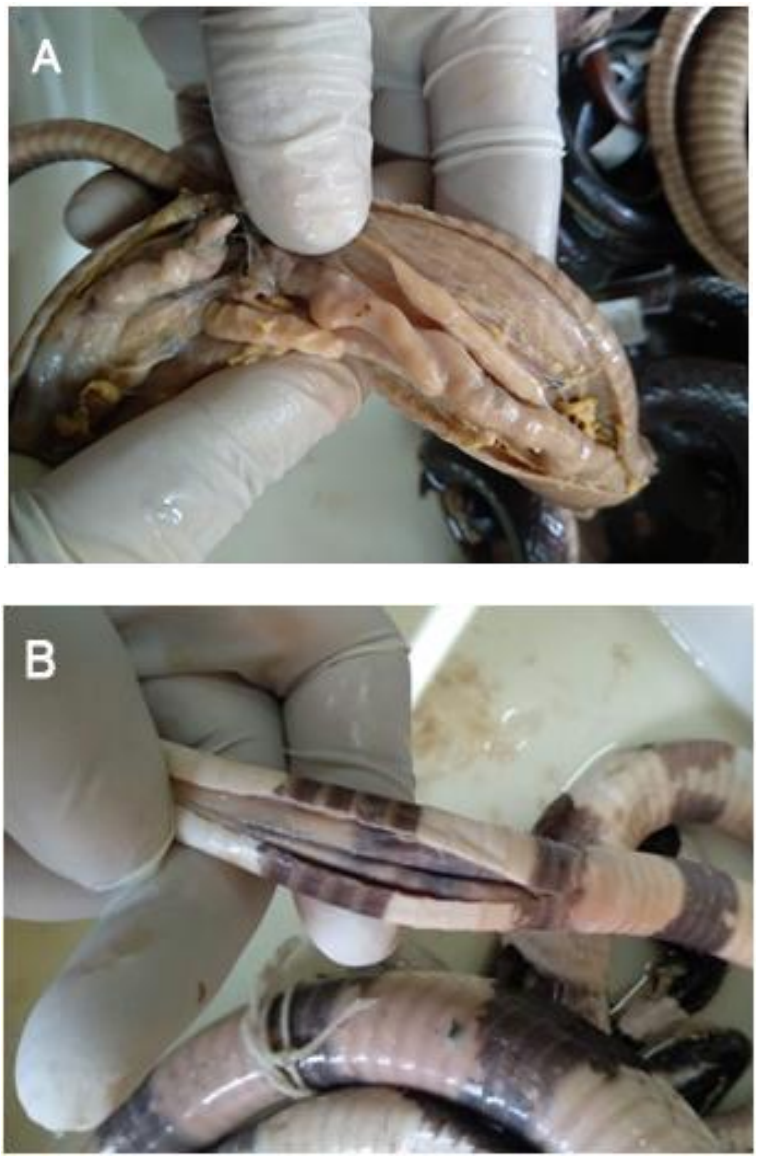

Fig 6: Dissecting snakes to see either its testis (A), or retractor muscles (B).

Molecular characterization

To analyze the phylogenetic status of different species under the genus *Bungarus* using COI marker gene, tissue and blood samples were used. DNA isolation was performed using QIAamp® DNA Mini Kit with the standard protocol provided.

PCR was performed using a Prolex™ 3x32-well PCR System (Applied Biosystematics). The standard PCR was carried in 25 µl volume containing 2.5 µl of 1 X *Taq* Buffer, 2.5 µl of 0.2 mM dNTP mix, 0.5 µl of 0.2 pmol each for forward and reverse primer, and 0.2 µl from 1 U of *Taq* Polymerase. The COI gene was amplified using universal primers LCO (F): (5'-TAA TAC GAC TCA CTA TAG GGG GTC AAC AAA TCA TAA AGA TAT TGG-3'), and HCO (R): (5'ATT AAC CCT CAC TAA AGT AAA CTT CAG GGT GAC CAA AAA ATC-3') suggested by Folmer et al. (1994), with the following PCR conditions: initial denaturation at 96˚C for 10 min, followed by 35 cycles of 96˚C for 1 min, 53˚C for 40 sec, 72˚C for 1 min, and with a final extension at 72 ˚C for 7 min.

The genomic and PCR products were checked on 0.8% and 1.2% Agarose gel respectively. The gel was visualized using a ChemiDoc™ XRS+ gel visualisation system (Fig 7). The PCR amplified products were sequenced with the Applied Biosystem Genetic Analyser 3500 in the DBT- Advanced level State Biotech Hub, Department of Biotechnology, Mizoram University. The resulting partial sequences of COI were aligned using BLAST algorithm and checked for ORF finding and translation. The sequences were searched against database for species identification and submitted to the NCBI GenBank. The generated sequences and the obtained

sequences from databases were aligned using ClustalW in MEGA X (Kumar et al., 2018). To derive molecular phylogenies, Maximum likelihood method with the substitution model of TN-93 (Tamura and Nei, 1993) was used, and the program MrBayes 3. 2. 7a x86_64 was also used for the Bayesian inference of phylogeny (Huelsenbeck and Ronquist, 2001). The Test of Phylogeny was performed by using Bootstrap method with a 2000 bootstrap replications. Genetic distances (Tamura et al., 2004) were calculated in MEGA X. The Transition/Transversion Bias was calculated by MEGA X using the substitution model of Tamura-Nei model with a statistic method of Maximum Composite Likelihood (Tamura et al., 2004). The codon usage bias from the desired sequences is computed using Relative Synonymous Codon Usage (RSCU) statistics by using MEGA X (Sharp et al., 1986; Nei and Kumar, 2000). The analysis on Tajima Relative Rate Test of Molecular Clock was conducted in MEGA X.

Abbreviations

HD: Head length

HW: Head width

ED: Eye diameter

END: Eye-notril distance

SL: Snout length

SW: Snout width

TL: Total length

SVL: Snout-vent length

TaL: Tail length

TBR: Tail-body ratio

Ve: Ventral

SC: Subcaudal scales

DSR: Dorsal scale row

IF: Infralabial

SL: Supralabial

SLE: Supralabial touching eye

IFSl: Infralabial touching sublabial

RTaL: Relative tail length

Hemp: Hemipenis

HpR: Hemipenis reach

Te: Temporal

PrO: Preocular

MZMU: Departmental Museum of Zoology, Mizoram University

IMG: Image voucher

asl.: above sea level

pers. obs.: Personal observation

VES: Visual Encounter Survey

COI: Cytochrome Oxidase subunit 1

DNA: Deoxyribonucleic acid

PoO: Postocular

BB: Body bands

BT: Bands on tail

NBW: Nuchal band width

PCR: Polymerase Chain Reaction

SDS: Sodium dodecyl sulphate

EDTA:Ethylenediaminetetraacetic acid

mM: millimolar

µl: microlitre

NaCl: Sodium chloride

PCI: Phenol chloroform isoamyl

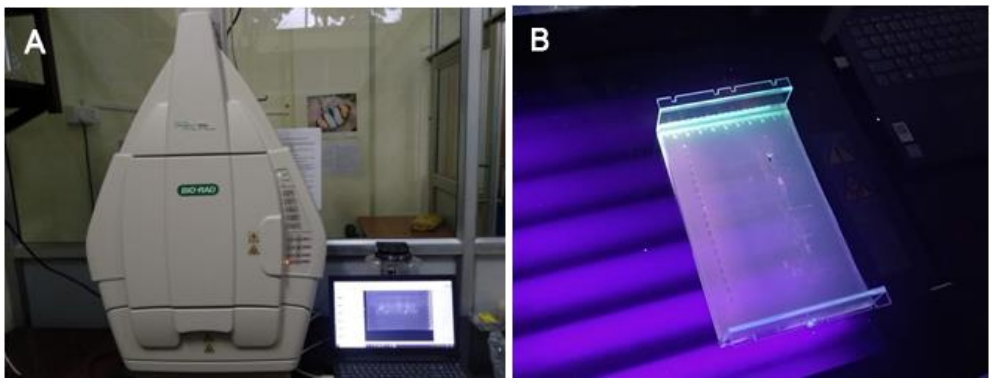

Fig 7: (A) Documentation of the PCR products electrophoresed on 1.2 % agarose gel; (B) Gel subjected for extraction to isolate a desired fragment.

CHAPTER 5

RESULTS

Diversity and Distribution of the genus *Bungarus* in Mizoram

In the present study, 2 species of *Bungarus* are confirmed in Mizoram. A total of 48 localities for *Bungarus niger* were documented in the statewide from as low as 42 m asl. in Buhchang up to 1646 m asl. in Champhai Vengsang. The distribution localities of the species are shown in Table 1 and Fig 8.

Table 1: Geo-coordinates of localities based on museum specimens, new collections and observations of *B. niger* in Mizoram.

Sl. no	Locality	District	Latitude	Longitude	Elevation	Museum / Photo Voucher number
1.	Bethlehem	Aizawl	23.727656 ˚N	92.721560 ˚E	966 m	IMG 5
2.	Buhchang	Kolasib	24.334716 ˚N	92.656246 ˚E	42 m	MZMU 975
3.	Champhai vengsang	Champhai	23.480273 ˚N	93.311896 ˚E	1646 m	MZMU 1315
4.	Chawngte	Lawngtlai	22.620997 ˚N	92.651198 ˚E	76 m	MZMU 973
5.	Darlawn	Aizawl	24.014760 ˚N	92.923608 ˚E	1050 m	MZMU 1388
6.	Durtlang Gosen	Aizawl	23.789400 ˚N	92.731236 ˚E	1107 m	MZMU 1416
7.	Durtlang ICFAI	Aizawl	23.799870 ˚N	92.728605 ˚E	1268 m	MZMU 1418
8.	Durtlang Mualveng	Aizawl	23.783473 ˚N	92.725798 ˚E	1211 m	MZMU 1482
9.	Falkawn	Aizawl	23.624767 ˚N	92.722927 ˚E	763 m	MZMU 986
10.	Hunthar	Aizawl	23.745383 ˚N	92.713316 ˚E	833 m	IMG 9
11.	Khawzawl	Champhai	23.529770 ˚N	93.173060 ˚E	1137 m	MZMU 993
12.	Mamit	Mamit	23.647816 ˚N	92.540045 ˚E	722 m	MZMU 1085
13.	Melriat	Aizawl	23.641867 ˚N	92.726138 ˚E	858 m	MZMU 1086
14.	Mission Veng	Aizawl	23.715408 ˚N	92.720023 ˚E	1066 m	MZMU 1337

15.	Mission Vengthlang	Aizawl	23.713471 ˚N	92.709380 ˚E	984 m	IMG 11
16.	Model Veng	Aizawl	23.712945 ˚N	92.716511 ˚E	957 m	IMG 10
17.	MZU	Aizawl	23.733526 ˚N	92.665941 ˚E	836 m	MZMU 978
18.	N. Vanlaiphai	Serchhip	23.133390 ˚N	93.055797 ˚E	1351 m	MZMU 1095
19.	Phainuam	Kolasib	24.470103 ˚N	92.781947 ˚E	64 m	IMG 2 MZMU 1585
20.	Phunchawng	Aizawl	23.767323 ˚N	92.679666 ˚E	561 m	MZMU 1338
21.	Reiek	Mamit	23.696715 ˚N	92.605320 ˚E	1160 m	MZMU 1527
22.	Rullam	Serchhip	23.445491 ˚N	92.999404 ˚E	1433 m	IMG 1
23.	Saihapui K	Kolasib	24.278606 ˚N	92.640933 ˚E	51 m	MZMU 1594
24.	Suangpuilawn	Aizawl	23.955617 ˚N	93.040710 ˚E	1043 m	IMG 4
25.	Tawipui South	Lunglei	22.673767 ˚N	92.845820 ˚E	975 m	IMG 13
26.	Thenzawl Vengthlang	Serchhip	23.289905 ˚N	92.772858 ˚E	757 m	IMG 8
27.	Thingdawl	Kolasib	24.170006 ˚N	92.698403 ˚E	652 m	MZMU 1339
28.	Thingsulthliah	Aizawl	23.692434 ˚N	92.859575 ˚E	892 m	MZMU 1340
29.	Tlangnuam	Aizawl	23.703608 ˚N	92.713966 ˚E	1008 m	IMG 15
30.	Tlungvel	Aizawl	23.606544 ˚N	92.852526 ˚E	1110 m	MZMU 1483
31.	Tuivamit	Aizawl	23.750836 ˚N	92.676276 ˚E	861 m	MZMU 1341
32.	Vaipuanpho	Mamit	23.709051 ˚N	92.641589 ˚E	451 m	IMG 14
33.	Vairengte	Kolasib	24.495901 ˚N	92.761032 ˚E	236 m	IMG 7; MZMU 1584
34.	World Bank road	Aizawl	23.723651 ˚N	92.737006 ˚E	752 m	IMG 12
35.	Zohnuai	Aizawl	23.739684 ˚N	92.708979 ˚E	949 m	MZMU 1342
36.	New Chalrang	Champhai	23.388079 ˚N	93.152522 ˚E	1276 m	MZMU 1549
37.	Lungleng	Aizawl	23.663132	92.664718	1009 m	MZMU

			˚N	˚E		1528
38.	Selesih	Aizawl	23.801772 ˚N	92.732673 ˚E	1134 m	MZMU 1525
39.	Serkawn	Lunglei	22.910081 ˚N	92.756471 ˚E	1142 m	MZMU 1343
40.	Kawnpui Hmarveng	Kawnpui	24.048000 ˚N	92.673353 ˚E	854 m	MZMU 1526
41.	Durtlang M Suaka Veng	Aizawl	23.781980 ˚N	92.724919 ˚E	1123 m	MZMU 1595
42.	Tlabung	Lawngtlai	22.914318 ˚N	92.474333 ˚E	183 m	MZMU 1570
43.	Tlabung Chawnpui	Lawngtlai	22.904479 ˚N	92.485194 ˚E	236 m	IMG 38
44.	Luangmual	Aizawl	23.738924 ˚N	92.710594 ˚E	943 m	IMG 36
45.	Kepran	Aizawl	23.945031 ˚N	92.934989 ˚E	1272 m	IMG 34
46.	Rawpuichhip	Mamit	23.788576 ˚N	92.561781 ˚E	804 m	IMG 35
47.	Chhiahtlang	Serchhip	23.385596 ˚N	92.844501 ˚E	971 m	IMG 33
48.	Lengte road	Mamit	23.801624 ˚N	92.617796 ˚E	196 m	IMG 39

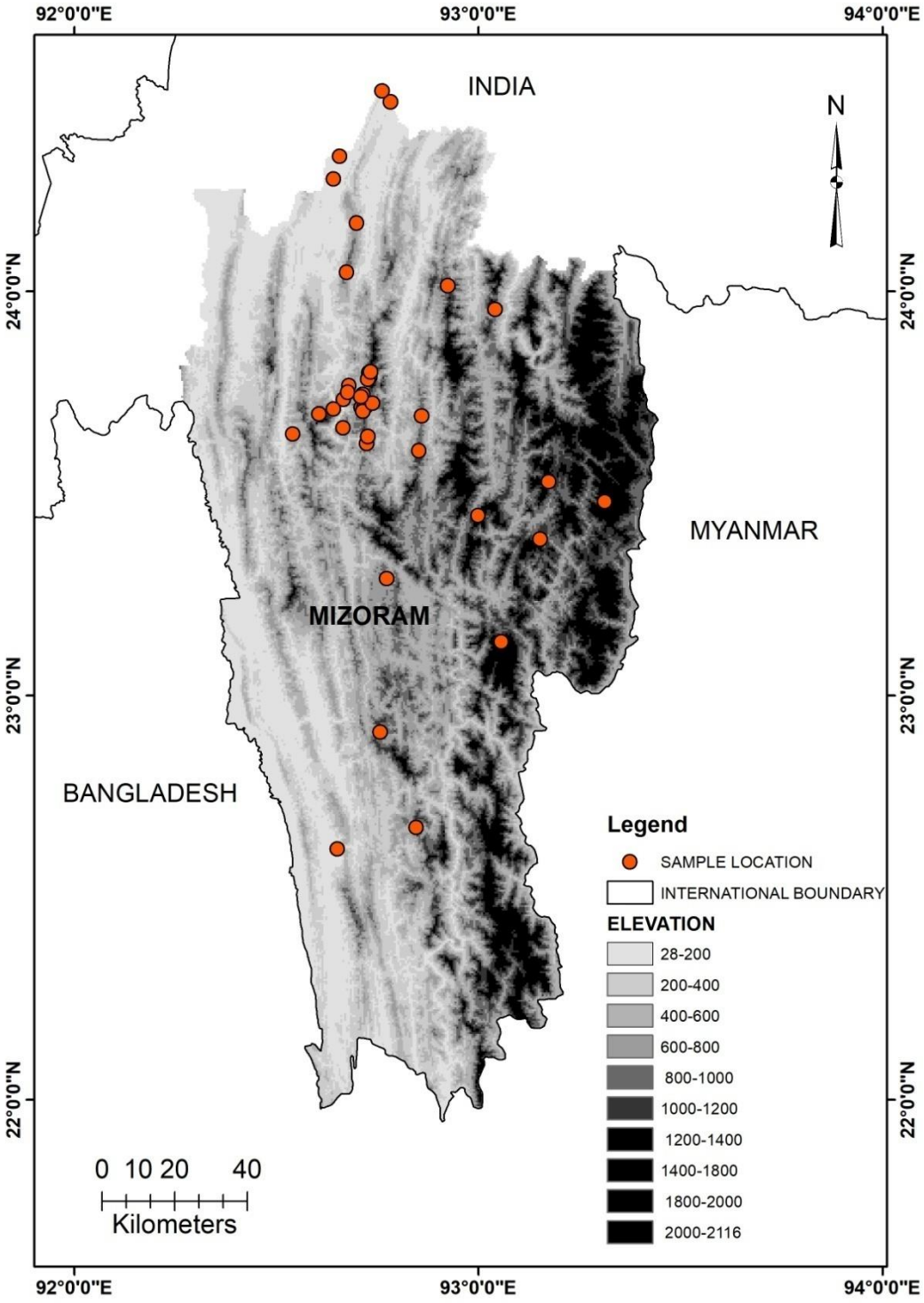

Fig 8: Digital elevation map of Mizoram showing location sites of *B. niger.*

For *Bungarus fasciatus*, 40 localities were documented in the statewide from as low as 49 m asl. in Buhchang up to 1426 m asl. in Champhai Jail veng. The distribution localities and geo-coordinates are shown in Table 2 and Fig 9. The mean of their elevational gradient distributions were subjected for statistical analysis using independent sample t-test (Fig 10 – 11).

Table 2: Geo-coordinates of localities based on museum specimens, new collections and observations of *B. fasciatus* in Mizoram.

Sl. no	Locality	District	Latitude	Longitude	Elevation	Museum / Photo Voucher number
1.	Ailawng	Mamit	23.690138˚ N	92.617108 ˚E	1172 m	Lalrinsanga, Pers. obs.
2.	Buhchang	Kolasib	24.318141˚ N	92.638588 ˚E	49 m	MZMU 1562
3.	Champhai Jail veng	Champhai	23.468161˚ N	93.317772 ˚E	1426 m	MZMU 1561
4.	Chawngte	Lawngtlai	22.620006˚ N	92.648507 ˚E	81 m	H. Laltlanchhuaha, Pers. obs.
5.	CTI Sesawng	Aizawl	23.745833˚ N	92.854751 ˚E	814 m	IMG 31
6.	Dapchhuah to W. Phaileng	Mamit	23.759784˚ N	92.492873 ˚E	447 m	IMG 21; MZMU 1417
7.	Durlui	Aizawl	23.899000˚ N	92.652129 ˚E	95 m	MZMU 934
8.	Durlui	Aizawl	23.899191˚ N	92.649556 ˚E	88 m	MZMU 933
9.	Khamrang	Kolasib	23.934774˚ N	92.655455 ˚E	232 m	MZMU 1550
10.	Khawrihnim	Mamit	23.621382˚ N	92.626148 ˚E	1057 m	IMG 22
11.	Khawruhlian	Aizawl	23.870982˚ N	92.876104 ˚E	924 m	Zohmingsangzuala, Pers. obs.
12.	Kolasib	Kolasib	24.245402˚	92.676890	613 m	H.T.

	Saidan		N	˚E		Lalremsanga, Pers. obs.
13.	Lengte road	Mamit	23.794119˚ N	92.605881 ˚E	296 m	Lalrinsanga, Pers. obs.
14.	Lawngtlai	Lawngtlai	22.528505˚ N	92.900511 ˚E	721 m	H. Laltlanchhuaha, Pers. obs.
15.	Mampui	Lawngtlai	22.526439˚ N	92.844184 ˚E	1056 m	H. Laltlanchhuaha, Pers. obs.
16.	Mission veng	Aizawl	23.716264˚ N	92.718944 ˚E	1084 m	Jeremy M, Pers. obs.
17.	Mualmam	Aizawl	23.805626˚ N	92.837221 ˚E	554 m	IMG 23
18.	Muthi park	Aizawl	23.783832˚ N	92.763273 ˚E	1054 m	IMG 24
19.	MZU	Aizawl	23.736143˚ N	92.667240 ˚E	848 m	MZMU 1572
20.	NeihbawihSihphir	Aizawl	23.834704˚ N	92.744017 ˚E	1323 m	H.T. Lalremsanga, Pers. obs.
21.	New Latoh	Saiha	23.368595˚ N	92.933221 ˚E	428 m	MZMU 1219
22.	Ngengpui WLS	Lawngtlai	22.441958˚ N	92.788998 ˚E	208 m	MZMU 1314
23.	Paikhai road	Aizawl	23.637089˚ N	92.735141 ˚E	745 m	IMG 26
24.	Palsang	Aizawl	24.283545˚ N	92.913105 ˚E	758 m	Lalrinsanga, Pers. obs.
25.	Phuldungsei	Mamit	23.474677˚ N	92.420315 ˚E	898 m	IMG 25
26.	Reiek	Mamit	23.690327˚ N	92.606771 ˚E	1280 m	Lalrinsanga, Pers. obs.
27.	Sakawrtuichhun	Aizawl	23.762003˚ N	92.653885 ˚E	477 m	IMG 27
28.	Sekhum	Lunglei	23.140123˚ N	92.752131 ˚E	851 m	H. Laltlanchhuaha, Pers. obs.
29.	Serchhip	Serchhip	23.336536˚ N	92.859369 ˚E	982 m	IMG 17
30.	Thenzawl	Serchhip	23.283152˚ N	92.782637 ˚E	773 m	IMG 20
31.	Thinghlun	Mamit	24.249253˚	92.379732	78 m	MZMU

			N	˚E		1548
32.	Tlungvel	Aizawl	23.608755˚ N	92.853249 ˚E	1058 m	H.T. Lalremsanga,Pers. obs.
33.	Tuivai	Aizawl	24.042688˚ N	93.253722 ˚E	449 m	IMG 19
34.	Near Tuirial	Aizawl	23.729786˚ N	92.777452 ˚E	666 m	IMG 30
35.	Tuirial	Aizawl	23.717744˚ N	92.799631 ˚E	171 m	IMG 29
36.	Tuivamit	Aizawl	23.753556˚ N	92.676859 ˚E	800 m	H. T. Lalremsanga, Pers. obs.
37.	Vaipuanpho base Camp	Mamit	23.709914˚ N	92.642254 ˚E	441 m	IMG 18
38.	Zawlnuam	Mamit	24.129764˚ N	92.337398 ˚E	76 m	H. Laltlanchhuaha, Pers. obs.
39.	Keitum	Serchhip	23.231403° N	92.912985 °E	630 m	MZMU 1421
40.	Borapansury	Lawngtlai	22.711888˚ N	92.524362 °E	55 m	IMG 32

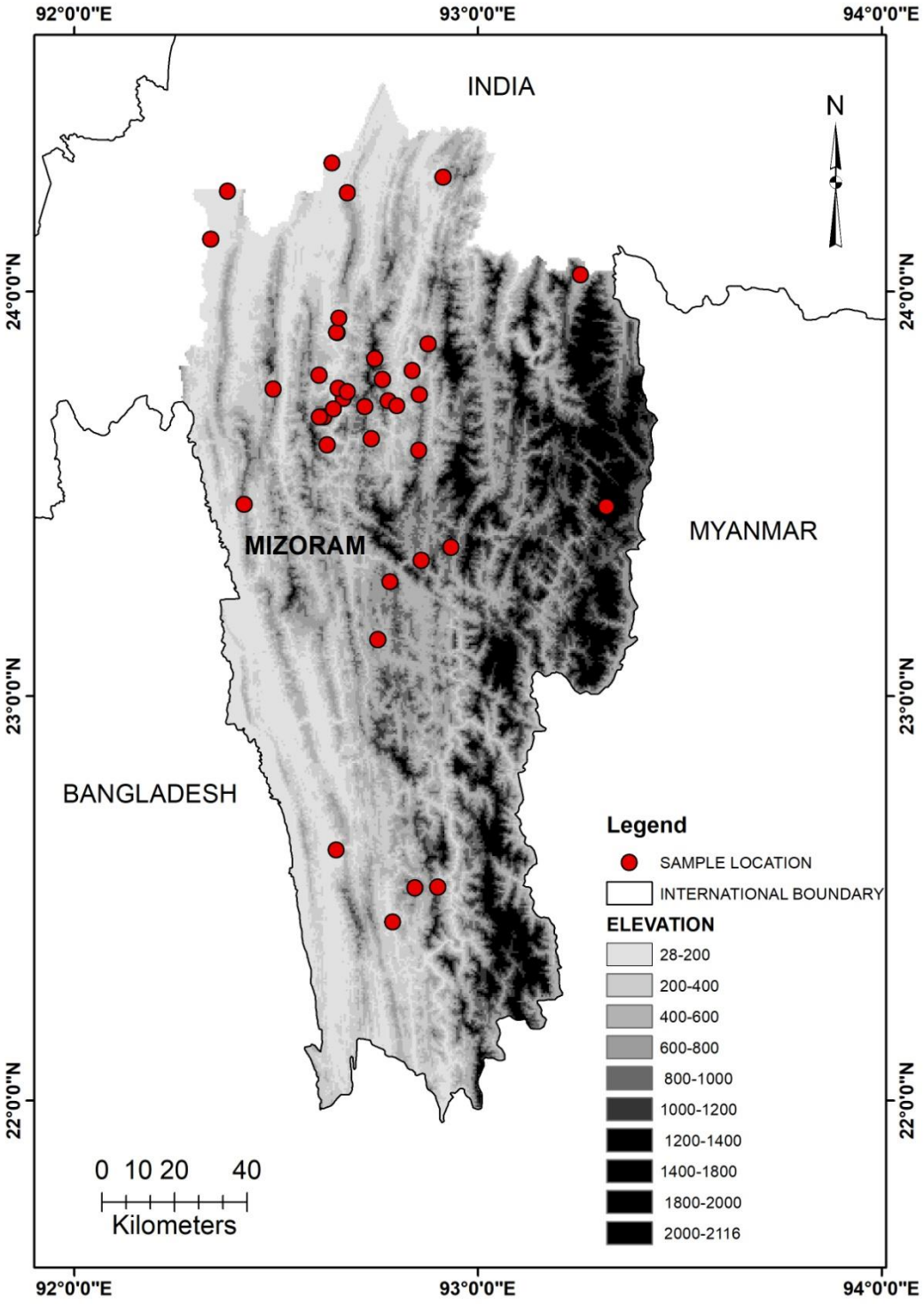

Fig 9: Digital elevation map of Mizoram showing location sites of *B. fasciatus*.

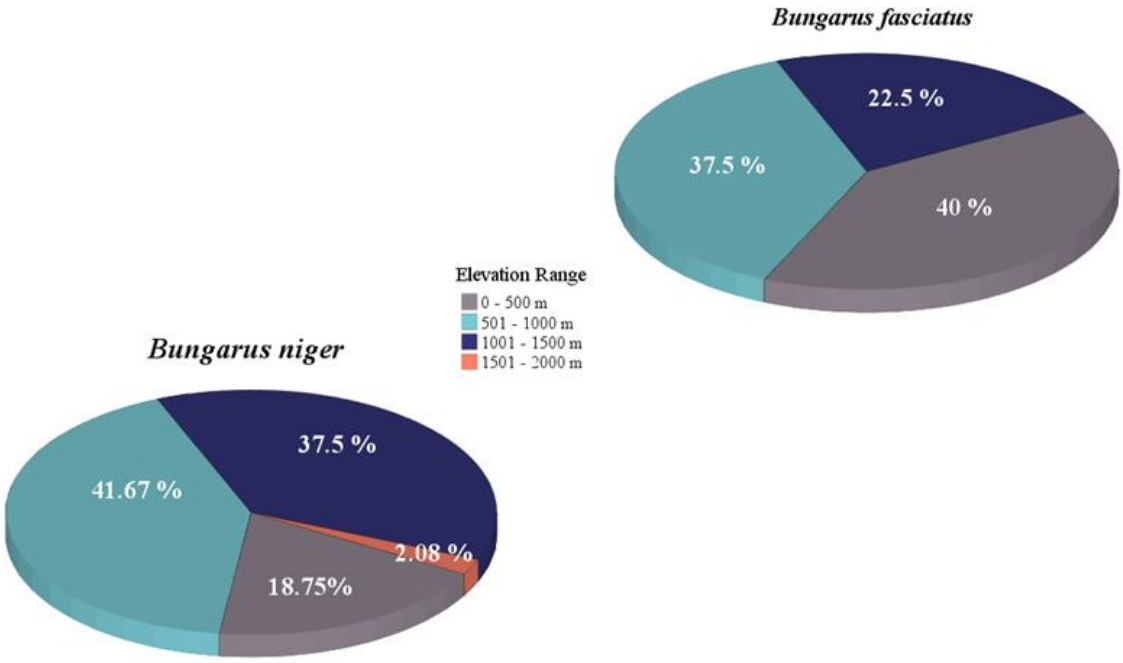

Fig 10: Pie diagrams showing the percentage of records on different elevational ranges of *B. niger* and *B. fasciatus.*

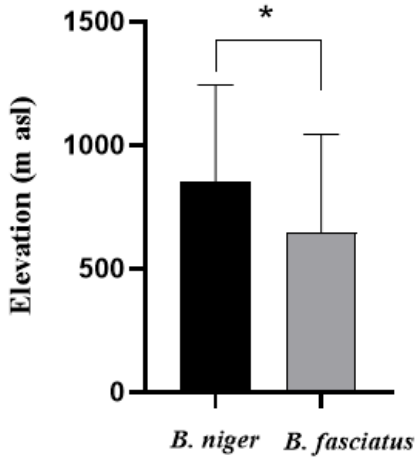

Fig 11: Difference between the mean elevational range of *B. niger* and *B. fasciatus.* * indicates significant level at 0.05. Where t = 2.479, df = 86, p = 0.015.

Morphological study

***Bungarus niger* Wall, 1908**

Bungarus niger Wall, 1908, *Journal of the Bombay Natural History Society.* 18 (4): 711-735 (Type locality: Tindharia, E. Himalaya).

Description of the species based on 32 specimens (Male = 26, Female = 6).

Body slender with uniform black or bluish- black dorsum; whitish ventral shields; head slightly distinct from neck; eyes small with round pupil; mean head length (range) in male is 19.14 mm , while 14.19 mm in female; mean head width (range) in male is 15.36 mm (7.26 – 22.46 mm), and 10.82 mm (6.78 – 15.09 mm) in female; the mean SVL (range) in male is 849.62 mm (319 – 1170 mm), and 597.33 mm (272 **–** 790 mm) in female; mean hemipenis length in male (range) = 22.54 mm (11.12 – 46.09 mm); RTaL (mean) in male is 0.168 (16.8 %), and 0.161 (16.1 %) in female; with a total length of 1325 mm in male, while 920 mm in female; smooth dorsals without apical pits; DSR in 15 rows throughout; ventral scales 214– 228 in male, 220– 228 in female; SC 48–58 in male, 48–56 in female. Detail morphometry and pholidosis data is shown in Table 3 – 7.

Hemipenis

The hemipenis extends up to 6^{th} – 12^{th} SC (11.12 – 46.09 mm); vaguely bilobed; about one- third of the distal is calyculate, followed by spinose from mid region with the size of spines decrease as they approach to the proximal area with ill-defined demarcation between calyculate and spinose region (Fig 12 – 13).

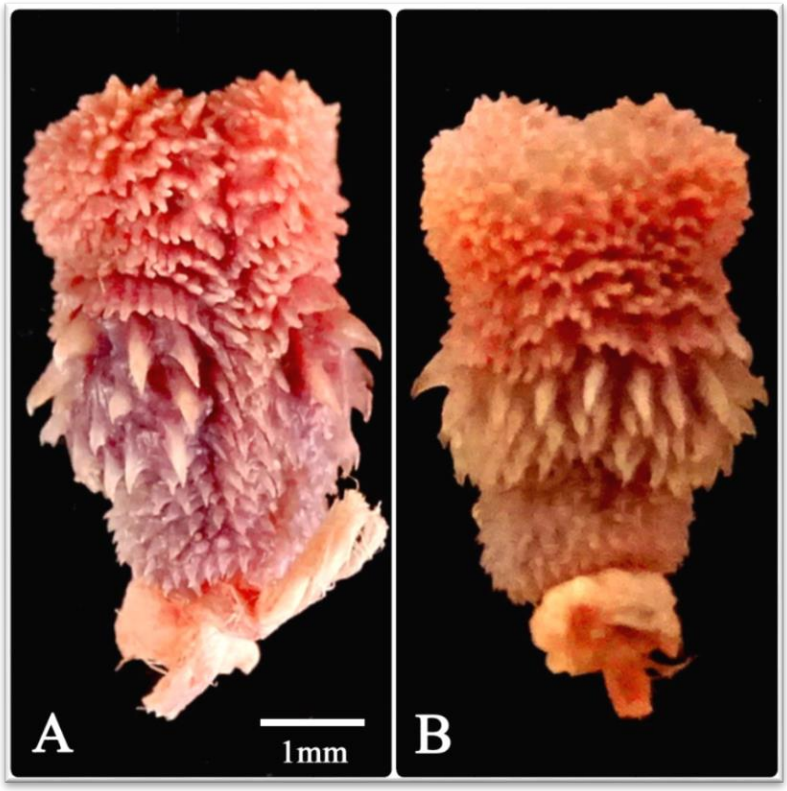

Fig 12: The hemipenis sulcus view (A) and asulcus view (B) of *B. niger*.

Fig 13: Everted hemipenes in male *B. niger*.

Table 3: Morphometry and pholidosis of *B. niger* (MZMU: 1324, 978, 975, 1315, 993, 986, 1337).

Museum number	MZMU 1324	MZMU 978	MZMU 975	MZMU 1315	MZMU 993	MZMU 986	MZMU 1337
Sex	M	M	M	M	M	M	M
Morphometrics (in mm)							
ED	3.04	3.84	2.48	2.37	2.42	2.40	3.12
END	3.50	5.96	3.10	3.22	3.33	3.38	4.96
TaL	151	171	132	122	123	110	175
SW	7.88	7.28	5.30	5.14	5.41	4.27	8.12
SL	2.82	2.82	1.46	1.40	1.68	1.32	1.34
HL	21.80	24.22	18.00	16.54	11.10	14.12	22.86
HW	19.56	20.26	12.94	13.28	12.50	11.10	19.82
SVL	1030	1050	779	684	695	635	1015
TBR	0.147	0.163	0.170	0.178	0.176	0.173	0.172
Scalation							
Ve	222	219	220	222	223	222	219
SC	50	53	51	54	55	56	53
DSR	15:15:15	15:15:15	15:15:15	15:15:15	15:15:15	15:15:15	15:15:15
SL	7/7	7/7	7/7	7/7	7/7	7/7	7/7
SLE	3-4th	3-4th	3rd-4th	3rd-4th	3rd-4th	3rd-4th	3rd-4th
IF	7/7	7/7	7/7	7/7	7/7	7/7	7/7
Te	1+2	1+2	1+2	1+2	1+2	1+2	1+2
PoO	2	2	2	2	2	2	2
PrO	1	1	1	1	1	1	1
HpR	up to 6 SC (20.45 mm)	up to 6 SC (19.80 mm)	up to 6 SC (19.54 mm)	up to 6 SC (11.12 mm)	up to 6 SC (15.94 mm)	up to 7 SC (17.30 mm)	up to 5 SC (20.54 mm)

Table 4: Morphometry and pholidosis of *B. niger* (MZMU: 1338, 1339, 1340, 1341, 1342, 1343, 1388).

Museum number	MZMU 1338	MZMU 1339	MZMU 1340	MZMU 1341	MZMU 1342	MZMU 1343	MZMU 1388
Sex	M	M	M	M	M	M	M
Morphometrics (in mm)							
ED	3.00	2.23	1.52	2.28	1.44	2.87	2.48
END	5.08	3.38	1.80	3.54	3.62	4.50	4.41
TaL	146	129	57	105	106	143	142
SW	7.50	6.52	3.50	5.56	5.02	7.59	6.88
SL	1.40	1.10	0.56	1.20	1.04	1.91	1.50
HL	22.02	19.48	9.80	16.52	13.96	21.81	20.66
HW	19.78	15.60	7.26	11.92	13.00	17.58	15.52
SVL	887	787	319	742	593	895	1170
TBR	0.165	0.164	0.179	0.142	0.179	0.160	0.121
Scalation							
Ve	226	223	222	222	222	214	216
SC	54	51	58	49	51	48	52
DSR	15:15:15	15:15:15	15:15:15	15:15:15	15:15:15	15:15:15	15:15:15
SL	7/7	7/7	7/7	7/7	7/7	7/7	7/7
SLE	3^{rd}-4^{th}	3^{rd}-4^{th}	3^{rd}-4^{th}	3^{rd}-4^{th}	3^{rd}-4^{th}	3^{rd}-4^{th}	3^{rd}-4^{th}
IF	7/7	7/7	7/7	7/7	7/7	7/7	7/7
Te	1+2	1+2	1+2	1+2	1+2	1+2	1+2
PoO	2	2	2	2	2	2	2
PrO	1	1	1	1	1	1	1
HpR	upto 7 SC (20.64 mm)	upto 7 SC (20.36 mm)	NA	upto 7 SC (18.01 mm)	upto 8 SC (17.52 mm)	upto 6 SC (19.74 mm)	NA

Table 5: Morphometry and pholidosis of *B. niger* (MZMU: 1418, 1416, 1453, 1527, 1085, 1086, 1482).

Museum number	**MZMU 1418**	**MZMU 1416**	**MZMU 1453**	**MZMU 1527**	**MZMU 1085**	**MZMU 1086**	**MZMU 1482**
Sex	F	M	F	M	F	F	M
Morphometrics (in mm)							
ED	2.46	2.95	1.27	2.95	2.46	2.36	2.72
END	3.73	4.44	1.40	4.61	3.44	2.81	3.98
TaL	128	143	46	159	120	110	140
SW	5.68	6.60	3.63	7.71	5.28	4.48	7.68
SL	1.12	1.32	0.85	1.30	1.24	1.32	1.62
HL	17.72	26.44	8.47	21.46	15.5	14.38	20.40
HW	15.09	22.46	6.78	17.28	11.2	11.85	15.38
SVL	722	934	272	1025	695	690	880
TBR	0.177	0.153	0.169	0.155	0.173	0.159	0.159
Scalation							
Ve	220	221	228	219	221	225	219
SC	48	52	56	51	52	53	52
DSR	15:15:15	15:15:15	15:15:15	15:15:15	15:15:15	15:15:15	15:15:15
SL	7/7	7/7	7/7	7/7	7/7	7/7	7/7
SLE	3^{rd}-4^{th}	3^{rd}-4^{th}	3^{rd}-4^{th}	3^{rd}-4^{th}	3-4^{th}	3-4^{th}	3-4^{th}
IF	7/7	7/7	7/7	7/7	7/7	7/7	7/7
Te	1+2	1+2	1+2	1+2	1+2	1+2	1+2
PoO	1	2	2	2	2	2	2
PrO	1	1	1	1	1	1	1
HpR		up to 6 SC (15.86 mm)		up to 10 SC (32.21 mm)			up to 9 SC (27.40 mm)

Table 6: Morphometry and pholidosis data of *B. niger* (MZMU: 1483, 1549, 1528, 1525, 1526, 1570, 1571).

Museum number	MZMU 1483	MZMU 1549	MZMU 1528	MZMU 1525	MZMU 1526	MZMU 1570	MZMU 1571
Sex	M	M	M	M	M	M	M
Morphometrics (in mm)							
ED	2.82	3.48	2.78	2.61	2.28	2.89	2.26
END	5.04	5.20	3.89	4.72	3.65	4.12	3.25
TaL	152	170	152	140	142	140	122
SW	7.96	8.30	6.31	6.31	6.37	6.58	5.72
SL	1.90	1.99	1.43	1.75	1.92	1.96	1.39
HL	23.80	24.87	19.96	21.96	20.18	15.93	17.02
HW	16.90	19.68	14.57	15.85	13.73	13.36	13.82
SVL	1060	1155	925	950	935	775	720
TBR	0.144	0.147	0.164	0.147	0.152	0.181	0.169
Scalation							
Ve	222	221	223	228	228	216	217
SC	51	49	55	50	52	56	51
DSR	15:15:15	15:15:15	15:15:15	15:15:15	7/7	7/7	7/7
SL	7/7	7/7	7/7	7/7	3-4th	3-4th	3-4th
SLE	3-4th	3-4th	3-4th	3-4th	7/7	7/7	7/7
IF	7/7	7/7	7/7	7/7	1+2	1+2	1+2
Te	1+2	1+2	1+2	1+2	2	2	2
PoO	2	2	2	2	1	1	1
PrO	1	1	1	1	7/7	7/7	7/7
HpR	up to 10 SC (29.82 mm)	up to 12 SC (46.09 mm)	up to 9 SC (26.0 mm)	up to 8 SC (27.13 mm)	up to 10 SC (29.27 mm)	up to 10 SC (20.88 mm)	up to 11 SC (25.39 mm)

Table 7: Morphometry and pholidosis of *B. niger* (MZMU: 1585, 1584, 1594, 1595).

Museum number	**MZMU 1585**	**MZMU 1584**	**MZMU 1594**	**MZMU 1595**
Sex	F	F	M	M
Morphometrics (in mm)				
ED	1.54	2.68	2.18	2.54
END	2.33	4.05	2.96	4.09
TaL	68	130	105	126
SW	4.22	6.64	6.03	7.98
SL	0.63	1.43	1.35	1.31
HL	11.39	17.7	14.81	17.87
HW	8.18	11.8	11.80	14.46
SVL	415	790	625	825
TBR	0.164	0.165	0.168	0.153
Scalation				
Ve	223	221	224	219
SC	48	54	54	46(tip broken)
DSR	15:15:15	15:15:15	15:15:15	15:15:15
SL	7/7	7/7	7/7	7/7
SLE	3-4th	3-4th	3-4th	3-4th
IF	7/7	7/7	7/7	7/7
Te	1+2	1+2	1+2	1+2
PoO	2	2	2	2
PrO	1	1	1	1
HpR	-	-	up to 9 SC (19.50 mm)	up to 10 SC (20.38 mm)

Sexual dimorphism

Two morphological features such as the relative tail length (TaL/SVL) and head dimension (HW/HL) were subjected for statistical analysis using the independent sample t-test at the alpha level of 0.05 to see whether there is sexual dimorphism on the selected features from the collected specimens of *B. niger* (Fig 14).

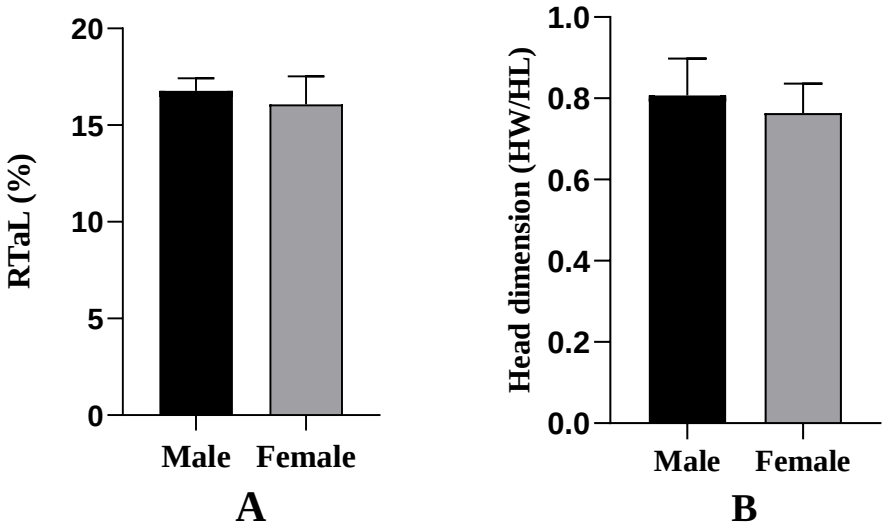

Fig 14: Comparison between the sexes in *B. niger;* (A) On the relative tail length, where *t* = 1.156, *df* = 30, *p* = 0.257, and (B) On the head dimension, where *t* = 0.083, *df* = 30, *p* = 0.287.

Correlation of various morphological features

The male *B. niger* were probed and measured the reach of the hemipenes in live as well as preserved specimens. The hemipenial reach was correalted with various morphological measurements such as SVL vs. hemipenis; RTaL vs.

hemipenis; TaL vs. hemipenis; and correlation was also tested between RTaL vs. SVL from the whole specimens in regardless of the sexes (Table 8–11; Fig 15–18).

Table 8: Correlation between relative tail length and hemipenis length in male population of *B. niger.*

Correlations			
		Hemipenis	**RTaL**
RTaL	Pearson Correlation	-.518**	1
	Sig. (2-tailed)	.010	
	N	24	24
Hemipenis	Pearson Correlation	1	-.518**
	Sig. (2-tailed)		.010
	N	24	24
**. Correlation is significant at the 0.01 level (2-tailed).			

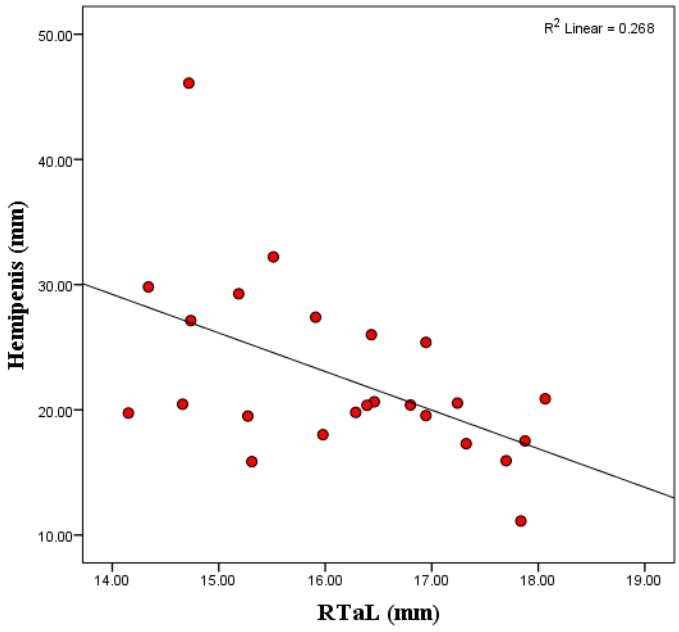

Fig 15: Scatter plot showing the correlation between relative tail length and hemipenis length in male population of *B. niger.*

Table 9: Correlation between hemipenis length and snout- vent length in male population of *B. niger*.

Correlations			
		Hemipenis	**SVL**
Hemipenis	Pearson Correlation	1	.641**
	Sig. (2-tailed)		.001
	N	24	24
SVL	Pearson Correlation	.641**	1
	Sig. (2-tailed)	.001	
	N	24	24
**. Correlation is significant at the 0.01 level (2-tailed).			

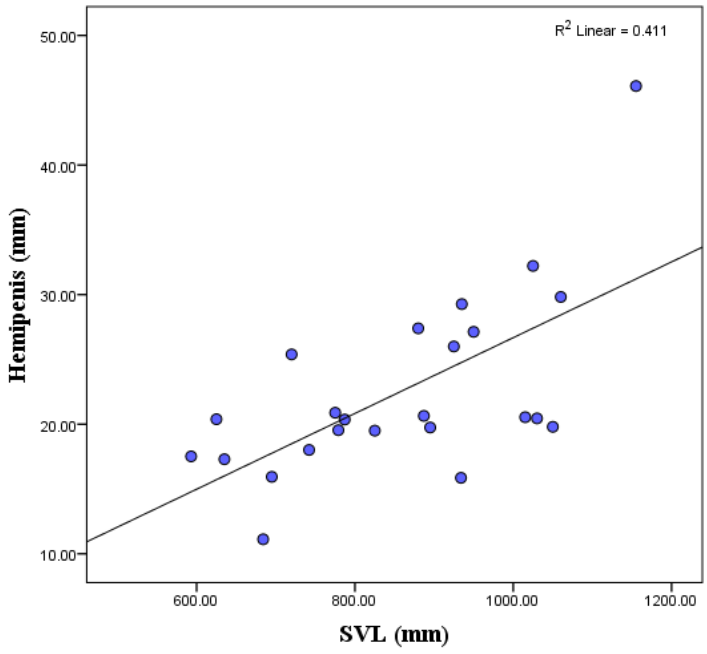

Fig 16: Scatter plot showing the correlation between snout-vent length and hemipenis length in male population of *B. niger*.

Table 10: Correlation between relative tail length and snout- vent length in *B. niger* with regardless of sex.

Correlations			
		RTaL (M & F)	**SVL**
RTaL (M+F)	Pearson Correlation	1	-.629**
	Sig. (2-tailed)		.000
	N	32	32
SVL	Pearson Correlation	-.629**	1
	Sig. (2-tailed)	.000	
	N	32	32
**. Correlation is significant at the 0.01 level (2-tailed).			

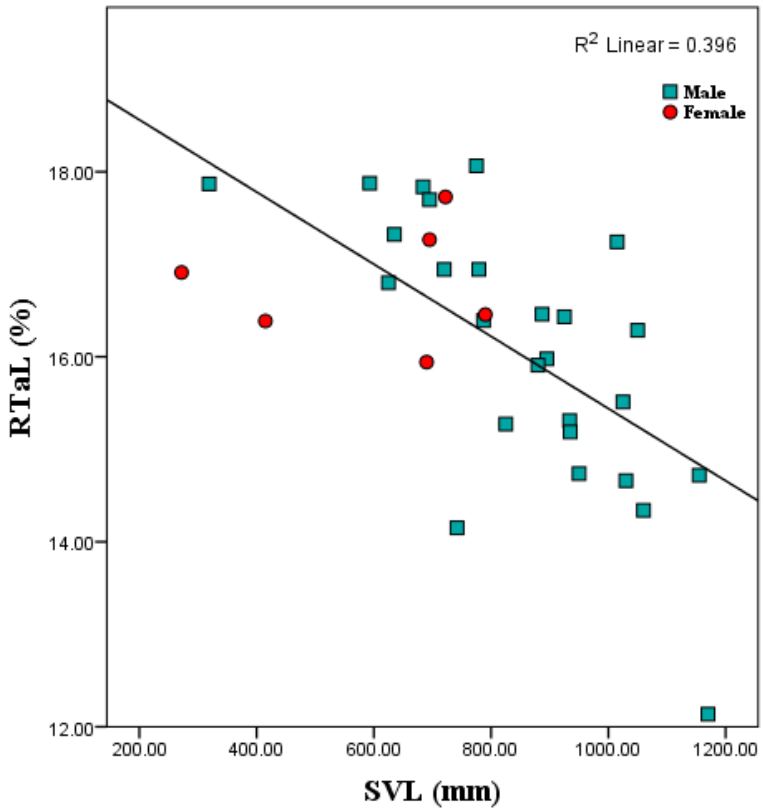

Fig 17: Scatter plot showing the correlation between snout-vent length and relative tail length in *B. niger* with regardless of sex.

Table 11: Correlation between hemipenis length and tail length in male population of *B. niger*.

Correlations			
		TaL	**Hemipenis**
TaL	Pearson Correlation	1	.519**
	Sig. (2-tailed)		.009
	N	24	24
Hemipenis	Pearson Correlation	.519**	1
	Sig. (2-tailed)	.009	
	N	24	24
**. Correlation is significant at the 0.01 level (2-tailed).			

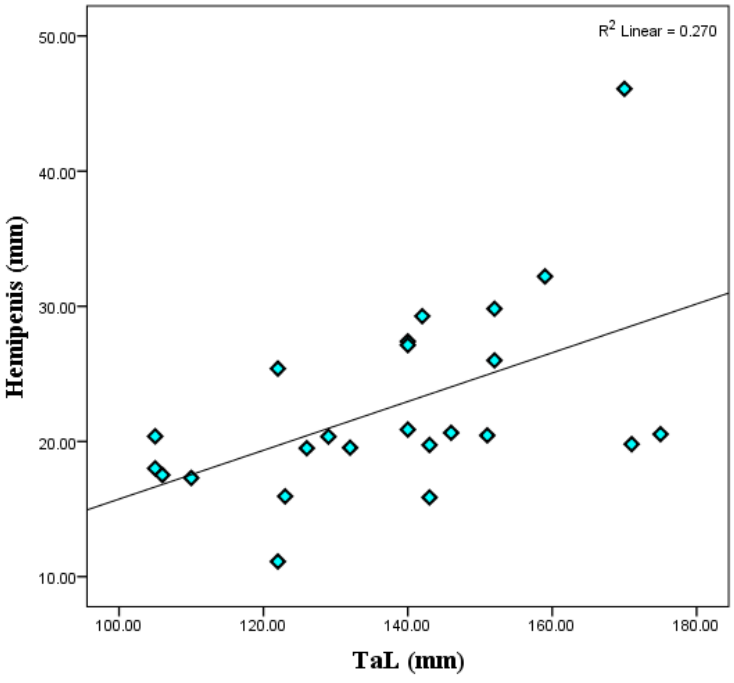

Fig 18: Scatter plot showing the relations between tail length and hemipenis length in male population of *B. niger*.

***Bungarus fasciatus* (Schneider, 1801)**

Pseudoboa fasciata Schneider, 1801, *Hostoriae Amphibiorum naturalis et literariae*. 2: 283. (Type locality: Bengal).

Description of the Species based on 13 specimens (Male = 5 and Female = 8).

Body robust with head distinct from neck, and triangular in cross-section; yellow or pale brown dorsum with black bands as broad as interspaces from nape up to the tail region; pale yellowish ventral shields with bands; eyes small with round pupil; mean head length (range) in male is 22.64 mm (12.8 – 26.6 mm) , while 19.79 mm (15.74 – 25.54 mm) in female; mean head width (range) in male is 19.67 mm (10.66 – 23.0 mm), and 15.76 mm (10.4 – 20.4 mm) in female; the mean SVL (range) in male is 970.2 mm (444 – 1220 mm), and 914.13 mm (700 – 1180 mm) in female; mean hemipenis length in male (range) = 25.6 mm (20.12 – 31.58 mm); RTaL (mean) in male is 0.112 (11.2 %), and 0.107 (10.7 %) in female; with a total length of 1353 mm in male, while 1299 mm in female; dorsals smooth; DSR in 15 rows throughout; Ve 222– 228 in male, 224– 231 in female; SC 35–36 in male, 32–36 in female. Detail morphometry and pholidosis data is shown in Table 12 – 13.

Hemipenis

The hemipenis extends up to 4th– 7th SC (20.12– 31.58 mm). About one- third of the distal is calyculate.Spinose from mid region. Size of the spines decrease as they approach to the proximal area. Sharply-defined demarcation between calyculate and spinose region (Fig 19).

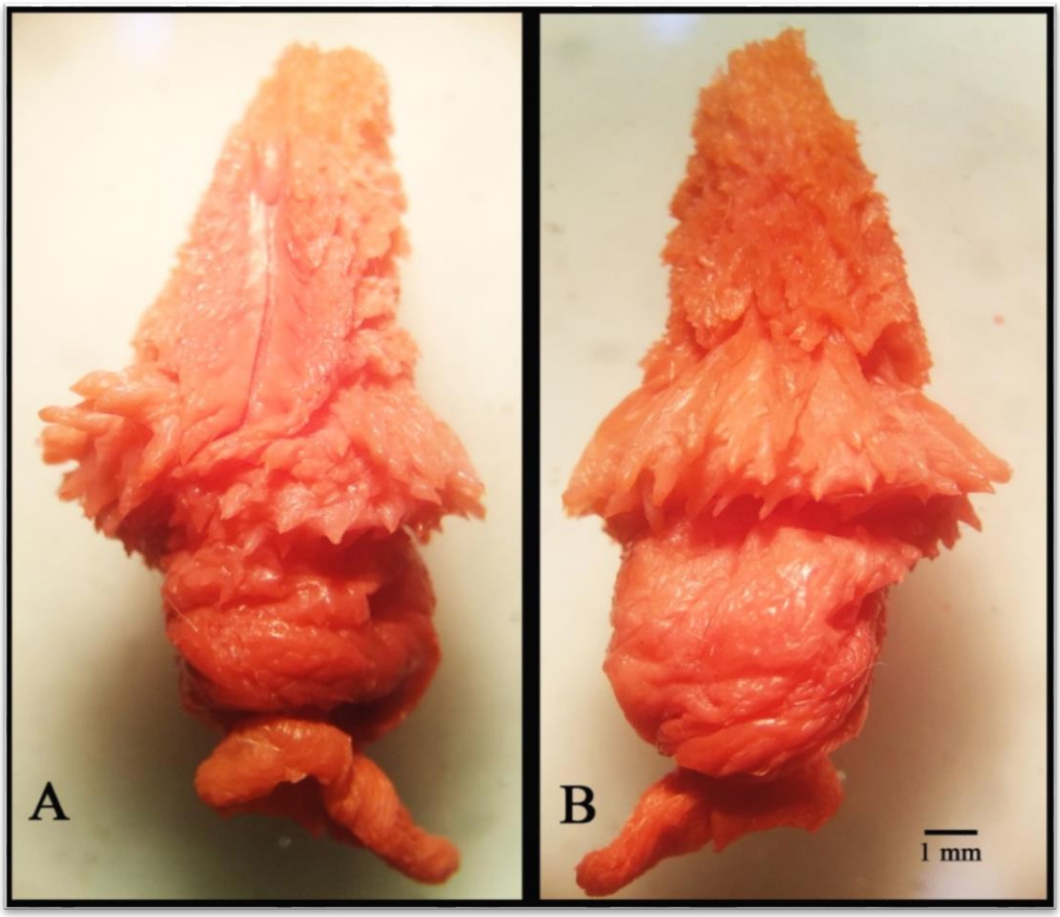

Fig 19: Weakly inflated hemipenis sulcate view (A) and asulcate view (B) of *B. fasciatus.*

Table 12: Morphometry and pholidosis data of *B. fasciatus* (MZMU: 933, 934, 1314, 1319, 1320, 1321, 1417).

Museum number	MZMU 933	MZMU 934	MZMU 1314	MZMU 1319	MZMU 1320	MZMU 1321	MZMU 1417
Sex	M	F	M	F	M	F	M
Morphometry (in mm)							
ED	3.60	2.9	3.98	4.76	4.54	4.80	3.42
END	4.90	NA	3.44	5.20	5.76	5.66	6.04
TaL	123	96	47	100	116	103	131
SW	7.90	Snout damaged	4.24	7.30	9.00	8.00	8.62
SL	1.70	NA	1.36	2.42	3.38	2.38	1.94
HL	26.60	NA	12.80	18.40	22.80	22.14	26.46
HW	22.10	10.4	10.66	18.90	23.00	20.40	22.46
SVL	1075	914	444	862	1019	935	1093
TBR	0.114	0.105	0.106	0.116	0.114	0.110	0.119
Ve	227	229	228	229	226	230	222
Scalation							
SC	35	33	36	34	35	32	36
DSR	15:15:15	15:15:15	15:15:15	15:15:15	15:15:15	15:15:15	15:15:15
IFSl	4^{th}-6^{th}	4^{th}-6^{th}	4^{th}-6^{th}	4^{th}-6^{th}	4^{th}-6^{th}	4^{th}-6^{th}	4^{th}-6^{th}
SL	7/7	7/7	7/7	7/7	7/7	7/7	7/7
SLE	3-4^{th}	3-4^{th}	3^{rd}-4^{th}	3^{rd}-4^{th}	3^{rd}-4^{th}	3^{rd}-4^{th}	3^{rd}-4^{th}
IF	7/7	7/7	7/7	7/7	7/7	7/7	7/7
Te	1+2	1+2	1+2	1+2	1+2	1+2/2+2	1+2
PoO	2	2	2	2	2	3/2	2
PrO	1	1	1	1	1	1	1
BB	26	22	27	27	25	26	23
BT	5	4	5	5	5	4	5
NBW	18	18	18	18	18	19	na
HpR	up to 7 SC (22.48 mm)				up to 7 SC (31.58 mm)		up to 7 (28.20 mm)

Table 13: Morphometry and pholidosis of *B. fasciatus* (MZMU: 1421, 1550, 1562, 1561, 1548, 1572).

Museum number	MZMU 1421	MZMU 1550	MZMU 1562	MZMU 1561	MZMU 1548	MZMU 1572
Sex	M	F	F	F	F	F
Morphometry (in mm)						
ED	3.04	2.92	4.19	3.26	4.40	3.29
END	5.36	3.26	3.31	3.24	4.44	4.28
TaL	133	76	82	89	118	119
SW	8.21	5.65	5.61	5.53	10.06	8.26
SL	1.55	1.37	1.37	1.61	2.2	1.66
HL	24.54	15.74	16.68	16.49	23.54	25.54
HW	20.12	12.46	12.80	13.04	19.70	18.34
SVL	1220	700	835	767	1120	1180
TBR	0.109	0.108	0.098	0.116	0.105	0.101
Scalation						
Ve	227	224	231	228	230	231
SC	36	33	33	36	34	35
DSR	15:15:15	15:15:15	15:15:15	15:15:15	15:15:15	15:15:15
IFSl	4^{th}-6^{th}	4^{th}-6^{th}	4^{th}-6^{th}	4^{th}-6^{th}	4^{th}-6^{th}	4^{th}-6^{th}
SL	7/7	7/7	7/7	7/7	7/7	7/7
SLE	3^{rd}-4^{th}	3^{rd}-4^{th}	3^{rd}-4^{th}	3^{rd}-4^{th}	3^{rd}-4^{th}	3^{rd}-4^{th}
IF	7/7	7/7	7/7	7/7	7/7	7/7
Te	1+2	1+2	1+2	1+2	1+2	1+2
PoO	2	2	2	2	2	2
PrO	1	1	1	1	1	1
BB	23	25	26	25	23	25
BT	5	5	4	4	4	4
NBW	19	17	15	15	20	19
HpR	up to 4 SC (20.12 mm)					

Sexual dimorphism

Two morphological features such as the relative tail length (TaL/SVL) and head dimension (HW/HL) were subjected for statistical analysis using the independent sample t-test at the alpha level of 0.05 to see whether there is sexual dimorphism on the selected features from the collected specimens of *B. fasciatus* (Fig 20).

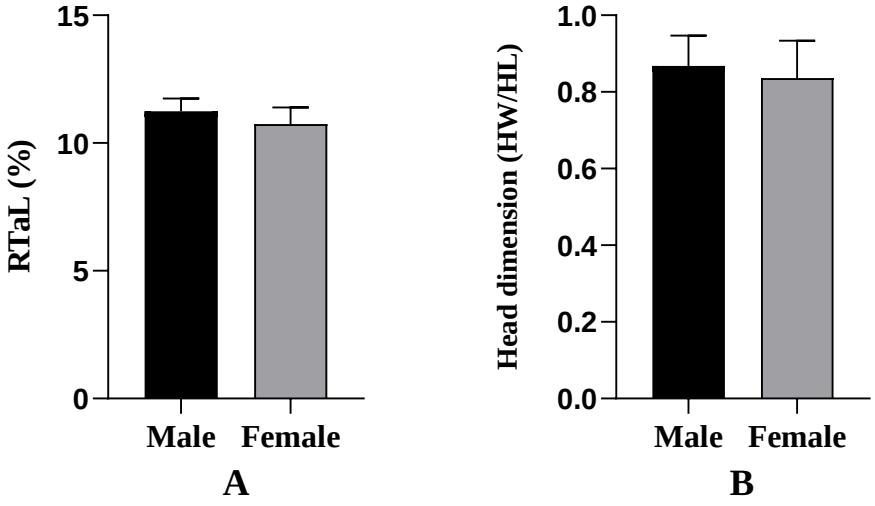

Fig 20: Comparison between the sexes in *B. fasciatus;* (A) On the relative tail length, where *t* = 1.467, *df* = 11, *p* = 0.17, and (B) On the head dimension, where *t* = 0.61, *df* = 11, *p* = 0.554.

Correlation on various morphological features

The relative tail length and SVL from the whole specimens in regardless of the sexes were tested for correlation (Table 14; Fig 21). Moreover, the subcaudal scales were also tested its correlation with the tail length (Table 15; Fig 22).

Table 14: Correlation between the snout-vent lengths and tail lengths in *B. fasciatus.*

Correlations			
		RTaL	**SVL**
RTaL	Pearson Correlation	1	-.047
	Sig. (2-tailed)		.885
	N	12	12
SVL	Pearson Correlation	-.047	1
	Sig. (2-tailed)	.885	
	N	12	12
Correlation is not significant.			

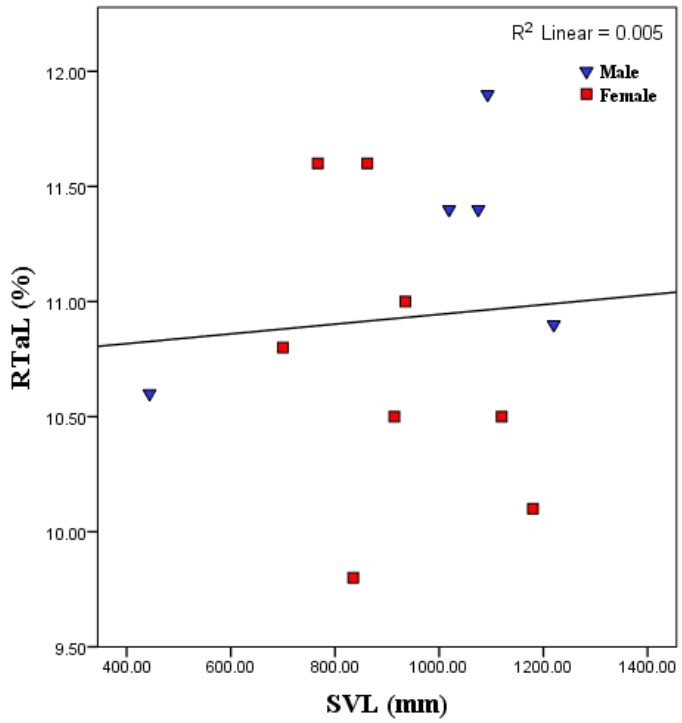

Fig 21: Scatter plot showing the absence of significant correlation between snout-vent lengths and relative tail lengths in *B. fasciatus.*

Table 15: Correlation between the subcaudals and tail length in *B. fasciatus.*

Correlations			
		SC	**TaL**
SC	Pearson Correlation	1	.614*
	Sig. (2-tailed)		.034
	N	12	12
TaL	Pearson Correlation	.614*	1
	Sig. (2-tailed)	.034	
	N	12	12
*. Correlation is significant at the 0.05 level (2-tailed).			

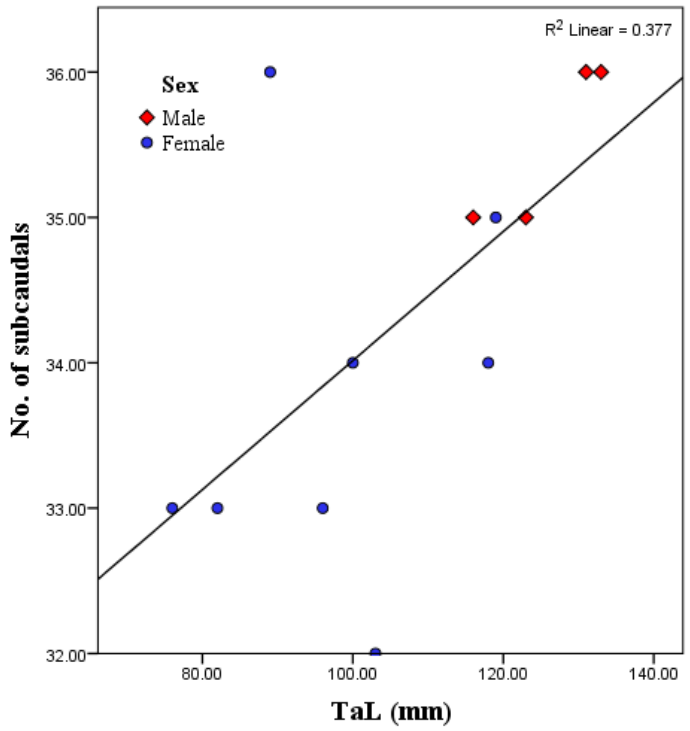

Fig 22: Scatter plot showing the correlation between subcaudals and tail length in *B. fasciatus.*

Natural History Notes on *Bungarus niger*

At the corner of a retaining wall near the building of Zoology Department, Mizoram University, India (23.737030˚N, 92.663397˚E, WGS 84, 798 m asl.), a male *Bungarus niger* (MZMU 978; SVL = 1100 mm) was observed preying on sub-adult *Coelognathus radiatus* (Copper-headed Trinket Snake). The *B. niger* biting the head of the *C. radiatus*, which biting back in order to escape (Fig. 23 A). The snakes struggled for several minutes (Fig. 23 B), with the *B. niger* coiling tightly around its prey (Fig. 23 C). Finally, the *B. niger* was able to start swallowing the *C. radiatus* from the head, which was completed in ca. 20 min (Fig. 23 D). The photos were deposited in Lee Kong Chian Natural History Museum, National University of Singapore, with the photo voucher number of ZRC (IMG) 2.410. In captivity it was also observed eating a *Argyrophis diardi*, and an adult *Psammodynastes pulverulentus.* While examining the preserved specimens, one female (MZMU 1086) collected from Melriat, Aizawl, Mizoram (23.641867˚N; 92.726138˚E, WGS 84, 858 m asl.) was found gravid with four eggs. The length and width of the eggs were measured (Table 16; Fig 24); mean length = 12.67 mm, and mean width = 5.30 mm.

Table 16: Egg measurements of *B. niger* (MZMU 1086).

Sl. no	Length (mm)	Width (mm)
1.	12.29	5.70
2.	11.89	4.77
3.	12.73	5.43
4.	13.76	5.27

Fig 23: Adult *B. niger* preying on sub-adult *C. radiatus* (Copper-headed Trinket Snake) in Mizoram University Campus, India [ZRC (IMG) 2.410].

Fig 24: A preserved female *B. niger* (MZMU 1086) with a clutch of four eggs.

Natural History Notes on *Bungarus fasciatus*

On the Buichali bridge, Sairang road, Mizoram, India (23.899261˚N, 92.652326˚E, WGS 84, 88 m elev.), a roadkilled adult male *B. fasciatus* (MZMU 933; SVL = 1174.2 mm) lying on the road was found (Fig 25; ZRC (IMG) 2.411). The abdomen was ruptured and the gut content of the snake was partially exposed. The *B. fasciatus* has recently fed on a *Boiga ochracea* (Tawny cat-snake), as indicated by the relatively intact condition of the prey. The species was also observed feeding on *Typhlops diardi* in captivity.

Fig 25: Roadkilled *B. fasciatus* with exposed gut contents at the Buichali Bridge in Mizoram [ZRC (IMG) 2.411].

Molecular Characterization

Amplification of the marker COI gene

Universal primer for COI (LCO-forward; HCO-reverse) with a product size of 720 bp was used. The PCR product of DNA was checked on 1.2 % agarose gel. Visualized and documented using Bio-Rad ChemiDoc^{TM} XRS+System (Fig 26).

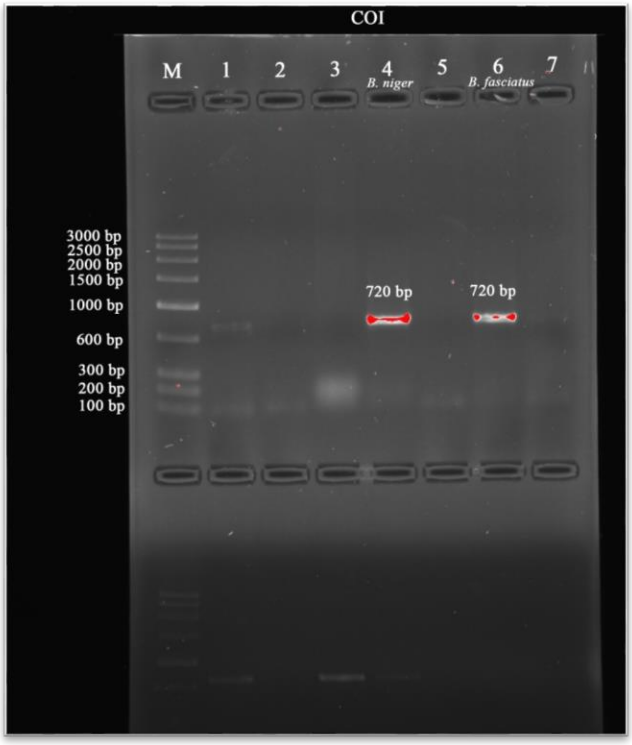

Fig 26: Gel image showing the PCR products of *B. niger* and *B. fasciatus.*

Sequence obtained

Bungarus niger (MZMU-1549), COI, 533 bp, partial CDS, GenBank Accesion number- MN722642.

>*Bungarus niger*

CTTTTAGGAAGTGACCAAATCTTTAACGTACTAGTTACTGCCCACGCATT
TATCATAATTTTCTTTATAGTCATACCAATCATAATCGGAGGATTTGGCA
ACTGACTTATCCCTTTAATAATCGGCGCCCCTGATATAGCCTTTCCCCGAA
TAAACAATATAAGCTTCTGGCTCCTCCCACCAGCACTACTCCTTCTCCTAT
CCTCCTCTTATGTAGAAGCCGGTGCCGGCACAGGTTGAACAGTCTACCCG
CCCCTATCGGGTAACCTAGTTCACTCAGGCCCATCAGTAGACTTAGCTAT
CTTCTCTCTACATTTAGCAGGAGCCTCCTCCATCCTAGGAGCAATCAATTT
TATTACAACATGCATTAATATAAAACCTAAATCAATACCAATATTTAATA
TTCCATTATTCGTTTGATCAGTATTAATCACAGCCATTATACTTCTTCTAG
CCCTGCCAGTTCTAGCTGCCGCAGTTACAATACTTTTAACCGATCGTAAT
CTCAATACATCCTTCTTTGACCCTTC

Bungarus fasciatus (MZMU-1421), COI, 426 bp, partial CDS, GenBank Accesion number- MN722643.

>*Bungarus fasciatus*

TAGTTATACCTATTATAATTGGCGGCTTTGGCAACTGACTTATCCCATTAA
TAATTGGAGCCCCAGATATAGCCTTTCCTCGAATAAATAATATAAGTTTC
TGACTACTCCCACCAGCACTTCTTCTTCTCCTATCATCTTCCTATGTAGAA
GCTGGAGCCGGCACAGGCTGAACAGTTTATCCGCCCCTATCGGGTAACTT
AGTTCATTCAGGCCCATCAGTAGATCTAGCTATTTTCTCTCTACACCTAGC
AGGAGCTTCTTCCATCCTAGGAGCAATCAACTTTATTACAACATGTATTA
ACATAAAACCTAAATCAATACCAATATTTAATATTCCATTATTTGTATGA
TCAGTCTTAATCACTGCTATTATACTTTTACTAGCCCTACCAGTACTAGCC
GCAGCAATCACTATACTTCTAACTA

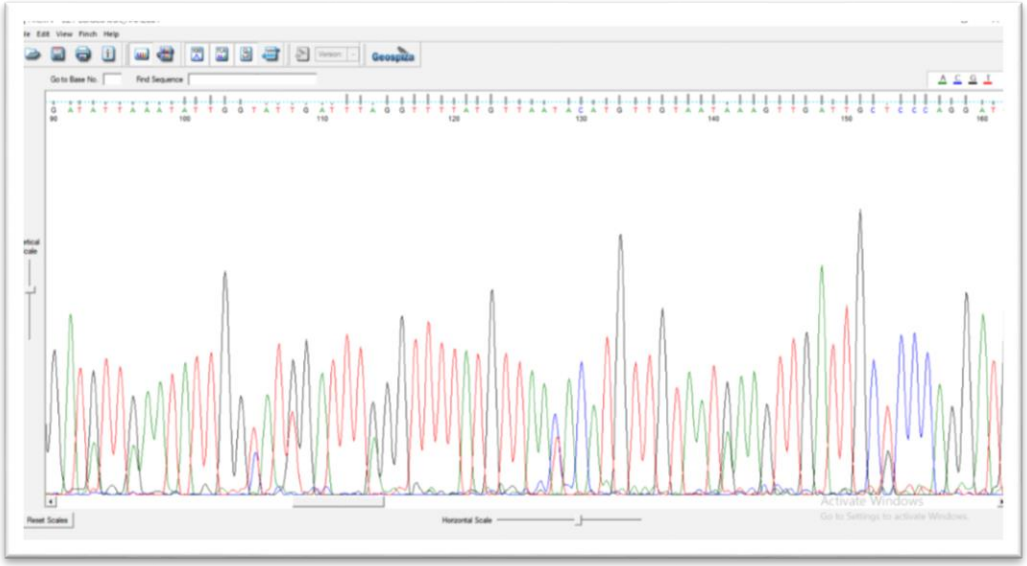

Fig 27: Chromatogram showing the sequence of *B. fasciatus.*

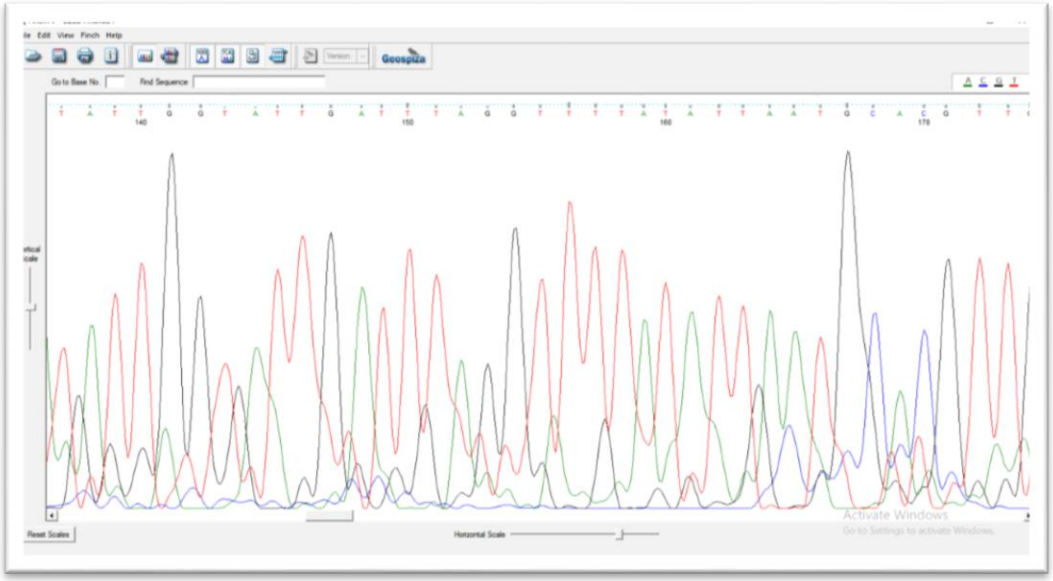

Fig 28: Chromatogram showing the sequence of *B. niger.*

Phylogenetic analysis

The evolutionary history was inferred by using the Maximum Likelihood method with a Tamura-Nei model. The tree with the highest log likelihood (-2863.34) is shown (Fig 29). The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 15 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. There were a total of 536 positions in the final dataset. Evolutionary analyses were conducted in MEGA X. The Bayesian inference tree was also prepared from MrBayes 3. 2. 7a x86_64 using the method of Makov Chain Monte Carlo (MCMC) to approximate the posterior probability of tree (Fig 30).

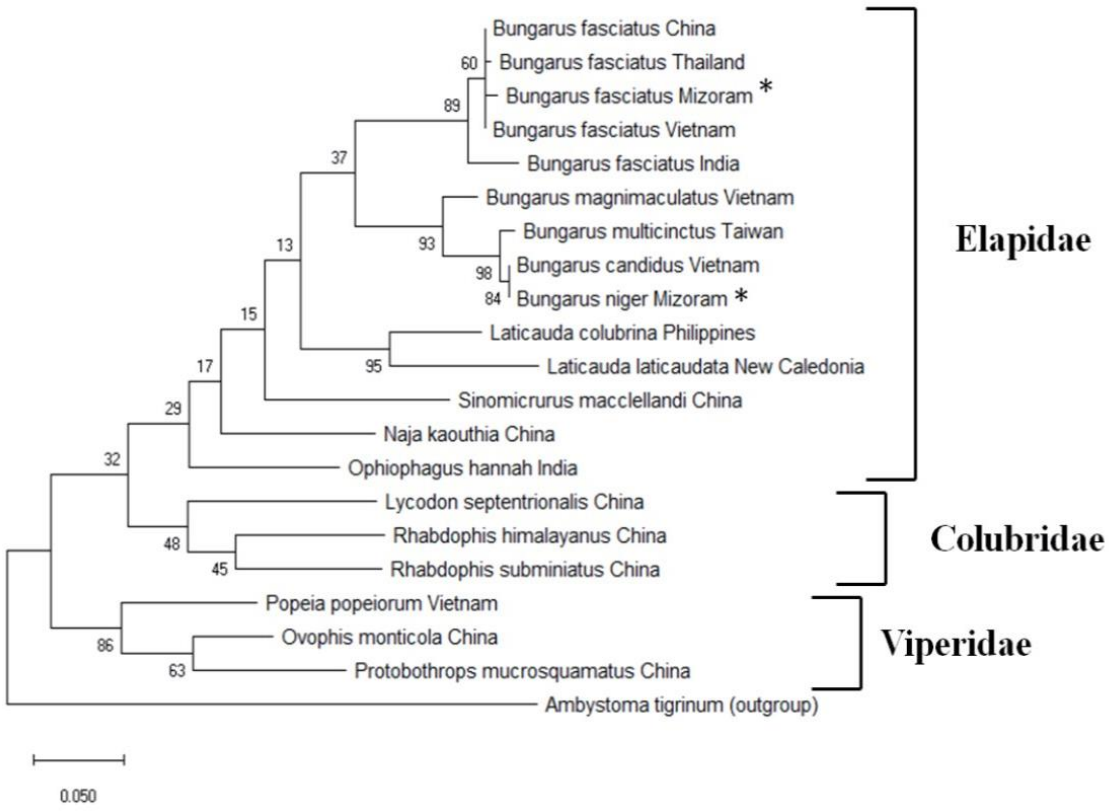

Fig 29: Maximum likelihood tree by using cytochrome c oxidase I sequences of the snake family Elapidae, Colubridae, and Viperidae, with emphasis on the genus *Bungarus*, and *Ambystoma tigrinum* as an outgroup (Mizoram samples are indicated in asterisk).

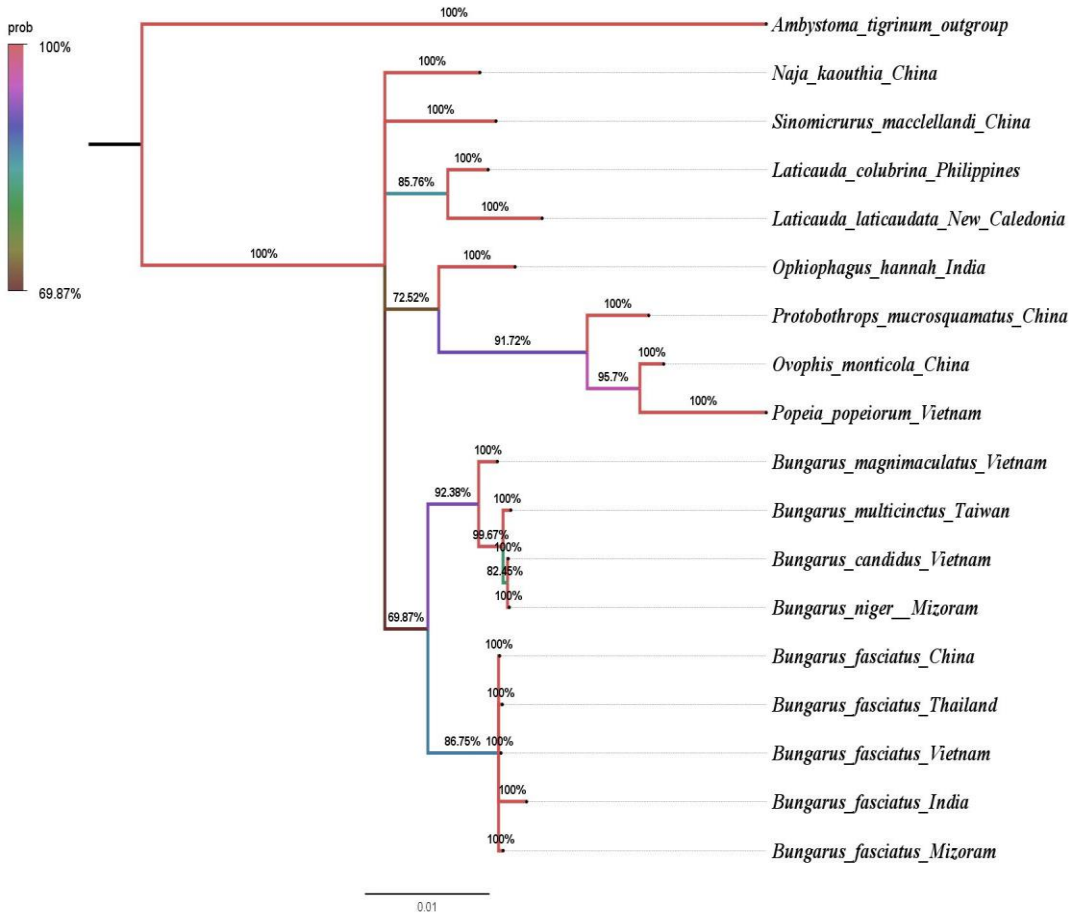

Fig 30: Bayesian inference tree based on cytochrome c oxidase I sequences of the snake family Elapidae with emphasis on the genus *Bungarus*, and *Ambystoma tigrinum* as an outgroup.

Estimates of Evolutionary Divergence

The number of base substitutions per site from between sequences is shown. Analyses were conducted using the Jukes-Cantor model (Jukes and Cantor, 1969). This analysis involved 15 nucleotide sequences (Table 17). Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 536 positions in the final dataset. Evolutionary analyses were conducted in MEGA X.

Table 17: Estimates of Evolutionary Divergence between Sequences (Jukes-Cantor model).

	B_niger_Mizoram	B_multicinctus_Taiwan	B_fasciatus_Mizoram	B_fasciatus_China	B_fasciatus_Thailand	B_fasciatus_Vietnam	B_fasciatus_India	B_candidus_Vietnam	Bu_magnimaculatus_Vietnam	N_kaouthia_China	S_macclellandi_China	O_hannah_India	L_colubrina_Philippines	L_laticaudata_New_Caledonia	A_tigrinum_(outgroup)
B_niger_Mizoram															
B_multicinctus_Taiwan	0.0142														
B_fasciatus_Mizoram	0.1378	0.1434													
B_fasciatus_China	0.1367	0.1480	0.0070												
B_fasciatus_Thailand	0.1416	0.1526	0.0117	0.0037											
B_fasciatus_Vietnam	0.1367	0.1480	0.0070	0.0000	0.0037										
B_fasciatus_India	0.1635	0.1635	0.0165	0.0377	0.0463	0.0377									
B_candidus_Vietnam	0.0000	0.0132	0.1378	0.1435	0.1480	0.1435	0.1635								
B_magnimaculatus_Vietnam	0.0543	0.0582	0.1350	0.1323	0.1367	0.1323	0.1535	0.0541							
N_kaouthia_China	0.1638	0.1734	0.1810	0.1949	0.1998	0.1949	0.2326	0.1688	0.1782						
S_macclellandi_China	0.1894	0.1949	0.1634	0.1853	0.1901	0.1853	0.1585	0.1925	0.1901	0.1758					
O_hannah_India	0.1689	0.1805	0.1577	0.1618	0.1664	0.1618	0.1738	0.1782	0.1829	0.1664	0.1853				
L_colubrina_Philippines	0.1465	0.1503	0.1693	0.1711	0.1758	0.1711	0.1535	0.1480	0.1572	0.1734	0.1711	0.1877			
L_laticaudata_New_Caledonia	0.1842	0.1925	0.1960	0.2047	0.2047	0.2047	0.1635	0.1901	0.1758	0.2022	0.1925	0.2120	0.1301		
A_tigrinum_(outgroup)	0.3163	0.3326	0.3390	0.3355	0.3385	0.3355	0.2904	0.3211	0.3125	0.3473	0.3714	0.3806	0.3385	0.3775	

Pattern of nucleotide substitution

The sum of r values is made equal to 100. Rates of different transitional substitutions are shown in **bold** and those of transversionsal substitutions are shown in *italics*. The nucleotide frequencies are 27.75% (A), 30.70% (T/U), 26.89% (C), and 14.66% (G). The transition/transversion rate ratios are k_1 = 6.013 (purines) and k_2 = 5.912 (pyrimidines). The overall transition/transversion bias is R = 3, where $R = [A*G*k_1 + T*C*k_2]/[(A+G)*(T+C)]$. This analysis involved 15 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 536 positions in the final dataset (Table 18).

Table 18: Maximum Composite Likelihood Estimate of the Pattern of Nucleotide Substitution using Tamura-Nei model.

	A	T	C	G
A	**-**	***3.86***	***3.38***	**11.08**
T	***3.49***	**-**	**19.98**	***1.84***
C	***3.49***	**22.81**	**-**	***1.84***
G	**20.98**	***3.86***	***3.38***	**-**

Codon usage Bias

The codon usage bias from the COI sequences of *Bungarus* are computed using Relative synonymous codon usage (RSCU) statistics (Sharp et al., 1986) conducted by using MEGAX (Table 19).

Table 19: Codon usage bias in the COI sequences of the snake genus *Bungarus.*

Codon	Count	RSCU	Codon	Count	RSCU	Codon	Count	RSCU	Codon	Count	RSCU
UUU(F)	4.4	1.36	UCU(S)	6	1.32	UAU(Y)	12.1	1.69	UGU(C)	1.7	0.83
UUC(F)	2.1	0.64	UCC(S)	2.6	0.56	UAC(Y)	2.2	0.31	UGC(C)	2.3	1.17
UUA(L)	0.9	0.36	UCA(S)	1.3	0.29	UAA(*)	5.1	2.38	UGA(*)	0.9	0.41
UUG(L)	0.8	0.32	UCG(S)	0.7	0.15	UAG(*)	0.4	0.21	UGG(W)	3.1	1
CUU(L)	6.2	2.55	CCU(P)	5.7	1.85	CAU(H)	4.8	1.39	CGU(R)	1.2	0.49
CUC(L)	3.1	1.27	CCC(P)	4.2	1.38	CAC(H)	2.1	0.61	CGC(R)	2.2	0.9
CUA(L)	1.1	0.45	CCA(P)	2	0.65	CAA(Q)	2.2	2	CGA(R)	0.4	0.18
CUG(L)	2.6	1.05	CCG(P)	0.3	0.11	CAG(Q)	0	0	CGG(R)	3.1	1.25
AUU(I)	4.8	1.47	ACU(T)	7.2	2.11	AAU(N)	11.8	1.53	AGU(S)	6.7	1.46
AUC(I)	5	1.53	ACC(T)	4.9	1.43	AAC(N)	3.7	0.47	AGC(S)	10.1	2.22
AUA(I)	0	0	ACA(T)	0.9	0.26	AAA(K)	1.9	0.94	AGA(R)	3.1	1.25
AUG(M)	1.3	1	ACG(T)	0.7	0.2	AAG(K)	2.1	1.06	AGG(R)	4.8	1.93
GUU(V)	0.9	2.29	GCU(A)	0.8	1.47	GAU(D)	0	0	GGU(G)	0	0
GUC(V)	0.6	1.43	GCC(A)	1.3	2.53	GAC(D)	0	0	GGC(G)	0.1	0.4
GUA(V)	0	0	GCA(A)	0	0	GAA(E)	0	0	GGA(G)	0	0
GUG(V)	0.1	0.29	GCG(A)	0	0	GAG(E)	0	0	GGG(G)	1	3.6

Tajima Relative Rate Test of Molecular Clock

The equality of evolutionary rate between sequences A (*Bungarus fasciatus* India) and B (*Bungarus fasciatus* Mizoram), with sequence C (*Bungarus niger* Mizoram) used as an outgroup in Tajima's relative rate test (Table 20). The χ^2 test statistic was 0.00 (*P* = 1.00000 with 1 degrees of freedom) *P*-value less than 0.05 is often used to reject the null hypothesis of equal rates between lineages. This analysis involved 3 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated (complete deletion option). There were a total of 184 positions in the final dataset.

Table 20: Tajima Relative Rate Test of Molecular Clock between the *B. fasciatus* (Mizoram), and *B. fasciatus* (India) with *B. niger* (Mizoram) as an outgroup.

Configuration	Count
Identical sites in all three sequences	**158**
Divergent sites in all three sequences	**1**
Unique differences in Sequence A	**1**
Unique differences in Sequence B	**1**
Unique differences in Sequence C	**23**

CHAPTER 6

DISCUSSION AND CONCLUSION

In the present study, 2 species of *Bungarus* i.e *B. niger* and *B. fasciatus* are confirmed. Further research is suggested since there is a high possibility on finding other species of krait especially the Lesser Black Krait (*B. lividus*) which was found sympatric with the greater Black Krait (*B. niger*) in the neighbouring states and other distribution ranges. In fact there is a photographic evidence of the North-Eastern Hill Krait (*B. bungaroides*) from Tuipang, Chhimtuipui District, Mizoram. But no state report confirmation has been made due to the lack of specimen for the time being. This work yields a couple of new information regarding the context of their morphology, distribution, natural history, as well as in enhancing the existing information on their molecular taxonomy. This work also represents a comparative study between the two species as far as practicable.

A total of 32 specimens (M = 26; F = 6) in *B. niger*, and 13 specimens (M = 5; F = 8) in *B. fasciatus* were morphologically studied. The pholidosis data on *B. niger* divulged a new Ve range for the species in the lower limit i.e 214– 228 vs. 216– 231, and in the upper limit on the range of SC i.e 48– 58 vs. 47– 56 (Purkayastha, 2013). In the case of *B. fasciatus,* all of the pholidosis falls within the range reported in the existing literatures (Eg: Smith, 1943; Purkayastha, 2013, etc.). Data analysis indicated that in both the sexes of *B. niger,* relative tail length is highest (ratio of tail length to SVL) in small snakes, then gradually decreases in larger snakes. Moreover, the morphological constraint hypothesis (King, 1989) prediction where male with relatively longer tail should have larger hemipenis was agrred in *B. niger* since the data analysis supports the prediction where the snake with longer tail have longer (but not wider) hemipenis. In this study, a detailed

description of the hemipenis in both the species was also provided. However, no significant difference is observed in the structure of the hemipenis within the conspecific male population. The statistical analysis for the test of sexual dimorphism on relative tail length and the head dimension for the two species are not statistically significant, but a significant result is not unexpectable from larger sample size in future.

The specimens collected from different localities within the statewide were represented by preserved museum specimens of Departmental Museum of Zoology, Mizoram University, newly collected specimens, as well as private collections. A new range of elevation for *B.niger* was recorded i.e 42 – 1646 m asl. vs.100 – 1500 m asl. (Ahmed et al., 2009), However, in *B. fasciatus* the altitudinal range falls within the range reported in literatures i.e 49 – 1426 m asl. vs. 40 – 2300 m asl. (Ahmed et al., 2009). In *B. niger*, 18.75 % of the records are found at 0 – 500 m asl., while 40 % of the *B. fasiatus* records falls within this range. Moreover, no record of *B. fasciatus* is available at the elevation range of above 1500 m asl. whereas in *B. niger*, 2.08 % of the records are found in this elavational range. The comparison between the two species mean elevation gradient distribution revealed a statistically significant difference at the alpha level of 0.05, where $t = 2.479$, $p = 0.015$, $df = 86$. Thus, it was assumed that *B. fasciatus* is more likely to be a lower elevation preferring species as compared to *B. niger* the sympatric congener species.

The observation of *B. niger* feeding on *C. radiatus, P. pulverulentus, A. diardi,* and *O. albocinctus*; as well as the documentation of roadkilled *B. fasciatus* with the exposed gut content containing *B. ochracea,* and the observation of its

feeding on *A. diardi* enlarged the dietary lists of the two krait species. New information on the reproductive habits of *B. niger* was also contributed with the documentation of clutch size which is 4 eggs with measuring 11.89 – 13.76 mm in length, and 4.77 – 5.7 mm in width.

The obtained sequences were inferred for the evolutionary history using Maximum Likelihood method and Bayesian inference phylogeny. In *B. fasciatus,* the obtained sequence from Mizoram is showing a minimal genetic distance with that of China and Vietnam, thus showing a negligible genetic diversity between them i.e 0.0070. However, the highest genetic distance is seen between the species of Mizoram and India (mainland) by a genetic diversity of 0.0165. Thus, according to the present study, the *B. fasciatus* of Mizoram is presumable to be genetically closer to the Southeast Asian population in comparing to that from India, but further extensive research with more samples (morphological and molecular data) from both mainland India and northeastern India is strongly suggested in order to clarify the complexities of the species as well as to the ideas on the dispersal of the species biogeographically.

In *B. niger,* the obtained sequence is showing minimal genetic distance to the congener species *B. candidus,* occupying the same clade which is most likely to be caused by the unavailabily of the conspecific sequence in the databases. However, the analysis of the obtained sequences revealed that the two sympatric congener species of krait from Mizoram i.e *B. niger* and *B. fasciatus* were showing a high level of genetic diversity between them i.e 0.1378. The analysis of COI sequences of *Bungarus* from generated and those obtained from Databases showed 445 nucleotide

positions as conserved, 91 nucleotide position as variable, and 71 nucleotide position as parsimony informative sites.

Tajima Relative Rate Test of Molecular Clock between the *B. fasciatus* of Mizoram and India (mainland) revealed that they have equal and constant rates of evolution. According to the present findings and existing literatures, biogeographically *B. niger* and *B. fasciatus* can be assumed as Gondwanan origin that later dispersed in this region around 40 million years ago.

The present study also documented the 9 tragic confirmed cases of fatal envenomation by *B. niger* from eight different localities (Sentlang, Khanpui, Darlawn, Vairengte, Mamit, Darlak, Mission Vengthlang, and Durtlang) within the last decade in Mizoram, which represents the new case reports other than the only published cases from Bangladesh (N = 5; Fatal = 2) and Nepal (Fatal = 1). Moreover, two cases of fatal envenomation by *B. fasciatus* from South Vanlaiphai, Lunglei District, and Lawngtlai, Lawngtlai District, were documented in Mizoram within the last decade, which represents a new case report other than the single known case of one guy bitten to death after 15 hours (Whitaker and Captain 2008).

CHAPTER 7

SUMMARY

- This work represents a comparative study between the sexes as well as on the two species as far as practicable.
- A total of 32 specimens (M = 26; F = 6) in *B. niger*, and 13 specimens (M = 5; F = 8) in *B. fasciatus* were morphologically studied.
- The pholidosis data on *B. niger* divulged a new Ve range for the species in the lower limit i.e 214– 228 vs. 216– 231, and in the upper limit of SC i.e 48– 58 vs. 47– 56 (Purkayastha, 2013).
- The morphological correlations on *B. niger* showed that snakes with longer body as well as longer tail has longer hemipenis, and the relative tail length gradually decreases with increase in body length.
- No significant correlation between body length and relative tail length in *B. fasciatus.*
- There is positive correlation between tail length and subcaudals in *B. fasciatus.*
- New elevational range of *B. niger* was recorded i.e 42 – 1646 m asl. (Lalbiakzuala et al., 2019) vs.100 – 1500 m asl. (Ahmed et al., 2009). The elevational range of *B. fasciatus* falls within the range of 40 – 2500 m asl. reported by Ahmed et al. (2009).
- The relative tail length is slightly longer in male for the two species but is statistically not significant.
- The head size of males is larger than in females for both the species but is also statistically not significant.
- The present study enlarged the dietary list of *B. niger* and *B. fasciatus.*

- New information on the clutch size of *B. niger* was recorded, where N = 4, mean (range) length = 12.67 mm (11.89– 13.76 mm); mean (range) width = 5.3 mm (4.77– 5.70 mm).
- A partial sequence of mitochondrial COI gene is obtained for *B. niger* (530 bp; GenBank Accession no. MN722642), and *B. fasciatus* (429 bp; GenBank Accession no. MN722643) from Mizoram.
- The phylogenetic analysis revealed that in *B. fasciatus* there is minimal genetic diversity between Mizoram and Southeast Asia.
- The sequence obtained from *B. niger* represents the first COI marker gene to be submitted for the species in NCBI GenBank.
- The COI sequences of *Bungarus* shows 445 nucleotide as conserved, 91 nucleotide position as variable, and 71 as parsimony informative sites.
- High genetic diversity is observed between the congeneric species *B. niger* and *B. fascia*tus (0.1378) in Mizoram.

CHAPTER 8

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Name of Exam	Year	Board	Subject	Percentage	Division
HSLC	**2010**	**MBSE**	**General**	**68 %**	**I**
HSSLC	**2012**	**MBSE**	**Science**	**59 %**	**II**
B.Sc	**2015**	**MZU**	**Zoology**	**81.3 %**	**I**
M.Sc	**2017**	**MZU**	**Zoology**	**70 %**	**I**
M.Phil (Course work)	**2019**	**MZU**	**Zoology**	**78.3 %**	**I**

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LALBIAKZUALA

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DEGREE : MASTER OF PHILOSOPHY

DEPARTMENT : ZOOLOGY

TITLE OF DISSERTATION : Study on the morphology, distribution and phylogenetic status of the genus *Bungarus* (Reptilia : Serpentes : Elapidae) in Mizoram, India

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PUBLICATIONS

Lalbiakzuala, Lalrinsanga, Vanlalchhuana, M. and Lalremsanga, H. T. (2019). *Preliminary survey on endoparasitism in Ophiophagus hannah (Reptilia: Serpentes: Elapidae) in Mizoram, India*. In: Lalchhandama. K (Eds.). Advances in Engineering Research. Vol. 178, Atlantic Press, Paris: pp. 221- 228.

Ashaharraza, K ., Rangasamy, V. , Lalremsanga, H. T., **Lalbiakzuala**, Sailo, J. and Charlton, T. (2019). A new state record of the Mandarin Rat *Snake Euprepiophis mandarinus* (Cantor, 1842) (Squamata: Colubridae: Coronellini) from Mizoram, India. *Amphibian and Reptile Conservation.* 13(1): 230- 234.

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Papers communicated

Lalbiakzuala, Vanlalhrima and Lalremsanga, H. T. (2019). *Pareas mizoramensis* (Reptilia: Squamata: Pareidae), a new species of pareid snake from Mizoram, Northeast India. *Journal of Threatened Taxa*. (Accepted).

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Lalbiakzuala, Lalrinsanga, Laltlanchhuaha, H. and Lalremsanga, H. T. (2019). Natural History Notes: *Trimeresurus erythrurus* (Reproduction). *Herpetological Review.* (Under review).

Lalbiakzuala, Lalremsanga, H. T., Ramhermawia, J., Lalrinkima, H., Lalramliana,Lalchhandama, K. and Malsawmtluangi. (2019). Natural History Notes: *Ophiophagus hannah* (Endoparasite). *Herpetological Review.* (Under review).

Lalmuansanga, Gospel, Z. H., **Lalbiakzuala**, Lalrinsanga and Lalremsanga, H. T. (2019). Notes on Geographic Distribution: *Theloderma nagalandensis* (A new state record for Mizoram). *Herpetological Review.* (Under review).

Remruatpuii, **Lalbiakzuala** and Lalremsanga, H. T. (2019). Natural History Notes: *Smithophis atemporalis* (Reproduction). *Herpetological Review.* (Under review).

Lalbiakzuala, Lalrinsanga, Lalremsanga, H. T., Romalsawma , Vanlalhrima, Sailo, V. and Laltlanchhuaha, H. (2019). Natural History Notes: *Bungarus niger* (Diet and Maximum/Minimum Elevation). *Herpetological Review.* (Accepted).

Lalbiakzuala, Lalrinsanga, Lalremsanga, H. T., Romalsawma , Vanlalhrima and Laltlanchhuaha, H. (2019). Natural History Notes: *Bungarus fasciatus* (Diet). *Herpetological Review.* (Accepted).

SEMINARS/WORKSHOPS/CONFERENCES PARTICIPATED

- Presented paper entitled **"Diversity and phylogenetic analysis of *Boiga cyanea* (Reptilia: Serpentes: Colubridae) with COX 1 gene sequence in Mizoram"** (12- 14th November, 2018) in the International Conference on Biodiversity, Environment and Human Health: Innovations and Emerging Trends (BEHIET 2018); organized by School of Life Sciences, Mizoram University, and Association of Biotechnology and Pharmacy (ABAP), India.
- Presented paper entitled **"Preliminary Survey on Endoparasitism *in Ophiophagus hannah* (Reptilia: Serpentes: Elapidae) in Mizoram"** (4-5th October, 2018) in the Mizoram Science Congress 2018 (MSC 2018); organized by Mizoram Science, Technology and Innovation Council (MISTIC) in association with Mizoram Science Society (MSS), Mizo Academy of Sciences (MAS), Geological Society of Mizoram (GSM), Science Teacher's Association of Mizoram (STAM), Mizoram Mathematics Society (MMS), and Biodiversity and Nature Conservation Network (BIOCONE).
- Presented paper entitled **"Inventory Survey on Snake Fauna of Hmuifang Community Reserved Forest"** (4-5th October, 2018) in the Mizoram Science Congress 2018 (MSC 2018); organized by Mizoram Science, Technology and Innovation Council (MISTIC) in association with Mizoram Science Society (MSS), Mizo Academy of Sciences (MAS), Geological Society of Mizoram (GSM), Science Teacher's Association of Mizoram (STAM), Mizoram Mathematics Society (MMS), and Biodiversity and Nature Conservation Network (BIOCONE).

- Attended **"National Symposium on Avian Biology & Comparative Physiology"** (22- 24th October, 2018) organized by Department of Zoology, Mizoram University.
- Attended **"National Workshop on A brief Introduction to Bioinformatics and System Biology"** (13- 14th December, 2018) organized by Bioinformatics Infrastructure Facility (BIF), Department of Biotechnology, Mizoram University.

ABSTRACT

STUDY ON THE MORPHOLOGY, DISTRIBUTION AND PHYLOGENETIC STATUS OF THE GENUS *BUNGARUS* (REPTILIA: SERPENTES: ELAPIDAE) IN MIZORAM, INDIA

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STUDY ON THE MORPHOLOGY, DISTRIBUTION AND PHYLOGENETIC STATUS OF THE GENUS *BUNGARUS* (REPTILIA: SERPENTES: ELAPIDAE) IN MIZORAM, INDIA

BY

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Submitted in partial fulfillment of the requirements for the degree of

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The present study deals with the morphological study, distribution and phylogenetic analysis on the snake genus *Bungarus*, Daudin, 1803, in Mizoram. Presently, there are 16 extant species under this genus out of which 4 species are recorded from northeastern India. In Mizoram, previous researchers reported 2 species i.e *Bungarus niger* (Greater Black Krait), and *Bungarus fasciatus* (Banded Krait). There is scanty of literature regarding the comparative morphological study within and between the two species as well as on their natural history. In spite of the fact that few localities for the species has been recorded within Mizoram, a detail survey or documentation is not available on the status of distribution pattern of the snake genus *Bungarus* in the statewide. No report or publication was available on the molecular characterization of snakes belonging to the genus *Bungarus* from Mizoram. Moreover, there is limited number of DNA marker gene sequence from these species in databases, thus the genetic diversity radiated by the two species is waiting to be unveiled. This work also represents a comparative study between the two species as far as practicable.

During this work, 2 species of *Bungarus* i.e *B. niger* and *B. fasciatus* are confirmed. Further research is strongly suggested since there is a high possibility on finding other species of krait especially the Lesser Black Krait (*B. lividus*) which was found sympatric with the greater Black Krait (*B. niger*) in the neighbouring states and other distribution ranges. In fact there is a photographic evidence of the North-Eastern Hill Krait (*B. bungaroides*) from Tuipang, Chhimtuipui District, Mizoram. But no state report confirmation has been made due to the lack of specimen for the time being. This work yields a couple of new information regarding the context of

their morphology, distribution, natural history, as well as in enhancing the existing information on their molecular taxonomy. The specimens collected from different localities within the statewide were represented by preserved museum specimens of Departmental Museum of Zoology, Mizoram University, newly collected specimens, as well as private collections. A new range of elevation for *B.niger* was recorded i.e 42 – 1646 m asl. vs.100 – 1500 m asl. (Ahmed et al., 2009), However, in *B. fasciatus* the altitudinal range falls within the range reported in literatures i.e 49 – 1426 m asl. vs. 40 – 2300 m asl. (Ahmed et al., 2009). In *B. niger,* 18.75 % of the records are found at 0 – 500 m asl., while 40 % of the *B. fasiatus* records falls within this range. Moreover, no record of *B. fasciatus* is available at the elevation range of above 1500 m asl. whereas in *B. niger,* 2.08 % of the records are found in this elavational range. The comparison between the two species mean elevation gradient distribution revealed a statistically significant. So, it was assumed that *B. fasciatus* is more likely to be a lower elevation preferring species as compared to *B. niger* the sympatric congener species.

A total of 32 specimens (M = 26; F = 6) in *B. niger,* and 13 specimens (M = 5; F = 8) in *B. fasciatus* were morphologically studied. The pholidosis data on *B. niger* divulged a new ventral range for the species in the lower limit, and in the upper limit on the range of subcaudals. In the case of *B. fasciatus,* all of the pholidosis falls within the range reported in the existing literatures. Data analysis indicated that in both the sexes of *B. niger,* relative tail length is highest in small snakes, then gradually decreases in larger snakes. Moreover, the morphological constraint hypothesis was agrred in *B. niger* since the data analysis supports the prediction where the snake with longer tail have longer (but not wider) hemipenis. A detailed

description of the hemipenis in both the species was also provided. However, no significant difference is observed in the structure of the hemipenis within the conspecific male population. The statistical analysis for the test of sexual dimorphism on relative tail length and the head dimension for the two species are not statistically significant, but a significant result is not unexpectable from larger sample size in future.

The observation of *B. niger* feeding on *C. radiatus, P. pulverulentus, A. diardi,* and *O. albocinctus*; as well as the documentation of roadkilled *B. fasciatus* with the exposed gut content containing *B. ochracea,* and the observation of its feeding on *A. diardi* enlarged the dietary lists of the two krait species. New information on the reproductive habits of *B. niger* was also contributed with the documentation of clutch size which is 4 eggs with measuring 11.89 – 13.76 mm in length, and 4.77 – 5.7 mm in width.

From both the species, the COI sequences were obtained and inferred for their evolutionary history using Maximum Likelihood method and Bayesian inference phylogeny. In *B. fasciatus,* the obtained sequence from Mizoram is showing a minimal genetic distance with that of China and Vietnam, thus showing a negligible genetic diversity between them i.e 0.0070. However, the highest genetic distance is seen between the species of Mizoram and India (mainland) by a genetic diversity of 0.0165. Thus, according to the present study, the *B. fasciatus* of Mizoram is presumable to be genetically closer to the Southeast Asian population in comparing to that from India, but further extensive research with more samples (morphological and molecular data) from both mainland India and northeastern India

is strongly suggested in order to clarify the complexities of the species as well as to the ideas on the dispersal of the species biogeographically.

In *B. niger*, the obtained sequence is showing minimal genetic distance to the congener species *B. candidus*, occupying the same clade which is most likely to be caused by the unavailabily of the conspecific sequence in the databases. However, the analysis of the obtained sequences revealed that the two sympatric congener species of krait from Mizoram i.e *B. niger* and *B. fasciatus* were showing a high level of genetic diversity between them i.e 0.1378. The analysis of COI sequences of *Bungarus* from generated and those obtained from Databases showed 445 nucleotide positions as conserved, 91 nucleotide position as variable, and 71 nucleotide position as parsimony informative sites. Tajima Relative Rate Test of Molecular Clock between the *B. fasciatus* of Mizoram and India (mainland) revealed that they have equal and constant rates of evolution. According to the present findings and existing literatures, biogeographically *B. niger* and *B. fasciatus* can be assumed as Gondwanan origin that later dispersed in this region around 40 million years ago.