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RESEARCH ARTICLE

Occurrence and Sexual Dimorphism of *Chalcosoma atlas* (Linnaeus, 1758) (Coleoptera : Scarabaeidae) in Mizoram, North-East India

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Abstract

Objectives: The present study aims to validate the presence of *Chalcosoma atlas* in Mizoram, India, and to provide morphological and molecular descriptions to reveal sexual dimorphism for future reference. **Methods:** *Chalcosoma atlas* was sampled during monsoon season in 2021 near Palak lake, Mizoram. Specimens were collected using light traps at night and were further preserved in 70% alcohol. Species identification and morphological measurements were done along with the extraction of male and female genitalia. Statistical analysis was performed using SPSS version 28.0. Student's T-test and Pearson's correlation were performed to determine significant differences between various morphological characters. The molecular data for the species was generated by performing maximum likelihood phylogenetic analysis using a partial mitochondrial 16S rRNA gene. **Findings:** Four male and two female *Chalcosoma atlas* were collected. The collected samples were measured using a slide calliper and their morphological characters along with the differences in male and female were recorded. The molecular analysis using the 16S rRNA gene shows that all the collected samples show similarity ($\geq$99%) with the database sequence of *C. atlas*. Analysis using Student's T-test shows that some characters of male and female have a significant relationship. Pearson's correlation shows that there is a significant positive relationship between different characters in both males and females while a significant negative correlation was observed only between some female characters. Male and female genitalia were taken out and photographed as they also serve as an important differentiating character. **Novelty:** *Chalcosoma atlas* was previously reported from Mizoram, India. However, this study validates the occurrence of *C. atlas* in Mizoram, India with detailed morphological and molecular descriptions required for the identification of the species and diagnosis of sexual dimorphism between male and female *C. atlas*.

Keywords: Correlation; Genitalia; Occurrence; Sexual dimorphism; Bio diversity hotspot

1 Introduction

The giant rhinoceros beetle, one of the largest beetles in Asia, belonging to the genus *Chalcosoma* (Hope, 1837) are the most unique and conspicuous group of insects widely distributed in tropical Asia and Australia [1]. They consist of five species namely *Chalcosoma argreggs* (Takeuchi, 2014), *C. chiron* (Olivier, 1789), *C. atlas* (Linnaeus, 1758), *C. moellenkampii* (Kolbe, 1900) and *C. engganensis* (Nagai, 2004) [2].

Chalcosoma atlas, in particular, are found in low to medium-elevation forests (mainly 0-1,200 m a.s.l) of Southeast Asia (except Java) viz. Palaearctic (Nepal), Oriental (Vietnam, Cambodia, Myanmar, Malaysia, Indonesia: Sumatra, Nias, Java, Sulawesi, Ambon, Borneo, Phillipines: Luzon, Mindanao, India: Mizoram) [1,3]. The species is diagnosed by having a trident-shaped horn that is broader than other species of the genus *Chalcosoma* [4].

A lot of research has been done to elucidate the evolutionary processes that underlie sexual dimorphism patterns between animals [5]. The direction and extent of the sexual differences in body shape and size in many insect species are greatly influenced by sexual dimorphism and particular developmental processes [6]. Beetles (Coleoptera) are cosmopolitan in distribution and exhibit great sexual dimorphism among different species. Scarabaeidae is one of the largest families of Coleoptera and are easily recognized by their distinctive lamellate antennae. For decades, beetle body size has been employed to study sexual dimorphism, population differentiation, and taxonomy differentiation [7,8].

The evolutionary foundation for the study of sex differences is provided by Darwin's sexual selection theory, which includes intrasexual competition for mates and discriminative mating partner choice (intersexual choice) [9]. Although niche differentiation between sexes through ecological character displacement can cause sexual dimorphism, its development has traditionally been attributed mostly to differences in the strength of sexual selection acting on males and females. [10]. Competition and selection dynamics not only result in the evolution of sex variations in trait size or degree of elaboration, but they also make these traits more sensitive to social and ecological pressures. Exposure to stressors will have a greater impact on the development and expression of sexually selected traits than on other traits. As a result, sex differences become smaller and more unpredictable than they would be under more favourable conditions [9].

A recent investigation in Mizoram resulted in the collection of *Chalcosoma atlas*, a species that was previously reported from Bilkhawthlir, Mizoram [11]. Previous reports on *C. atlas* lacked detailed morphological information, and even, the original description has been vague, such scarcity of information resulted in difficult taxonomic identification. The molecular data for the species was also generated using the mitochondrial 16S rRNA gene. For further species identification, the male and female genitalia were also diagnosed and photographed. The purpose of this study is to validate the occurrence of *C. atlas* from Mizoram as well as to provide the description and diagnosis required for the identification of male and female *C. atlas* using different morphological characters and to reveal sexual dimorphism in the species.

2 Methodology

2.1 Sampling and preservation

Four male and two female *Chalcosoma atlas* beetles were collected from Palak lake which is located inside Palak Wildlife Sanctuary, Siaha (22°20′25″N 92°56′33″E) and is the largest and biggest natural lake in Mizoram, North-East India. The lake is oval and covers about 1 square km. It is located within the Indo-Myanmar biodiversity hotspot and is surrounded by forests rich in flora and fauna. The specimens were collected at night using light traps during monsoon season in the year 2021. The collected beetles were preserved in 70% ethanol until they are taken for morphological analysis and brought to Research and Instrumentation Centre, Pachhunga University College, Aizawl for further identification.

2.2 Morphological measurement and identification

Species identification is based on an accessible online generic guide to new world scarab beetles [12] and Rowland and Miller [13]. Morphological measurements and terminology followed Kawano [14]. The collected specimens were measured with a slide-calliper to the nearest 0.1mm for body length (BL), the distance from the front of the head [excluding horns or mandibles] to the tip of the body along the center of the line, mandible length (ML), the distance from the base to the tip, body width (BW) or breadth at mid elytra (the width of the body/elytra at its widest point), the head length (HL) and head width (HW) or breadth of the head, pronotum width (PW), the width of the pronotum at its widest point, pronotum length (PL), elytral length (EL), humeral width (HuW), antennal length (AL), pro, meso and meta femoral width (FWp, FWms, FWmt), pro, meso and meta femoral length (FLp, FLms, FLmt), pro, meso and meta tibia width (TWp, TWms, TWmt), pro, meso and meta tibia length (TLp, TLms, TLmt), eye diameter (ED). The segment from where the antenna is club, the tarsal formula along with the length

of the 1st, 2nd, 3rd, 4th, and 5th tarsal segment of pro, meso, metatarsus (Tlp, Tlmt, Tlms) was also recorded.

In male, the head horn length (the straight-line distance from the base to the tip), the left and right pronotum horn (distance from base to tip) and the anterior pronotum horn length were also measured. To retrieve the genitalia, the abdomen of one male and female specimen was separated from the body, and the external male and female genitalia were extracted, washed, and softened in a bowl of hot water. It was then washed in a hot water solution containing 10% potassium hydroxide (KOH). All male genitalia were cleansed in 95% ethanol and kept in a glass vial containing 70% alcohol[15]. Photos were taken with the help of a binocular microscope (Leica S9i KL300 Stereo Zoom). The length of the male and female genitalia was measured with a slide-calliper to the nearest 0.1mm.

2.3 Phylogenetic analyses

The genomic DNA was extracted from the ethanol-fixed soft tissue of six specimens collected from Palak Wildlife Sanctuary. Genomic DNA extraction was performed using Phenol Chloroform Isoamyl Alcohol (PCI) method[16]. Extracted gDNA was used as a template for the amplification of the partial 16S rRNA mitochondrial gene.

PCR amplification was conducted in a 25-μL reaction mix with Emerald Amp GT PCR Master Mix (Takara Bio, India) and 2 μL genomic DNA will be used as a template and partial 16s rRNA primers: forward (16SF13398 - CGCCTGTTTATCAAAAACAT) and reverse (16SR12887 — CTCCGGTTTGAACTCAGATCA)[17]. The PCR amplification was performed using ProFlex^{TM} thermocycler (3x32-Well PCR System, Applied Biosystems) with the thermal regimes; 4 minutes at 95°C for initial denaturation, followed by 35 cycles of 50 seconds at 94°C for second denaturation, 1 minute for annealing at 54°C, elongation for 1 minute at 72°C, and a final elongation for 7 minutes at 72°C. PCR products were checked using 1.5% agarose gel electrophoresis.

Samples were then sequenced using Sanger's dideoxy method, and sequencing reactions were carried out only for forward direction on a sequencer (Eurofins Genomics India Pvt. Ltd, Bangalore, India). All the generated sequences were submitted to NCBI GenBank. The phylogenetic analyses included 11 nucleotide sequences consisting of 511 bp for 10 ingroup taxa and one outgroup taxon. All phylogenetic analyses were performed in MEGA X[18]. The alignments were performed using ClustalW[19] with default parameter settings and pairwise distances were calculated (Table 1). The Maximum Likelihood tree was constructed using Hasegawa-Kishino-Yano model with gamma distribution (HKY+G)[20] (Figure 1).

Table 1. Uncorrected pairwise genetic distance of *Chalcosoma atlas* and its congeners retrieved from the NCBI database

No	Species	P- distance										
		1	2	3	4	5	6	7	8	9	10	11
1	Chalcosoma atlas OQ000257											
2	Chalcosoma atlas OQ000258	0										
3	Chalcosoma atlas OQ000259	0	0									
4	Chalcosoma atlas OQ000260	0	0	0								
5	Chalcosoma atlas OQ000261	0	0	0	0							
6	Chalcosoma atlas OQ000262	0	0	0	0	0						
7	Chalcosoma atlas JX994028	0.004	0.004	0.004	0.004	0.004	0.004					
8	Chalcosoma atlas mantetsu AB758833	0.012	0.012	0.012	0.012	0.012	0.012	0.008				
9	Chalcosoma caucasus JX994029	0.026	0.026	0.028	0.026	0.028	0.028	0.033	0.038			
10	Chalcosoma caucasus chiron AB758852	0.025	0.025	0.025	0.025	0.025	0.025	0.02	0.025	0.022		
11	Allomyrina dichotoma tunobosonis LC075184	0.067	0.067	0.067	0.067	0.066	0.067	0.064	0.064	0.064	0.06	

2.4 Statistical analysis

Various statistical analyses were performed using SPSS version 28.0[21]. Student's t-test was performed to determine significant differences between various morphological characters between males and females. Pearson's correlation coefficient (r) was performed to determine if there are significant relationships between various morphological characters in both males and females.

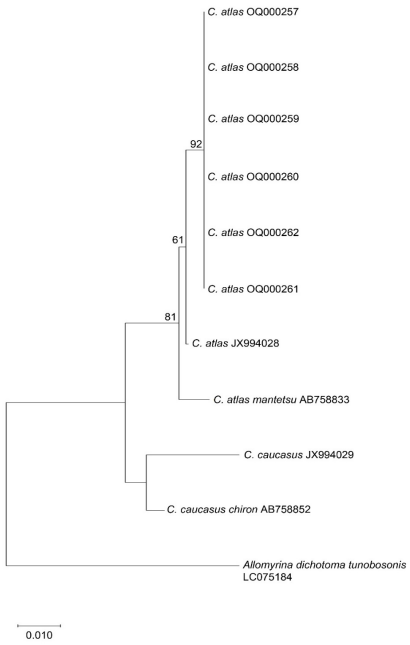

Fig 1. Maximum likelihood phylogenetic tree of *Chalcosoma* constructed based on the partial 16S rRNA gene and the comparison with its congeners retrieved from the NCBI database; number at nodes indicate bootstrap proportion support value (50% or more, 1000 replicates). Numbers after each species indicate the GenBank Accession number.

3 Results and Discussion

3.1 Systematic account

Family : Scarabaeidae Latrielle, 1802
Subfamily : Dynastinae MacLeay, 1819
Tribe : Dynastini MacLeay, 1819
Subtribe : Chalcosomina Rowland and Miller, 2012
Genus : *Chalcosoma* Hope, 1837
Species : *Chalcosoma atlas* Linnaeus, 1758

3.2 Diagnosis

The males have a larger and greater body size which is a general feature of sexually dimorphic species. The males of *C. atlas* have long, large forwardly dorsolateral horns on the prothorax, and the apex of the cephalic head horn acuminates. The pronotum of male are glossy and smooth while females have a punctured and glossy pronotum. Males have a smooth elytron while females have small setae present along the length of the sutural elytral margin which are large on the midline and tends to be smaller towards the lateral sides. The femur of males has less hair as compared to females which have a hairy femur (Figure 2). In males, the mean length of the head horn is 37.68mm, the mean length of the right pronotum is 37.55mm, the mean length of the left pronotum horn is 37.30mm and the mean length of the anterior horn is 3.75mm.

3.3 Description

Chalcosoma atlas has tarsal formula 5-5-5, and they have olive green eyes. Antennae 10-jointed, they are club between 8^{th} to 10^{th} segment in both males and females and are capable of being closed together. The antennal insertion is not visible from above with mandibles exposed from dorsal view. The mandibles and labrum do not project anteriorly beyond clypeus. Procoxae transverse. The base of pronotum and elytra are subequal in width. There are always two spurs on the apex of the posterior tibia.

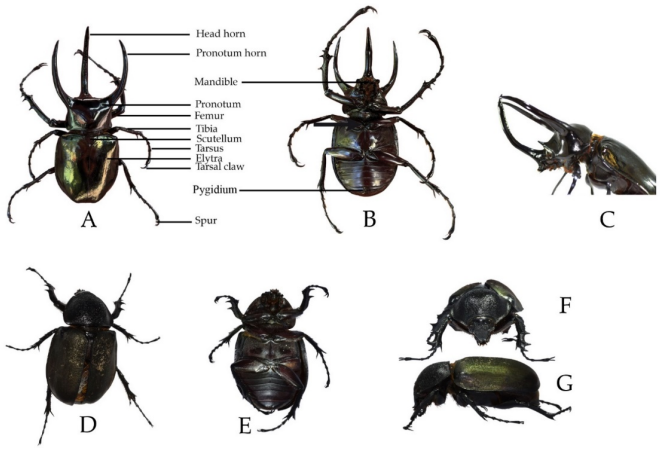

Fig 2. ***Chalcosoma atlas*** **(Male & Female) Dorsal view (A&D), Ventral view (B&E), Lateral view (C&G), Frontal view (F).**

The mesotibial spurs at the apex are simple and not pectinate. The metatibial spines are adjacent and are not separated by the base of the tarsomere. The scutellum is visible, normal, and not enlarged. They have an oblong body and cannot be rolled into a sphere. Their middle and posterior tibiae are dilated and significantly flattened. They have 6 ventral sclerites in their abdomen with 8th abdominal segment lacking spiracle. The abdominal sternites are normal and not narrowed at midline with the length of all sternites longer than the length of metasternum. Their dorsal surface is variably sculptured and shining. Their elytra are not shortened and are not widely divergent at apex with exposed pygidium. The claws of the middle and posterior tarsi are simple, not independently movable and are both equal in length.

The adult specimens collected were carefully measured. The mean body length, body width, length and breadth of head, mandible length, pronotum length and width, elytral length, humeral width, antennal length, eye diameter in males were found to be larger and bigger than in females, and they were found to be statistically significant (t-test at $p<0.05$) except for pronotum width, antennal length, and eye diameter (t-test at $p>0.05$). The mean pro, meso and meta femoral width, femoral length, tibia width, tibia length, 1st, 2nd, 3rd, 4th, and 5th tarsus length of male and female were also measured and were found to be statistically significant (t-test at $p<0.05$) except for pro and meta femoral width, meso tibia width, meta tibia length, pro and meta 1st tarsus length (t-test at $p>0.05$). Thus, T-test indicated that various morphological characters in males and females are significantly ($p<0.05$) different (Figure 3).

Beetles are cosmopolitan and widely distributed. The climatic conditions as well as geographical features enable India to harbor and sustain high diversity. Numerous works had been done to reveal the diversity of beetles in India by many researchers. The occurrence of *C. atlas* in India was previously reported with no illustrated keys for identifying this species. Since then, no work or report had been made regarding the occurrence as well as providing their morphological characters. The study of morphological characters is necessary as this will provide better knowledge for the classification of this species. Sexual dimorphism occurs in many species of beetles, ranging from completely indistinguishable to huge in size and form with all degrees and types of intermediates [13]. Most species of the subfamily Dynastinae display a very strong sexual dimorphism [22]. The mean body length and body width of male *C. atlas* along with different characters were found to be greater than females (Figure 3).

The males of *C. atlas* are larger than females where males can reach up to 60 mm and females about 25 mm [3]. The male and female of most beetle species are only distinguishable by slight (often microscopic) physical differences. The development of horns in *C. atlas* is generally restricted to males. They have extravagant horns which comprise cephalic (head) horn with a broad end, a large recurved horn on the head and two large directed horns on the prothorax. Conspicuous male characters in *C. atlas* serve largely as a mechanism for intraspecific male-male competition [4]. Male alternative mating strategies exist in a variety of animals, with larger individuals outperforming conspecific males and occupying better feeding or mating areas, whereas smaller individuals often lose in combat and occupy marginal places [14]. Thus, the larger male of *C. atlas* will be superior as compared to the smaller male and will have a better chance of mating with females.

Pearson's correlation was performed to observe the relationship between different characters in males and females. A significant positive correlation was observed between different characters in both males and females. This shows that the increase of one character will result in the increase of other characters in both males and females. A significant negative

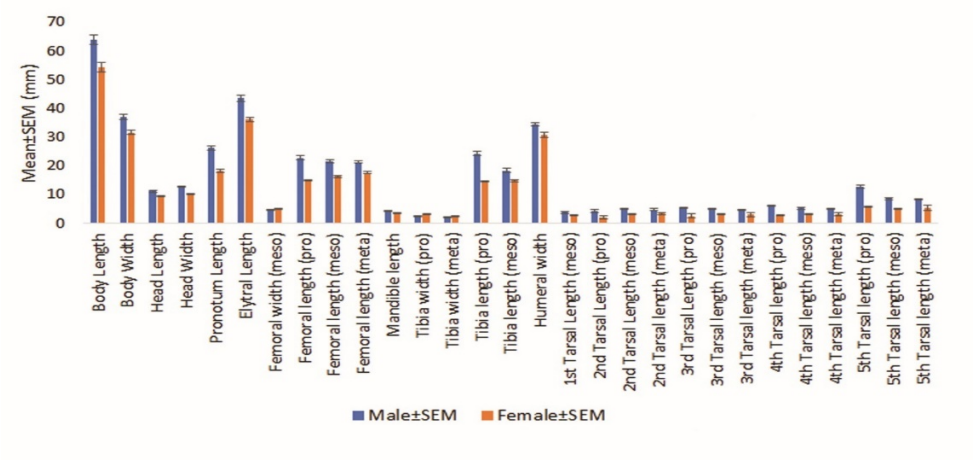

Fig 3. t-Test between male and female morphological characters of *Chalcosoma atlas*. *Statistically significant at 0.05 level.

correlation was not observed between male characters while it was observed in females between HL and 5thTLp as well as 1stTlms and 5thTlms. This indicates that with the increase or growth in HL, the 5thTLp of females will decrease and with the increase of 1stTlms, the 5thTlms of females will also decrease (Figures 4 and 5).

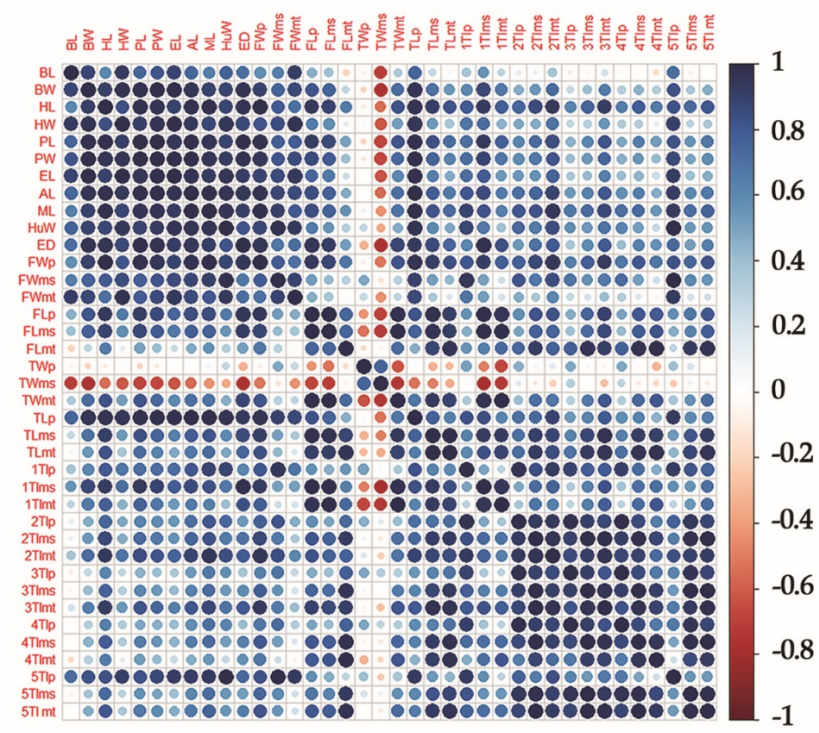

Fig 4. Pearson's correlation coefficient (r) between various morphological characters in male (Darker colour shows significant correlation while faint colour shows less correlation between different characters). *Statistically significant at 0.05 level.
**** Statistically significant at 0.01 level.**

The six generated sequences (OQ000257 — OQ000262) exhibited 99.59% and 98.73% similarity with the database sequence of *C. atlas* (JX994028) and *C. atlas mantetsu* (AB758833) respectively with the intraspecific distance of 0.4% — 1.2%. The intraspecific distance among the developed sequences was found to be 0.0%. However, the generated sequences show an inter-specific distance of ≥2.5% with the database sequence of another *Chalcosoma* sp. available from NCBI GenBank. This shows

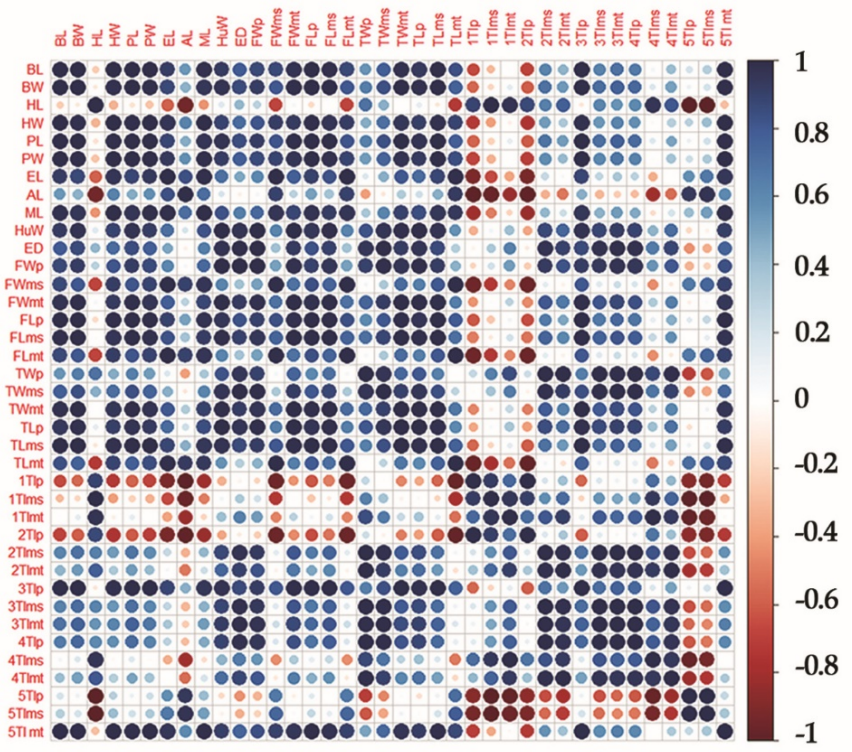

Fig 5. Pearson's correlation coefficient (r) between various morphological characters in female (Darker colour shows significant correlation while faint colour shows less correlation between different characters). *Statistically significant at 0.05 level. ** Statistically significant at 0.01 level.

that *C. atlas* forms a sister taxon with *C. chiron*. However, the wider end of the cephalic (head) horn distinguishes *C. atlas* beetle from other *Chalcosoma* species (such as *C. chiron*) [4]. The maximum likelihood tree of *C. atlas* inferred from the partial 16S rRNA gene was shown in Figure 1 and the estimated pairwise distance was shown in Table 1. The lack of appropriate molecular information for *Chalcosoma* in the data bank may have an impact on the phylogenetic position of *C. atlas* in our research. We anticipate that this new information will advance the current scant molecular knowledge of this species group.

After retrieving the genitalia, the length of the male genitalia was 14.4 mm and the length of the female genitalia was 3.6 mm. One of the most crucial diagnostic features in many investigations has been the male genitalia, which has been utilized to establish higher taxonomic levels and, primarily, to delimitate species. Male genitalia consists of two parts: the movable parameres and the phallobase. It has been revealed that variations in paramere length affect how the female is held mechanically during copulation. [23]. As a result, the length of the male penis plays an important role in mating success. In the male genitalia, the length of the phallobase were twice shorter than the parameres. Parameres were broad at the base and slightly tapered distally and curved ventrally with pointed tips and slightly bulging out at the middle. The female genitalia consist of an oval-shaped vaginal palp with a valvifer located at its top (Figure 6).

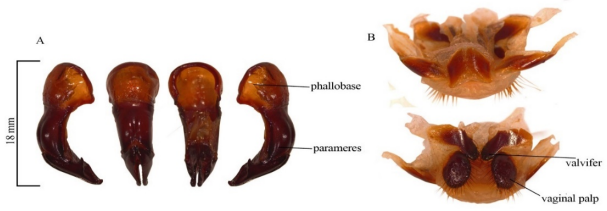

Fig 6. A. Male genitalia showing parameres and phallobase. B. Female genitalia showing valvifer and vaginal palp

4 Conclusion

The description and diagnosis of different morphological characters were provided. The significant variations observed between male and female characters in this study explained the occurrence of sexual dimorphism in this species. This will be useful for identifying male and female species, as well as for elucidating issues concerning the evolutionary relationships among this group. Analysis of intraspecific variation would also help to clarify the origins of each trait, and a comparison of intraspecific variation in sexually dimorphic traits at various phylogenetic levels would help to clarify the evolutionary history of sexual dimorphism. This study validated the presence of *Chalcosoma atlas* in Mizoram, India, and confirmed the existence of sexual dimorphism by providing detailed morphological and molecular descriptions which are expected to be useful for future references.

5 Declaration

Presented in 4th Mizoram Science Congress (MSC 2022) during 20th & 21st October 2022, organized by Mizoram Science, Technology and Innovation Council (MISTIC), Directorate of Science and Technology (DST) Mizoram, Govt. of Mizoram in collaboration with science NGOs in Mizoram such as Mizo Academy of Sciences (MAS), Mizoram Science Society (MSS), Science Teachers' Association, Mizoram (STAM), Geological Society of Mizoram (GSM), Mizoram Mathematics Society (MMS), Biodiversity and Nature Conservation Network (BIOCONE) and Mizoram Information & Technology Society (MITS). The Organizers claim the peer review responsibility

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