Advances in Entomology, 2024, 12, 130-142
https://www.scirp.org/journal/ae
ISSN Online: 2331-2017
ISSN Print: 2331-1991

Advanced Insights into *Goliathus orientalis* (Coleoptera: Scarabaeidae) of Tanzania

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How to cite this paper: Rwegasira, G.M. and Kayombo, P.M. (2024) Advanced Insights into *Goliathus orientalis* (Coleoptera: Scarabaeidae) of Tanzania. *Advances in Entomology*, **12**, 130-142.
https://doi.org/10.4236/ae.2024.122010

Received: February 5, 2024
Accepted: April 26, 2024
Published: April 29, 2024

Abstract

Members of the *Goliathus* group in the order Coleoptera, family Scarabaeidae and the sub-family Cetoniidae is one among essential ecological insects worldwide with scanty documentation in Tanzania. The country was among the first point of collection of the specimen by Moser (1909) but subsequent attempts to locate and collect the specimen were unsuccessful possibly due to changed habitat and behaviour of Goliathus. Much of published work about the insect are based on the morphological characteristics of collected specimen which led to designation of six major species namely; *Goliathus goliatus*, *G. cacicus*, *G. regius*, *G. albosignatus*, *G. orientalis* and *G. usambarensis,* the last two being considered to exist in Tanzania. The current study reports on successful identification of habitats using biological cues and subsequently collected several *Goliathus* specimen that had been difficult to trace for more than a century. New insights into correct identity of the species by using a combination of morphological and molecular techniques have been set. The study has resolved that only one species, *Goliathus orientalis* with five sub-species exist in Tanzania.

Keywords

Coleoptera, *Goliathus* Species, Morphological Features, Barcoding, Tanzania

1. Introduction

Goliathus orientalis presents one diverse arthropods of the order Coleoptera, sub-order Polyphaga, superfamily Scarabaeoidae, in the family Scarabaeidae and the sub-family Cetoniidae. Several genera are known to exist in Africa with a great diversity that has been documented under five different tribes namely, tribe Cetoniini, tribe Diplognathini, tribe Goliathini, tribe Gymnetini and tribe

Xiphoscelidini [1]. These insects are distributed throughout sub-Saharan Africa where they play several ecological roles including being part of the wider ecological niche, nutrient re-cycling and serving as biological indicator of naturally well-preserved forest habitats. The physical characteristics of Goliath beetles paint a portrait of true magnificence. Their colossal size and intricate, vibrant designs showcase the sheer diversity and artistry that nature has crafted over eons. As living testaments to the wonders of evolution, these beetles continue to captivate the imaginations of many scientists and serve as a reminder of the boundless beauty that the natural world has to offer [2].

Early works conducted on *Goliathus* in Tanzania covering the Eastern and Southern parts of Tanzania (Tanganyika by then) dated back to the 1900s whereby a private collector Moser managed to collect a giant beetle with a striking appearance and named the specimen as *Goliathus orientalis* Moser [3], the name being presumed to have been influenced by the old biblical terms where the name Goliath was attributed to Giant human being hence all giant creatures got associated with the name Goliath. Given the peculiarities and rareness of the specimen it was given special attention and kept in the Germany Museum in Berlin (the Museum Far Naturkunde Berlin German) [4]. Several attempts that were made by various professional collectors to unveil the species or close relatives proved futile.

The available vital publications inclusive of Knirsch [5]; Wiebes [6]; Lachaume [7]; Sakai & Nagai [8]; Beinhundner [9]; Mawdsley [10] have been made without a proper resolve of the actual identification of the *Goriathus orientalis* species. According to De Palma *et al.* [4] six species of *Goliathus* are recognized namely; *Goliathus goliatus* (Drury 1770), *Goliathus cacicus* (Olivier 1789), *Goliathus regius* (Klug 1835), *Goliathus albosignatus* (Boheman 1857 with doubtful subspecies *Goliathus a. kirkianus* Gray 1864), *Goliathus orientalis* (Moser 1909) and *Goliathus usambarensis* (Preiss, 1933). Such recognition of the species has not been without confusion. Wiebes [6] considered *Goliathus meleagris* and *G. usambarensis* as junior synonym of *G. orientalis* under *G. goliatus* while Sakai & Nagai [8] treated *G. goliatus*, *G. orientalis* and *G. regius* as separate species. Moreover, confusion still surrounds the identity of *G. orientalis* and *G. usambarensis* and how the two are related to *G. melaegris* [4]. Unlocking the huddle on the two species *G. orientalis* and *G. usambarensis* believed to originate in Tanzania [1] [3] [4] [12] which could possibly resolve much of the unknown with other species was considered paramount by a team of scientist from Sokoine University of Agriculture that undertook an extensive research on the *Goliathus species.* Apart from lack of clear separation among species there was another challenge in locating the specimen after several attempts for almost a century failed to locate *G. orientalis* let alone the rarely found *G. usambarensis*. After a four years extensive research that span from 2019 to 2023, the present team managed to establish vital information about the giant beetle. Here we report the findings which considered the classical and molecular analysis of about 1339 specimen that aided the re-classification of the *Goliathus* species particularly the *G. orien-*

talis and *G. usambarensis*.

2. Material and Methods

2.1. Habitats Identification and Collection of Specimen

Extensive literature search was made to establish the ecological preferences of different species of *Goliathus* and develop the possible correlation with the location of the first collection of the specimen in Tanzania. Based on the presented description it was established that the first sample by Moser in 1909 was collected somewhere between Lindi and Ruvuma regions in Tanzania. The team took initiatives to assess the fauna and flora composition of the two regions whereby flora dominated by Miombo and Acacia trees were the main indicators for possible presence of *Goliathus* insects. Regions with similar flora and fauna composition namely Morogoro, Mtwara and Tanga were also considered for a new detailed search for the possible existence of Goliathus. Another aspect considered by the team was the possible changes in Fauna and Flora over the century that could have influenced the existence, dispersion and abundance of the *Goliathus* such that it could have influenced changes in the feeding behavior. As such, the key ecological cues that were regarded essential by the team included the dominant vegetation, presence of animals such as *Heterohyras spp* and *Dendrohyrax spp* whose wastes (urine and dungs) are preferred by various beetle species such as *Rhabdotis corpulenta* (Beinhundner 2008), *Rhabdotis Kayomboi* (Leonard 2019), *Argyrophegger kolbei* (Kraatz 1985), *Hegemus vittatus* (Bates 1888) and *Lonsbergia (Trapezorhina) Sordida albolineata* (Beinhundner 2013). Identification of the suitable collection site was random based on existing flora and fauna cues. Consideration of both above ground and underground composition of different localities was also made during the survey. Digging was made on the soil under the ground suspected to contain the insects and the beetles were picked by hands and preserved in ventilated jars and later sent to the entomological laboratory at SUA for detailed assessment and identification. A total of 1339 specimen were collected during the whole search conducted in four years.

2.2. Identification of Collected Specimen

Specimen vials were well labeled and dry-preserved prior to sending them to the molecular laboratory at Sokoine University of Agriculture for further assessment. Using the species-specific references [4] the collected specimen were identified based on the observable morphological characteristics. Further undertaking necessitated the molecular analysis to ascertain the observations made whereby some features had suggested the possible existence of new species.

2.3. Molecular Analysis of the Collected Specimen

2.3.1. DNA Extraction

Deoxyribonucleic acid (DNA) was extracted from the clubbed antennae of collected specimen from each of eleven prior established groups of Goliath beetles (based on morphological features) through a series of steps. The antennae were subjected to CTAB (Cetyl trimethyl ammonium bromide) treatment. Each sample underwent two washes with NE buffer (NaCl and EDTA) and was then homogenized in a 1.5 ml microcentrifuge tube containing 700 ul of 2× CTAB, Mercaptoethanol, and Polyvinylpyrrolidone. The mixture was vortexed and incubated at 65˚C for 30 minutes with slight shaking. Subsequently, 700 µl of Chloroform Isoamyl alcohol (24:1) was added, vigorously shaken for 2 minutes, and incubated at −20˚C. The supernatant obtained after centrifugation was collected, stored in a new micro-centrifuge tube and treated with 5 µl of RNase (10 mg/ml) at 37˚C for 1 hour. This was followed by the addition of 36 µl of Ammonium acetate and 420 µl of ice-cold isopropanol, mixed by inversion for 10 minutes at room temperature. The DNA pellet, obtained after centrifugation at 14,000 rpm for 20 minutes, underwent two washes with 500 µl and 150 µl of ice-cold 70% Ethanol. The pellet was air-dried on sterilized filter paper for 15 minutes, rehydrated with 50 µl of nuclease-free water, and stored at −20˚C until downstream analysis. The quality and concentration of DNA were evaluated using a Nanodrop spectrophotometer (Biochrom Ltd., Voltage 18VDC).

2.3.2. PCR Amplification

DNA obtained was subjected to Polymerase Chain Reaction (PCR) amplification using the Agilent SureCycler 8800 thermocycler. Each 10 µl PCR reaction mixture comprised 12.5 µl of OneTaq Quick Load 2× Master (New England BioLab) Mix with standard buffer, 1 µl of each primer (10 µM), 1 µl of DNA template, and 5.5 µl of Nuclease-free water (Fisher BioReagents). The region of the goliath beetle was amplified using the primer pair LCO1490 (GGTCAACAAATCATAA AGATATTGG) and HCO2198 (TAAACTTCAGGGTGACCAAAAAATCA). The thermocycling conditions included an initial denaturation at 94˚C for 1 minute, followed by 5 cycles of denaturation at 94˚C for 1 minute, annealing at 45˚C for 1 minute and 30 seconds, and extension at 72˚C for 1 minute and 30 seconds. This was followed by 35 cycles of 94˚C for 1 minute, 50˚C for 1 minute and 30 seconds, 72˚C for 1 minute, and a final extension at 72˚C for 5 minutes. The amplified products were stained with Ethidium Bromide before visualization on a 1% agarose gel under UV light. Positive PCR amplicons were purified using the ZR-96 DNA Cleanup kit from Zymogen Company and subsequently sent overseas for Sanger sequencing.

2.3.3. Sequence Processing

Initial Sanger sequences were imported into MacVector Software, Assembly Version 18.6.1, and subjected to default conditions. Cleaning involved trimming unreliable ends, and Chromatography view validation ensured accurate base pair calls. Consensus sequences, derived from both forward and reverse sequences for each isolate, were saved as FASTA files. These files were then queried against the Basic Local Alignment Search Tool (BLAST) to identify top hits based on e-value and query coverage. Selected matching sequences were downloaded and employed as reference sequences for subsequent analysis.

2.3.4. Multiple Sequence Alignment and Similarity Matrix

Consensus and reference sequences underwent further analysis using MacVector software. Employing ClustalW parameters under default settings, multiple sequences were aligned. The alignment revealed nucleotide insertions, deletions, and single nucleotide polymorphisms. A nucleotide similarity matrix was calculated to assess sequence similarities. The resulting aligned sequences were exported as a FASTA file for the construction of evolutionary relationship trees. Phylogenetic trees were generated using the Maximum Likelihood parameter with 1000 bootstrapping values in MEGA XI software.

3. Results

3.1. Specimen Collection Points and Assigned Names

Several 1339 *Goliathus* specimen were collected from two out of five regions that were surveyed. These were mainly Lindi and Tanga regions. Comparatively, more spacemen were collected from Lindi than Tanga regions confirming the fact that the first specimen collected by Moser in 1909 was truly from Lindi. Basing on the morphological features, the obtained results suggested an existence of several *Goliathus* species in Tanzania as detailed (**Table 1**).

3.2. Characterization of the Collected Specimen

Morphological features coupled with molecular resolution by DNA bar coding and the resultant phylogram (**Figure 1**) delineated five different clans (designated as sub-species) out of the 11 groups originally conceived based on the morphological features of the specimen. Subsequently the bootstrapping based on phylogenetic resolution led to the qualification of the clans into five sub-species of the species *Goliathus orientalis* (**Figure 2**). The five different sub-species being suggested from the present work includes the following; 1) *Goliathus orientalis tanzaniensis* (Kayombo & Rwegasira 2023), *Goliathus orientalis rwegasirai* (Rwegasira 2023), *Goliathus orientalis kayomboi* (Kayombo 2023), *Goliathus orientalis kayomutai* (Kayombo & Rwegasira 2023), and the re-named *Goliathus oriaentalis moser* (Moser) (Kayombo & Rwegasira 2023).

3.2.1. *Goliathus orientalis tanzaniensis* (Kayombo & Rwegasira, 2023)

The specimen bearing the name exhibits two oval shaped thick lines on the thorax coupled with two extra lines on each side to form a total of six lines curved towards the central axis (**Figure 3**). On the elytra, there are dark (black to chocolate) colored lines extending from near the base of the wings to the terminal position. The clear white bands dividing the dark colored lines are wide extending from the base to the terminal of the wings. All specimens bear the V mark at the base of the elytra. There are slight variations among specimen whereby some have dark chocolate colored lines which are densely packed (TZA09-E), which

Table 1. Information on the major categories of collected specimen and proposed new names.

Specimen Code	Origin (Sample location)	Scientific Name	Remarks
TZA03-4	Usambara Mountain	*Goliathus orientalis tanzaniensis*	The specimen were collected from multiple locations in Usambara in North eastern Tanzania and several locations in Lindi in the South-eastern Tanzania disqualifying the former naming of subspecies *G. orientalis usambarensis.* Further DNA bar coding confirmed the specimen to be genetically similar hence the resolve to re-name it as ***Goliathus orientalis tanzaniensis.***
TZA09-E	Lindi-Liwale (Ng'ende)	*Goliathus orientalis tanzaniensis*	
TZA05-G	Lindi-Liwale (Ng'ende)	*Goliathus orientalis tanzaniensis*	
TZA11-I	Lindi-Liwale (Ng'ende)	*Goliathus orientalis tanzaniensis*	
TZA08-1	Usambara Mountain	*Goliathus orientalis tanzaniensis*	
TZA10-C	Lindi-Liwale (Ng'ende)	*Goliathus orientalis rwegasirai*	The specimen collected from multiple locations in Lindi bore distinguishing morphological features but proven further to be genetically similar by DNA bar coding hence designated as a new subspecies ***Goliathus orientalis rwegasirai*** named after the collector.
TZA04-D	Lindi-Liwale (Ng'ende)	*Goliathus orientalis rwegasirai*	
TZA06-H	Lindi-Liwale (Ng'ende)	*Goliathus orientalis rwegasirai*	
TZA07-F	Lindi-Liwale (Ng'ende)	*Goliathus orientalis kayomutai*	The peculiar morphological and DNA characteristics led to designation of the new subspecies ***Goliathus orientalis kayomutai*** named after the two scientists who collected the specimen.
TZA02-B	Lindi-Liwale (Ng'ende)	*Goliathus orientalis kayomboi*	The peculiar brown color of the elytra and DNA characteristics totally different from other specimen led to the designation of the new subspecies ***Goliathus orientalis kayomboi***.
TZA01-A	Lindi-Liwale (Ng'ende)	*Goliathus orientalis moser*	The specimen had morphological resemblance with the holotype collected by Moser in 1909 and DNA bar coding designated the isolate into an independent cluster but not much distinct to qualify a new species hence named as subspecies ***Goliathus orientalis moser***.

may appear more or less similar to specimen (TZA08-1) but the only difference is that the dark-chocolate colored lines in the later are more spaced with clear orientation from the base to the terminus position of the elytra. Another variation which is apparent on specimen (TZA03-4) is prominence of cream-white (off white) bands with dark zigzag lines that extends from some distance off the base of the elytra to the terminal position. The dark lines on the extreme ends (margins) of each side of elytra extend from the base to the terminus position. Generally, this specimen is less densely colored and the dark lines are thin and less apparent compared to the rest of the specimen of the same species.

The specimen (TZA05-G) exhibits chocolate cored lines separately extending from the base of the elytra but conjoins later towards the terminus position of the elytra. The three lines on each side conjoins into less separate lines making them appear as if are joined towards the tip. The far lines on the margins of the wings are black and clearly extend from the base to the terminus position. The

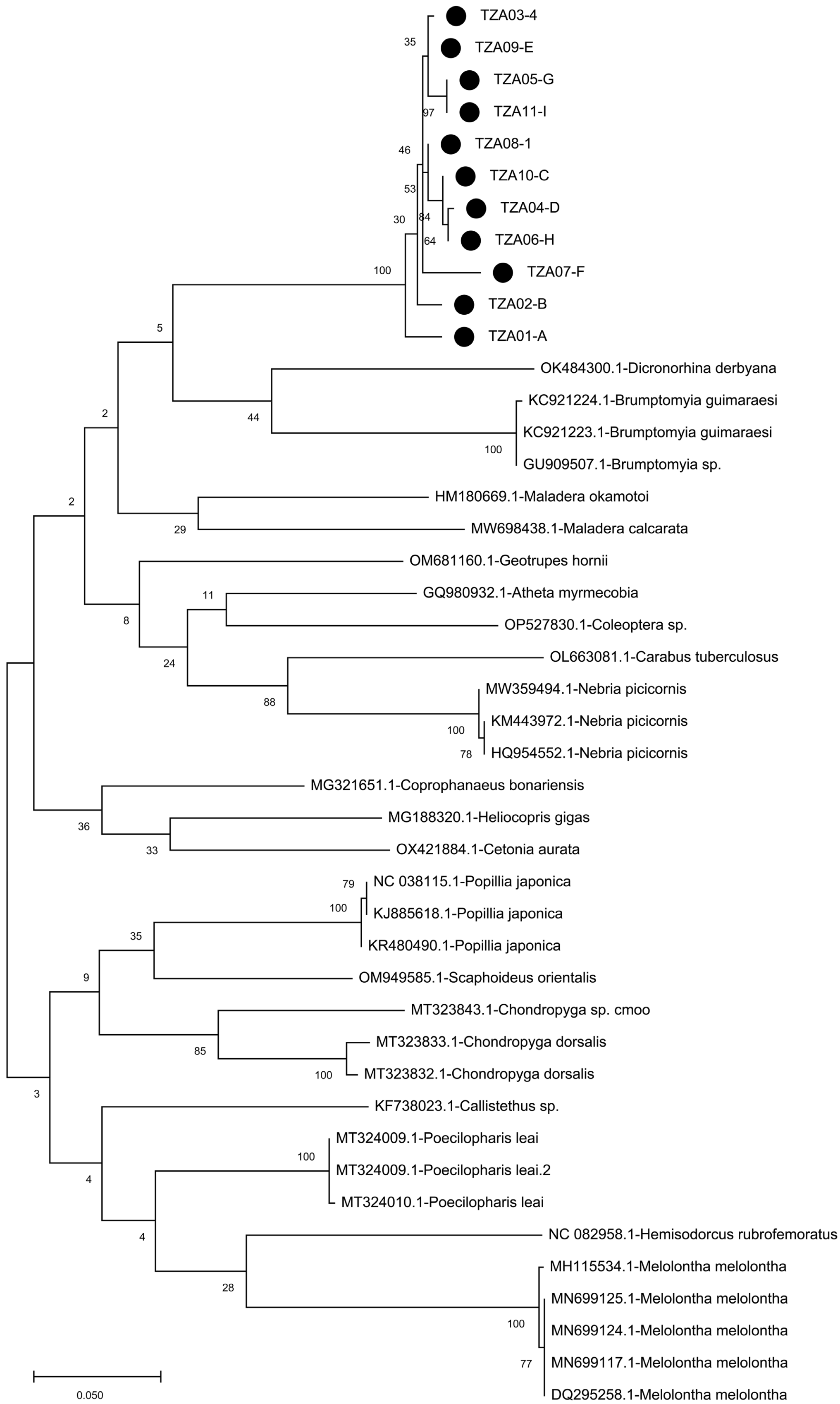

Figure 1. Phylogram for the collected *Goliathus* specimen with reference to the database.

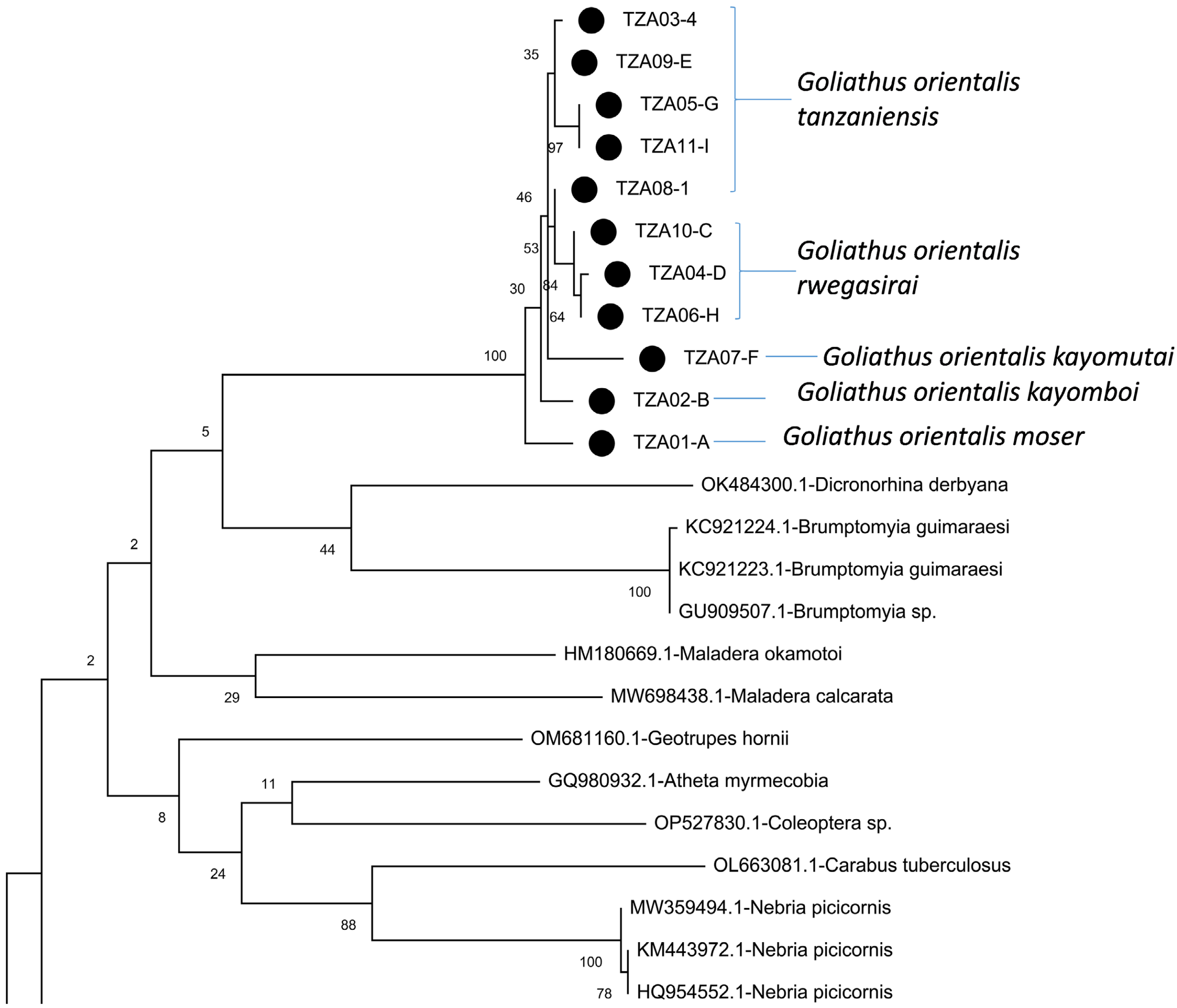

Figure 2. Portion of the phylogram indicating names for the new *Goliathus orientalis* subspecies.

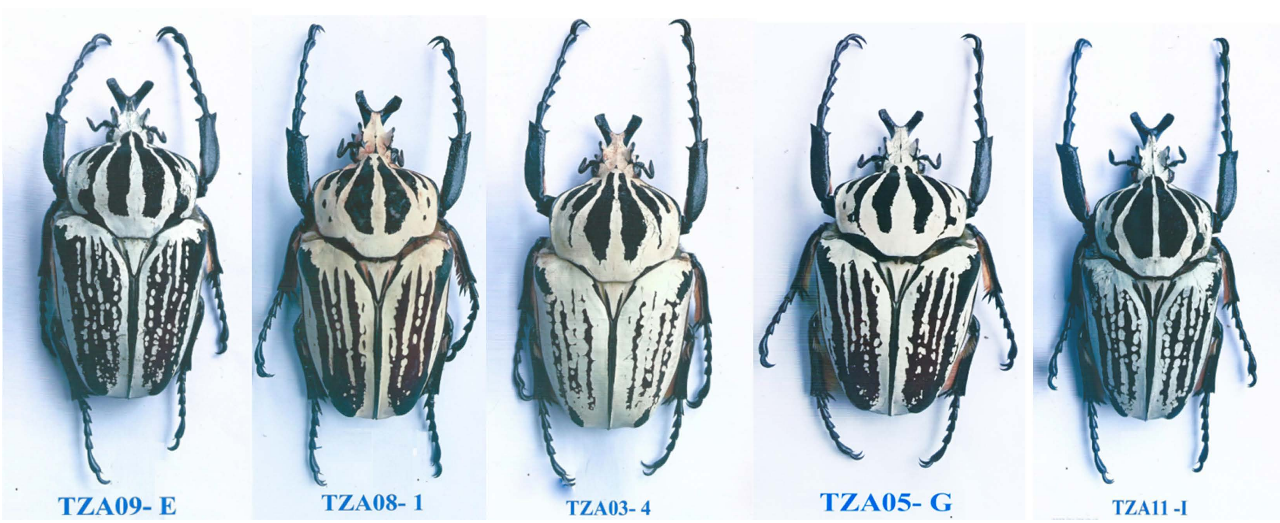

Figure 3. Various specimen of the subspecies *Goliathus orientalis tanzaniensis* (Kayombo & Rwegasira, 2023).

last specimen (TZA11-I) exhibits very thick, dark colored, oval-shaped central lines on the thorax with the line next to the inner ones being relatively longer extending from the head to the base of the thorax. The dark lines on the elytra extends from the base to the terminus but with some vein-like extensions along the way which inter-links the different lines forming a network of somewhat separate but intermittently connected black lines. Despite these morphological variations indicated, the molecular resolution suggested these five specimens to belong to the same species. Moreover, given the fact that the specimen were collected from various sites in Lindi and Tanga regions, the team logically considered against the proposal by Preiss [12]) who had originally suggested the name *Goliathus orientalis usambaraensis* as well as Beinhundner [1] who changed the name to *Goliathus usambaraensis* and concluded to re-name the specimen as *Goliathus orientalis tanzaniaensis* (Kayombo & Rwegasira, 2023).

3.2.2. *Goliathus orientalis rwegasirai* (Rwegasira, 2023)

The specimen bearing the name exhibits a thick dark line separating the thorax from the abdomen (**Figure 4**). Like other *Goliathus orientalis*, the specimen possess two oval shaped thick lines on the thorax coupled by two extra lines on either sides to form a total of six lines curved towards the central axis. The far lines on the peripheral are very thin and less apparent. On the elytra, there are darks (black to chocolate) colored lines extending from near the base of the wings to the terminal position. The clear lines dividing the dark colored lines are wide extending from the base to the terminus of the wings with varying intensity from sparse to dense ones. All specimens bear the V mark at the base of the elytra. The slight variations among specimen includes very densely packed dark chocolate colored plates with few dotted white lines (TZA 10-C), somewhat densely packed dark-brownish chocolate plates with fairly distributed dotted white lines (TZA 04-D) as well as apparently white (off white) colored plate of the elytra (TZA 06-H) blended with dark or black lines with irregular or rough-shaped margin lines extending fairly away from a base of elytra to near end of the terminus position.

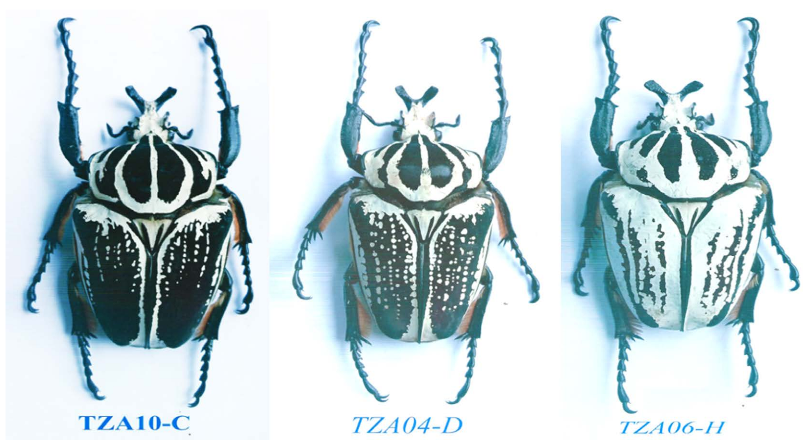

Figure 4. Various specimen of the subspecies *Goliathus orientalis rwegasirai* (Rwegasira, 2023).

3.2.3. *Goliathus orientalis kayomboi* (Kayombo, 2023)

The specimen bearing the name *Goliathus orientalis kayomboi* exhibits thick dark chocolate plate forming the elytra with few thin lines on the margins of the wings (Figure 5). The thorax is marked with two central oval-shaped lines that are thicker at the tip and relatively thinner at the base. The two lines next to the central thick lines on either side of the thorax are semi-circular (C-shape) extending to both tips while the far third outer lines are very thin and less apparent (TZA 02-B).

3.2.4. *Goliathus orientalis kayomutai* (Kayombo & Rwegasira, 2023)

The specimen bearing the name *Goliathus orientalis kayomutai* (TZA07-F) has very apparent characteristics (Plate 1) which could possibly be confused with *Goliathus orientalis moser* by a novice or irrational observer. Unlike other related species, the specimen bears limited decorations of lines with manifestation of six short lines (the two inner lines thicker than the outward ones) at the base of the thorax while much of the thoracic part is clear (with neither marks nor lines). Likewise, the elytra have fewer lines extending from near the base towards the

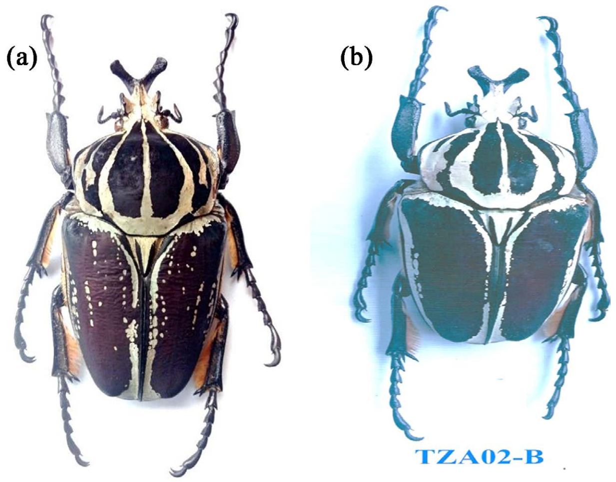

Figure 5. The subspecies *Goliathus orientalis kayomboi* (Kayombo, 2023).

Plate 1. The subspecies *Goliathus orientalis kayomutai* (Kayombo & Rwegasira, 2023).

terminal positions. The low intensity in which the lines are marked but with somewhat network of vein-like connections appearing like boxes which makes the elytra look beautiful like painted image with a typical creamy white appearance.

3.2.5. *Goliathus oriaentalis* Moser (Kayombo & Rwegasira, 2023)

Specimens in this group have a network of dark lines beautifying the whole of the elytra (Figures 6(a)-(d)). The vein-like network of lines differentiates this species from the rest of *Goliathus orientalis*. In addition, there is clear space between the decorated portion of the elytra and the non-decorated one making the specimen appear like a painted picture with some utmost precisions. The thorax exhibits thick dark lines with the thickest likes at the center forming an inverted V-like separation as they extend from the base to the tip (towards the abdomen). The other lines on the thorax are well spaced with reducing sizes as they extend away from the central thick lines. When compared with the available information, this was the only specimen that matched with the one in the Berlin Museum by 100 percent (Figure 6(a)).

4. Discussion

The present study has provided the proof that *Goliathus orientalis* Moser still exist in Tanzania and the first collection made [3] was indeed a valid collection from Lindi in southern Tanzania. In the current work, the team of researchers managed to further establish biological indicators inclusive of fauna (*Heterohyras* spp and *Dendrohyrax* spp) and flora (*Acacia* spp and several tree species of the Miombo woodland) that were effectively used to locate *Goliathus* specimen habitat. Establishment and successful use of these indicators were regarded major breakthrough in several attempts [1] to locate and collect *Goliathus* specimens in Tanzania. The present study managed to fix the knowledge gap between 1909 to the present times during which the whereabouts of *Goliathus* spp in Tanzania was never reported. Moreover, the present finding provides the last resolve to speculations that *G. orientalis* Moser was probably collected between Lake Usagara and Malawi or Mahenge [4].

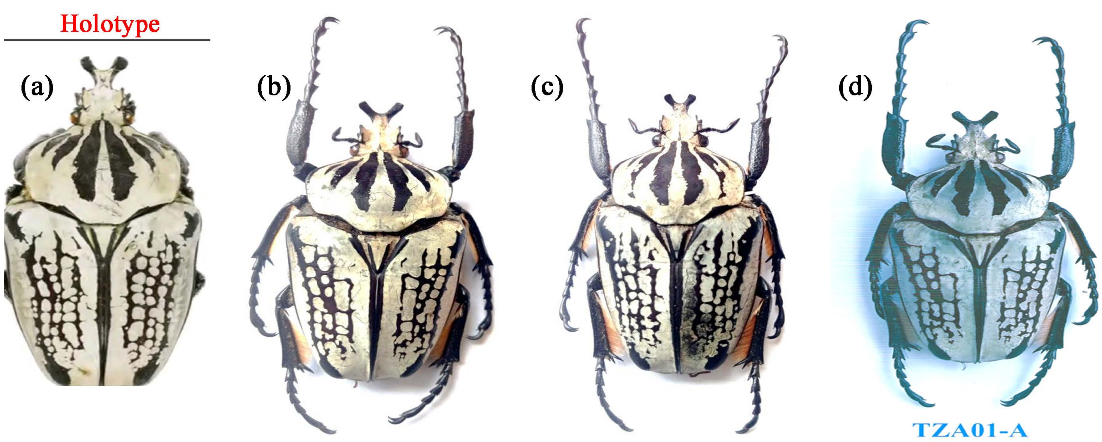

Figure 6. *Goliathus oriaentalis moser* (Kayombo & Rwegasira, 2023).

Thorough assessment of morphological features of the collected specimen revealed a lot of variation among them. However, further analysis of the Deoxy-ribo Nucleic Acid through bar coding suggested the relatedness of the species that was sufficient to place them in one species. Thus the observed boostrap distances along the phylogram only suggested the variation within the species hence the decision to name some isolates as sub-species. The present observation negates the prior report that *Goliathus usambarensis* [12] and *Goliathus orientalis* [3] were two separate species. Assessment of the bar coding implied the two to belong to the same clan of separate lineage with highest likelihood of the two being sub-species of *Goliathus orientalis*. Notwithstanding the descriptions and illustrations by the first author [12] that *G. usambarensis* had much in common with *G. orientalis* and *G. meleagris,* the combination of classical and molecular analysis of the specimen has confirmed beyond doubt that *G. usambarensis* is indeed a close relative to *G. orientalis*, hence a subspecies *Goliathus orientalis tanzaniensis.*

Furthermore, the species *Goliathus usambarensis* [13] specimen were collected from various sites of Lindi and Usambara mountains disqualifying the perception that it only belonged to the Usambara mountain ranges. The morphological resemblance among collected specimen and the DNA bar coding proved that despite being collected from different locations, they belonged to the same genetic origin. These evidences led to the decision to re-name the species as *Goliathus orientalis tanzaniensis*.

The five sub-species *Goliathus orientalis rwegasirai, Goliathus orientalis kayomboi, Goliathus orientalis kayomutai, Goliathus orientalis tanzanien*sis and *Goliathus orientalis moser* established during the present study by a combination of classical and molecular methods provided new insights into the understanding of previously unresolved issues brought about by reliance on morphological features to classify *Goliathus* species. It was apparent in the present study that some colors of the cuticle, orientation of lateral lines and bands on the elytra and the thorax may not only be adequate to effectively designate and name the *Goliathus* species. Thus, the combination of the two approaches (Classical and molecular analysis) to classification of *Goliathus* specimen like previously reported [4] should possibly be considered as standard approach in designation of new species.

5. Conclusion

The finding in the present study unveiled facts about *Goliathus* species in Tanzania which has not been recovered for more than a century. It confirmed the information on *Goliathus* that it was originally collected from Lindi in the Southern part of Tanzania. The confirmation by DNA barcoding that only one *Goliathus orientalis* species exists in Tanzania with five sub-species could possibly suggest that previous classification of *Goliathus* specimens in the rest of Africa should be reviewed with backups from genetic analysis by the Bar coding methods. Tanzania has been proven to be a home to *Goliathus orientalis* species with several variations that make up subspecies as detailed in the present study.

Acknowledgements

Authors would like to acknowledge the assistance received from Messer; Ramadhan Kulita, Washa S. Salage, Hosea Kibona, Omary Mlima, Kephas Sinkonde, Orasto Mgaya and Makarius Kayombo during field data collection, sorting and preservation of the specimen. Laboratory work was accomplished with technical assistance from Dr. Protas Deogratius (COSTECH) and Dr. Hellen Kanyagha (SUA).

Funding Information

The present work was solely funded through researchers' own sources and no support was received from any organization.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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