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# A possible new species of the *Maculabatis gerrardi* complex (Dasyatidae: Urogymninae) in the Indian Ocean coast off Southeastern Africa

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## Abstract

The genus *Maculabatis* is a group of batoid rays from the Dasyatidae family, consisting of two main complexes: the *gerrardi* (spotted species) and the *pastinacoides* (plain species). This study investigated the diversity within the *Maculabatis gerrardi* complex, revealing the presence of two distinct geographical lineages, with a potential new species captured off the coast of Mozambique. Molecular analysis showed a significant divergence: COI sequences from Mozambique specimens exhibited over 99% similarity with *M. gerrardi* from South Africa but more than 2% divergence from those in the Indo-Pacific. Phylogenetic analysis identified two distinct subclades, suggesting at least two hidden lineages within the genus *Maculabatis* and consequently possible new undescribed species within *M. gerrardi* complex. These findings emphasize the importance of conducting additional research that integrates both morphological and molecular methods to better understand the group's diversity and evolutionary dynamics, ultimately supporting the development of effective conservation strategies.

## Introduction

The genus *Maculabatis* Last, Naylor and Manjaji-Matsumoto, 2016, whose species were formerly classified within the genus *Himantura* Müller & Henle 1837, is a group of batoid rays belonging to the family Dasyatidae (Last *et al.*, 2016). Based on morphological and molecular data, the genus *Maculabatis* comprises two major complexes: the *gerrardi* complex (containing spotted species) and the *pastinacoides* complex (featuring plain species) (Last *et al.*, 2016).

*Maculabatis* species and other closely related species have experienced multiple taxonomic revisions because of new insights gained from morphological and molecular research. Consequently, species such as *Dasyatis bennetti* (Müller & Henle 1841), *Himantura gerrardi* (Gray 1851) and *Himantura walga* (Müller & Henle 1841) have been reclassified as *Hemitrygon bennettii*, *Maculabatis gerrardi* and *Brevitrygon walga*, respectively (Fricke *et al.*, 2024; Froese and Pauly, 2024).

Currently, nine species are recognized within *Maculabatis* (Last *et al.*, 2016). Research focusing on the study of *M. gerrardi* complex is still scarce and the taxonomic studies have always been suggested to try to resolve and better understand the group (Naylor *et al.*, 2012). In general, the species within *M. gerrardi* complex (formerly *H. gerradi*) are classified and characterized as large-sized whipray with a rhomboidal disc and narrowly rounded apices; anterior margin of the disc is almost straight; snout broadly triangular with small tip; not extending to tail base (in juveniles restricted to central disc) some enlarged denticles before and after pearl-like tubercular thorn, widely spaced; tail very slender, elongate, whip-like beyond sting, no cutaneous folds on tail; surface of disc usually paler greenish grey/greyish brown with numerous white spots posteriorly (rarely plain), tail banded beyond sting (Kizhakudan *et al.*, 2018).

The sharpnose stingrays *M. gerrardi* are predominantly present in coastal settings, including estuaries, within the Indo-West Pacific area, inhabiting depths up to 60 m (Last *et al.*, 2010, 2016). Species within *M. gerrardi* complex are frequently harvested by tangle net, bottom trawl, and trammel net fishing methods, where its flesh is consumed and hide utilized for leather production (White *et al.*, 2006; Last *et al.*, 2010). *M. gerrardi*, constitutes a set of cryptic species spread throughout the Indo-West Pacific region, displaying considerable differences both within and between species, regardless of the specific geographic area they occupy (Ward *et al.*, 2008; Bineesh *et al.*, 2016).

However, despite the existence of these classifications, there is still a great challenge in identifying specific species within the complex, mainly due to the morphological similarities that are observed between them, considering the colouring and in several cases the presence of small white spots on the posterior disc and with black and white banding to varying degrees on the tail which are the most common characteristic (Henderson, 2020).

Because of this, there is still a lot of inconsistency and difficulties in distinguishing these species, leading to errors in identifying them. The complexity for the identification within the *M. gerrardi* complex occurs along the Gulf of Oman descending to the Indo-Pacific region and northern Indian Ocean (Henderson, 2020). Last *et al.* (2012), in one of their works, had incorrectly examined and classified a species *Himantura randalli* (now *Maculabatis randalli*) due to its morphological characteristics.

Naylor *et al.* (2012) in their exhaustive work within the group had already suggested that it is important to make collections including samples from the Mozambique channel to try to gather a greater number of samples from the group in order to achieve a taxonomic resolution, since he identified several subgroups of the *gerrardi* complex in the Mozambique Channel on the sides of Madagascar.

Possibly the research that identifies the species of *M. gerrardi* on the coast of Tanzania is another one of these cases of wrong identification (and may be unknown specie of the complex) due not only to the complexity of the group, but also because it did not use multiple markers or morphological characteristic to resolve the group (Nehemia *et al.*, 2024).

These revisions have raised several questions and highlighted the need for further studies to clarify the taxonomic relationships and distribution patterns within the genus, as well as to better understand their ecological roles, conservation status and potential threats (Fricke *et al.*, 2024). In addition, the decline in elasmobranch stocks, particularly rays, coupled with their low fecundity and increased capture rates, have rendered these species increasingly vulnerable to extinction (Borsa *et al.*, 2012). Recent evaluations by the International Union for Conservation of Nature (IUCN) indicate that *M. gerrardi* now falls under the endangered species category, primarily due to the intensification of overfishing and the deterioration of its natural habitat (Sherman *et al.*, 2020; Dulvy *et al.*, 2021). Consequently, continued research is necessary to better understand these species ecology, population dynamics, and responses to anthropogenic threats, ultimately aiding in their conservation and management (Dulvy *et al.*, 2021).

In this study, through new sequences generated and molecular analyses, we verified the presence of two lineages in the *M. gerrardi* complex, with a possible new species distributed off the coast of Mozambique.

## Materials and methods

Four individuals of *M. gerrardi* were gathered off the coast of southeastern Africa, specifically in the Inhambane province, Vilankulos district in Mozambique, by local fishermen who employed artisanal fishing trawls. These collections occurred in July 2022. The four specimens evaluated, are part of the barcode sequences generated by Muhala *et al.* (2024). All specimens captured had their bodies altered with visible incisions in the form of slices. Therefore, no photographs were taken in the field. Previous morphological identification was based on the shape and colour of the tail, which has a zebra-like camouflage of alternating black and white bands, and the posterior margin of the disc with small, sparse white spots as described by Last *et al.* (2010). Samples of tissue extracted from the specimens were conserved in a solution of 96% ethanol for laboratory preservation purposes.

The isolation of genomic DNA was carried out using a Wizard Genomic DNA Purification kit (Promega) according to the manufacturer's protocol specifically designed for extracting muscle tissue. The Nanodrop 2000 spectrophotometer (Thermo Scientific, CA, USA) was utilized to assess the quality and concentration of the obtained DNA. To amplify partial sequences of the cytochrome oxidase subunit I (COI) gene, polymerase chain reaction (PCR) was performed using the FishF1 and FishR1 primers (Ward *et al.*, 2005).

PCRs were conducted in a concluding volume of 15 µl that encompassed 2.5 µl of dNTPs (at 1.25 mM), 1.5 µl of buffer at 10× concentration, 0.7 µl of $MgCl_2$ (with a concentration of 50 mM), 0.5 µl of every primer (10 pmol $\mu l^{-1}$), 1.0 µl of all-inclusive genomic DNA (100 ng $\mu l^{-1}$), 0.2 µl of Taq DNA polymerase (5 U $\mu l^{-1}$), and a necessary quantity of sterile water to reach the intended volume of the reaction. The augmentation protocol was initiated by a 3-min denaturation process at 95°C, which led to 35 rounds of 40-s denaturation at 94°C, 40-s hybridization at 50°C, 60-s extension at 72°C, and concluded with a final 5-min extension at 72°C. Post-amplification, the PCR derivatives were purified using a 20% solution of polyethylene glycol (Lis and Scheilef, 1975), and the sequencing was carried out via the Sanger method (Sanger *et al.*, 1977) using the BigDye Terminator v3.1 Cycle Sequencing kit (provided by Applied Biosystems, Foster City, CA, USA) following the directives set out by the manufacturer. Finally, the positive PCR samples were sequenced on the ABI 3500 XL device (from Thermo Fisher, CA, USA).

The obtained sequences were examined and analysed using chromatograms, and subsequently aligned using Geneious software, version 9.0.5 (accessible at https://www.geneious.com). In order to make the most comprehensive comparisons of *Maculabatis* species, all sequences available in GenBank and BOLD Systems (83 sequences) were included in the final database. We added as many *Maculabatis* individuals as possible to visualize the resulting clusters and confirm the previous morphological identification. Finally, a sequence from *Plesiobatis daviesi* (access BOLD/GenBank NCBI: ANGBF11484-15/KF899649), was used as an outgroup. The accession numbers and all used sequences are available in Supplementary Table S1. The final bank of sequences had a length of 514 bp.

Bayesian inference (BI) and maximum likelihood (ML) methods were used to explore the phylogenetic relationships of the species making use of MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001) and RAxML 7.2.7 (Stamatakis, 2006), respectively. For the BI process, four chains along with two self-governing runs each consisting of 10 million generations were incorporated. After every batch of 10 generations, 25% of the original trees were removed during the burn-in phase to eliminate any initial biases. JModelTest 2.1.10 (Darriba *et al.*, 2012) was used to verify the GTR-GAMMA evolutionary model for ML analysis. The analysis was performed with 1000 bootstrap pseudo-replicates to provide reliable statistical support.

To explore the genetic structure patterns, haplotype networks were assembled utilizing Haploviewer (Salzburger *et al.*, 2011). The calculation of genetic distances between distinct groups was achieved by determining the count of net nucleotide substitutions per site using the Da 10.21 formula (Nei, 1987), as well as the unadjusted genetic distance (also known as *P*-distance) using DNAsp software (Rozas *et al.*, 2017). The subsequent modifications to the trees were performed employing FigTree version 1.4.3 (Rambaut, 2017).

## Results and discussion

Comparison of our data with sequences stored in the GenBank and BOLD Systems databases showed that the samples from

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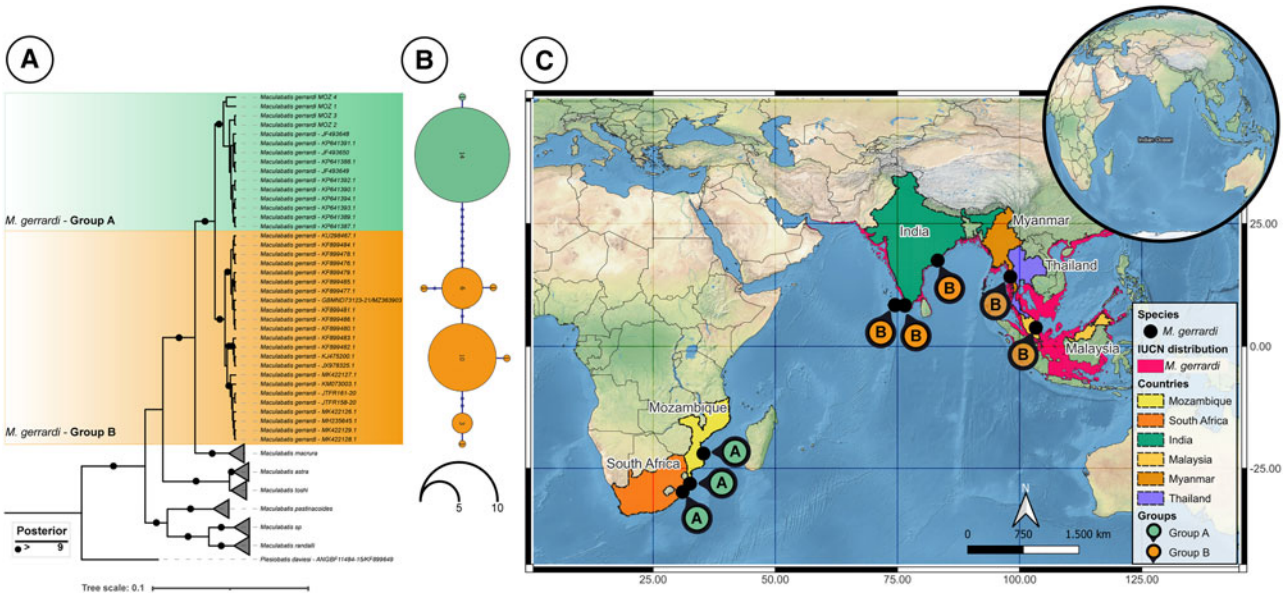


**Figure 1.** (A) Phylogenetic relationships from Bayesian inference analysis. (B) Haplotype network derived from mtDNA haplotypes of *M. gerrardi* and (C) Pinpointing the new presence of *M. gerrardi* in the southern coastal region of Mozambique, specifically in the province of Inhambane, Vilankulos district, including sightings in South Africa. The map represents the distribution acknowledged by the IUCN – International Union for Conservation of Nature. The two identified lineages of *M. gerrardi*, along with their locations, are depicted as Group A and Group B.

this study formed a single group with the *M. gerrardi* samples (Figure 1A). However, there was a notable divergence in the degree of similarity across two distinct geographical regions. The COI sequences derived from samples amassed in the current study displayed over 99% similarity with those of *M. gerrardi* originating from South Africa but exhibited more than 2% divergence with those from the Indo-Pacific region (Supplementary Table S1). Furthermore, the *M. gerrardi* samples from Mozambique did not cluster with any other *Maculabatis* species, which is significant because the samples were misidentified by fishermen at the time of collection. The phylogenetic analysis based on mtDNA unveiled two distinctive subclades within *M. gerrardi*, which possibly correspond to two different lineages of species: the first subclade, which consists of samples from South Africa and Mozambique, and the second subclade, representing individuals from the Indo-West Pacific region (Figure 1A–C). The degree of genetic differentiation between the two subgroups, as measured by both uncorrected p-distance and Da distance, is 0.01049 and 0.01645, respectively. Hence, the data imply the presence of at least two hidden lineages within the genus *Maculabatis*.

*Maculabatis gerrardi* is a species complex with at least five species that show genetic distances between each other, but do not show an exclusive morphological pattern that clearly separates the five species (Naylor *et al.*, 2012). Our specimens were grouped with specimens from South Africa that we believe to be specimens of the species also found by Naylor *et al.* (2012) and previously named *Himantura* cf. *gerrardi* 4.

At the time the biological tissues were collected, the samples used in this study were not whole, which prevented an in-depth analysis of morphological aspects. Although Naylor *et al.* (2012) pointed out that the *M. gerrardi* complex does not show clear morphological differences between the subgroups, an analysis combining morphological and molecular methods is urgently needed. This is necessary to determine whether *M. gerrardi* represents a complex of distinct cryptic species or is undergoing a process of recent speciation, with a yet undescribed species present off the coast of Mozambique.

Recognizing and differentiating species is crucial for better management, especially in a group with many endangered species, such as elasmobranchs. Different but closely related species, as is often the case with cryptic species, belong to different evolutionary lineages and may have different ecological, behavioural or genetic requirements (Burns and Bloom, 2020; Gómez-Corrales and Prada, 2020). Correct identification of these species is essential, as failures to do so can lead to an underestimation of diversity and the application of inadequate conservation measures. Without this recognition, distinct populations may be treated as a single species, which can result in ineffective management for unidentified lineages (Hohenlohe *et al.*, 2021).

The diversity of species within the *gerrardi* complex is not strange, as it was pointed out by Naylor *et al.* (2012) when he analysed samples from the Mozambique Channel (Madagascar) and concluded that there was enough genetic difference to consider a possible new species within the complex.

This study provides new evidence for a possible undescribed species of the *M. gerrardi* complex in Mozambique. Understanding the true species diversity of the complex is fundamental for better management and conservation, as *M. gerrardi* is recognized as endangered by the IUCN Red List of Threatened Species (Sherman *et al.*, 2020).

Although the results are preliminary, they indicate the presence of two exclusive lineages within this complex, highlighting the need for more extensive research covering morphological aspects with molecular diversity using distinct markers. In this way, we can truly verify the biodiversity and evolutionary dynamics within this group in a complementary taxonomic approach.

**Supplementary material.** The supplementary material for this article can be found at https://doi.org/10.1017/S0025315424000973

**Author Contributions.** V. M. wrote the first draft of the manuscript and reviewed the manuscript. A. G.-C. and I. E. M. designed research. Í. L., A. R. B. S. and L. P. R. analysed data and wrote the first draft of the manuscript. O. J. H. and G. E. -G. involved in material preparation and data collection. M. V. and I. S. obtained funding and laboratory requirements. All authors read and approved the final manuscript.

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**Conflict of Interest.** None.

**Ethical Standards.** Not applicable.

**Data Availability.** All data are available upon request.

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