Poster Identification Number: 426

Integrative Taxonomy of the Crinoids of the Shallow-Waters of KwaZulu-Natal, South Africa

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Abstract

Marine biodiversity of eastern Africa is relatively poorly known with great disparities in taxonomic and geographical coverage and with large gaps in taxonomic data. The last comprehensive taxonomic revision for southern African echinoderms for instance dates from 1976. The coastline of KwaZulu-Natal in the South East of South Africa has been reasonably well explored for echinoderms and has recently resulted in a number of taxonomic revisions, but not for crinoids. This study deals with the crinoid specimens that were sampled during five recent expeditions (1999 to 2016) to the shallow-waters (50 m depth max) of KwaZulu-Natal. We combined classical comparative morphology with DNA barcoding to delimitate the collected species. Such integrative taxonomy allowed us to raise the number of shallow-water crinoids of KwaZulu-Natal from 4 to a putative 10 species. The 96 barcodes generated during this study are the first DNA barcodes of crinoids for Eastern South Africa and represent modern standards to characterize this neglected, but ecologically important, component of biodiversity.

Material and methods

Origin of the specimens

113 specimens were collected during five different expeditions (VIII.1999, VII.2000, II.2001, IX.2003 and I.2016) undertaken in the shallow-waters (max 50 m depth) of KwaZulu-Natal (Fig. 1.). Specimens were anesthetized with fresh water or with a 4% $MgCl_2.6H_20$ solution. Fixation was done with 80% ethanol. The specimens were then rinsed with 70% ethanol, and finally preserved for long term storage within 70% ethanol or dried. Analyzed tissue samples were taken from both wet and dry preserved specimens.

Morphological species identifications

As with the other echinoderm classes, the bulk of taxonomic information is derived from the skeleton. Skeletal elements were studied by light stereoscope. Additionally, three selected characters (pinnulars of first pinnule; cirrals of cirri and brachials of brachitaxis) were further investigated using a SEM.

DNA barcoding

Eight primers extracted from the literature were used, targeting the mitochondrial COI Folmer region. Most similar COI sequences from BOLD (Fig. 2.) and GenBank were retrieved using BLAST. They were then aligned and used for a neighbour-joining tree reconstruction (Kimura 2-parameter method (Kimura, 1980), 1000 bootstrap replicates (Felsenstein, 1985)).

Fig. 1. Origin of the studied specimens. Samples are mainly derived from a region North (Sodwana and Kosi Bay, [1]) and South (Trafalgar and Aliwal Shoal [2]) of Durban, KwaZulu-Natal.

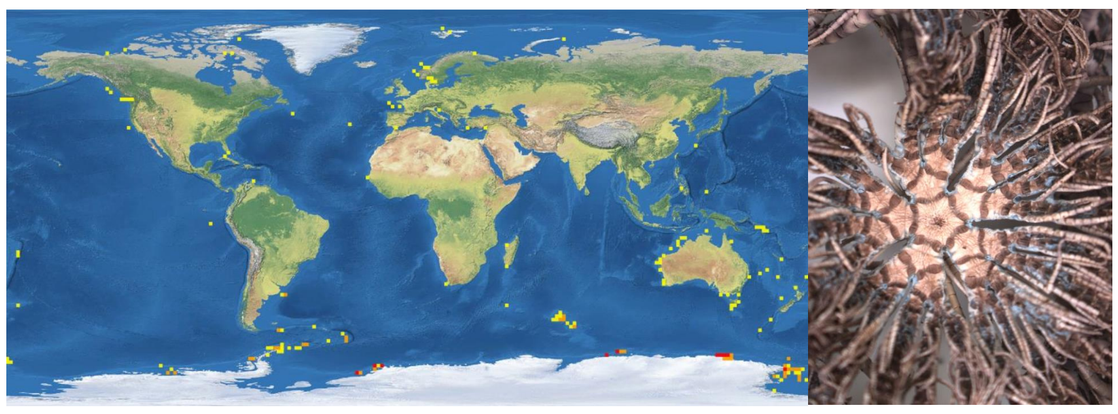

Fig. 2. Left: geographic distribution of the crinoid barcodes available in BOLD. Right: Oral view of *Lamprometra palmata*, a species known from KZN, but *hithertho* without associated barcode.

Results

Sequencing output

Of the 113 samples, 96 ethanol and dry preserved specimens could be successfully barcoded (max size 839 bp). However, near 43% of the samples yielded incomplete Folmer COI barcodes. The IX.2003 sample batch had a very low success rate (27%). This could be attributed to the fact that this batch has been stored in a jar that was contaminated by formaldehyde.

Integrative taxonomy

DNA barcoding identification based on BOLD and GenBank were performed up to the generic level so as not to bias morphological identifications, which were done using the species identification keys of Clark & Rowe (1971), Clark & Courtman-Stock (1976), as well as non monographic works such as Clark (1951, 1972) and Marshall & Rowe (1981). Morphological identifications allowed to identify nine species. These could largely be matched to the clusters recognized on the NJ tree, with recognition of subspecies in two of them (Fig. 3.).

Towards a new checklist

Prior to this study, only four crinoid species were known from the shallow-waters of KwaZulu-Natal, namely: *Comanthus wahlbergii* (Müller, 1843), *Decametra durbanensis* (Clark, 1951), *Oligometra serripinna occidentalis* (Carpenter, 1881) and *Tropiometra carinata carinata* (Lamarck, 1816). Additionally to these four species, two were recorded from nearby southern Mozambican waters, being: *Comanthus parvicirrus* (Müller, 1841) and *Heterometra delagoa* (Gislén, 1938). We confirmed the presence of the here-above listed species, except for *Heterometra delagoa*. We complemented this list with five additional species: *Stephanometra indica* (Smith, 1876), *Cenometra emendatrix* (Bell, 1892), *Lamprometra palmata* (Müller, 1841), *Annametra occidentalis* (Clarck, 1915) and a non-morphologically identifiable *Antedon* sp.

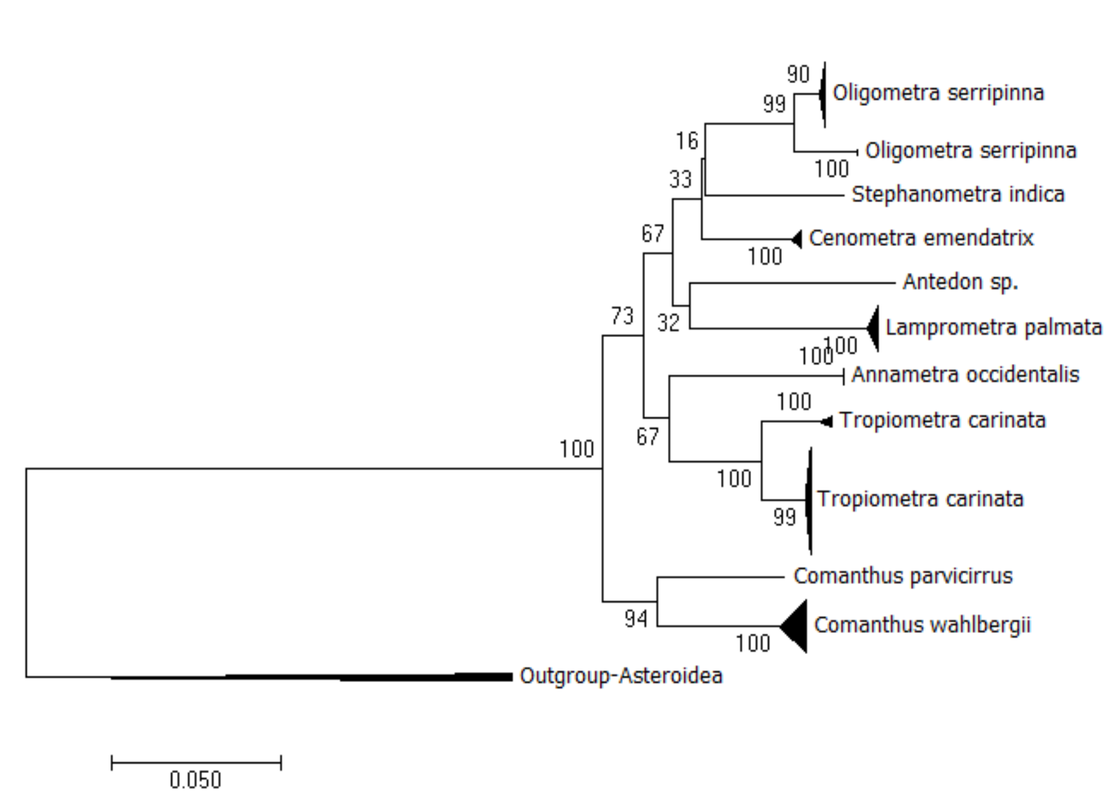

Fig. 3. Neighbour-joining tree reconstruction performed with MEGA. Bootstrap values are shown at the branches.

Perspectives

Further integrative taxonomic research will reveal if *O. serripinna and T. carinata* should be (re)split into sub-taxa and if *C. parvicirrus* (Müller, 1841) is a junior synonym of *C. wahlbergii multibrachia* Gislén, 1938 which is currently known only from the single holotype from False Bay, S. Africa?

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Funding Sources/Acknowledgements

Funding for this work came from the Flemish Community [Bilateral (International) Scientific and Technological Cooperation, project numbers BIL98/84 and BIL01/46), the Fund for Scientific Research Flanders, the Research Council of the Free University Brussels (VUB), the Belgian GTI and the King Leopold III Fund for Nature Exploration and Conservation (YS), the University of Gent (GS), and from the JEMU project funded by the Belgian Science Policy and supported by the Royal Belgian Institute of Natural Sciences (RBINS, Brussels) and the Royal Museum for Central Africa (RMCA, Tervuren).

We are grateful to the late P. Timm of Triton Diving, M. Addison of Blue Wilderness Diving and the Dive unit of Ezemvelo KZN Wildlife. We also like to thank Dr. D. VandenSpiegel for the skeletal characterization of the specimens through SEM performed at the Royal Museum for Central Africa.