

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% MgCl₂·6H₂O 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)).

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* (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), *Cenometa emendatrix* (Bell, 1892), *Lamprometra palmata* (Müller, 1841), *Annametra occidentalis* (Clarck, 1915) and a non-morphologically identifiable *Antedon* sp.

References

- Clark, A.M., Rowe, F.W.E. (1971); Monograph of Shallow-water Indo-West Pacific Echinoderms; i-vii: 1-238, 31 pls.; Trustees of the British Museum (Natural History) London.
 Clark, A.M., Courtman-Stock, J. (1976); The Echinoderms of Southern Africa; i-ii, 1-277; Trustees of the British Museum (Natural History) London.
 Clark, A.M. (1951); On some Echinoderms in the British museum; Annals and Magazine of Natural History Vol.40; p 1256-1268; London
 Clark, A.M. (1972); Some Crinoids from the Indian Ocean; Bulletin of the British Museum (Natural History) Zoology Vol.24; 73-156; London
 Felsenstein J. (1985); Confidence limits on phylogenies: An approach using the bootstrap; Evolution; 39:783-791.
 Kimura M. (1980); A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences; Journal of Molecular Evolution; 16:111-120.
 Marshall & Rowe (1981); Crinoids of Madagascar; Bulletin Musée National d'Histoire Naturelle Série 4; p 379-413; Paris

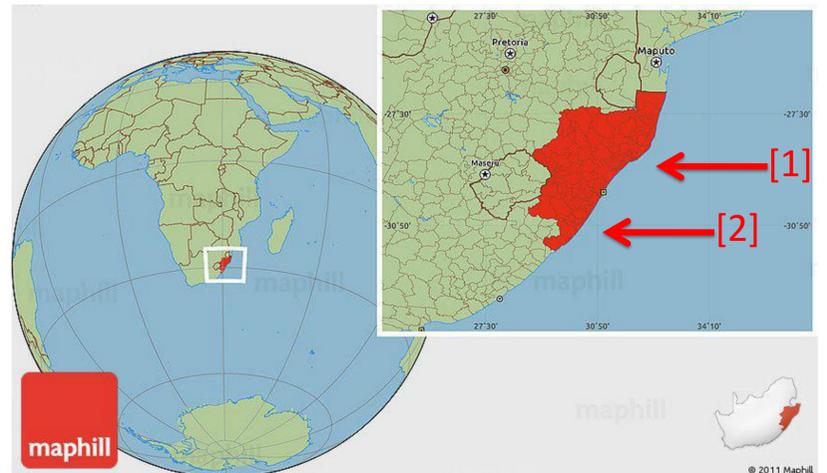


Fig. 1. Origin of the studied specimens. Samples are mainly derived from a region North (Sodwana and Kosi Bay, [1]) and South (Trafalgar and Aliwal Shoal [2]) of Durban, KwaZulu-Natal.

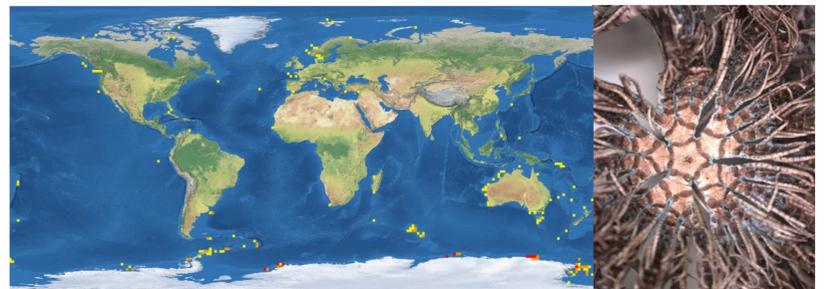


Fig. 2. Left: geographic distribution of the crinoid barcodes available in BOLD. Right: Oral view of *Lamprometra palmata*, a species known from KZN, but *hitherto* without associated barcode.

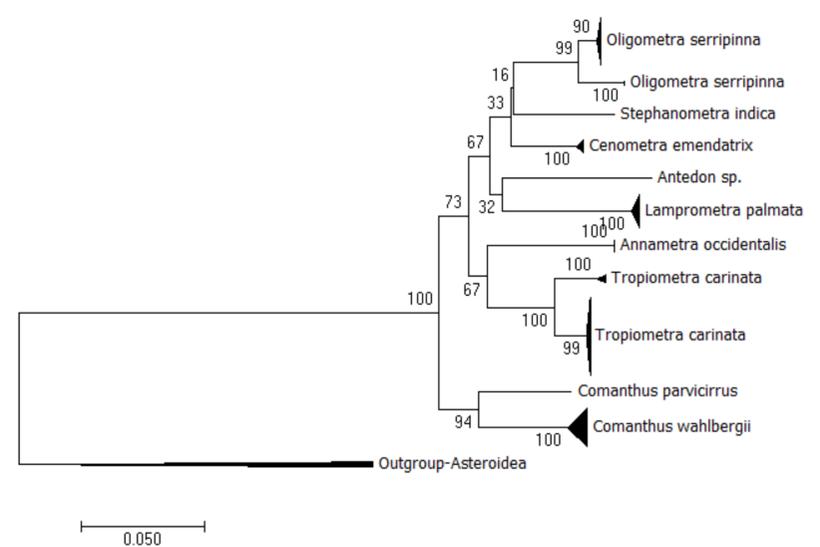


Fig. 3. Neighbour-joining tree reconstruction performed with MEGA. Bootstrap values are shown at the branches.

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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