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The *Ophiocoma erinaceus* complex: another case of cryptic speciation in echinoderms

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ABSTRACT: After two centuries of careful morphological description and comparison, echinoderms have been generally viewed as having a reasonably stable taxonomy, with species well differentiated. However, recent molecular and taxonomic studies have challenged this view. Comprehensive phylogenies based on molecular data have frequently revealed undescribed cryptic species, many within what were considered well-known clades. Species are often sympatric and difficult to distinguish, differing only in very subtle colour or morphological differences, or by a shift in reproductive strategy. A new example of a species complex (*Ophiocoma erinaceus*: Ophiocomidae: Ophiuroidea) is given here. The existence of cryptic species has profound implications for the study of echinoderm ecology, conservation biology, biogeography and fisheries management.

1 INTRODUCTION

Scientists and naturalists have long been attracted to echinoderms, perhaps drawn by their large size, bright colours and dramatic form. They were amongst the first animals to be scientifically described and there is a prolific taxonomic literature dating back to the early nineteenth century. By the mid-twentieth century these earlier reports had been drawn together in a series of comprehensive monographs, focusing on a taxonomic group (e.g. Mortensen 1928–1951 for echinoids, A.H. Clark 1931–1950 and A.H. Clark & A.M. Clark 1967 for crinoids) or region (e.g. H.L. Clark 1946 for Australia, A.M. Clark & Rowe 1971 for the tropical Indo-West Pacific). These advances have led to assumptions that echinoderm taxonomy is relatively well-known and stable. For example, echinoderms have never been a priority taxon for Australia's primary source of funding for taxonomic research: the Australian Biological Resources Study.

However, recent phylogenetic or phylogeographic studies based on molecular data have frequently reported undescribed cryptic species, many within what were considered well-known clades (Table 1). Cryptic or sibling-species are defined as those that are difficult to distinguish morphologically or were recognised using non-morphological characters (Knowlton 1993). Their presence appears to be ubiquitous in marine habitats (Knowlton 1993).

As an example, this paper describes a species complex within the ophiuroid genus *Ophiocoma*. Ophiocomids are common in littoral tropical habitats. They are large, robust and mobile, generally hiding within crevices or under rocks during the day and emerging at night to feed. Within the genus *Ophiocoma*, black individuals from the Indo-West Pacific with alternating numbers of arm spines on successive segments, are generally referred to the species *O. erinaceus*. Taxonomists have sometimes distinguished specimens with one tentacle scale on each tube foot pore as a separate species *O. schoenleinii*, restricting *O. erinaceus* to specimens with two tentacle scales (e.g. Clark & Rowe 1971). In a comprehensive taxonomic review of *Ophiocoma*, Devaney (1970) found other characters that appeared to distinguish *O. schoenleinii*, including the presence of three arm spines on the fourth segment (compared to four for *O. erinaceus*), the extension of granules on the ventral disc surface almost to the oral shields (compared to them being restricted to a small wedge near the ventral margin), and longer upper arm spines. Nevertheless, Devaney noted that these differences were not absolute, with some specimens having some but not all of these characters. He concluded that the two forms are "... polymorphs of the same species having phenotypically linked morphological characters".

This paper uses a range of molecular, morphological and day/night colour change data to distinguish

Table 1. Some recent multi-species phylogeographic studies on echinoderms, with the total number of ingroup species (Total), the number of previously unrecognised species (New) and the number of identified synonyms (Syns). An asterisk denotes unresolved heterogeneity within species complexes.

Taxon	Reference	Total	New	Syns
Cryptasterina	Hart et al. (2003), Byrne et al (in press), Dartnall et al. (in press)	4	2	–
Patiriella '*regularis*'	O'Loughlin et al. (2002)	2	1	–
Patiriella '*gunnii*' complex	O'Loughlin et al. (in press)	4	2	–
Coscinasterias	Waters & Roy (2003)	5	1	–
Leptasterias	Hrincevich et al. (2000)	5	*	–
Asterias	Wares (2001)	3	–	–
Linckia spp.	Williams (2000)	5	1	1
Echinometra	McCartney et al. (2000)	7	–	–
Echinometra	Landry et al. (2003)	6	1	–
Eucidaris spp.	Lessios et al. (1999)	4	–	1
Ophiactis savignyi	Roy & Sponer (2002)	2	1	–
Amphipholis squamata	Sponer & Roy (2002)	4	3	–
Ophiocoma '*erinaceus*' complex	This paper	3	1	–

at least two and possibly three species, within the *O. erinaceus* species complex.

2 MATERIALS AND METHODS

Specimens of *Ophiocoma* were collected from under coral slabs on the reef flat around Raine Island (11° 35′ S 144° 02′ E) and Moulter Cay (11° 24′ S 144° 01′ E), off the tip of Cape York in northern Queensland in December 2001. Specimens spawned spontaneously in a bucket or were induced to spawn using a temperature/light shock treatment (Selvakumaraswamy & Byrne 2000). Embryos were cultured in seawater at air temperatures for two weeks. Colour-change was observed by placing animals in a covered bucket overnight and photographing the animals in the morning. Specimens were eventually preserved in 70% ethanol and stored in the Museum Victoria collections. The morphology of other specimens collected throughout the Indo-West Pacific was examined in the collections of the Natural History Museum (London), the Zoological Society of India (Kolkata), and Museum Victoria (Melbourne).

DNA was extracted from tube foot tissue of 16 specimens from Raine Island and Moulter Cay using the GenomiPhi DNA Amplification kit (Amersham Sciences, Mamone 2003) and a portion of the mitochondrial COI gene amplified using the universal metazoan COI primers, COI F (forward) and COI A (reverse) (Kessing et al. 1989), a modified version of COIf (5′ – ttt ggt cat cct gaa gtt tat at – 3′), and two internal primers L6551 (5′ – tac cag ggt tcg gta tta ttt ccc a – 3′) and H7040 (5′ – ggg aaa aat gtt aag tta act ccg a-3′).

PCR amplifications were carried out in a reaction volume of 12.5 μl (25 μl were later used for sequencing) containing the following: 2.5 μm commercial reaction buffer (Promega), 3–4.5 mM $MgCl_2$, 0.5 units *Taq* polymerase (Promega), 0.2 μM of each dNTP and 0.16 μM of each primer. PCR parameters were as follows: (1) initial denaturation at 95°C for two minutes, (2) 40 cycles of 94°C for 40 seconds, annealing at 45°C for 40 seconds, and extension at 72°C for 45 seconds and (3) final extension at 72°S for 2 minutes. Gel isolation (GFX^{tm} 96 PCR Purification Kit, Amersham Biosciences) was used according to the manufacturer's protocol for samples with persistent secondary products. PCR products were sequenced using a MegaBACE^{tm}, a fluorescence based DNA analysis system that utilizes capillary electrophoresis to sequence DNA. The resulting sequences were checked against the chromatograms using Chromas (v.1.45) and aligned by eye using Proseq (PROcessor of SEQuences, v.2.9).

A 538 bp sequence from each individual was analysed using Mega2 (Molecular Evolutionary Genetic Analysis v.2.1) and MrBayes (v.3.0B4) software that were used to create Neighbour-Joining (using Kimura-2 distances)/Maximum Parsimony and Bayesian Maximum-Likelihood Consensus trees, respectively (burnin value = 100).

3 RESULTS

The grey-black *Ophiocoma* brittlestars collected from Raine Island and Moulter Cay could be readily divided in the field into two groups based on the colour of the tube feet: bright red compared to dark grey (sometimes with a light tip). Even after preservation in ethanol this difference was usually evident; as the red tube feet bleach white, while the dark tube feet retain their colour. In general the specimens with dark tube feet were also found to be more robust and less mobile than those with red tube feet. As specimens with red tube feet mostly had two tentacle scales they were labelled *O. erinaceus*, whereas specimens with grey tube feet mostly had one tentacle scale and were labelled *O. schoenleinii*.

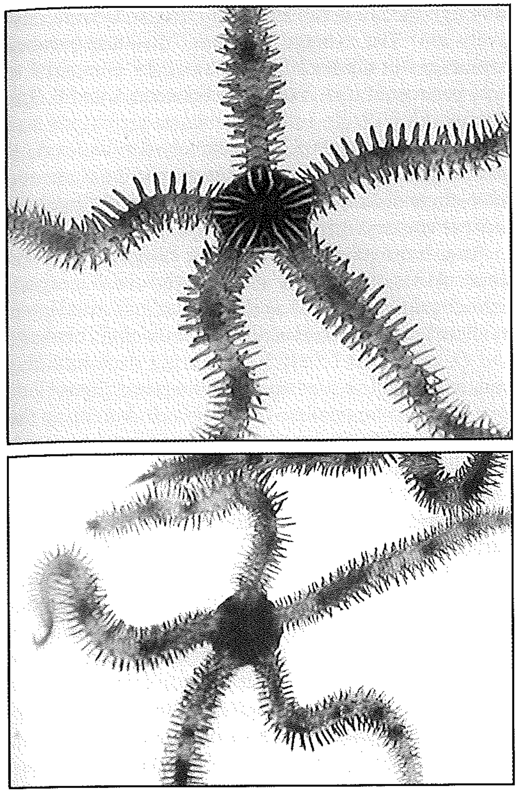

Figure 1. Night-time colour patterns, *Ophiocoma erinaceus* (top) and *O. schoenleinii* (bottom). Disc diameter is approximately 15 mm.

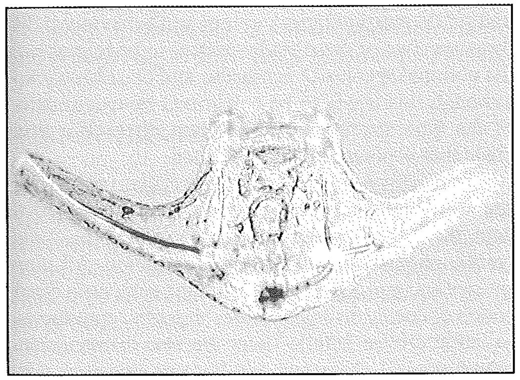

Figure 2. Four-day old ophiopluteus of *Ophiocoma erinaceus*.

Both forms were found to alter their body wall colour in dark and light conditions, analogous to that reported from Caribbean species of *Ophiocoma* (Hendler 1984, Hendler & Byrne 1987, Aizenberg et al. 2001). During the day, all specimens at Raine Island were predominantly black on the dorsal side, sometimes with white/grey banding on distal parts of the arm. The ventral side was usually paler, with variable white markings on the proximal part of each ventral arm plate near the disc, becoming limited to the narrow space between ventral arm plates distally. In contrast, in the dark they displayed prominent white/grey markings on the disc and dorsal arm surface. The colour pattern was subtly different between the two forms (Fig. 1). In *O. erinaceus* the arms were banded black and white/grey with patches being 3–4 segments long, particularly near the base of the arms. The distal edge of many dorsal arm plates within the lighter bands was also dark, particularly near the arm tip. In addition, there were often three spoke-like white stripes on the dorsal disc surface near each pair of radial shields, two longer ones on the interradial side of each radial shield (extending from the margin to about 1/4 the disc diameter) and a shorter one between the radial shield pair (extending about half that length). There was often a white stripe around the interradial disc margin with a bell shaped marking proximal to the centre of each marginal stripe. Sometimes the markings on the disc can be seen faintly in preserved material, particularly on small specimens, and the arm spines often have distinct longitudinal white lines.

On *O. schoenleinii* specimens the arms were also banded, but there was a dark marking in the centre of each dorsal arm plate in addition to the line along the distal edge. The disc had white patches near the radial shield, but in this case they covered most of the radial shields, sometimes overlain with a black dot near the distal end of the radial shield.

The eggs of both forms were 95–100 microns in diameter and had a decorated fertilisation envelope; the surface was not smooth being covered in small spikes. The larvae (Fig. 2) were very similar, both being typical ophioplutei with a small pre-oral lobe.

Examination of many museum specimens from around the Indo-West Pacific appeared to confirm Devaney's (1970) observations: that although specimens with one tentacle scale generally had longer arm spines, fewer arm spines on the fourth segment and more extensive granulation on the ventral disc surface, these difference are not always consistent. The colour of the tube feet in these preserved specimens was also not always consistent. On average every third specimen had one character that was inconsistent with the number of tentacle scales, and one in every 25 specimens had two inconsistent characters.

In the Natural History Museum (London) there are specimens with a single tentacle scale (*O. schoenleinii*) from the Andaman Islands in the Sea of Bengal and from Madagascar which extend the known distribution of this species from the East-Indo-West Pacific Ocean (Devaney 1970, Clark & Rowe 1971).

The analysis of mitochondrial sequences from the 16 specimens identified 10 unique haplotypes within the *Ophiocoma erinaceus* species complex. Phylogenetic analyses of these sequences using Neighbour-Joining (NL, not shown), Maximum Parsimony (MP, not shown), and a Bayesian consensus (BML, Fig. 3) of 4900 maximum likelihood trees yielded largely congruent topologies. The NL and MP topologies differed from the consensus tree in placing all the *Ophiocoma* haplotypes as a sister group to the outgroup. In all trees, the *Ophiocoma* haplotypes formed three major clades. The specimens with red tube feet (identified here as *O. erinaceus*) formed a single well-supported clade. However, specimens with dark tube feet (identified here as *O. schoenleinii*) formed two distinct clades (A and B). Interestingly, the *O. schoenleinii* specimens were paraphyletic with respect to *O. erinaceus* in all analyses, *O. schoenleinii* clade B being sister group to *O. erinaceus* (clade E), although the bootstrap values for this node were relatively low. The average Kimura-2 distance between haplotypes in clades B and E was 0.19 compared to 0.21 between A and B and 0.25 between A and E. The average intra-clade distance between haplotypes was 0.016, 0.034, and 0.022 respectively for clades A, B and E. The large difference between intra-clade and inter-clade distances is indicative of there being three distinct species (Avise 2000).

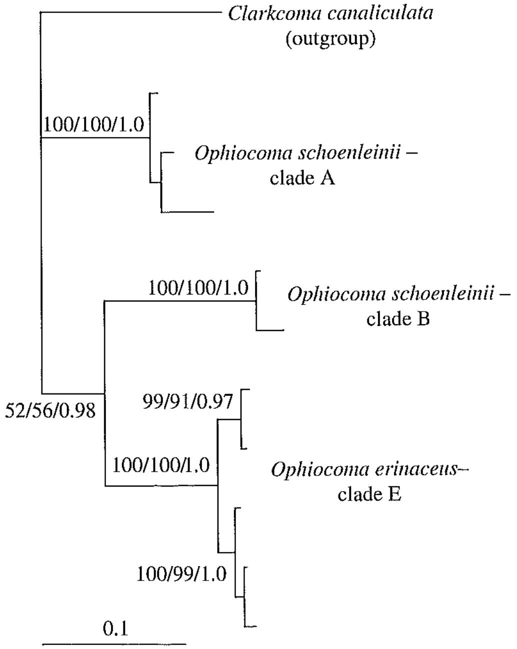

Figure 3. Bayesian maximum-likelihood (BML) consensus tree for ten haplotypes of the *Ophiocoma erinaceus* species complex (n = 16) from Raine Island. Numbers next to the branches indicate Neighbour-Joining and Maximum Parsimony bootstrap values and BML posterior probabilities.

Re-examination of specimens assigned to these three clades revealed a surprisingly clear morphological pattern. *O. schoenleinii* clade B was intermediate between the classic *O. schoenleinii* (clade A) and *O. erinaceus* (clade E) in having dark tube feet and two tentacle scales on most arm pores. Thus clade B is likely to be confused with *O. schoenleinii* in the field and with *O. erinaceus* in preserved museum material. Fortunately, adult specimens of clade B can also be distinguished from both other clades by the almost complete lack of granules on the ventral disc surface (granules are often absent from all forms in juveniles). The discovery of this new clade accounts for much of the perceived variation in the morphological characters found in museum specimens.

Unfortunately, specimens from clade A and B were not differentiated in the field and further work is required to identify the colour and life history characters that distinguish these two taxa.

4 DISCUSSION

There now appears to be abundant evidence that there are three clades of black *Ophiocoma* in the Indo-Pacific which can be distinguished by molecular data and subtle morphological, colour and behavioural differences. Two of these clades appear to be referable to the nominal species *O. erinaceus* and *O. schoenleinii*, although this needs to be confirmed by examination of the type specimens. In addition, there is a third clade that is potentially undescribed.

The evidence suggests that these clades are separate species. While only one gene has been sequenced to date, the clades are separated by large Kimura-2 distances, compared to within-clade variation. All three species occur at Raine Island, and the sympatric distribution of deeply divergent clades suggests reproductive isolation (Avise 2000). There are also distinguishing morphological characters that became apparent once some specimens had been placed into separate clades by molecular analysis.

The apparent existence of a third species of black *Ophiocoma* was a surprising result; one not predicted from field surveys, experiments, or prior examination of preserved specimens. Consequently, many of the field characters of "*O. schoenleinii*" remain unclear and must await further study. This includes the colour change, egg and larval characters reported in this paper.

This case study is a good example of molecular data revealing previously unrecognised cryptic speciation. Cryptic speciation is apparently ubiquitous in the marine environment (Knowlton 1993). Recent phylogenetic and phylogeographic studies suggest that cryptic species may also be common in the Echinodermata (Table 1). The majority of these studies have reported at least one unrecognised species. These species are generally distinguishable by subtle morphological, colour or life history differences that become clear after the molecular analysis has been performed.

The implications of unresolved cryptic speciation are profound. In this case, experiments and surveys that were designed to resolve the subtle differences between *O. erinaceus* and *O. schoenleinii* have been confounded by the subsequent discovery of sympatric cryptic taxa. Unresolved cryptic speciation has the potential to confound ecological, behavioural, reproductive, and biogeographic studies that include these animals.

Based on the limited data reported in Table 1, the species-level diversity of echinoderms may have been considerably underestimated. Some of these new species may be short-range endemics that may require management action to secure their long-term conservation. Some exploited species may prove to be heterospecific, invalidating fishery models. It is imperative to understand the phylogeography of exploited species before populations are relocated for aquaculture or natural stock enhancement.

A new effort to validate historical echinoderm taxonomy using modern molecular methods is required. Echinoderm taxonomy is still incompletely known and is deserving of appropriate biodiversity funding.

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