2. Taxonomic study of Ophiuroidea

2.1. Collecting

Collecting brittle and baskets stars can be challenging, as they often 'fall to pieces' when handled. With careful handling and gentle manipulation, they generally can be collected by hand without damaging them. Lifting specimens with a scraper or knife or by lifting them by the disc can assist in handling.

They occur in a wide variety of habitat types, under rocks, inside crevices and crannies (Fig. 3), within sediment, on open reef (Hyman 1955), and amongst algae and other organisms, such as jellyfish, soft corals and sponges. In some cases, brittle and basket stars may be cryptic and nocturnal, but are relatively easy to find and collect by hand by breaking rocks and by lifting boulders or rocks.

When collecting, these shelters should be carefully returned to their original position to avoid crushing other organisms and to minimize any damage or disturbance to their habitat. The marine environment is under immense pressure so it is important, when collecting, to only take what you need, do not waste samples and take proper care of your samples to maximise the data obtained.

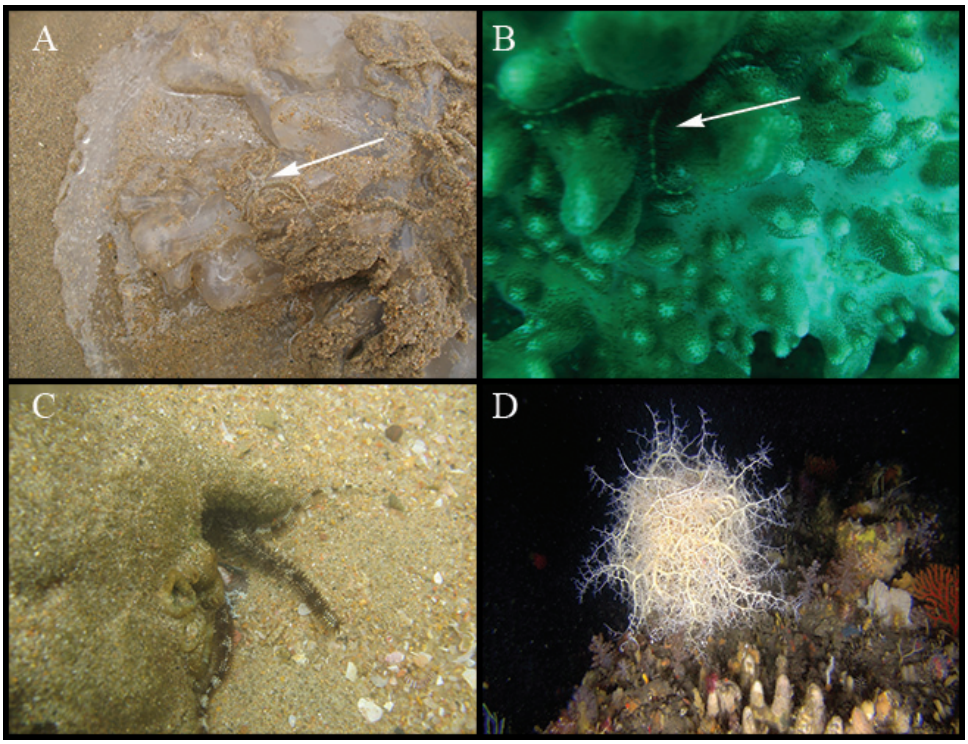


Fig. 3. Ophiuroidea are found in many habitat types and together with other organisms. **A**. On jellyfish. **B**. On soft coral. **C**. In crevices, under rocks and boulders. **D**. On open reef.

In the field, buckets, plastic resealable bags, collecting mesh bags and plastic bottles are good temporary storage items. When collecting, plastic bags are effective in that they form a protective water-balloon around the specimens. Mesh bags can also be used, but basket stars become tangled in the mesh and may prove difficult to remove from the bag. It is preferable to keep specimens separated from each other to avoid antagonistic effects.

If specimens need to be studied alive in the laboratory, it is preferable they are returned alive once the study is complete. However if the specimens have been kept in a laboratory together with alien species, or the laboratory has had a recent disease outbreak, it is better to destroy the specimens or have them lodged in a museum for taxonomic studies. Specimens should not be returned to sites other than the one from which they were collected, as this can spread diseases, result in disruption of genetic structure of populations or spread species to sites in which they would not naturally occur.

When specimens are collected for purposes where they are required to be killed or specimens which die during a study, it is important to deposit representative samples in a natural history museum, where they will be preserved and used for future reference.

2.2. Photography

Photographs of specimens in their environment are incredibly valuable and hold an immense amount of information, but the cryptic nature of these animals is such

Fig. 4. Photographing the specimens alive or soon after being collected to capture the natural colour, together with labels and scale bars.

that photography is not always possible. Where specimens are required to be preserved, they should be photographed soon after collection (Fig. 4) before they lose their natural colouration. When photographing, a scale bar should be placed adjacent to the specimen. Photographs taken with the specimen placed beneath some water, may enhance the quality of the image. To avoid reflections on the surface of the water, place light source at a 45 degree angle.

Photographs of this nature can add immense value to online platforms such as iSpot (https://www.ispotnature.org/communities/southern-africa) and EchinoMAP (http://vmus.adu.org.za/), which hold distribution records and assist in species distributions.

2.3. Relaxation, fixation and preservation

Relaxing specimens can be somewhat time-consuming, but is worth the effort as this greatly enhances the scientific value of the specimens. It is essential to anesthetise or relax the specimens before fixation. Often they contort, crunch up or release their arms when chemicals are added to the water while they are un-anesthetised. To relax them, several methods exist, but adding Magnesium Chloride ($MgCl_2$) or Magnesium sulphate ($MgSO_4$; 4% being the desired concentration) to a basin of sea water allows the specimens to expire. If the concentration of $MgCl_2$ is too high, they will crunch their arms, but this can be counteracted by diluting the solution with more sea water and by placing pressure on the arms until the specimens relax. As the specimen begins to expire and relax more, slowly add more $MgCl_2$ to the solution until the specimens perish; ii) use a fresh and sea water solution, in the same manner as above iii) place specimens in the refrigerator overnight. All three processes can take a relatively long time, i.e., a few hours.

Relaxation is complete once the tube feet or arms no longer react to nudging or prodding. At this point, they need to be preserved in either 70-99% ethanol (C_2H_6O) or in 4% formaldehyde (CH_2O) solution. Ethanol (EtOH) is the preferred chemical because formaldehyde is acidic, hazardous to human health and damages the integrity of DNA, obstructing future molecular studies. After fixation, the fixative needs to be replaced with the preservation fluid, i.e., 70-80% ethanol. One can also opt to dry the specimens, a procedure that is less costly and saves precious museum shelf space. The disadvantage of dry preservation is the internal anatomy becomes largely unaccessible and the molecular integrity is reduced for study. Furthermore, if preserved dry, fixation in formaldehyde is preferred. Dry specimens are prone to insect infestations while collections in warm and humid climates are prone to mould, both of which, can destroy a specimen.

2.4. Molecular studies

For molecular studies, the specimens are required to be subsampled, which is recommended practice for all collection trips. This should take place after relaxation but before fixation and preservation. Depending on the size of the specimen, a small piece of the arm is cut off with sterilised scissors or scalpel and

placed in 99% ethanol. These are stored separately from the specimen in vials or Polymerase Chain Reaction (PCR) vials for further processesing. It is imperative that subsamples can be tracked back to the original specimen, see labelling below.

2.5. Labelling and record keeping

Data should be logged from the moment a collection trip begins until the specimens are preserved for long term storage in a museum. Every specimen should be accompanied with a label detailing collection information. Specimen labels (Fig. 5) should contain at least the following information: genus and species (if known), unique number, expedition name, locality, GPS coordinates, depth, habitat, collection date, collector name, collection method and if identified, who by. Special alcohol and water-proof paper must be used (i.e., Xerox NeverTear paper), while the label must be written using a soft lead pencil or in indelible Indian ink or printed with ink that will not dissolve in alcohol.

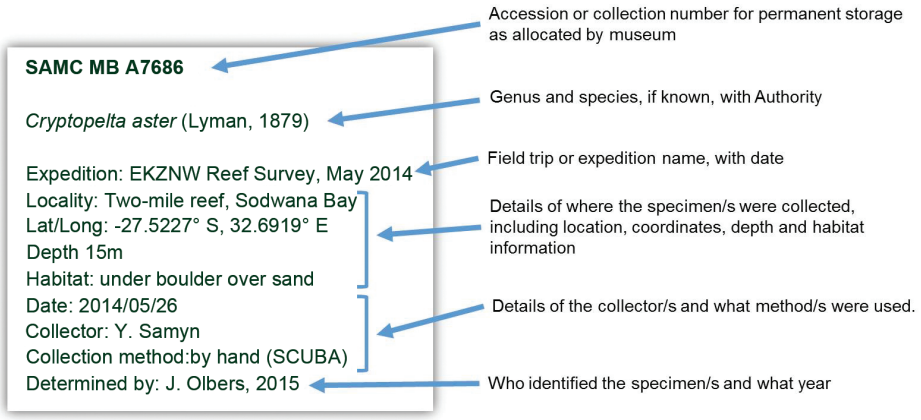


Fig. 5. Example of label with essential information.

2.6. Transportation

If the specimens are being transported locally, then storing them in ethanol in plastic resealable bags in a bucket is acceptable. Labels with data are required for each specimen or lot of specimens, even during transportation or temporary storage.

If specimens are to be couriered or posted, then specimens will require correct packaging procedures (Fig. 6), relevant permits (transportation, import, export) and will be required to meet shipping standards and codes, as per shipping regulations and the courier company.

The International Air Transport Association (IATA) has shipping standards which specimens are required to be packaged in accordance with. Natural history

Fig. 6. Packing specimens for transportation requires correct procedures. **A**. Each specimen / lot to be packaged separately in a bag of the correct size, this can be made with plastic and a heat sealer. **B**. Seal three sides of the bag for the specimens. **C**. Insert specimens, ethanol (not more than 30 ml in inner package) and label. **D**. Seal bag and check for leaks. **E**. Make and seal specimen in a triple bag, placing some absorbent material in the third bag. **F**. Wrap in bubble wrap. **G**. Place in box with packaging material. **H**. Place additional packaging material around specimen. **I**. Seal the box and attach necessary documentation or permits.

specimens are considered to be in the IATA category of 'dangerous goods' when in ethanol. Specimens will require an IATA SP A180 shipping declaration to accompany the package. An example of a shipping declaration, to be placed on the outside of the package, is given on page 22.

2.7. Storage

Once the specimens reach their destination for identification and/or permanent storage, it is imperative that specimens are not muddled up and the labels with data are kept meticulously together with the samples and/or specimens

Museums and institutions around the world have different storage techniques and facilities. Brittle and basket stars can be stored wet or dry. If wet, they should be

Cape Town, 18 December 2018

SHIPPING DOCUMENTATION/CUSTOMS DECLARATION

"scientific research specimens, not restricted, special provision A180 applies"

Full description of goods: Ophiuroidea specimens preserved in a minimal quantity of 70 % ethanol. These biological samples are non-toxic, non-pathogenic and are derived from non-CITES listed species. They are on loan from the Iziko South African Museum in Cape Town to the Royal Belgian Institute of Natural Sciences in Brussels (Belgium).

The scientific research specimens are not restricted and have been packed according to IATA SP A180 (triple heat-sealed packing, no more then 30 mL of free ethanol in inner package, outer packing not exceeding 1 L, absorbent material included)". Class 3/UN1170/PG III.

Declared value: ZAR 100

Iziko South African Museum
Collections Manager
25 Queen Victoria Street
Cape Town
8001
South Africa
Email address:
Tel: +2721 481 3800

Important

Postal inspectors: This package contains dead, preserved material for scientific research without commercial value. If this shipment is inspected, it is absolutely imperative that the packages are sealed tightly again. If not, the material will dry rapidly and become useless for scientific research. We thank you very much for taking good care of this important resource.

Très important

**Précautions à Prendre à l`inspection postale**: Ce colis contient du matériel biologique fixé dans un produit de conservation et est destiné à des études scientifiques. S`il est ouvert pour une inspection il est très important que les sacs en plastiques doivent de nouveau être soigneusement scellés. Si non, le matériel biologique dessèchera rapidement et deviendra alors inutile pour étude. Nous vous en remercions beaucoup.

Convention on International Trade in Endangered Species of Wild Fauna and Flora: *Include Institutional CITES Number*
HS-Code: 9705.00 (Collections of zoological/botanical/ mineralogical/archaeological/paleontological interest)

preserved in 70-96% ethanol, which covers the specimen, in well-sealed jars, after which the ethanol regularly needs to be topped up, if evaporation occurs. If the climate is not excessively humid then specimens can be dried for storage, which is often the case when large specimens are being stored. Drying brittle and basket stars can compromise the integrity of the DNA and the morphology of many internal features, conversely, examination of skelatal features is often enhanced.

2.8. Permits and legislation

South Africa is a signatory to the Nagoya Protocol on Access and Benefit Sharing and collecting any marine plant or animal can be undertaken with two types of permits. A recreational permit allows for collection for food, bait and/or for use in home aquaria, this is for personal use only. The second permit is for scientific research which needs to be applied for from the Department of Environment, Forestry and Fisheries. In some cases, permission from the marine protected area management authority is also required.

All collecting requires a permit, regardless of whether the specimen will be released alive after the study or not. The National Environmental Management Act (107 of 1998) creates the framework for environmental law in South Africa together with its associated regulations and Specific Environmental Management Acts. For all marine species, including brittle and basket stars, the following legislation applies:

– National Environmental Management: Biodiversity Act 10 of 2004;
– National Environmental Management: Protected Areas Act 57 of 2003;
– National Environmental Management: Integrated Coastal Management Act 24 of 2008; and the
– Marine Living Resources Act 18 of 1998.

Permits for scientific research are applied for to the Permits for scientific research are applied for to the Department of Environment, Forestry and Fisheries, at the following email address: researchpermits@daff.gov.za.

For further queries:

Dr. Kim Prochazka
Director: Resources Research
Tel: +27 (0)21 402-3546
Email: kimpro@environment.gov.za

Ms Melleney Cope
Personal Assistant to Dr. Kim Prochazka
E-mail: melleneyC@daff.gov.za

2.9. Morphology, biology and taxonomic terminology of brittle and basket stars

There are 33 families arranged into six orders as per O'Hara *et al.* (2018): Euryalida, Ophiurida, Ophioscolecida, Ophiacanthida, Ophioleucida and Amphilepidida. All six orders and 26 families are represented in the South African Ophiuroidea fauna.

The Ophiuroidea are most similar in body shape to the Asteroidea and can be differentiated from them by a number of features, but most importantly because the arms of an asteroid are usually confluent with one another and the body cavity between the arm and disc is open. To identify Ophiuroidea, knowledge of the terminology used to describe their anatomy is necessary. A glossary of terms used can be found at the end of this guide.

The morphology of the ophiuroid is illustrated in Fig. 7 with additional figures below. It is important to understand the location of features when referring to the body plan of an ophiuroid. Proximal is closest to the centre of the disc and distal is furthest from the centre or the disc.

The water vascular, nervous and haemal systems are similar to those of asteroids. Each arm contains a small coelom, a radial nerve, and a radial canal of the water vascular system. In contrast to other echinoderms, the ambulacral grooves are enclosed and covered by plates. A pair of tube feet are present on each arm joint on the ventral surface, which in many cases are protected by one or more modified spines or tentacle scales.

Five pairs of invaginations called bursae open toward the ventral surface through genital slits at the bases of the arms. They can be variable in size and shape but they generally extend from the disc margin to the oral shields, supported either side by a genital plate. These plates may be distinct, but the shield adjacent to the arm base is usually indistinct. The genital slit edges may be smooth, have scallops, or bear genital papillae. Externally, these slits may be long and narrow, short and wide, or be divided into pairs. Water circulates in and out of the bursae for exchange of gases. Gonads occur on the coelomic walls of each bursa and

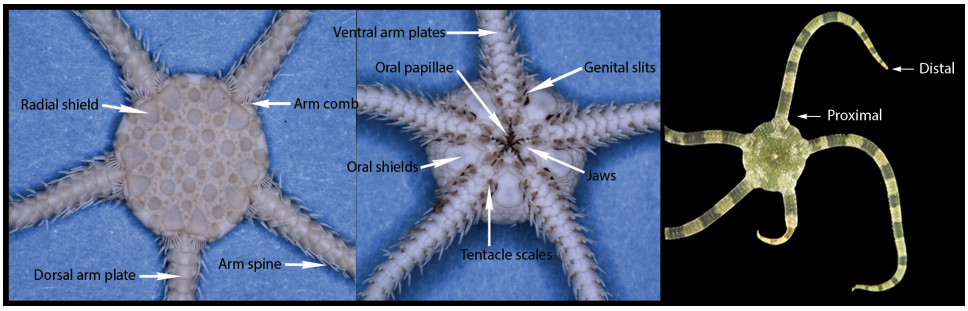


Fig. 7. General morphology of an ophiuroid, also indicating position of distal and proximal in relation to disc.

discharge their ripe sex cells, passing through the genital slits into the water for fertilization.

In most cases, the organs are confined to the disc, with the stomach being sac-like. There is no anus, thus any indigestible material is expelled through the mouth. The disc can be round or pentagonal, flat or puffy, excavated or indented radially or interradially. The disc is covered in plates and may be covered with thickened skin, scales, spines, granules, stumps, or a combination of these.

The main taxonomic characters on the dorsal disc are the radial shields (Fig. 8) and the primary scales or primary rosettes, including the central scale, which may or may not be distinct (Fig. 9). The armament of the dorsal disc is also of prime importance and may include granules, spines or tubercles. Figure 8 shows some of the dorsal characters and a composite of the dorsal disc armament of some common families.

The ventral surface of the disc (Fig. 10) holds more taxonomically informative characters. Adjacent to the jaws, the main characters visible are the genital slits, with some taxa also bearing genital papillae.

On the arms, the dorsal, ventral and lateral arm plates are taxonomically significant. The arm plates are taxon-indicative in width: length ratio and the curvature of the plates distally and/or proximally. Lateral arm plates support the arm spines, in

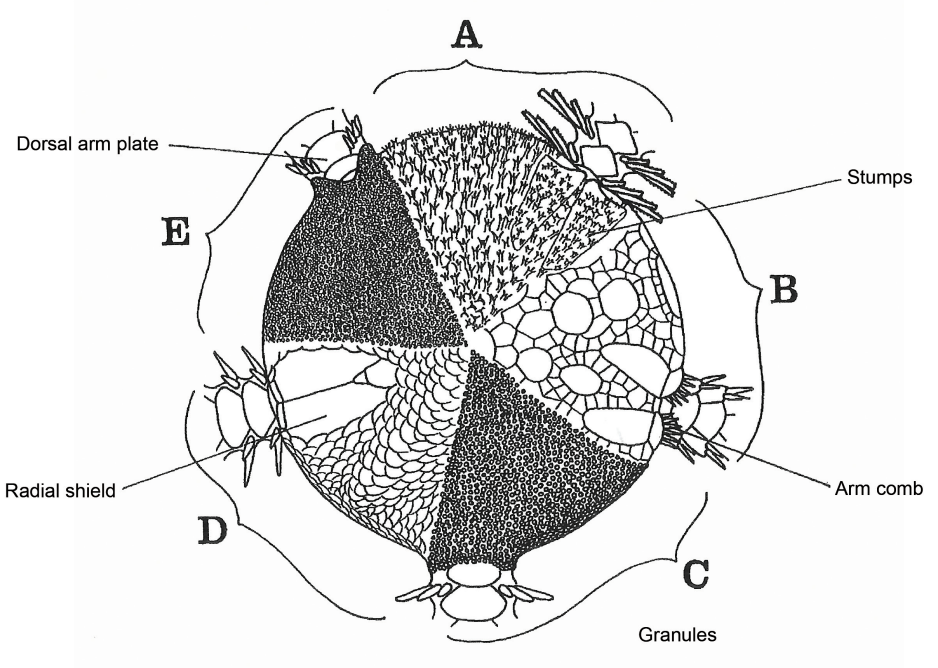


Fig. 8. Composite diagram showing characters of the dorsal surface of the disc in the following families. **A**. Ophiotrichidae. **B**. Ophiuridae. **C**. Ophiocomidae. **D**. Amphiuridae. **E**. Ophiodermatidae. From Clark & Rowe (1971).

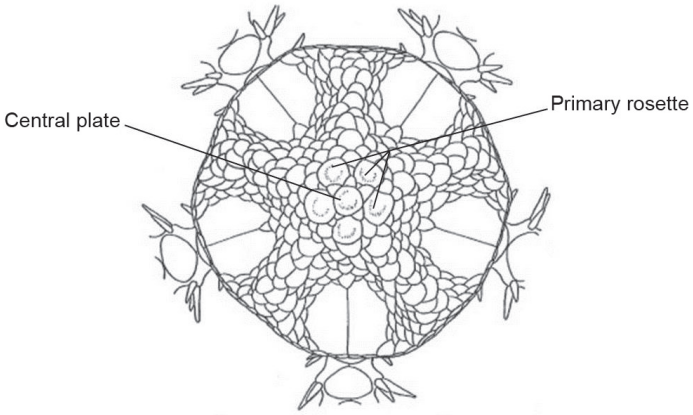


Fig. 9. Plates forming part of the primary rosette including the central plate. Adapted From Clark & Rowe (1971).

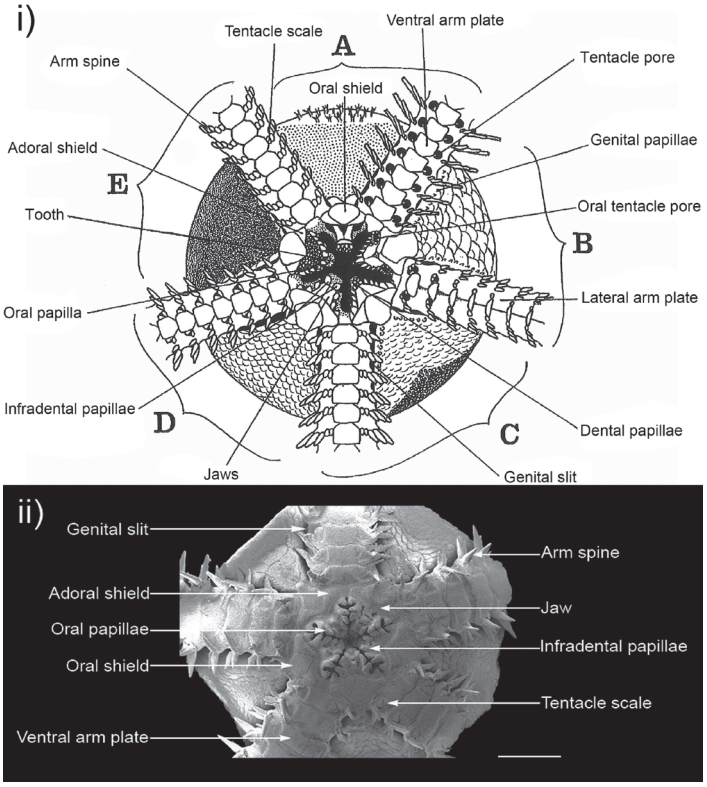


Fig. 10. Composite diagram (i) showing characters of the ventral surface of the disc in the following families. **A**. Ophiotrichidae. **B**. Ophiuridae. **C**. Ophiocomidae. **D**. Amphiuridae. **E**. Ophiodermatidae. From Clark & Rowe (1971). Ventral disc of an amphiurid (ii). Scale bar: 1 mm. Photo: Didier VandenSpiegel.

which some species, the number and sequence have been used as important features (Devaney 1970).

The ventral interradial areas may also be covered in a combination of granules, spines, tubercles and scales. Figure 10 shows the main ventral characters and a composite of typical ventral disc armament for some common families.

In this guide, the primary characters by which most families are distinguished from each other are with the jaws. Jaw features include the oral papillae, dental papillae, oral tentacle pores, oral tentacle scales, teeth, oral shields, dental plates at the tip of the jaws, and adoral shields, which flank the oral shields on either side. Figure 11, a side view of the jaw, shows the placement of the dental plate, teeth and dental papillae, while Figure 12 shows the placement of teeth, dental papillae

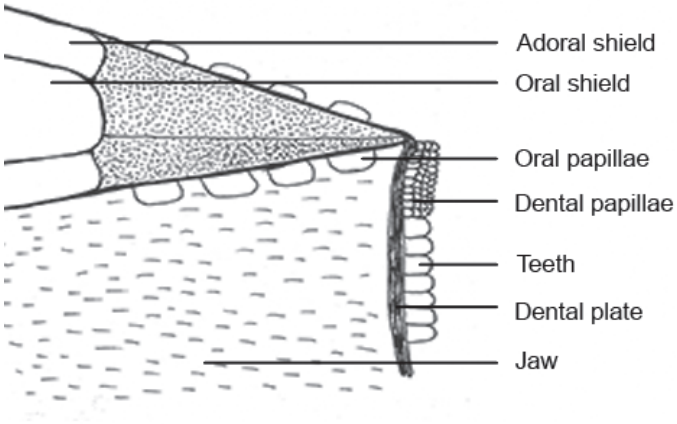


Fig. 11. Side view of the jaw, indicating placement of the dental plate, dental papillae, teeth, oral shield, adoral shields and oral papillae.

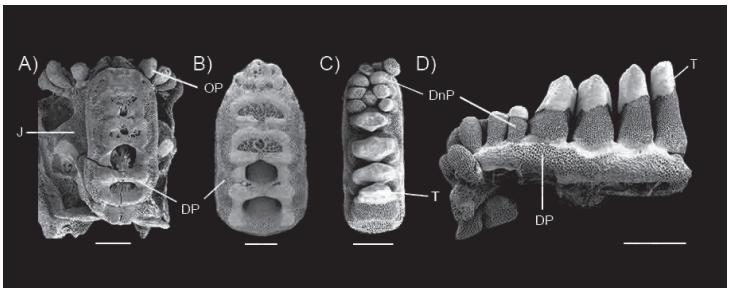


Fig. 12. Placement of dental plate, dental papillae, oral papillae and teeth. **A**. Dental plate attached to jaw (*Breviturma brevipes*). **B**. Dental plate with no teeth or dental papillae attached, showing structures to which teeth and papillae would attach (*O. scolopendrina*). **C**. Dental plate with teeth and dental papillae attached (*Ophiocoma erinaceus*). **D**. Side view of dental plate with teeth and dental papillae attached (*Ophiocoma erinaceus*). Abbreviations: J = jaw; OP = oral papillae; DP = dental papillae; DnP = dental papillae; T = teeth.
Scale bar: 0.5 mm. Photos: Didier VandenSpiegel.

and oral papillae on the jaw. The madreporite, a modified oral shield, is located in the vicinity of the mouth. In combination with the jaws, the arrangement, number, shape and size of various other external characters determine genera and species. The dental plate requires dissection to view and is used as a taxonomic character for some species.

There are usually five arms, but sometimes more, and these can be long and slender, short and stout and may be smooth or spiny. While the majority of species have simple arms, basket stars have branching arms, producing a network of tree-like branches. To the eye, the ophiuroid arms appear to be segmented, but these correspond to internal articulated ossicles or vertebrae (Fig. 13) which are connected by soft tissue. They are usually covered dorsally, ventrally and laterally by arm plates (Fig. 14).

In some families or genera, there are supplementary plates or shields adjacent to the dorsal arm plates, ventral arm plates or the oral shields (Fig. 15).

The distal, lateral and/or proximal shape of the arm plate edges are of significance in taxonomy. The plate edges may be concave (curving in), convex (curving out) or straight (Fig. 16).

Most often the lateral arm plates bear arm spines, varying in number, forming a vertical series. The arm spines may be positioned at right angles, or they can be appressed to the arm. The arm spines (Fig. 17) can vary in length and shape and may be tapering, pointed, blunt, clavate or hooked. In addition, the spines can be smooth, serrated, or bear hooks to varying degree.

In some cases (except in the girdle hooks of Gorgoncephalidae) arm spines transform into hooks in various forms and number. The terminal or primary tooth is on the distal end of the hook, while the secondary, additional teeth and the lamina of the structure are proximal to the base of the hook or spine (Fig. 18).

Radial shields (dorsal side of the disc) and oral shields (ventral side of the disc) are described in length and width (Fig. 19) and in some cases in colouration and armament (e.g., granules, spines). The distinction between width and length is important for both these skeletal structures.

The terminology used for describing the shape and form of various plates, shields and papillae is illustrated in Figure 20.

Various terms used to describe disc armament, arm spines and protrusions are illustrated in Figure 21.

As explained above, the combination of jaw characters are important in distinguishing between many Ophiuroidea families. Table 2 illustrates the position and arrangement of the oral papillae, dental papillae, oral tentacle pores, oral tentacle scales, teeth, oral shields and adoral shields in 26 Ophiuroidea families where the differences are most obvious.

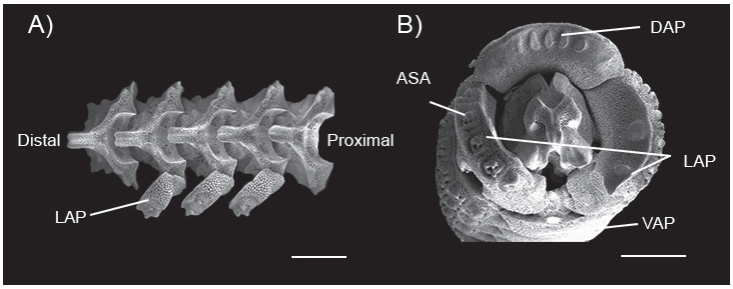


Fig. 13. Arm vertebrae of Ophiuroidea. **A**. Series of vertebrae, dorsal view, three lateral arm plates attached (Ophiotrichidae). **B**. Section of arm with arm plates attached around vertebra (*Ophiarachnella* sp.). Abbreviations: ASA = Arm spine articulation; DAP = dorsal arm plate; LAP = lateral arm plate; VAP = ventral arm plate. Scale bars: 1 mm. Photos: Didier VandenSpiegel.

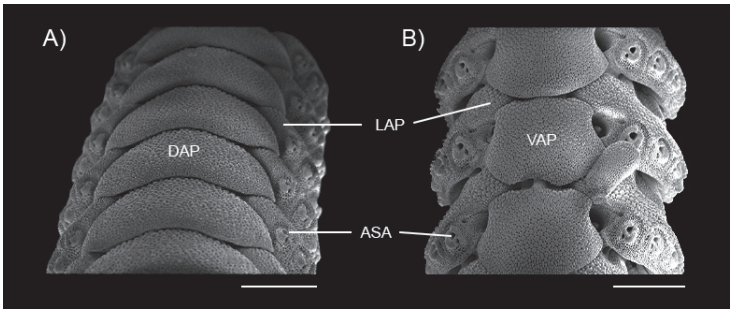


Fig. 14. View of dorsal and ventral arm of *Ophirachnella* sp. **A**. Dorsal and lateral arm plates. **B**. Ventral and lateral arm plates. Abbreviations: ASA = Arm spine articulation; DAP = Dorsal arm plates; VAP = Ventral arm plates; LAP = Lateral arm plates. Scale bars: 1 mm. Photos: Didier VandenSpiegel.

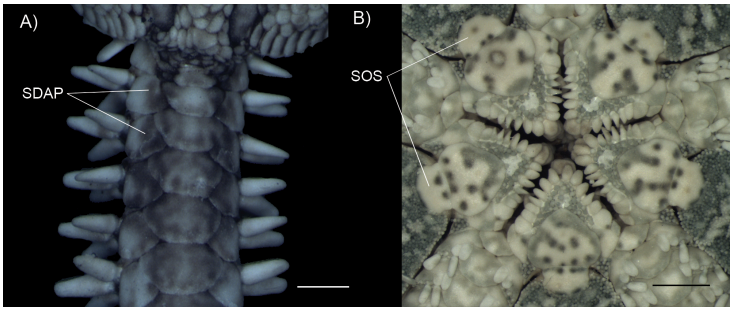


Fig. 15. Placement of supplementary plates and shields. **A**. Supplementary dorsal arm plates (*Ophionereis porrecta*) on dorsal arm. **B**. Supplementary oral shields (*Ophiarachnella gorgonia*) adjacent to oral shields. Abbreviations: SDAP = Supplementary dorsal arm plates; SOS = Supplementary oral shields. Scale bars: A = 1 mm; B = 2 mm. Photos: Didier VandenSpiegel.

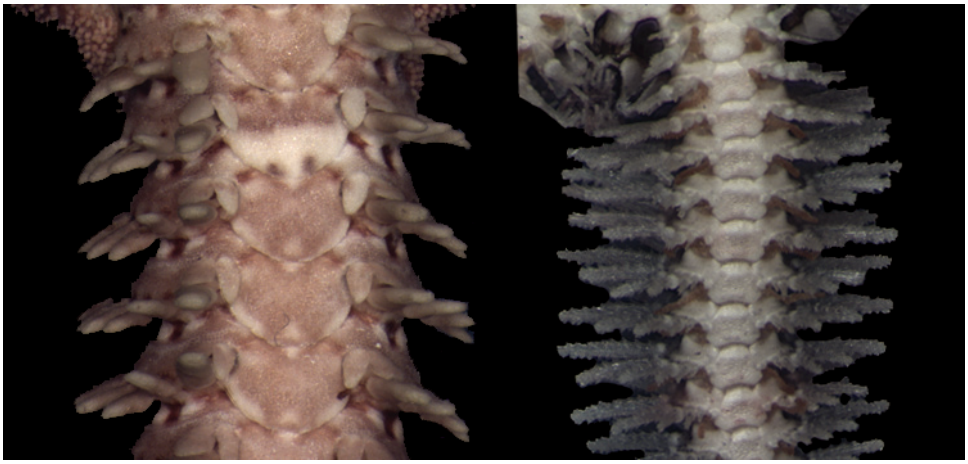


Fig. 16. Section of ventral arms showing arm plate edges which are distally convex (left) and distally concave (right). Photos: Didier VandenSpiegel.

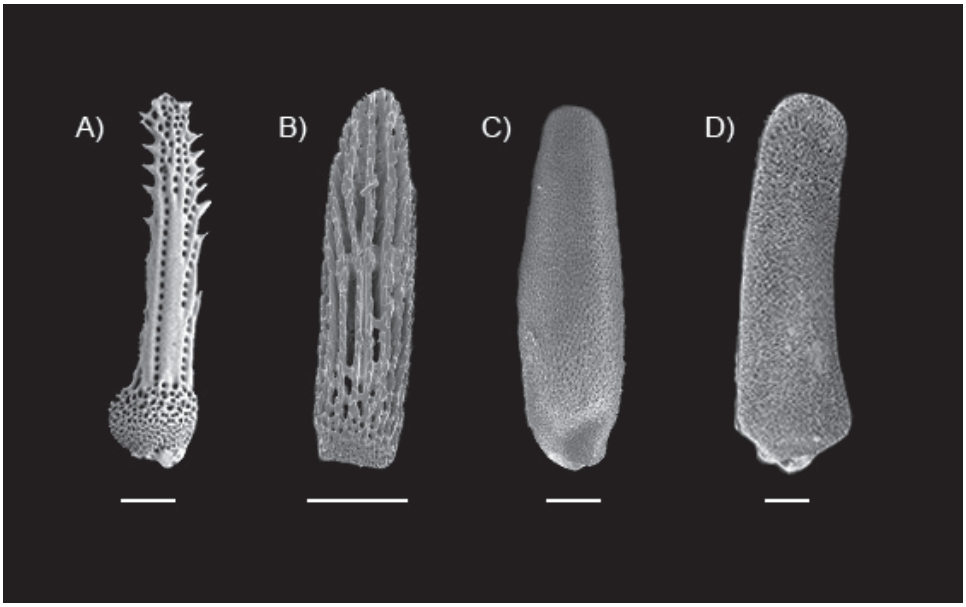


Fig. 17. Arm spines illustrating the different forms and shape. Tapering (A, B and C), pointed (A and B), blunt (C, D), cigar (D), smooth (C, D) and serrated (A). **A**. *Macrophiothrix* sp. **B**. *Ophioconis cupida*. **C**. *Ophiocoma* sp. **D**. *Ophionereis* sp. Scale bar: A, C, D = 200 µm; B = 50 µm. Photos: Didier VandenSpiegel.

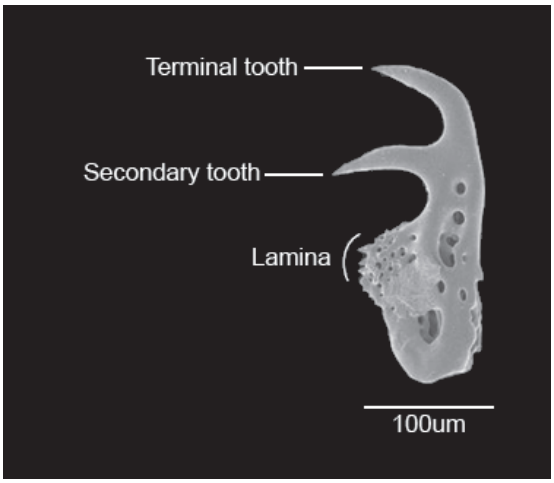


Fig. 18. Hooked arm spine showing placement of terminal tooth, secondary tooth and lamina. Photo from Okanishi *et al.* (2013).

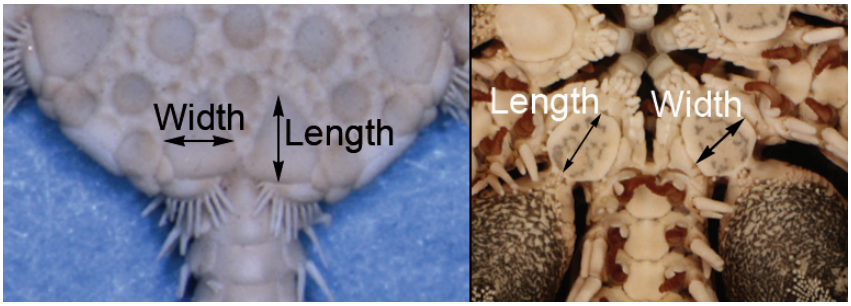


Fig. 19. A pair of radial shields (left) and two jaws with their oral shields (right) illustrating their width and length.

Table 2. Representative jaws and key features of South African Ophiuroidea families. Papillae and teeth are colour coded: Yellow: infradental papillae, blue: apical papillae, green: oral papillae, purple: dental papillae, orange: teeth, pink: oral tentacle scales. Illustrations adapted from Mortensen (1933a), Clark & Rowe (1971), Clark & Courtman-Stock (1976) and O'Hara *et al.* (2018).

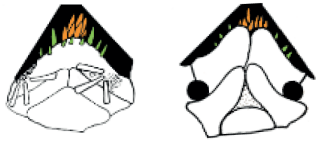
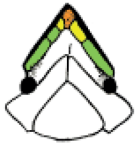
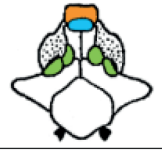
Asteronychidae Multiple columns of spiniform oral papillae. 	**Euryalidae** Single column of teeth and sometimes some papillae either side which may or may not be considered as true oral papillae. 	**Gorgonocephalidae** Multiple, more or less spiniform, oral papillae in irregular columns. 	**Ophiomusaidae** Oral papillae almost appear fused, structure of each papilla still visible. 
Astrophiuridae Single apical papillae with numerous oral papillae either side. 	**Ophiuridae** Numerous oral and apical papillae, oral tentacle scales both sides of second oral tentacle pore, often within series. 	**Ophiopyrgidae** Oral papillae close together or may appear fused, lowermost tooth distinct or apical papillae present. 	**Ophioscolecidae** Both teeth and oral papillae serrated, may be broad or narrow and appear similar. Oral papillae either side of jaws, teeth apical 
Ophiotomidae Clusters of short oral papillae, sometimes papilliform. 	**Ophiacanthidae** Single pointed apical papilla flanked by three or more oral papillae, often with distalmost papillae being enlarged. Teeth below apical papillae. No clusters. 	**Ophiodermatidae and Ophiopezidae** Teeth not particularly broad, oral papillae in a vertical series down sides of the jaw and, if present, in series with oral tentacle scale towards the distal end. 	**Ophiomyxidae** Both teeth and oral papillae serrated, may be broad or narrow and appear similar. Oral papillae either side of jaws, teeth apical. 

Table 2 (continued).

Ophiocomidae Teeth broad and square (not illustrated here) with a number of dental papillae and oral papillae present. 	**Ophiernidae & Ophioleucidae** Oral papillae may be pointed or opercular. Teeth present, tapering to blunt point. 	**Ophiolepididae** Rounded oral papillae with distalmost being broadest and in series with single tentacle scale. 	**Hemieuryalidae** Oral papillae, distalmost being broadest, remaining papillae elliptical leaf-shaped, apical papillae bluntly pointed. Teeth four, rounded (not illustrated here). 
Amphilimnidae Apical oral papillae asymmetrical, distalmost oral papillae on edge of adoral shield. Teeth single, broad. 	**Ophionereididae** Broad quadrangular teeth blunt angled apices. The four or more oral papillae may be in series or overlap the tentacle scale of second oral pore. 	**Ophiopsilidae** Oral papillae separated from apical tooth by diastema. 	**Amphiuridae** Two infradental papillae spaced apart and symmetrical, teeth may be broad or tapering and flanked by one, two or three oral papillae either side. 
Amphilepididae Two infradental papillae spaced apart, oral papillae, distalmost elongated and much longer than proximal-most. Teeth triangular and long. 	**Ophiothamnidae** Numerous oral papillae, distalmost broad and opercular, single large apical papillae. 	**Ophiactidae** Single, apical, broad and blunt papillae and broad, rounded or rectangular teeth with a diastema between these and the one or two oral papillae. 	**Ophiotrichidae** Cluster of dental papillae superficial to the broad rectangular teeth (not illustrated here) and no oral papillae present. 

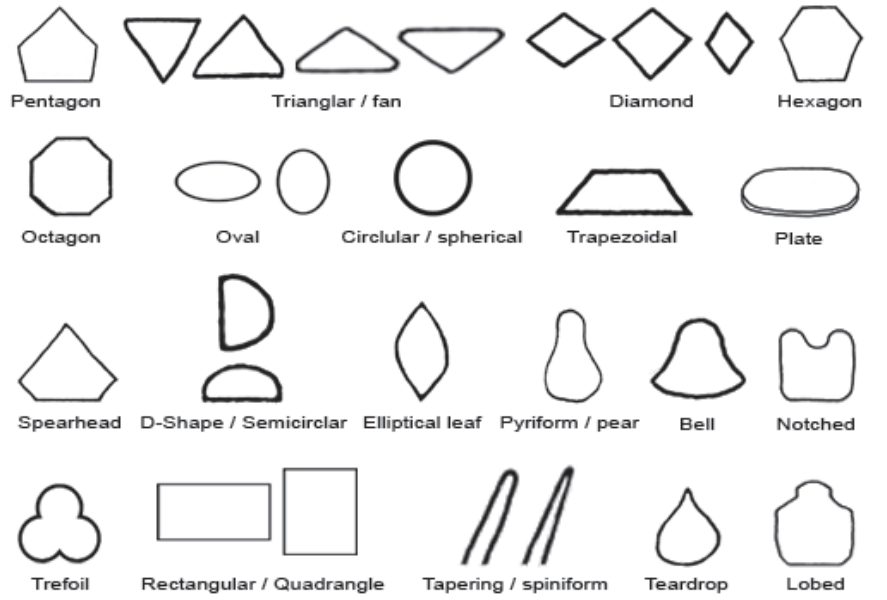


Fig. 20. Terms describing various shapes of plates, shields and papillae.

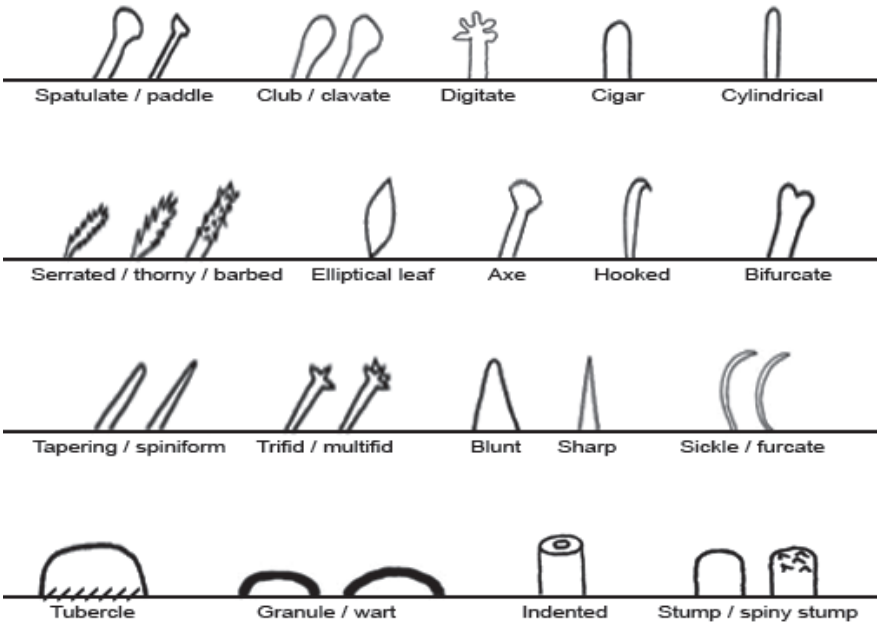


Fig. 21. Terms describing various disc armament, arm spines and protrusions.