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10. Taxon Groups: Medusozoa and Ctenophora

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Darwin Tree of Life



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This is a working protocol that may be subject to changes in the future.

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Abstract

This is part of the **collection** "DTOL Taxon-specific Standard Operating Procedures (SOPs) for Marine Metazoa", lead by the Other Metazoa Working Group. The SOP collection contains guidance on how to process the various marine Metazoa species within the scope of the Darwin Tree of Life project. The guidance specifically refers to the tissue samples needed for DNA barcoding (which takes place at the Natural History Museum (NHM) and at the Marine Biological Association (MBA)) and outlines the dissected tissues required for whole genome sequencing, which takes place at the Wellcome Sanger Institute. Every specimen is submitted for DNA barcoding first before potentially being sent to the Wellcome Sanger Institute.

Definition: Medusozoans are distinguished by having a medusa stage in their complex life cycle. A medusa is typically an umbrella-shaped body with stinging tentacles around the edge. With the exception of some Hydrozoa (and Polypodiozoa), all are called jellyfish in their free-swimming medusa phase. Ctenophora, also known as comb jellies, sea gooseberries, sea walnuts, or Venus's girdles, are typically predators. Unlike medusozoans, with which they share several superficial similarities, they lack stinging cells.

Including: Scyphozoa, Staurozoa, Ctenophora, Cubozoa.

Excluding: Hydrozoa, Polypodiozoa. Specimens under 5mm.

Please note, some medusozoan species have stinging cells. Species which are known to be dangerous to humans should be avoided unless adequate safety measures are in place. This SOP does not cover precautions against stings and other potential risks.

See the Guidelines for important details and checklists.



Guidelines

Field sampling:

1. Environment to be sampled: Marine

2. Trap/method of sampling: Direct removal from substrate (for stalked jellyfish/ Staurozoa (stauromedusae)) or water column. May be caught as bycatch in fishing nets, or collected by divers by hand or by net. Some may be found in shallower water, or off a marina or other structure and can be collected via net. Some species (Staurozoa (stauromedusae)) can attach to other living things and may need to be collected alongside their associated organism.

Note

Each specimen, regardless of species, must have its own relevant unique identifier (e.g. QR code) which will be attached to any subsequent tubes, genome or barcoding results.

For genome sequencing:

3. Specimens can be sampled live. Most specimens will not survive very long out of their natural habitat and should be processed quickly, they are also easily damaged and need to be handled carefully.

Photography:

4. Recommendations listed below:



Note

Scyphozoa

Including Coronatae, Semaestomeae and Rhizostomeae

Overall shot of body showing shape of umbrella, and placement/organisation of radial canals, oral arms, tentacles (if present), manubrium and gonads. For smaller organisms, close-ups of marginal vestical arrangement if present.

Staurozoa

Bell with arms (if present) and tentacles (including primary tentacles if present), showing number and arrangement of these features. Close ups of gonad shape and position. Peduncle in relation to bell. Close ups of tentacles may be needed for some species.

Include in-situ shot (if possible) as this can help with identification.

Cubozoa

Photograph the shape of the bell, position of interradial tentacles and pedalia, as well as the periradial sense organ.

Ctenophora

Photograph overall shape of the body and comb rows. Tentacles (if present), tentacle pouches (if present).

5. The image should be taken in the highest quality resolution - a macro lens is recommended. The photos should be of high enough resolution to be diagnostic, when possible.

Photograph to include a unique identifier (e.g. QR code, specimen barcode) where possible; where no voucher specimen parts are retained the photograph will serve as voucher and should include identifying features.

Note

Further tips

Medusozoans/ctenophores can be particularly hard to photograph as some are translucent. These species often best show their features when placed against a dark background. Most will photograph better when they are in water to display tentacles and features.

Dissection for DNA barcoding:

6. Tentacles can be useful for barcoding. Avoid gonad and any stomach contents, or a small section of body tissue. – rinse in filtered sea water and blot.



Once the tissue for barcoding is removed, put the tissue in 100% ethanol. The rest of the frozen/live organism can then be dissected.

Dissection for whole genome sequencing:

7. Use any soma/body tissue, avoiding any obvious digestive tissue, would be appropriate for whole genome sequencing. it is recommended to blot out any slime, either through rinsing in filtered sea water or blotting.

Dissect up to ten, lentil-sized pieces in separate tubes if possible.

Tissue should be frozen at at least -80°, for example in dry ice, a liquid nitrogen charged dry shipper or in a -80° freezer.

Storage of frozen tissue:

8. If barcoded tissue passes the DNA barcoding stage, subsequent frozen tissue of specimen to be sent to Wellcome Sanger Institute.

Note

Please refer to [DNA barcoding SOP v2.1](#).

9. Leftover tissue from specimens must be sent to NHM for vouchersing and long term storage.

Storage of voucher:

10. Vouchers to be sent to/kept at NHM.

11. Vouchered tissue to be eventually preserved in 70-90% ethanol.

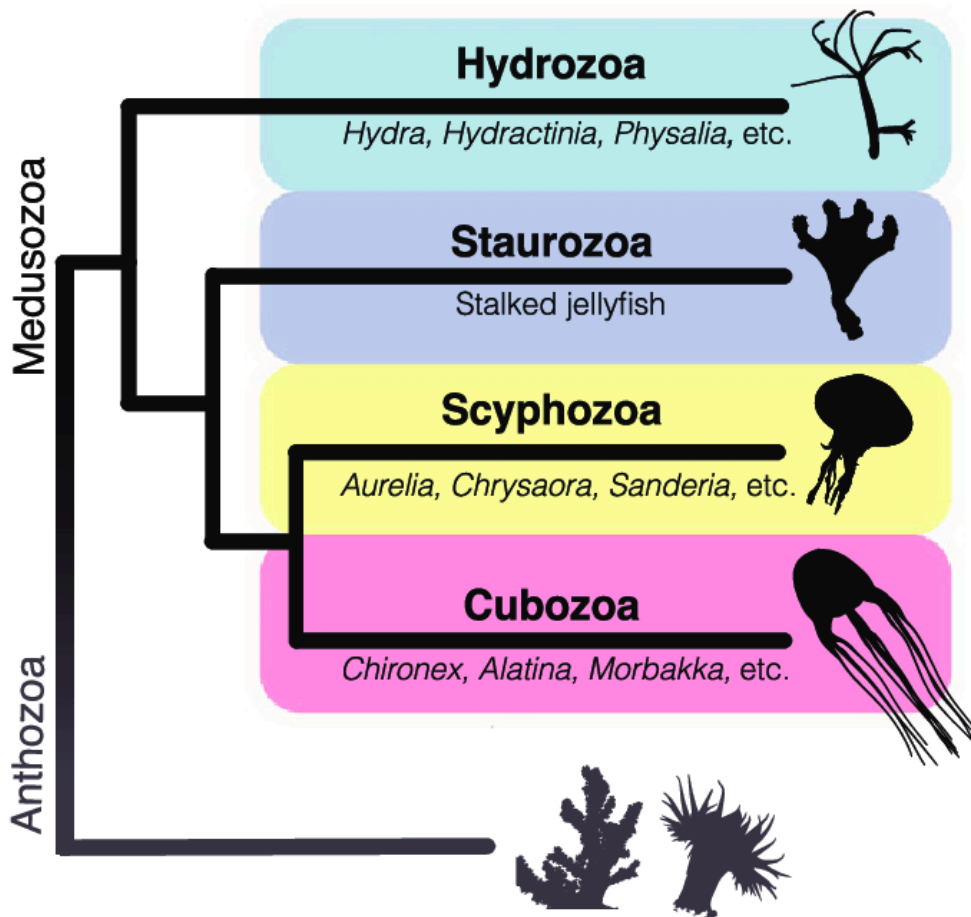
Note

Please note, soft body invertebrates (such as Medusozoa) retain their morphology much more effectively when preserved in formalin, as opposed to in ethanol. Ethanol is recommended as the preservative in this instance due to the fact it increases the potential for further tissue sequencing, however, there is the option to preserve representative specimens in formalin with the goal of maintaining their structure.

Troubleshooting

Protocol references

Basic Cnidaria taxonomic tree



Taken from: Klompen, Anna ML, et al. "Phylogenetic and selection analysis of an expanded family of putatively pore-forming jellyfish toxins (Cnidaria: Medusozoa)." *Genome Biology and Evolution* 13.6 (2021): evab081.