# CSI – Cuttlefish Sepion Investigation: overview of cuttlebones found on Aotearoa New Zealand shores and analysis of predation and scavenging marks

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The information presented herein provides more details of the analysed material and methodology, as well as additional results that complement the main text.

## **1. MATERIAL & METHODS**

### 1.1. Material studied

All the studied specimens, as well as additional sources of data are listed below. Museum acronyms are as follows: AWMM, Auckland War Memorial Museum (Auckland, New Zealand); CM, Canterbury Museum (Christchurch, New Zealand); GNS, GNS Science (Lower Hutt, New Zealand); NIWA, National Institute of Water and Atmospheric Research (Wellington, New Zealand); NMNZ, Museum of New Zealand Te Papa Tongarewa (Wellington, New Zealand); OM, Otago Museum (Dunedin, New Zealand); SMAG, Southland Museum and Art Gallery (Invercargill, New Zealand).

### 1.1.1. Specimen data

Below are listed all museum specimens analysed for the present work and their collection information, including GenBank registration numbers when applicable. Specimens with 'no further data' are presumed to have been found in New Zealand. No specimens could be found in the collection of NIWA. Photographs of the specimens are available in the Supplementary Material 2.

*Sepia apama* Gray, 1849: AWMM MA126095 (Auckland, Muriwai Beach, 29 km along the beach; A.W.B. Powell col. ix/1948), GenBank MZ934380; CM M5553 (Canterbury, New Brighton; pres. Miss Bellwood); CM M5555 (Wellington, Waikanae); NMNZ M.016880 (Otago, Dunedin, Tomahawk Beach; F. Bruce col. 1963); M.032420 (Wellington, Paekakariki Beach; P. Collins col. 08/xi/1973); NMNZ M.330266 (Wellington, Peka Peka Beach, 40°50.03'S 175°3.15'E; A.J.D.

Tennyson col. 29/iv/2020), GenBank MZ934375; NMNZ M.330267 (Wellington, Paraparaumu Beach, 40°52.67'S 174°59.47'E; A.J.D. Tennyson col. 06/v/2020), GenBank MZ934374; NMNZ M.330268 (Wellington, Peka Peka Beach, 40°49.28'S 175°3.70'E; A.J.D. Tennyson col. 01/v/2020), GenBank MZ934379; OM IV4869 (Southland, Colac Bay); SMAG E74.132 (Southland, Oreti Beach; x/1973, G. King & B. King leg.); SMAG 8366 (immature; no further data). Sepia cf. apama Gray, 1849: AWMM MA126096 (Waikato, Waikawau Bay; T.H. Race 09/iii/1975), GenBank MZ934373. Likely Sepia apama Gray, 1849: NMNZ M.010926 (Waikato, Kawhia Harbour; C.A. Fleming col. xii/1956); NMNZ M.330087 (Wellington, Waikanae Beach; A.J.D. Tennyson col. 27/iv/2002); NMNZ M.330090 (Wellington, Waikanae Beach; A.J.D. Tennyson col. 15/ix/2019); NMNZ M.330093 (Wellington, Te Horo Beach, 40°46.78'S 175°5.37'E; A.J.D. Tennyson col. 13/v/2020). Sepia grahami A. Reid, 2001: SMAG 8367 (no further data). Possibly Sepia latimanus Quoy & Gaimard, 1832: SMAG 8360 (Stewart Island, Mason Bay?; 1949-1959?). Sepia sp.: AWMM MA124201 (Auckland, Whatipu, west of Ninepin Rock; H.R. Grenfell & W.M. Blom col., 21/x/2017); AWMM MA126093 (Auckland, Muriwai Beach); AWMM MA126094 (Auckland, Muriwai Beach; A.W.B. Powell col. 07/xi/1920); CM M5554 (Canterbury, New Brighton; 11/vi/1892); CM unnumbered (no further data); GNS HS4205 (Bay of Plenty, Tauranga; ex H. Suter colln.); NMNZ M.009831 (Stewart Island, Easy Harbour; R.K. Dell col. 21/v/1956); NMNZ M.011229 (Northland, Spirits Bay; W.R.B. Oliver col. 26/xi/1916; ex Oliver colln.); NMNZ M.018735 (Wellington, Waikanae Beach, north of Waimeha Stream; 1955; ex Ponder colln.); NMNZ M.330086 (Wellington, Waikanae Beach; A.J.D. Tennyson col. 21/xii/2019); NMNZ M.330088 (Wellington, Waikanae Beach; A.J.D. Tennyson col. 03/x/2019); NMNZ M.330089 (Wellington, Peka Peka Beach, 40°48.42'S 175°4.33'E; A.J.D. Tennyson col. 11/v/2020); NMNZ M.330091 (Wellington, Waikanae Beach; A.J.D. Tennyson col. 15/ix/2019); NMNZ M.330092 (Wellington, Te Horo Beach, 40°46.78'S 175°5.37'E; A.J.D. Tennyson col. 13/v/2020); NMNZ M.330094 (Wellington, Pukerua Bay, 41°1.78'S 174°52.43'E; A.J.D. Tennyson col. 01/vi/2020); NMNZ M.330095 (Waikato, Raglan; A. Hamilton col. i/1912); NMNZ M.330096 (Kermadec Islands, Raoul Island, Denham Bay; E.B. Davidson col. viii/1937); NMNZ M.330099 (Wellington, Pukerua Bay, 41°1.80'S 174°52.78'E; A.J.D. Tennyson col. 01/vi/2020); NMNZ M.330269 (Wellington, Waikanae Beach; A.J.D. Tennyson col. 18/viii/2019); NMNZ M.330270 (Wellington, Waikanae Beach, 40°51.87'S 175°1.03'E; A.J.D. Tennyson col. 11/x/2018); NMNZ M.330638 (Wellington, Waikanae River estuary, 40°52.60'S 175°0.031E; A.J.D. Tennyson & S.G. Tennyson col. 01/x/2020); NMNZ M.330650 (Wellington, Otaki Beach, 40°43.97'S 175°6.731'E; A.J.D. Tennyson & S.G. Tennyson col. 30/ix/2020); NMNZ M.330651 (2 specimens; Wellington, Waikanae Beach, 40°40.02'S 175°0.781E; A.J.D. Tennyson col. 26/ix/2020); OM IV60442 (Southland, Riverton Beach; 2019); SMAG 8358 (Stewart Island, Mason Bay; 1949–1959?); SMAG 8359 (Stewart Island, Mason Bay?; 1949–1959?); SMAG 8361 (Stewart Island, Mason Bay?; 1949–1959?); SMAG 8362 (no further data); SMAG 8363 (no further data); SMAG 8364 (no further data).

### 1.1.2. Literature data

The following data were extracted from the literature and represents localities for which no voucher specimens are known. As we cannot confirm their identification, we treated all as *Sepia* sp.: Northland: North Cape, Waikuku Beach, 1932 (Powell, 1940, as *S. plangon*); Ninety Mile Beach

(Powell, 1979; as *S. apama*). Waikato: Mercury Bay, Whitianga (Dell, 1952, as *S. apama*, listed as being part of the AWMM collection, but not found).

### 1.1.3. iNaturalist data

The following data were extracted from the website iNaturalist (<u>https://www.inaturalist.org</u>) on 01/vii/2021. The identity of each record was confirmed by us. The entries are listed according to their internal iNaturalist observation numbers. Each iNaturalist observation number needs to be added to the end of the command "https://www.inaturalist.org/observations/" to become a functioning URL for accessing each observation on their website. Two records had their coordinates obscured and were not included on the map.

Sepia apama Gray, 1849: 2823789 (Northland, Te Paki, Waikuku Beach, Te Kanakana Stream, -34.423538 173.011799; pjd1, 21/iii/2016). Sepia sp.: 3873138 (Auckland, Muriwai Beach, -36.69 174.33; heni, 13/viii/2016); 8938448 (Manawatu-Wanganui, Foxton Beach, -40.39212167 175.22574; arnim, 25/xi/2017); 14935608 (Auckland, Karekare Beach, -36.98891042 174.4709773; jacqui-nz, 29/vii/2018); 15967425 (Northland, Ninety Mile Beach, Oneroa a Tohe, -34.65171324 172.8721565; tangatawhenua, 27/viii/2018); 16120041 (Wellington, coordinates obscured; wild wind, 25/vii/2018); 26484147 (Auckland, Huia, -36.99996543 174.5714708; jacqui-nz, 06/vi/2019); 26589745 (Taranaki, Ohawe Beach, -39.58683708 174.1954185; stbobservations, 08/vi/2019); 42292319 (Auckland, coordinates obscured; jacqui-nz, 16/iv/2020); 63618056 (Taranaki, New Plymouth, -39.06125382 174.0203546; commoncopper, 20/x/2020); 65483066 (Auckland, Waiheke Island, -36.7784665 175.0629461; mike-duke, 14/xi/2020); 68518525 (Manawatu-Wanganui, Foxton Beach. -40.46069333 175.2148233; arnim, 24/i/2021). Problematica: One iNaturalist record (60933858; Stewart Island, -46.9647033 167.7083475) shows two fresh-looking cuttlebones side by side belong to two different species: Sepia smithi Hoyle, 1885 and Sepia pharaonis Ehrenberg, 1831. However, the observation was made in retrospect: it was created on 27/x/2020, but refers to an observation from 27/xii/2010. It was flagged by the iNaturalist community as potentially not being natural. There are no further specimens or data to corroborate this observation.

### 1.1.4. Associated biota

Below are listed the specimens removed from recently collected cuttlebones, all housed in the NMNZ collections.

Cnidaria, *Obelia dichotoma* (Linnaeus, 1758): NMNZ CO.007142 (Wellington, Waikanae Beach; A.J.D. Tennyson col. 18/viii/2019; removed from cuttlebone specimen NMNZ M.330269); NMNZ CO.007143 (Wellington, Paraparaumu Beach, 40°52.67'S 174°59.47'E; A.J.D. Tennyson col. 06/v/2020; removed from cuttlebone specimen NMNZ M.330267). Crustacea, *Lepas anatifera* Linnaeus, 1758: NMNZ CR.025588 (Wellington, Peka Peka Beach, 40°50.03'S 175°3.15'E; A.J.D. Tennyson col. 29/iv/2020; removed from cuttlebone specimen NMNZ M.330266); NMNZ CR.025589 (Wellington, Peka Peka Beach, 40°49.28'S 175°3.70'E; A.J.D. Tennyson col. 01/v/2020; removed from cuttlebone specimen NMNZ CR.025590 (Wellington, Waikanae Beach; A.J.D. Tennyson col. 18/viii/2019; removed from cuttlebone specimen NMNZ CR.025590 (Wellington, Waikanae Beach; A.J.D. Tennyson col. 18/viii/2019; removed from cuttlebone specimen NMNZ CR.025590 (Wellington, Waikanae Beach; A.J.D. Tennyson col. 18/viii/2019; removed from cuttlebone specimen NMNZ M.330269).

#### 1.1.5. Tissue samples

Below is listed the information regarding the AM specimens from which the tissue samples were obtained, including GenBank registration numbers.

*Sepia apama* Gray, 1849: AM C.474138.002 (Australia, South Australia, Upper Spencer Gulf, -33.92 137.80; S. Donellan col., ix/2011), GenBank MZ934382; AM C474139.002 (Australia, South Australia, Upper Spencer Gulf, -33.92 137.80; S. Donellan col., ix/2011), GenBank MZ934384; AM C474140.002 (Australia, South Australia, Upper Spencer Gulf, -33.92 137.80; S. Donellan col. ix/2011), GenBank MZ934377; AM C.476607.003 (Australia, New South Wales, North Bondi, -33.88 151.28; A. Schnell col., 29/vi/2012), GenBank MZ934376; AM C476608.002 (Australia, New South Wales, Sydney, Cronulla, off Oak Park, -34.080261 151.195803; 28/vi/2012), GenBank MZ934381; AM C476613.003 (Australia, New South Wales, Sydney, Manly, off Fairy Bower, -33.8022 151.2917; A. Schnell col., 23/iii/2012), GenBank MZ934383; AM C.479177.001 (Australia, Western Australia, north of Bunker Bay, Geographe Bay, -33.5086 115.0439, A. Sampey col., 21/v/2007), GenBank MZ934378. *Sepia* cf. *apama* Gray, 1849: AM C479179.002 (Australia, Western Australia, Houtman Abrolhous Islands, off Pelsart Island, -28.7311 114.0444; A. Sampey col., 26/iv/2007), GenBank MZ934372. *Sepia* latimanus Quoy & Gaimard, 1832: AM C.476101.002 Timor-Leste, Dili; A. Reid col., 19/ix/2012), GenBank MZ934371.

### 1.1.6. Additional GenBank data

Below are listed the additional sequences extracted from GenBank and used in the phylogenetic analysis, with data on their collection locality when available.

*Octopus vulgaris* Cuvier, 1797: AB052253. *Sepia apama* Gray, 1849: AB675092 (Australia, Gulf St Vincent); AP013073. *Sepia latimanus* Quoy & Gaimard, 1832: AB192338 (Japan, Okinawa, Makishi); AB430406 (Indonesia); AP013074; KF009663 (Philippines, Aurora, Region 3); LC552688 (Indonesia). *Sepia officinalis* Linnaeus, 1758: AB240155. *Sepia pharaonis* Ehrenberg, 1831: AB430408 (Taiwan, Taichung); AP013076; HM164538 (Iran, Gulf of Oman); HQ846093; LR777651.

### 1.2. Barcoding & phylogenetic analysis

#### 1.2.1. DNA extraction

We removed a piece of the dorsal shield of the sepion, which is richer in organic compounds than the ventral chambered portion (Le Pabic et al. 2017), gently scrubbed it with bleach and washed it with distilled water to remove any surface contaminants. We also trialled the ventral carbonate portion of one specimen.

DNA extractions and polymerase chain reactions (PCRs) were set up in the ancient DNA laboratory of the NMNZ, which is physically isolated from where modern DNA and PCR products are handled. Potential contamination was monitored by the use of negative extraction and PCR controls. Sepion shield fragments were ground to a fine powder using a mortar and pestle. Mortar and pestles were scrubbed with detergent, followed by soaking in bleach and UV-sterilisation between

each use. DNA was extracted from the powdered material by incubation overnight at 55°C in 945  $\mu$ l of 0.5 M EDTA (pH 8.0), 20  $\mu$ l of 10% SDS, and 3  $\mu$ l of 20 mg/ml Proteinase-K, followed by extraction of the supernatant using the DNEasy® Blood & Tissue Kit (Qiagen) using the manufacturer's instructions except that the final elution was in 35 $\mu$ l of Buffer AE.

For the ethanol-preserved tissue samples, the standard protocol of the DNEasy® Blood & Tissue Kit was followed.

### 1.2.2. PCR

PCR mix was done using 6  $\mu$ l of MyTaq Red Mix (Bioline), 2  $\mu$ l of 10% BSA (bovine serum albumin), 2  $\mu$ l of molecular grade water, and 0.5  $\mu$ l of each primer of the pair. PCR amplification steps were: initial denaturation at 95°C (5 min); followed by 35 cycles of denaturation at 94°C (40 s), annealing at 48°C (primer pair LCO and Ill\_C\_R) or 46°C (primer pair HCO and Ill\_B\_F) (1 min), and extension at 72°C (30 s); finishing with a final extension at 72°C (5 min). For the ethanol-preserved tissue samples, PCR settings were the same as above, with an annealing temperature of 48°C. All PCR products were quantified via agarose gel electrophoresis, cleaned with ExoSAP-IT<sup>TM</sup> (Affymetrix Inc.) and sent out for sequencing.

#### 1.1.3. Phylogenetic analysis

Phylogenetic analyses were performed using PAUP\* v.4.0b10 (by D. L. Swofford, Sinauer Associates, Sunderland, MA) with maximum parsimony (MP), the PhyML v.3.0 web server (<u>http://www.atgc-montpellier.fr/phyml</u>, accessed 26 August 2021; Guindon et al. 2010) with maximum likelihood (ML), and MrBayes v.3.2.7 (Huelsenbeck and Ronquist 2001) for Bayesian inference (BI).

The MP analysis was performed using a heuristic search algorithm with 100 random addition sequence replicates, and tree bisection reconnection (TBR) branch-swapping. Branch support was assessed using 1,000 bootstrap pseudoreplicates, each with 10 random addition replicates. For ML analysis the best-fit models of sequence evolution were determined with Smart Model Selection (GTR + I + G) and the Akaike information criterion (Lefort et al. 2017). A heuristic search was performed with 10 random addition sequence replicates and subtree-pruning-regrafting (SPR) branch-swapping. Branch support was assessed with 1,000 bootstrap pseudoreplicates. For BI, two concurrent analyses were run, each with four Markov chains of 10 million generations that were sampled every 1,000 generations, and with nst = 6, rates = invgamma and the default priors. Tracer v.1.7.1 (Rambaut et al. 2018) was used to confirm that stationarity had been reached and the first 25% of samples were discarded as 'burn-in'.

### 1.3. Mapping

Coordinates were estimated for the historical specimens and literature data (see Item 1.1 above) using Google Earth (Google LLC). The original and derived coordinates, can be seen in Table S1 below. The map was created using the QGIS software (v.3.8.1; QGIS Development Team 2019).

| Fable S1. Coordinates used for map | o and source of data. Derived | coordinates are indicated in red font. |
|------------------------------------|-------------------------------|--|
|------------------------------------|-------------------------------|--|

| Specimen reg. nr. | Locality                            | Date        | Species   | Latitude   | Longitude  |
|-------------------|-------------------------------------|-------------|-----------|------------|------------|
| AWMM MA124201     | Whatipu, W of Ninepin Rock          | 21-Oct-2017 | indet     | -37.051507 | 174.496313 |
| AWMM MA126093     | Muriwai Beach                       |             | indet     | -36.835209 | 174.426598 |
| AWMM MA126094     | Muriwai Beach                       | 7-Nov-1920  | indet     | -36.835209 | 174.426598 |
| AWMM MA126095     | Muriwai Beach                       | Sep-1948    | apama     | -36.835209 | 174.426598 |
| AWMM MA126096     | Waikawau Bay                        | 9-Mar-1975  | cf. apama | -36.596376 | 175.524057 |
| CM M5553          | New Brighton                        |             | apama     | -43.507014 | 172.731987 |
| CM M5554          | New Brighton                        | 11-Jul-1892 | indet     | -43.507014 | 172.731987 |
| CM M5555          | Waikanae                            |             | apama     | -40.862445 | 175.020263 |
| GNS HS4205        | Tauranga                            |             | indet     | -37.631388 | 176.179277 |
| NMNZ M.009831     | Stewart Island, Easy Harbour        | 21-May-1956 | indet     | -47.154331 | 167.575796 |
| NMNZ M.010926     | Kawhia Harbour                      | Dec-1956    | apama     | -38.066120 | 174.822729 |
| NMNZ M.011229     | Spirits Bay                         | 26-Nov-1916 | indet     | -34.426744 | 172.857749 |
| NMNZ M.016880     | Tomahawk Beach                      | 16-May-1905 | apama     | -45.906346 | 170.539771 |
| NMNZ M.018735     | Waikanae Beach, N of Waimeha Stream | 1955        | indet     | -40.864881 | 175.016419 |
| NMNZ M.032420     | Paekakariki Beach                   | 8-Nov-1973  | apama     | -40.987436 | 174.949653 |
| NMNZ M.330086     | Waikanae Beach                      | 21-Dec-2019 | indet     | -40.8608   | 175.0232   |
| NMNZ M.330087     | Waikanae Beach                      | 27-Apr-2002 | apama     | -40.862911 | 175.019927 |
| NMNZ M.330088     | Waikanae Beach                      | 3-Oct-2019  | indet     | -40.8608   | 175.0232   |
| NMNZ M.330089     | Peka Peka Beach                     | 11-May-2020 | indet     | -40.8070   | 175.0722   |
| NMNZ M.330090     | Waikanae Beach                      | 15-Sep-2019 | apama     | -40.8683   | 175.0108   |
| NMNZ M.330091     | Waikanae Beach                      | 15-Sep-2019 | indet     | -40.8683   | 175.0108   |
| NMNZ M.330092     | Te Horo Beach                       | 13-May-2020 | indet     | -40.779667 | 175.0895   |
| NMNZ M.330093     | Te Horo Beach                       | 13-May-2020 | apama     | -40.779667 | 175.0895   |
| NMNZ M.330094     | Pukerua Bay                         | 1-Jun-2020  | indet     | -41.0296   | 174.874    |
| NMNZ M.330095     | Raglan                              | Jan-1912    | indet     | -37.798048 | 174.883134 |
| NMNZ M.330096     | Raoul Island, Denham Bay            | Aug-1937    | indet     | -29.261803 | 177.947682 |
| NMNZ M.330099     | Pukerua Bay                         | 1-Jun-2020  | indet     | -41.0301   | 174.8798   |
| NMNZ M.330266     | Peka Peka Beach                     | 29-Apr-2020 | apama     | -40.833833 | 175.0525   |
| NMNZ M.330267     | Paraparaumu Beach                   | 6-May-2020  | apama     | -40.877833 | 174.991167 |
| NMNZ M.330268     | Peka Peka Beach                     | 1-May-2020  | apama     | -40.821333 | 175.061667 |
| NMNZ M.330269     | Waikanae Beach                      | 18-Aug-2019 | indet     | -40.862911 | 175.019927 |
| NMNZ M.330270     | Waikanae Beach                      | 11-Oct-2018 | indet     | -40.8645   | 175.017167 |
| NMNZ M.330638     | Waikanae River estuary              | 1-Oct-2020  | indet     | -40.876667 | 175.0005   |
| NMNZ M.330650     | Otaki Beach                         | 30-Sep-2020 | indet     | -40.732833 | 175.112167 |
| NMNZ M.330651     | Waikanae Beach                      | 26-Sep-2020 | indet     | -40.667    | 175.013    |
| OM IV4869         | Colac Bay                           |             | apama     | -46.364657 | 167.884354 |
| OM IV60442        | Riverton Beach                      | 2019        | indet     | -46.363821 | 168.020552 |
| SMAG 8358         | Stewart Island, Mason Bay           | 1949–1959   | indet     | -46.922334 | 167.766400 |
| SMAG E74.132      | Oreti Beach                         | Oct-1973    | apama     | -46.438187 | 168.229323 |

| Literature      | Locality                          | Date        | Species | Latitude   | Longitude  |
|-----------------|-----------------------------------|-------------|---------|------------|------------|
| Dell (1952)     | Mercury Bay, Whitianga            |             | indet   | -36.821842 | 175.701588 |
| Powell (1940)   | North Cape, Waikuku Beach         | 1932        | indet   | -43.287271 | 172.722328 |
| Powell (1979)   | Ninety Mile Beach                 |             | indet   | -34.718693 | 172.928286 |
| iNaturalist nr. | Locality                          | Date        | Species | Latitude   | Longitude  |
| 2823789         | Waikuku Beach, Te Kanakana Stream | 21-Mar-2016 | apama   | -34.423538 | 173.011799 |
| 3873138         | Woodhill Forest                   | 13-Aug-2016 | indet   | -36.69     | 174.33     |
| 8938448         | Foxton Beach                      | 25-Nov-2017 | indet   | -40.392122 | 175.225740 |
| 14935608        | Karekare Beach                    | 29-Jul-2018 | indet   | -36.988910 | 174.470977 |
| 15967425        | Ninety Mile Beach                 | 27-Aug-2018 | indet   | -34.651713 | 172.87216  |
| 26484147        | Huia                              | 06-Jun-2019 | indet   | -36.999965 | 174.571471 |
| 26589745        | Ohawe Beach                       | 08-Jun-2019 | indet   | -39.586837 | 174.19542  |
| 63618056        | New Plymouth                      | 20-Oct-2020 | indet   | -39.061254 | 174.020354 |
| 65483066        | Waiheke Island                    | 14-Nov-2020 | indet   | -36.778466 | 175.062946 |
| 68518525        | Foxton Beach                      | 24-Jan-2021 | indet   | -40.460693 | 175.214823 |

# 2. RESULTS

## 2.1. Associated biota

The gooseneck barnacles are *Lepas anatifera* Linnaeus, 1758 (Lepadidae), identified by their overall size and shape and yellow membranes (A. Hosie, pers. comm.). Individuals of this common species can have smooth or radially striated valves (Foster 1978) and both forms were present in our material.

The hydroid material was degraded, most likely due to the time spent out of the water on the beach before collection and preservation, however, the characters that remain clear appear to match the genus *Obelia* Péron & Lesueur, 1810, specifically and most likely *Obelia dichotoma* (Linnaeus, 1758) (Obeliidae). *Obelia* is a problematic taxon and there are many species described only from the polyp stage, resulting in many synonymies over the years and uncertainty in the taxonomic validity of some species (Schuchert 2021). *Obelia* medusae are common throughout the world, although no useful characteristics are so far known to distinguish them (Russel 1953; Bouillon and Barnett 1999; Vervoort and Watson 2003), adding to the taxonomic confusion. Diagnostic morphological characters of the polyp stage such as colony size, branching pattern, hydrotheca length, and pedicel rings are often intermediate or shared between species. Previous work has shown that these variations can be attributed to environmental variables such as flow rate and direction, nutrition, substrate, latitude, and water temperature (Ralph 1956; Cornelius 1975; Cunha et al. 2020). Phylogenetic studies of this species suggest several cryptic lineages with overlapping morphological characters (Cunha et al. 2017, 2020).

In our specimens, the hydrothecae were often not clearly visible due to the above reasons and were abraded, so the presence or absence of cusps could not be determined. Some specimens, where the hydrothecae were visible, showed them to be unusually long. Free medusae were not present in the sample. However, the shape and branching of the material agrees with the currently accepted

morphological description for *Obelia dichotoma* sensu lato. The colonies were sympodial, nearly straight, with monosiphonic stems rising from a stolonal, tubiform network attached to cuttlebone surface. This material did not appear to have any polysiphonic stems. The perisarc was of a yellow-brown colour in sample NMNZ CO.007142, and light yellow to colourless in sample NMNZ CO.007143. The hydrocaulus was irregularly branched, with slightly curved internodes that had about two or three annulations basally. Hydrothecae were on a pedicel with about 10 annulations, often interrupted in the middle with a smooth part, arising from branches. Gonothecae were on a pedicel with about three to five annulations, more than twice as long as hydrotheca, and were elongated and conical in shape widening towards a ring-shaped aperture on a low collar.

Furthermore, the dorsal shield sample we took from cuttlebone specimen AWMM MA124201 for DNA extraction was not entirely scrubbed clean of contaminants, and we accidentally acquired DNA from dry hydroid fragments. The resulting COI sequence (GenBank number MZ052083) has excellent Phred Quality Scores (30–40) and, when compared to GenBank data, resulted in 98% identity with a specimen of *Obelia dichotoma* from Hawaii (MW277711). Identity to other *Obelia* spp. COI sequences in GenBank ranged from 88 to 93% among the following species: *O. bidentata* Clark, 1875, *O. geniculata* (Linnaeus, 1758), and *O. longissima* (Pallas, 1766).

## **3. REFERENCES**

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