

Identification of *Cassiopea* sp. in Lake Macquarie, Australia and revision of the taxonomic status of *Cassiopea maremetens* Gershwin, Zeidler & Davie, 2010 (Cnidaria: Scyphozoa: Cassiopeidae) (#113132)

1

First submission

Guidance from your Editor

Please submit by **13 Feb 2025** for the benefit of the authors (and your token reward) .



Structure and Criteria

Please read the 'Structure and Criteria' page for guidance.



Custom checks

Make sure you include the custom checks shown below, in your review.



Raw data check

Review the raw data.



Image check

Check that figures and images have not been inappropriately manipulated.

If this article is published your review will be made public. You can choose whether to sign your review. If uploading a PDF please remove any identifiable information (if you want to remain anonymous).

Files

Download and review all files from the [materials page](#).

11 Figure file(s)
6 Table file(s)
2 Raw data file(s)
1 Other file(s)



Custom checks

DNA data checks



Have you checked the authors [data deposition statement](#)?



Can you access the deposited data?



Has the data been deposited correctly?



Is the deposition information noted in the manuscript?

Field study



Have you checked the authors [field study permits](#)?



Are the field study permits appropriate?



Structure and Criteria

Structure your review

The review form is divided into 5 sections. Please consider these when composing your review:

1. BASIC REPORTING
2. EXPERIMENTAL DESIGN
3. VALIDITY OF THE FINDINGS
4. General comments
5. Confidential notes to the editor

 You can also annotate this PDF and upload it as part of your review

When ready [submit online](#).

Editorial Criteria

Use these criteria points to structure your review. The full detailed editorial criteria is on your [guidance page](#).

BASIC REPORTING

-  Clear, unambiguous, professional English language used throughout.
-  Intro & background to show context. Literature well referenced & relevant.
-  Structure conforms to [Peerj standards](#), discipline norm, or improved for clarity.
-  Figures are relevant, high quality, well labelled & described.
-  Raw data supplied (see [Peerj policy](#)).

EXPERIMENTAL DESIGN

-  Original primary research within [Scope of the journal](#).
-  Research question well defined, relevant & meaningful. It is stated how the research fills an identified knowledge gap.
-  Rigorous investigation performed to a high technical & ethical standard.
-  Methods described with sufficient detail & information to replicate.

VALIDITY OF THE FINDINGS

-  **Impact and novelty is not assessed.** Meaningful replication encouraged where rationale & benefit to literature is clearly stated.
-  All underlying data have been provided; they are robust, statistically sound, & controlled.
-  Conclusions are well stated, linked to original research question & limited to supporting results.



The best reviewers use these techniques

Tip

Example

Support criticisms with evidence from the text or from other sources

Smith et al (J of Methodology, 2005, V3, pp 123) have shown that the analysis you use in Lines 241-250 is not the most appropriate for this situation. Please explain why you used this method.

Give specific suggestions on how to improve the manuscript

Your introduction needs more detail. I suggest that you improve the description at lines 57- 86 to provide more justification for your study (specifically, you should expand upon the knowledge gap being filled).

Comment on language and grammar issues

The English language should be improved to ensure that an international audience can clearly understand your text. Some examples where the language could be improved include lines 23, 77, 121, 128 – the current phrasing makes comprehension difficult. I suggest you have a colleague who is proficient in English and familiar with the subject matter review your manuscript, or contact a professional editing service.

Organize by importance of the issues, and number your points

1. Your most important issue
2. The next most important item
3. ...
4. The least important points

Please provide constructive criticism, and avoid personal opinions

I thank you for providing the raw data, however your supplemental files need more descriptive metadata identifiers to be useful to future readers. Although your results are compelling, the data analysis should be improved in the following ways: AA, BB, CC

Comment on strengths (as well as weaknesses) of the manuscript

I commend the authors for their extensive data set, compiled over many years of detailed fieldwork. In addition, the manuscript is clearly written in professional, unambiguous language. If there is a weakness, it is in the statistical analysis (as I have noted above) which should be improved upon before Acceptance.

Identification of *Cassiopea* sp. in Lake Macquarie, Australia and revision of the taxonomic status of *Cassiopea maremetens* Gershwin, Zeidler & Davie, 2010 (Cnidaria: Scyphozoa: Cassiopeidae)

Claire E Rowe ^{Corresp., 1}, Shane T Ah Yong ^{1, 2}, Will F Figueira ³, Ingo Burghardt ^{1, 4}, Stephen J Keable ¹

¹ Australian Museum Research Institute, Australian Museum, Darlinghurst, New South Wales, Australia

² School of Biological, Earth & Environmental Sciences, University of New South Wales, Kensington, New South Wales, Australia

³ School of Life and Environmental Sciences, University of Sydney, Sydney, New South Wales, Australia

⁴ Aquatic Ecology, Sydney Water, West Ryde, New South Wales, Australia

Corresponding Author: Claire E Rowe

Email address: Claire.Rowe@australian.museum

Scyphozoans of the genus *Cassiopea* are notable for their unusual benthic habit of lying upside-down with their exumbrella resting on the substrate and oral arms facing upwards, resulting in their common name “upside-down jellyfish”. *Cassiopea* includes species that are model examples of invasives that have been historically confused because of taxonomic ambiguity. These can have significant economic and environmental consequences as their blooms are known to impact fisheries, tourism, and trophic structures. *Cassiopea* medusae were first reported in temperate Lake Macquarie, New South Wales, Australia in 2017, though historically these jellyfish have a more northern tropical distribution in eastern Australia. Owing to the invasive nature of *Cassiopea* and their potential impacts, correct species identification is crucial for future management. To address this knowledge gap, this study used genetic comparison through the COI barcoding gene and morphometric analysis, together with revision of type and topotype material of *Cassiopea maremetens* Gershwin, Zeidler & Davie, 2010, an incompletely known nominal species from eastern Australia, to investigate the identity of *Cassiopea* occurring in Lake Macquarie. The morphometric analysis was also used to identify key features that distinguish the Lake Macquarie species from a second species, designated *Cassiopea* sp.3, that is also expanding its range south in eastern Australia and which may be sympatric in some areas. The results of this study show the species occurring in Lake Macquarie is *Cassiopea xamachana* Bigelow, 1892, originally described from Jamaica and subsequently widely reported from the Western Atlantic and the Indo-West Pacific. Additionally, we demonstrate that *Cassiopea maremetens*, described in 2010 from southern Queensland, Australia, is a junior synonym of *C. xamachana*. Morphological

characters that can be most readily used to distinguish mature specimens of *C. xamachana* from *C. sp.3*, which has an overlapping distribution on the Australian east coast, are: 1) the number of large appendages on the oral disc, which is much higher in *Cassiopea sp.3* (at least 1 but up to 14) versus a maximum of two in *C. xamachana*; 2) the oral arm branching pattern, which is usually alternating for *C. xamachana*, but a combination of alternating, bifurcating and pinnate for *Cassiopea sp.3*; 3) the length of the large appendage on the oral arm, which is proportionally longer relative to the bell diameter in *C. xamachana*.

Identification of *Cassiopea* sp. in Lake Macquarie, Australia and revision of the taxonomic status of *Cassiopea maremetens* Gershwin, Zeidler & Davie, 2010 (Cnidaria: Scyphozoa: Cassiopeidae)

Claire E. Rowe^{*,1}, Shane T. Ahyong^{1,2}, Will F. Figueira³, Ingo Burghardt^{1,4}, Stephen J. Keable¹

1. Australian Museum Research Institute, Australian Museum, Darlinghurst, New South Wales,
2010, Australia

2. School of Biological, Earth & Environmental Sciences, University of New South Wales,
Kensington, New South Wales, 2052, Australia

3. School of Life and Environmental Sciences, University of Sydney, Sydney, NSW, 2006,
Australia

4. Aquatic Ecology, Sydney Water, West Ryde, New South Wales, 2114, Australia

* Claire.Rowe@Australian.Museum

Keywords: invasive, jellyfish, taxonomy

18 Abstract

19 Scyphozoans of the genus *Cassiopea* are notable for their unusual benthic habit of lying
 20 upside-down with their exumbrella resting on the substrate and oral arms facing upwards,
 21 resulting in their common name “upside-down jellyfish”. *Cassiopea* includes species that are
 22 model examples of invasives that have been historically confused because of taxonomic
 23 ambiguity. These can have significant economic and environmental consequences as their
 24 blooms are known to impact fisheries, tourism, and trophic structures. *Cassiopea* medusae were
 25 first reported in temperate Lake Macquarie, New South Wales, Australia in 2017, though
 26 historically these jellyfish have a more northern tropical distribution in eastern Australia. Owing
 27 to the invasive nature of *Cassiopea* and their potential impacts, correct species identification is
 28 crucial for future management. To address this knowledge gap, this study used genetic
 29 comparison through the COI barcoding gene and morphometric analysis, together with revision
 30 of type and topotype material of *Cassiopea maremetens* Gershwin, Zeidler & Davie, 2010, an
 31 incompletely known nominal species from eastern Australia, to investigate the identity of
 32 *Cassiopea* occurring in Lake Macquarie. The morphometric analysis was also used to identify
 33 key features that distinguish the Lake Macquarie species from a second species, designated
 34 *Cassiopea* sp.3, that is also expanding its range south in eastern Australia and which may be
 35 sympatric in some areas. The results of this study show the species occurring in Lake Macquarie
 36 is *Cassiopea xamachana* Bigelow, 1892, originally described from Jamaica and subsequently

widely reported from the Western Atlantic and the Indo-West Pacific. Additionally, we demonstrate that *Cassiopea maremetens*, described in 2010 from southern Queensland, Australia, is a junior synonym of *C. xamachana*. Morphological characters that can be most readily used to distinguish mature specimens of *C. xamachana* from *C. sp.3*, which has an overlapping distribution on the Australian east coast, are: 1) the number of large appendages on the oral disc, which is much higher in *Cassiopea sp.3* (at least 1 but up to 14) versus a maximum of two in *C. xamachana*; 2) the oral arm branching pattern, which is usually alternating for *C. xamachana*, but a combination of alternating, bifurcating and pinnate for *Cassiopea sp.3*; 3) the length of the large appendage on the oral arm, which is proportionally longer relative to the bell diameter in *C. xamachana*.

Introduction

Upside-down jellyfish (*Cassiopea* spp.) are unusual scyphozoans because they spend the majority of the medusa phase of their lifecycle in a sedentary state, resting on the benthos, with the bell facing downwards, and the oral arms extended above (Ohdera et al., 2018). *Cassiopea* occurs world-wide in tropical to sub-tropical regions, in shallow and protected habitats, such as coral reefs, mangrove forests or seagrass beds (Ohdera et al., 2018). Some species of *Cassiopea*, however, are considered to be globally invasive with a number of range expansions into novel environments (Bolton & Graham, 2006; Holland et al., 2004; Keable & Ahyong, 2016; Rowe et

al., 2022b). This can occur localised dispersal, or through direct anthropogenic interventions including shipping traffic, ballast water, or release through the aquarium trade (Bolton & Graham, 2006; Graham & Bayha, 2008). Characteristics considered important in their success as invaders include tolerance of a broad range of environmental parameters, high reproductive rate and the ability to reproduce both sexually and asexually (Holland et al., 2004; Keable & Ahyong, 2016; Maggio et al., 2019; Morandini et al., 2016; Schiariti et al., 2014). Additionally, *Cassiopea* can have reproductive blooms in high densities (Morandini et al., 2017; Stoner et al., 2014) and under increasingly favourable environmental conditions associated with climate change, these blooms may occur more frequently (Brotz & Pauly, 2016; Ohdera et al., 2018; Richardson et al., 2009; Rowe et al., 2022a).

Cassiopea is the only genus in the family Cassiopeidae. The most recent synopses of the genus recognise 12 valid species (Collins, Jarms & Morandini, 2022; Jarms et al., 2019) : 1, *C. andromeda* (Forskål, 1775); 2, *C. culionensis* Light, 1914; 3, *C. depressa* Haeckel, 1880; 4, *C. frondosa* (Pallas, 1774); 5, *C. maremetens* Gershwin, Zeidler & Davie, 2010; 6, *C. mayeri* Gamero-Mora, Collins, Boco, Geson III & Morandini, 2022; 7, *C. medusa* Light, 1914; 8, *C. mertensi* (Brandt, 1838); 9, *C. ndrosia* Agassiz & Mayer, 1899; 10, *C. ornata* Haeckel, 1880; 11, *C. vanderhorsti* Stiasny, 1924; and 12, *C. xamachana* Bigelow, 1892. However, species of the genus can be extremely difficult to distinguish based on morphological characters alone because of their conservative morphology, with few reliable morphological taxonomic characters

(Gamero-Mora et al., 2022; Holland et al., 2004). Such a situation is commonplace in scyphozoan systematics, which has traditionally based descriptions on morphological characters that may have high plasticity (Arai et al., 2017; Gohar & Eisawy, 1960; Holland et al., 2004). This is especially the case for *Cassiopea*, where analysis based solely on morphological characters is challenging because a number of features vary between different habitats and stages of growth (Hopf & Kingsford, 2013; Maggio et al., 2019). As a result, it has been suggested that an integrative approach, using both morphological and genetic data will provide more reliable taxonomic separation (Arai et al., 2017; Dawson, 2003; Holland et al., 2004; Maggio et al., 2019).

Molecular study has often revealed unrecognised species in many marine taxa, suggesting that marine biodiversity is higher than previously thought, and speciation is more frequent than originally recognised (Dawson & Jacobs, 2001; Knowlton, 2000). For example, the scyphozoan, *Aurelia aurita* (Linnaeus, 1758), long thought to be a single widespread species based on morphology has been revealed, based on molecular data, to be a complex of at least 10 species (Dawson & Jacobs, 2001; Lawley et al., 2021). One of the first molecular studies of *Cassiopea* focussed on Hawaiian fauna (Holland et al., (2004), and tentatively concluded that there were six species including: 1) *C. frondosa*, which originates from the western Atlantic and at that time considered by Holland et al., (2004) to be the only morphologically well characterised species; 2) *C. andromeda*, initially reported from the Red Sea and subsequently the Western Atlantic; 3) *C.*

ornata, reported from Indonesia, Palau and Fiji; and 4–6) species that were not associated with named species based on their morphological characters. Subsequently, genetic analysis has been used as a tool for identifying species of *Cassiopea*, such as *C. andromeda* in shrimp farms in Brazil (Thé et al., 2020) and from Italy (Maggio et al., 2019), respectively. Additionally, utilising genetic techniques, new species have been identified, including two different lineages from Palau (*Cassiopea* sp.5 and sp.6, Arai et al., 2017), which were not sequenced by Holland et al. (2004). A recent synoptic phylogenetic study of *Cassiopea* (Gamero-Mora et al., 2020) recognised at least 17 species of which only six can be aligned with formal names, the most recently described being *C. mayeri* and *C. culionensis* (Gamero-Mora et al., 2022; Gamero-Mora et al., 2020). There are still at least six named species, whose identity is ambiguous, indicating significant revision of the genus is required (Gamero-Mora et al., 2022; Gamero-Mora et al., 2020).

In Australia, *Cassiopea* has typically been recorded from sub-tropical and tropical latitudes, occurring north of 27°58'S on the east coast and 16°08'S on the west coast, with the exception of an apparently introduced population established near a warm water outlet from a power station at Angas Inlet, Adelaide, South Australia (approximately 34°48'S, 138°32'E, Keable & Ahyong, 2016; Southcott, 1982, Figure 1). To date, four named species of *Cassiopea* (*C. andromeda*, *C. maremetens*, *C. ndrosia*, *C. ornata*) and two undescribed species have been reported from Australia (Gershwin, Zeidler & Davie, 2010; Keable & Ahyong, 2016). *Cassiopea maremetens*, however, is the only species described from Australia (type locality: Pelican

Waters, Queensland, 26°49'42"S, 153°06'48"E); other Australian species were described from elsewhere. To date no genetic data for *C. maremetens* have been available but it morphologically closely resembles *C. andromeda* and *C. ndrosia* (Gershwin, Zeidler & Davie, 2010; Keable & Ahyong, 2016).

Keable & Ahyong (2016) reported a southward range expansion of two species of *Cassiopea* on the east Australian coast into New South Wales, identifying *C. cf. maremetens* in Wallis Lake (32°11'45"S, 152°29'56"E) and *C. ndrosia* in Lake Illawarra (34°31'36"S, 150°51'53"E). A further southern population of *Cassiopea* was discovered in 2017 in Lake Macquarie (33°04'00"S, 151°32'42"E), approximately mid-way between Lake Illawarra and Wallis Lake (Rowe et al., 2022b, Figure 1). This raises questions about which species occurs in Lake Macquarie and from where it may have originated. This is especially important because of the invasive potential of *Cassiopea* and its environmental impacts.

This study aims to determine the identity of the species of *Cassiopea* occurring in Lake Macquarie using molecular and morphological data, and to re-assess the taxonomic status of *C. maremetens* in relation to *Cassiopea* from New South Wales.

Methods

Taxon Sampling

Specimens were collected under NSW Department of Primary Industries permit FP23/41 from four localities on five occasions in Lake Macquarie, New South Wales, Australia: Lake Petite, 33°7'3"S, 151°32'6"E, on 4 June 2017, 9 February 2018, and 9 May 2019; Kilaben Creek, 33°1'43"S, 151°35'1"E, on 9 May 2019; Karignan Creek, 33°10'32"S, 151°34'0"E, on 15 May 2018 and 6 May 2019; and Mannering Bay, 33°9'28"S, 151°31'41"E, on 7 May 2019; and Myuna Bay, 33°03'59"S, 151°32'43"E, on 2 December 2019 (Figure 1).

Additionally, specimens for comparative analysis were collected in Wallis Lake (32°11'58"S, 152°30'39"E) on 18 September 2014, 28 April 2015, 8 April 2016, and 10 May 2019, Port Hacking (34°04'51"S, 151°08'01"E) on 31 May 2021, and Lake Illawarra (34° 31' 36" S, 150° 51' 52"E) on 1 June 2021. Specimens from southeastern Queensland were collected from Coombabah Creek (27°54'26"S, 153°22'57"E) on 8 March 2021, and Pelican Waters (26°50'01"S, 153°06'44"E), the type locality of *C. maremetens*, on 10 March 2021.

Specimens were collected using hand-nets, either from a paddling vessel or by wading into the water. For further details see the Taxonomy section and Supplementary Files Tables S1-S3.

Tissue samples from each specimen were dissected from the gonads and tip of one of the oral arms; these were fixed and preserved in 95% ethanol. The remainder of the specimen was fixed in 10% formalin mixed with seawater to maintain the morphological characteristics. All

New South Wales specimens are deposited in the collections of the Australian Museum, Sydney (AM).

Additional Genetic and Morphological Samples

Additional tissue samples were sequenced from specimens made available by the Queensland Museum, Brisbane (QM), South Australian Museum, Adelaide (SAM), and ReefHQ, Townsville (S1). Additionally, available genetic sequences of *Cassiopea* from Australian and overseas localities were downloaded from GenBank, including two scyphozoan outgroups, *Catostylus mosaicus* and *Aurelia aurita*, following Holland et al. (2004) (S1 and S2).

Collections preserved at the AM, QM and SAM were examined for morphological comparison (S3). Due to uncertainty regarding many characters of *C. maremetensis*, this included type material from QM to redescribe the species (see Taxonomy section).

Genetic Analysis

DNA extraction, PCR amplification and sequencing

DNA was extracted from gonad or oral arm tissue using an Isolate II Genomic DNA Kit following manufacturer's instructions. An approximately 500-bp fragment of the mitochondrial gene cytochrome *c* oxidase subunit I (COI) was sequenced with the primers Lobo Forward (KBTCHACAAAYCAYAARGAYATHGG) and Lobo Reverse (TAAACYTCWGGRTGWCCRAARAAYCA) (Lobo et al. 2013). Polymerase chain reactions

(PCRs) were performed in a total volume of 20 µl with Invitrogen 10× PCR buffer (2 µl), both primers (0.4 µl each), Invitrogen MgCl₂ (1.5 µl), dNTPs (1.5 µl), Invitrogen Taq DNA Polymerase (0.1 µl), Milli-Q H₂O (13.1 µl) and the sample (1 µl). Reaction conditions for COI were as follows: 94°C for 3 minutes; 40 cycles: at 94°C (30s), 52°C (30s) and 72°C (60s) a final extension at 72°C (5 minutes). PCR products were assessed using agarose gel electrophoresis stained with GelRed (Biotium), with the inclusion of a 1000 bp DNA ladder (ThermoFischer). PCR products were sent to Macrogen (Macrogen Inc. Seoul, Republic of Korea) for sequencing.

Molecular data analysis

A total of 38 COI sequences were obtained from various populations of *Cassiopea* from around Australia for this study (S1). The sequences were viewed and edited using Geneious (V.2020.0.5) and matched against other sequences in the GenBank database using the BLAST search. All of the mitochondrial COI sequences from populations in Australia obtained in this study and from GenBank were aligned using MEGA10: Molecular Evolutionary Genetics Analysis version 10 (MEGA) (Stecher, Tamura, & Kumar, 2020) using the MUSCLE algorithm (total of 48 sequences, S1). MEGA was then used to compute pairwise distances between sequences using Kimura 2-parameter model (K2P), followed by the construction of a Maximum Likelihood (ML) tree using Tamura 3-parameter, which was selected as a result of the lowest BIC value when comparing models in MEGA. Bootstrap values were calculated using 1,000

pseudoreplicates implemented in MEGA. Analyses were rooted to *Catostylus mosaicus* and *Aurelia aurita* (see Australian results).

This method was repeated using a sequence from each locality in Australia, compared to populations worldwide, including two sequences originating from Israel and Singapore, respectively, sequenced in this study, and *Cassiopea* CO1 sequences available on GenBank (total of 97 sequences, S2, see Global results).

Additionally, a species delimitation analysis was completed using Bayesian implementation (bPTP) of the Poisson tree process model to infer putative species boundaries on a phylogenetic input tree (Zhang et al., 2013). The input tree used in the bPTP analysis was the resulting trees from both the regional and global analysis. The bPTP was run as a rooted tree with 100,000 generations, 10% burn-in and outgroups were removed.

Morphological Analysis

Results from the molecular analysis were used to inform specimen sampling for morphometric analysis. A suite of morphological traits were identified and measured following those examined by Ohdera et al. (2018); Morandini et al. (2017); Mayer (1910); Kramp (1961); Hummelinck (1968); Gershwin, Zeidler & Davie (2010); Keable & Ahyong (2016); Jarms et al. (2019). Morphological observations (Figure 2) were made using a magnification lamp and dissecting microscope. In most cases the exumbrella diameter was measured across the widest point to the nearest millimeter and used to compare size ratios of different features between

specimens. However, some specimens have a brittle exumbrella that would not fully open, in which case their condition noted. Exumbrella diameter, overall height and oral disc height were measured with the oral arms of the specimen lying on top of their exumbrella and with the aboral surface of the exumbrella oriented in the ventral position as when the specimen is naturally at rest on the substrate when encountered in the field (i.e., the typical ‘upside-down’ position). Overall height was measured from the base of the exumbrella to the top of the oral disc. Oral disc height was measured from the intersection of oral disc with the bell to the top of the oral disc. The number of rhopalia and lappets, and the number of lappets per paramere were counted, and their shape noted (i.e., round or flat edge, each lappet distinct with grooves between them or connected with a smooth edge, short or long). While the shape and colour of large appendages have been found uninformative in identifying *Cassiopea* species in other analyses (Gamero-Mora et al., 2020), it was observed that their position and size consistently differed between populations occurring in this study on the east coast of Australia. Therefore, the number and shape of large appendages along the oral arms and on the oral disc were recorded. We also measured the length of largest appendage on the oral arm and oral disc from the base to the tip, as well as the width at the midlength. Additionally, the specimen was inverted so that the dorsal surface of the exumbrella was exposed, and the exumbrella was folded back so the oral arms were visible, allowing the length of the oral arms to be recorded from the proximal base at the oral disc to the distal tip of the main trunk. The main oral arm trunk and lateral oral arm

branching patterns were also recorded as this is considered to be useful in diagnosing species of *Cassiopea* (Gamero-Mora et al., 2020), with three different patterns observed in this study: 1, alternating — the lateral branches switch sides and are separated along the main oral arm trunk; 2, pinnate — the lateral branches are arranged on either side of the main oral arm trunk in pairs opposite each other; 3, bifurcating — the main trunk of the oral arm is divided into two subequally broad distal branches (in this case the distance from the oral disc to the proximal base of the bifurcation, and also whether each branch was equal in length, was recorded).

Standardisation of body size

To ensure that all count data were not skewed by body size, a regression was completed for each continuous variable against exumbrella diameter to determine if there was a positive relationship. If a significant relationship was detected, the variable was divided by the exumbrella diameter for each individual. If a significant relationship was still detected, the z-score (Curtis et al., 2016) was calculated for each individual using the following formula:

$$Z = \frac{x - \text{mean}}{\text{standard deviation}}$$

Where x is the continuous variable for a specimen and after which, all data was standardised.

Morphometric analysis

To determine if there was any clustering separating putative species based on morphological characters, and if this differed between populations, a non-metric multidimensional scaling

(nMDS) was constructed based on a Bray-Curtis similarity matrix using both the continuous data that was standardised for bell diameter, and categorical variables. This was completed using both the ‘vegan’ package and ‘ggplot2’ in R (v2021.09.01). To test for morphological differences a one-way permutation multivariate analysis of variance (PERMANOVA) was completed, with putative species as the fixed factor (3-levels: presumptive *C. xamachana*, *Cassiopea* sp.3, *Cassiopea* sp.2, as listed in Supplementary Table S3). Additionally, a Ward hierarchical clustering (Cluster) analysis was undertaken to determine morphological similarities between individual samples. Any morphological differences between populations within species for putative *C. xamachana* and *Cassiopea* sp.3 were tested using one-way PERMANOVAs with the location of the populations as a fixed factor (3-levels for each species). If there was a significant difference between groups of specimens a cluster analysis was completed to determine how individuals clustered across populations and in which localities these are morphologically similar.

To establish which features distinguish putative species, a similarity percentage analysis (SIMPER) was applied using the ‘vegan’ package, and the six most influential features were identified. All statistical analyses were completed in R (v2021.09.01).

Results

Genetic Analysis

261 *Australian results*

262 The ML tree (Figure 3) based on COI of *Cassiopea* from all sampled localities in Lake
 263 Macquarie, Wallis Lake, Pelican Waters, Gold Coast and Moreton Bay formed a strongly
 264 supported clade (bootstrap support 100%); all were genetically identical ($K2P \pm S.E.: 0.00 \pm$
 265 0.00 , Table S4) suggesting they represent a single species, distinct from those of Lake Illawarra
 266 (mean between populations $K2P \pm S.E.: 0.318 \pm 0.00$), Port Hacking (0.318 ± 0.00), Coombabah
 267 Creek (0.324 ± 0.00), and the Northern Territory (0.32 ± 0.00), which were closely related to
 268 each other (mean within species $K2P \pm S.E.: 0.021 \pm 0.00$). Additionally, the Lake Macquarie
 269 specimens differed from those from South Australia (0.078 ± 0.00), Lizard Island (0.294 ± 0.00),
 270 and Port Douglas (0.273 ± 0.00). The bTP analysis for species delimitation estimate the presence
 271 of 6 to 36 species with a mean of 21.64. Five species are recognised herein, including *Cassiopea*
 272 from Lake Macquarie, Wallis Lake and Pelican Waters as one species, those from Lake
 273 Alexander, Lake Illawarra, Port Hacking and Coombabah Creek as a second species, and each of
 274 the populations from Angas Inlet, Port Douglas and Lizard Island, as separate species (see
 275 Discussion section and Figure 1).

276 *Global results*

277 The global ML tree (Figure 4) recovered the Lake Macquarie, Wallis Lake and Pelican
 278 Waters specimens in the same clade and genetically invariant, with low divergence from
 279 populations in Hawaii, Brazil, Panama and the Florida Keys (mean within species $K2P \pm S.E.:$

0.016 ± 0.00, Table S5), previously identified as *Cassiopea xamachana* (Holland et al., 2004; Gamero-Mora et al., 2019; Stampar et al., 2021, S2, see Discussion). The low COI divergence suggests that these populations from different localities all represent the same widespread species. Additionally, specimens from Lake Illawarra, Coombabah Creek, Port Hacking and the Northern Territory (herein referred to as *Cassiopea* sp.3) form a separate clade and are closely related to another species from Japan, Hawaii and Papua New Guinea (Figure 4, Table S5). The bTP analysis for species delimitation indicates an estimated 16 to 38 species with a mean of 25.13.

Morphometric Analysis

The nMDS ordination of the specimens of *Cassiopea* examined is illustrated in Figure 5. The stress level associated with this two-dimensional plot was 0.176, demonstrating that there were some morphological characters distinguishing the species (Dexter et al. 2018). The nMDS ordination plot formed clearly separated groups based on morphological dissimilarities (Figure 5). The PERMANOVA confirmed that there are significant morphological differences between species ($R^2 = 0.395$, pseudo-F = 30.089 $p < 0.01$). Additionally, with the exception of three *Cassiopea* sp.3 specimens (AM G.20076 from Port Hacking, G.20060 from Coombabah Creek and AM G.18075 from Lake Illawarra) and two *Cassiopea* sp.2 specimens (AM G.17387 and AM G.17370) from Papua New Guinea, the cluster analysis confirmed these results (Figure 6), and grouped specimens into two morphological groups, with material corresponding to putative

299 *Cassiopea* sp.3 and *Cassiopea* sp.2 in the first cluster and presumptive *C. xamachana* in the
 300 second. The PERMANOVA between Australian populations of *Cassiopea* from Lake
 301 Macquarie, Wallis Lake and Pelican waters indicates there are morphological differences
 302 between locations ($R^2 = 0.077$, pseudo- $F = 2.587$, $p < 0.05$). The follow-up cluster analysis
 303 (Figure 7A) indicated no *C. xamachana* location clusters driving this significance with the two
 304 significant clusters containing specimens from all three locations. However, morphological
 305 differences were detected between populations of *Cassiopea* sp.3 from Lake Illawarra,
 306 Coombabah Creek, Port Hacking, and Lake Alexander ($R^2 = 0.498$, pseudo- $F = 6.605$, $p <$
 307 0.001). Additionally, the follow up cluster analysis (Figure 7B) confirmed clear morphological
 308 clusters between locations of *Cassiopea* sp.2, including cluster 1 containing specimens from the
 309 Port Hacking, cluster 2 from Coombabah Creek with two specimens from the Port Hacking,
 310 cluster 3 from Northern Territory, with one specimen from Lake Illawarra, and cluster 4
 311 containing specimens from Lake Illawarra.

312 SIMPER indicated the three most informative features contributing to separation of
 313 putative *C. xamachana* and *Cassiopea* sp.3 according to their cumulative contribution are: the
 314 number of large appendages on the oral disc; oral arm branching pattern; and length of large
 315 appendages on the oral arm (Table 1, Figures 6–8). Branching pattern is also a distinguishing
 316 feature between *C. xamachana* and *Cassiopea* sp.2, as well as between *Cassiopea* sp.2 and
 317 *Cassiopea* sp.3, along with number of ring canals and large appendage distribution (Table 1).

The features that were considered as less useful when distinguishing between taxa include the shape of the subgenital pit, the number of ring canals, and the shape of the oral arm.

Discussion

Although the genus *Cassiopea* is easily recognised, the species have had a confused taxonomic history owing to subtle morphological distinctions and general morphological conservatism in the genus (Holland et al., 2004; Hopf & Kingsford, 2013; Maggio et al., 2019; Ohdera et al., 2018). This has resulted in a need for molecular data combined with morphology to identify species of *Cassiopea* in new locations (Holland et al., 2004; Maggio et al., 2019; Ohdera et al., 2018). Additionally, some species of *Cassiopea* are invasive, with new distribution records around the world, and so correctly identifying the species of *Cassiopea* is crucial to track and manage their spread (Holland et al., 2004; Keable & Ahyong, 2016; Maggio et al., 2019; Morandini et al., 2017; Ohdera et al., 2018; Thé et al., 2020).

Sequences of *Cassiopea* from eastern Australia (Lake Macquarie, Wallis Lake and Pelican Waters) group with, and are indistinguishable at species level from, those from Hawaii, Brazil, Palau, Panama, Florida Keys and an additional locality in the Northwest Pacific Ocean (Figure 4). Terminals within this clade are referable to *C. xamachana* based on genetic comparisons, morphology, and redescription of material that is to be designated as the neotype of the species (Gamero-Mora et al., 2019; Gamero-Mora et al., 2020; Stampar et al., 2020). However, *Cassiopea* occurring in Lake Macquarie and Wallis Lake, namely *C. xamachana*, are

also genetically indistinguishable from those from Pelican Waters. This result is particularly significant because Pelican Waters is the type locality of *C. maremetens* (Gershwin, Zeidler & Davie, 2010). Morphological congruence was also found between specimens from Lake Macquarie and Pelican Waters, including the holotype of *C. maremetens*. This is the first time that specimens morphologically corresponding to *C. maremetens* from the type locality of the species have been evaluated based on genetic data.

This indicates that *C. maremetens* is conspecific with *C. xamachana*, with the latter having nomenclatural priority as the older name. This was confirmed by morphological revision of type material of *C. maremetens* and comparison with additional specimens including topotypes (see Taxonomy and Morphometric Analysis sections). As such, we formally synonymise *C. maremetens* with *C. xamachana*, below. Additionally, material from Panama in this clade previously identified by Daglio & Dawson (2017) as *C. frondosa* appears to be a misidentification and is actually *C. xamachana*. Conversely, a specimen from the same study identified as *C. xamachana* and also from Panama was placed here in the *C. frondosa* clade (Daglio & Dawson, 2017), and is apparently *C. frondosa* based on the identity assigned in other studies to the material in this clade.

Cassiopea xamachana was first described by Bigelow (1892) from the Caribbean Sea and has subsequently been reported from a wide variety of locations (see Remarks in Taxonomy section). This species and has been taxonomically confused with *C. andromeda* (type locality:

Red Sea), both of these species apparently overlap in distribution in Florida with a third species, *C. frondosa* (type locality: Caribbean Sea, Muffett & Miglietta, 2023). Our study supports those of Gamero Mora et al. (2020) and Muffett & Miglietta (2023) in recognising *C. xamachana* is more closely related to *C. andromeda* (type locality: Red Sea) with a mean pairwise divergence of 7.2%, than to its Caribbean congener, *C. frondosa* (mean pairwise divergence: 19%). Some studies suggest that *C. xamachana* and *C. andromeda* might be conspecific given their morphological and genetic similarities (Arai et al., 2017; Gamero-Mora et al., 2020; Jarms et al., 2019), but Gamero-Mora et al. (2022) treated these as separate species because of significant divergence and monophyly on the basis of 28S ribosomal sequences; this was supported by Muffett & Miglietta (2023). Like *C. xamachana*, *C. andromeda* has subsequently been reported from the tropical and subtropical western Atlantic, Mediterranean Sea, the Indo-Pacific (including Australia, Gershwin, Zeidler & Davie, 2010, this study) and recently, the eastern Atlantic (Gueroun et al. 2024). For these two species, geography should not be used for species identification given that both can be sympatric in parts of their range as result of both natural and artificial processes, and the additional possibility that other species may be present (Fitt et al., 2021; Muffett & Miglietta, 2023).

Specimens of a second species occurring in eastern Australia analysed here from Lake Illawarra, New South Wales, and Coombabah Creek, Queensland, are found to be closely related to specimens from Japan (Abboud, Daglio & Dawson, 2018), Papua New Guinea and Hawaii

(Holland et al., 2004), reported as *Cassiopea* sp.3 by Holland et al. (2004). While there is some genetic variation between Australian populations, including Lake Alexander, NT, and those from overseas (mean pairwise divergence: 6.7%), they are here considered a single species due to the pairwise divergence being lower than the minimum between closely related species recognised by Gamero-Mora et al. (2022) (i.e. ~7.7% between *C. xamachana* and *C. andromeda*). Additionally, while Keable & Ahyong (2016) identified the population in Lake Illawarra as *C. ndrosia*, based on morphology and previous records using this name, the morphological features of these populations require comparison with those from the type locality of *C. ndrosia*, Suva Harbour, Fiji.

Morphometric analysis detected significant morphological differences between the two species occurring on the east coast of Australia and also *Cassiopea* sp.2 from Papua New Guinea. These consistent differences corroborate their previous treatment as separate species (Chiaverano, Bayha & Graham, 2016; Holland et al., 2004; Lessios, 2008), which is also indicated by the genetic analysis in this study. Three main features significant in the morphometric separation of *C. xamachana* and *Cassiopea* sp.3 are: 1) the number of large appendages on the oral disc, which is much higher in *Cassiopea* sp.3 (at least 1 but up to 14 in medusae > 10 cm), with *C. xamachana* having a maximum of two large appendages; 2) the oral arm branching pattern, which is usually alternating for *C. xamachana*, but a combination of alternating, bifurcating and pinnate for *Cassiopea* sp.3; 3) the length of the large appendage on

the oral arm (average of 2.6 cm, which is 0.2 bell diameter, compared to 1.4 cm for *Cassiopea* sp.3, which is 0.12 bell diameter).

The large appendages in *Cassiopea* are shown here to be useful and reliable character to separate some species, and they are believed to have two main functions. First, they provide additional surface area for zooxanthellae, and second, they contain nematocysts in clusters known as *cassiosomes*, which are used for feeding to capture prey or released in defence (Ames et al., 2020; Larson, 1997; Stampar et al., 2020). Historically, clear and consistent information about large appendages has not been included in descriptions of *Cassiopea*, and as a result there is no standardised terminology, leading to taxonomic confusion (Gamero-Mora et al., 2020). Whereas, the present study recorded variations in size, colour, and shape of large appendages within species, their distribution and the location of the longest large appendage proved particularly useful for comparing Australian populations of *C. xamachana* and *Cassiopea* sp.3.

The characters that we identify as consistent across *C. xamachana* populations along the east coast of Australia are also consistent with the original description of specimens from Jamaica, Caribbean Sea, by Bigelow (1892) and the revision of this species by Gamero-Mora et al., (2020), respectively. These features include generally five lappets per paramere; ribbon shaped large appendages located along the oral arms, and one on the bifurcation of each branch. However, Bigelow (1892) and Gamero-Mora et al., (2020) record 5-13 large appendages on the oral disc, which is far more than we found in Australian specimens. Conversely, Gamero-Mora

et al., (2020) also record variations between populations of *C. xamachana*, noting that the Atlantic Mexican population only has one appendage located on the oral disc if present at all, which is consistent with the Australian material observed here. Additionally, characters highlighted in the original description of *C. maremetens* by Gershwin, Zeidler & Davie (2010) (see Taxonomy section) and comparison with other species by Gamero-Mora et al. (2020) are consistent with the distinguishing features identified in this study, including five lappets per paramere, up to two large appendages on the oral disc, and a leaf shaped large appendage on the bifurcation of the oral arm. While Gershwin, Zeidler & Davie (2010) also recorded the absence of large appendages on some of the paratypes of *C. maremetens*, those specimens are not from the type locality, but from localities within Moreton Bay and without molecular data, and so were not examined in this study. As a result, molecular comparisons of specimens from these localities need to be made with known *C. xamachana* and *Cassiopea* sp.3 populations to confirm the identification of these paratypes.

Keable & Ahyong (2016) identified the species occurring in Lake Illawarra as *C. ndrosia*, in comparison with the taxonomic revision by Gamero-Mora et al. (2020) and the specimens in this study assigned to *Cassiopea* sp.3, some of the features are consistent with this identification as *C. ndrosia*. This includes the range in numbers of rhopalia (14-23) and the presence of large appendages on the oral disc which are conversely absent from the oral arms. Despite morphological similarities between *Cassiopea* sp.3 and the description of *C. ndrosia* by Gamero-

Mora et al. (2020), a molecular clade has not yet been recognised containing material definitively identified as *C. ndrosia*. Therefore, both genetic and morphological comparisons need to be made between *Cassiopea* sp.3 from Australia, Japan, Hawaii, Papua New Guinea, and specimens from the type locality of *C. ndrosia*, Suva Harbour, Fiji, before a positive match for *Cassiopea* sp.3 can be made with an available taxonomic name.

Although further revisionary study of *Cassiopea* occurring in Australia is needed, recognition of the presence of *C. xamachana* in Australia provided by the data presented here is significant given the attention this species has received at other localities as an invasive species.

As a potentially new arrival, rather than a native species increasing its range, the expanding distribution records within Australia are of greater importance than previously recognised.

Additionally, further study to determine vectors by which this species may be being translocated and other factors driving range expansions would be beneficial.

Taxonomy

Order RHIZOSTOMEAE Cuvier, 1817

CASSIOPEIDAE Tilesius, 1831

***Cassiopea* Péron & Lesueur, 1810**

***Cassiopea xamachana* Bigelow, 1892**

Fig 10–11.

- 451 *Cassiopea xamachana* Bigelow, 1892: 212–221.—Bigelow, 1893: 301.—Bigelow, 1900: 191,
452 figs A–L, pl. 31–38. —Mayer, 1910: 499–735, pl. 56–76.— Kramp, 1961.—
453 Hummelinck, 1968: 1–57. —Holland et al., 2004: 1119.—Morandini et al., 2017: 321. —
454 Ohdera et al., 2018: 1.— Gamero-Mora et al., 2019: 1.—Jarms & Morandini, 2019: 504.
- 455 *Cassiopea maremetens* Gershwin, Zeidler & Davie, 2010: 91, fig. 6C–F.—Templeman &
456 Kingsford, 2015:1–8. —Epstein et al. 2016: 340–346.—James & Morandini, 2019: 492.
457 —Gamero-Mora, et al., 2020: 113, fig 43. —Rowen et al., 2017:143–148.—McKenzie et
458 al., 2020:1–7.—Templeman et al., 2021:1. [New synonymy]
- 459 *Cassiopea* cf. *maremetens*. — Keable & Ahyong, 2016: 26, figs 2E–H, 3C–D, 4D–F.
- 460 *Cassiopea* sp. —Rowe et al., 2022a: 2.—Rowe et al., 2022b: 1.
- 461 *Cassiopea medusa*. — Durieux et al., 2023: 9 [not *C. medusa* Light, 1914, erroneous citation of
462 Rowe et al. (2022a) who indicate *Cassiopea* medusae, i.e. medusae of *Cassiopea* sp.,
463 subsequently identified here as *C. xamachana*].
- 464 ?*Cassiopea andromeda*. — Stephensen, 1962: 94 [doubtfully *C. andromeda* Forsskål, 1775].
465
- 466 **Type material of *C. maremetens* examined.** Holotype: QM G.326486, female (17.5 cm
467 diameter), off Lamerough Canal, Lake Magellan, Pelican Waters, Queensland, Australia,
468 26°49'47"S, 153°6'36"E, D. Potter and G. Cranitch, 24 May 2007. Paratypes: QM G.6645, 8
469 specimens (9.8, 10, 10.9, 10.3, 11, 11.5, 11.5, 11.5 cm), Mud Island, Moreton Bay, Queensland,

470 Australia, 27°19'47"S, 153°15'0"E, C. Wallace, 4 August 1972. QM G.327932, 2 females (8.8,
471 17.2 cm), off Lamerough Canal, Lake Magellan, Pelican Waters, Queensland, Australia,
472 26°49'47"S, 153°6'36"E, D. Potter and G. Cranitch, 24 May 2007. QM G.327969, 2 females
473 (11.9, 14.5 cm), estuary on NW side of Bentick Island, Queensland, Australia, 17°3'35"S,
474 139°29'24"E, P. Davie, 20 November 2002.

475 **Additional material examined (all from Australia).** Pelican Waters, Queensland: AM
476 G.20068-20069, 2 females (15.5, 16.1 cm), 26°50'01"S, 153°06'44"E, C.E. Rowe, 10 March
477 2021. Gold Coast, Queensland: QM G.339123–339125, 3 specimens (5.9, 7.7, 12.1 cm), 28°3'S,
478 153°24'26"E, M. Ekins and I. Jamieson, 8 August 2019. QM G.339126–339130, 5 females (6.7,
479 7.2, 8, 10.3, 17.3 cm), 28°10'S, 153°24'37"E, M. Ekins and I. Jamieson, 8 August 2019.

480 SeaWorld Culture, Queensland: AM G.18699–18701, 3 specimens (6.1, 6.5, 10.4 cm), most
481 probably originating from a small inlet on southern end of South Stradbroke Island, Gold Coast
482 Council Region, 25°55'3"S, 153°25'15"E, 22 January 2019. Wallis Lake, New South Wales:
483 AM G.18137–18139, 13 specimens (6.4, 7.7, 7.7, 7.7, 9.2, 10.4, 10.9, 11.2, 11.3, 12.1, 12.7 cm),
484 in channel splitting Godwin Island approximately one-third distance from southern shore,
485 32°11'45"S, 152°29'55"E, R. Pearce, 15 August 2014. AM G.18143–18156, 12 specimens (2.9,
486 3.6, 4.4, 5.2, 5.6, 7.2, 8.4, 8.8, 8.8, 9.4, 9.6, 9.7, 10.7 cm), behind Smugglers Cove Caravan Park,
487 Pipers Creek, "The Keys", 32°12'0"S, 152°30'39"E, R. Pearce, 18 September 2014. AM
488 G.18181–18183, 4 specimens, inlet on south-east side of Mather Island, 32°11'26"S,

489 152°29'36"E, S.J. Keable and A.D. Hegedus, 28 April 2015. AM G.18184, 1 specimen, Pipers
 490 Creek within Smugglers Cove Caravan Park, 32°11'58"S, 152°30'39"E, S.J. Keable and A.
 491 Murray, 30 April 2015. AM G.18736–18755, 20 females (8.9, 11, 12, 12.5, 12.8, 13.1, 13.3, 15,
 492 15.1, 15.1, 16.7, 17.3, 18.7, 19.1, 19.4, 19.4, 20.8, 20.9, 22.3, 24,cm), Pipers Creek within
 493 Smugglers Cove Caravan Park, 32°11'58"S, 152°30'39"E, S.J. Keable and C.E. Rowe, 10 May
 494 2019. Lake Macquarie, New South Wales: AM G.18362–18365, 12 specimens (7.1, 10, 12.6,
 495 13.1, 13.3, 13.4, 16.7, 17, 17.4, 17.5, 19.6, 19.7 cm), Lake Petite, 33°06'59"S, 151°32'04"E, S.J.
 496 Keable, D.J. Keable, S. Jones, D. Jones, and E.M. Keable, 4 June 2017. AM G.18428, 1
 497 specimen (13.4 cm), Lake Petite, 33°07'00"S, 151°31'58"E, S.J. Keable and A. Hay, 9 February
 498 2018. AM G.18528, 1 specimen (6.1 cm), Karignan Creek, 33°10'36"S, 151°34'03"E, C.E.
 499 Rowe and S.J. Keable, 16 May 2018. AM G.18711–18712, 2 specimens (11.9, 12.4 cm),
 500 Mannering Bay, 33°09'28"S, 151°31'41"E, C.E. Rowe and S.J. Keable, 7 March 2019. AM
 501 G.18716–18723, 8 females (10.3, 14.3, 12, 15.5, 16.4, 17, 19.6, 20 cm), Karignan Creek,
 502 33°10'32"S, 151°34'00"E, S.J. Keable and C.E. Rowe, 6 May 2019. AM G.18724–18728, 5
 503 females (11.3, 12, 12.2, 12.9, 14.4 cm), creek north of Kilaben Creek, 33°01'43"S, 151°35'01"E,
 504 S.J. Keable and C.E. Rowe, 9 May 2019. AM G.18729–18735, 7 females (14.2, 16.1, 17.2, 19.2,
 505 19.7, 19.8, 20.9 cm), Lake Petite, 33°07'03"S, 151°32'06"E, S.J. Keable and C.E. Rowe, 9 May
 506 2019.

507 **Comparative material examined.** *Cassiopea* sp.3: Northern Territory, Australia: AM G.17363
508 and G.17374, 3 specimens (6.3, 6.8, 6.8 cm), Lake Alexander, Darwin, 12°24'S, 130°49'E, M.
509 Dawson, 15 November 2003. Queensland, Australia: AM G.20057–20061, 5 females (14.9, 15.4,
510 16.2, 16.3, 18.2 cm), Kangaroo Avenue, Coombabah Creek, Gold Coast, 27°05'26"S,
511 153°22'57"E, C.E. Rowe and J. Lawley, 8 March 2021. AM G.20064–20067, 4 specimens (2.3,
512 5.1, 9.9, 10.7 cm), SeaWorld culture originating from Coombabah Creek and South Stradbroke
513 Island, Gold Coast Region, 25°55'3"S, 153°25'15"E, 9 March 2021. AM G.13568, 3 specimens
514 (10.1, 12.2, 13.3 cm), off Hayman Island, Whitsunday Passage, 20°03'S, 148°53'E, JA. McNeill,
515 17 April 1934 (material referred to by Stiasny (1931) and Keable & Ahyong (2016) as *C.*
516 *ndrosia*). New South Wales, Australia: AM G.18075, 1 specimen (9.2 cm), canal through jetties
517 by the lake at Windang Road, Lake Illawarra, 34°31'36"S, 150°51'52"E, M. Cameron, 8 May
518 2013 (material referred to by Keable & Ahyong (2016) as *C. ndrosia*). AM G.20077, 11
519 specimens (3, 4.2, 5.4, 6.9, 7.2, 7.3, 8.2, 9.8, 10.5, 10.8, 15.1 cm), canal through jetties by the
520 lake at Windang Road, Lake Illawarra 34°31'36"S, 150°51'52"E, S.J. Keable and C.E. Rowe, 1
521 June 2021. AM G.20076, 2 specimens (12.7, 12.8 cm), channel between rocky shore and sand
522 spit, north east of entrance to Cabbage Tree Basin, Port Hacking 34°04'44"S, 151°07'58"E, S.J.
523 Keable and C.E. Rowe, 31 May 2021.

524 *Cassiopea* sp.2: Papua New Guinea: AM G.17370 and G.17385, 6 specimens (4.6, 7.3,
525 7.7 8.9, 9, 10.5 cm), outer slope, west side of Mascot Channel mouth, 2°40'04"S, 150°25'58"E,

526 P.L. Colin, 3 July 2003. AM G.17387, 1 specimen (5.2 cm), atoll ~70 nautical miles south-south-
 527 east of Manus Island, Sherburne Reef, 3°19'59"S, 148°01'03"E, D. de Mara, 20 June 2002.
 528 Queensland, Australia: AM G.18344, 1 specimen (6.3 cm), north east coast, Lizard Island,
 529 14°40'01"S, 145°27'37"E, A. Hoggett, 21 February 2016.

530 *Cassiopea andromeda*: South Australia: SAM H3568, H3577–78 and H3581, 4
 531 specimens (6.2, 6.2, 7.9, 9.7 cm), boat ramp, Garden Island Yacht Club, Garden Island, Angas
 532 Inlet, 34°48'11"S, 138°31'55"E, M. Bossley and A. Crowther, 4 May 2022.

533 **Description.** (Where variation occurs, value for holotype of *C. maremetans* given in brackets).

534 Exumbrella: marginal outline circular; aboral surface mostly flat with small concavity; maximum
 535 diameter 24 cm, mean 11.5 cm (holotype: 17 cm); height 0.2–2.7 cm (0.4 cm), mean 0.9 cm.

536 *Colour pattern* brown, yellow, green (greenish yellow). *Markings* include small white spots
 537 around bell margin, triangle facing towards each ocellus on live specimens (markings lost in
 538 preservation). *Rhopalia* 13–23 (19), mean 15, located in incised notches; ocelli present.

539 *Marginal lappets* 32–120 (104), mean 83 depending on the condition of the specimen, with 3–7
 540 per paramere (4–6), not always distinct, distal edge with rounded low lobes; 1–5 (3) velar
 541 lappets located between 2 ocular lappets. *Ring canals* 1 in total, raised.

542 Oral disc 0.2–2 cm height, with a mean of 0.94 cm, 0.5–8.2 cm width, and with a mean
 543 of 3.88 cm, typically 1.6–5.4 × the bell diameter, with a mean of 0.4 × bell diameter. *Subgenital*

544 *pit* circular or rhomboid, 0.1–0.9 cm width, with 0.65 cm mean, $0.09\text{--}0.58 \times$ oral disc height,
 545 with 0.3 mean. *Large appendages* present on specimens > 8 cm; 1 or 2 in centre of oral disc (1),
 546 fusiform, 0.7–3.4 cm in length (2.5 cm), averaging 1.3 cm, $0.17\text{--}0.67 \times$ oral disc width, but
 547 typically $0.25 \times$ oral disc width, width of large appendage ranges from 0.06 to $0.4 \times$ length of
 548 large appendage (0.4), typically 0.27 length of large appendage.

549 Oral arms: 7–10 (8), usually 8 (except AM G.18733, G.18728, QM G.327932, G.6645,
 550 G.32769). 0.9–14.5 cm in length (~ 12.3) with mean of 6.38 cm, 0.75 to $1.54 \times$ bell radius ($1.5 \times$
 551 bell radius) with mean $1.2 \times$ bell radius, extending radially beyond bell margin, cylindrical in
 552 shape. *Lateral branches* 3–7 per oral arm (4 or 5), cylindrical; alternating branching pattern;
 553 secondary lateral branches with alternating branching pattern of equal strength. Trunk of oral
 554 arm ending in unequal bifurcation. *Small appendages* numerous, flat, fusiform, transparent,
 555 distributed evenly along oral surface of oral arm and lateral branches. *Large appendages* present;
 556 largest located at bifurcation of oral arm, 0.2–5 cm in length (1.7 cm) with mean of 2.50 cm,
 557 $0.07\text{--}0.63 \times$ oral arm length with mean $0.3 \times$ oral arm length, and width $0.05\text{--}0.36 \times$ length, but
 558 typically 0.22 length. Number of large appendages on oral arm varying with size, with 2 large
 559 appendages near distal bifurcation in smaller specimens (e.g., AM G.18754 (11 cm), G.18744
 560 (12.8 cm), G.18723 (12 cm)) and up to 17 evenly distributed along the oral arm in larger
 561 specimens (e.g., AM G.18365 (17 cm) and G.18362 (19.7 cm), holotype QM G.326486 with 2, 1

located at distal bifurcation and other half way along oral arm, but this may be an artefact of damage, see Remarks), always present at distal bifurcation, fusiform.

Gonads: Always visible, cruciform, as mature gonads indicating sex, but definition varying with size and condition of preserved specimen; developed in specimens > ~10 cm in diameter.

Remarks

The diagnostic characters of Australian specimens, including the type material of *C. maremetens* and additional material from the type locality, are indistinguishable from those of *C. xamachana* from the western Atlantic (Bigelow, 1892). These include the number of lappets per paramere (4–6) and their indistinct, rounded shape; the alternating lateral branches along the oral arm, which end with an unequal bifurcation; and the longest large appendage located at the bifurcation in the oral arm, with the number of large appendages on the oral arm increasing with size (1–17), with maximum 2 located at the centre of the oral disc. These features are consistent across all specimens examined from Wallis Lake, Lake Macquarie and Pelican Waters, which are also genetically invariant in COI. Additionally, the morphometric analysis supports these as consistent features distinguishing *C. xamachana* from *Cassiopea* sp.3. As such, *C. maremetans* and *C. xamachana* are indistinguishable and we recognise the former as a junior synonym of the latter.

Variation in Australian *C. xamachana*:

No significant morphological differences were detected between *C. xamachana* occurring along the east coast of Australia. However, small variations were evident, such as the number of lappets per paramere (within and between specimens), the number of oral arms and lateral branches, and, in the larger specimens, whether one or two large appendages are present in the centre of the oral disc.

Our examination found some morphological differences between specimens studied and the original description of *C. maremetens* by Gershwin, Zeidler & Davie (2010). For example, the original diagnosis and the holotype description indicate absence of ocelli, but these were found to be present in the holotype, additional material examined in this study and specimens examined by Keable & Ahyong (2016) from Wallis Lake. Additionally, the original diagnosis recorded one large appendage at the base of the oral arm pairs, and one at the distal bifurcation of each arm. Although the location of the large appendage in the holotype is consistent with our observations, other specimens we examined had a much higher number of large appendages on the oral arms, with up to 17 recorded on larger specimens. However, we note that the holotype is in relatively fragile condition, so some large appendages may have been damaged or lost. Additionally, the original description is based on specimens from multiple locations in the Moreton Bay region, including the paratypes QM G.327969 from Bentick Island, and QM G.6445 from Mud Island. These specimens are also in poor condition so many characters cannot be accurately assessed, but we were still able to observe numerous large appendages around the

oral disc. This is a feature of *Cassiopea* sp.3, which also occurs in the Moreton Bay region.

Therefore, the paratype series of *C. maremetens* may also include misidentified specimens of

Cassiopea sp.3. As a result, these localities should be resampled so morphology and genetic

information can be used to confirm the identification of these specimens.

Comparison of Australian and overseas *C. xamachana*:

Cassiopea xamachana was first described from Kingston Harbour, Jamaica (Bigelow,

1892), and has since been reported widely around the world, most commonly in the Western

Atlantic from Florida (Verde & McCloskey, 1998) south to Brazil (Gamero-Mora et al., 2019),

as well as several Indo-West Pacific localities including Hawaii (Holland et al., 2004) and Palau

(Arai et al., 2017; Jarms et al., 2019). The species was re-described by Gamero-Mora et al.

(2020), who set forth plans to designate a neotype from Fort Jefferson, Dry Tortugas, Florida

Keys, Florida, USA, as the original type material is lost. Both the original description and

redescription note that the characteristic features of this species include: 5 lappets per paramere,

which are obtuse and separated by grooves in the bell margin; there are usually 16 rhopalia; 4

pairs of oral arms that contain 10–15 lateral branches that are alternating and contain additional

secondary branches; and large appendages located in the axil of each oral arm, whose length can

be up to one-fourth the bell diameter. Additionally, they record that the centre of the oral disc

contains 5–13 of the large appendages, but in the prospective neotype, they are also scattered

along the oral arms. The number and location of the large appendages on the oral arms of the

prospective neotype, and the number of lappets and rhopalia are consistent with distinguishing features identified in *C. maremetens*. These similarities, combined with our wider genetic and morphometric analysis of Australian material corroborate the conclusion that *C. maremetens* is conspecific with *C. xamachana*.

It has been reported that there are morphological variations, even in clonal populations of *Cassiopea* (Hummelinck, 1968; Mayer, 1910; Morandini et al., 2017; Stampar et al., 2020). Therefore, some morphological variation among other populations of *C. xamachana* can be expected, especially given the wide distribution of the species. This includes the absence of grooves occurring between the lappets, which in the Australian specimens are rounded and connected. Additionally, the original description of *C. xamachana* indicates that the specimens have 5–13 large appendages on the oral disc, but the Australian specimens have a maximum of two. However, Gamero-Mora et al. (2020) concluded that there are morphological differences in the Mexican population of *C. xamachana*, noting specimens had 6–10 oral arms, that were up to $1.6 \times$ the bell radius, and the number of large appendages range from zero to 25 and are either on the distal part or scattered over the oral arms, with at least one located on the oral disc, which resembles the condition of specimens from Wallis Lake, Lake Macquarie and Pelican Waters, Australia.

Comparison of *C. xamachana* to other species of *Cassiopea* in Australia:

The identification of *C. ndrosia* reported by Stiasny (1931) and Keable & Ahyong (2016) needs to be examined more closely to confirm their identification with sequences matched to morphology (see Discussion). Therefore, in this study, specimens have been designated *Cassiopea* sp.3 according to previous placement of molecular sequences (Holland et al. 2004). Key distinguishing features that separate *C. xamachana* from *Cassiopea* sp.3 include fewer large appendages on the oral disc (1 or 2 compared to ~6), the location of the large appendages (oral arms compared to oral disc), length of the large appendage on the oral arm (usually 0.2 the bell diameter compared to 1.2), oral arm length (usually 0.57 the bell diameter compared to 0.67), lappets per paramere (usually 5 compared to 4), and the location of the large appendages on the oral arm (evenly distributed and always at the bifurcation, compared to sometimes at the bifurcation, but often absent).

Further comparisons need to be made with the South Australian population in the vicinity of Angas Inlet. Although Southcott (1982) reported Angas Inlet specimens as *C. ndrosia*, our results identified *C. andromeda*, which is closely related to *C. xamachana* and morphologically similar, including sharing these features: a large appendage on the fork of the oral arms; 1–2 central large appendages on the oral disc; and an alternating oral arm branching pattern. However, the specimens are juvenile (<10 cm), so many of the other key characters useful to distinguish species of *Cassiopea* were not fully developed. Given Southcott's (1982)

identification of material from Angas Inlet as *C. ndrosia*, the potential presence of another species, formerly or currently in the area, needs to be considered.

Conclusion

We are able to confirm that the species of *Cassiopea* occurring in Lake Macquarie, Wallis Lake and Pelican Waters (type locality of *C. maremetens*) is genetically and morphologically referable to *C. xamachana*. We provide a detailed description of Australian *C. xamachana*, including a comparison with the type and topotypic material of *C. maremetens*, synonymised herein. Additionally, we provide evidence that a second species, *C. sp.3*, is expanding its range south along the east coast of Australia and identify the morphological features that distinguish it from *C. xamachana*. As the two species continue to expand their range southwards in eastern Australia, which is expected under climate change scenarios (Rowe et al., 2022a), these diagnostic features will facilitate their identification, especially in monitoring and management of their invasion front.

Acknowledgements

The authors would like to thank Dr. Merrick Ekins, and Dr. Andrea Crowther, the Collection Managers at Queensland Museum and South Australian Museum respectively, for the loan of specimens. Additionally, we would like to thank Alex Hegedus, Professor Kylie Pitt, Dr.

Jonathan Lawley, and Dr. Mike Bossley for their assistance in collecting specimens for this study. Thank you to the Australian Museum Research Institute for their support through the 2020/21 AMF/AMRI Postgraduate Award. We would also like to thank Dr. Edgar Gamero-Mora for guidance and discussions about upside-down jellyfish genetics and morphology.

References

- Abboud SS, Daglio LG, Dawson MN. 2018. A global estimate of genetic and geographic differentiation in macromedusae implications for identifying the causes of jellyfish blooms. *Marine Ecology Progress Series* 591:199–216. DOI: 10.3354/meps12521.
- Agassiz A, Mayer AG. 1899. Acalephs from the Fiji Islands. *Bulletin of the Museum of Comparative Zoology* 32:157–189.
- Ames CL, Klompen AM, Badhiwala K, Muffett K, Reft AJ, Kumar M, Janssen JD, Schultzhaus JN, Field LD, Muroski ME. 2020. Cassiosomes are stinging-cell structures in the mucus of the upside-down jellyfish *Cassiopea xamachana*. *Communications Biology* 3:1–15. DOI: 10.1038/s42003-020-0777-8.
- Arai Y, Gotoh RO, Yokoyama J, Sato C, Okuizumi K, Hanzawa N. 2017. Phylogenetic relationships and morphological variations of upsidedown jellyfishes, *Cassiopea* spp. inhabiting Palau Island. *Biogeography* 19:133–141. DOI: 10.11358/biogeography.19.133

- 692 Bigelow RP. 1892. On a new species of *Cassiopea* from Jamaica. *Zoologischer Anzeiger*
693 15:212–214.
- 694 Bigelow RP. 1893. *Some observations on Polyclonia frondosa*. Baltimore, MD: Johns Hopkins
695 University Circulars, 106.
- 696 Bigelow RP. 1900. The anatomy and development of *Cassiopea xamachana*. *Boston Society of*
697 *Natural History*:191–236.
- 698 Bolton TF, Graham WM. 2006. Jellyfish on the rocks: bioinvasion threat of the international
699 trade in aquarium live rock. *Biological Invasions* 8:651–653.
- 700 Brandt JF. 1838. Ausführliche Beschreibung der von C. H. Mertens auf seiner Weltumsegelung
701 beobachteten Schwimmpollen. *Mémoires de l'Académie impériale des sciences de St-*
702 *Petersbourg* 6:237–412.
- 703 Brotz L, Pauly D. 2016. Studying jellyfish fisheries: toward accurate national catch reports and
704 appropriate methods for stock assessments. In: Mariottini GL, ed. *Jellyfish: ecology,*
705 *distribution patterns and human interactions Nova Publishers, New York*, 313–329.
- 706 Chiaverano LM, Bayha KW, Graham WM. 2016. Local versus generalized phenotypes in two
707 sympatric *Aurelia* species: understanding jellyfish ecology using genetics and
708 morphometrics. *PloS one* 11:e0156588. DOI: 10.1371/journal.pone.0156588.
- 709 Collins AG, Jarms G, Morandini AC. 2022. World List of Scyphozoa. *Cassiopea Péron &*
710 *Lesueur*, 1810. Available at

<https://www.marinespecies.org/aphia.php?p=taxdetails&id=13525> (accessed May 2022).

Curtis AE, Smith TA, Ziganshin BA, Elefteriades JA. 2016. The mystery of the Z-score. *Aorta* 4:124–130. DOI: 10.12945/j.aorta.2016.16.014

Daglio LG, Dawson MN. 2017. Species richness of jellyfishes (Scyphozoa: Discomedusae) in the Tropical Eastern Pacific: missed taxa, molecules, and morphology match in a biodiversity hotspot. *Invertebrate Systematics* 31:635–663. DOI: 10.1071/IS16055.

Dawson M. 2003. Macro-morphological variation among cryptic species of the moon jellyfish, *Aurelia* (Cnidaria: Scyphozoa). *Marine Biology* 143:369–379. DOI: 10.1007/s00227-003-1070-3.

Dawson MN, Jacobs DK. 2001. Molecular evidence for cryptic species of *Aurelia aurita* (Cnidaria, Scyphozoa). *The Biological Bulletin* 200:92–96.

Durieux DM, Scroggins GD, Fender C, Lewis DB, Deban SM, Gemmell BJ. 2023. Benthic jellyfish act as suction pumps to facilitate release of interstitial porewater. *Scientific Reports* 13:3770. DOI: 10.1038/s41598-023-30101-4.

Epstein HE, Templeman MA, Kingsford MJ. 2016. Fine-scale detection of pollutants by a benthic marine jellyfish. *Marine Pollution Bulletin* 107:340–346. DOI: 10.1016/j.marpolbul.2016.03.027.

- 729 Fitt WK, Hofmann DK, Kemp DW, Ohdera AH. 2021. Different Physiology in the Jellyfish
730 *Cassiopea xamachana* and *C. frondosa* in Florida Bay. *Oceans* 2:811–821. DOI:
731 10.3390/oceans2040046.
- 732 Forskål P. 1775. Descriptiones animalium, avium, amphibiorum, piscium, insectorum, vermium;
733 quae in itinere orientali observavit Petrus Forskål. *Hauniae*:1–64.
- 734 Gamero-Mora E, Collins A, Boco S, Geson III S, Morandini A. 2022. Revealing hidden diversity
735 among upside-down jellyfishes (Cnidaria: Scyphozoa: Rhizostomeae: *Cassiopea*):
736 distinct evidence allows the change of status of a neglected variety and the description of
737 a new species. *Invertebrate Systematics* 36:63–89. DOI: 10.1071/IS21002.
- 738 Gamero-Mora E, Halbauer R, Bartsch V, Stampar SN, Morandini AC. 2019. Regenerative
739 capacity of the upside-down jellyfish *Cassiopea xamachana*. *Zoological Studies* 58:e37.
740 DOI: 10.6620/ZS.2019.58-37.
- 741 Gamero-Mora E, Maronna MM, Lawley JW, Collins AG, Dawson MN, Morandini AC. 2020.
742 Hidden diversity and insights into the origin and biogeogrpahy of the upside-down
743 jellyfishes (Cnidaria: Scyphozoa). *PhD Thesis*.
- 744 Gershwin L, Zeidler W, Davie PJ. 2010. Medusae (Cnidaria) of Moreton Bay, Queensland,
745 Australia. *Memoirs of the Queensland Museum* 54:47–108.

- 746 Gohar H, Eisawy A. 1960. The biology of *Cassiopea andromeda* (from the Red Sea)(with a note
747 on the species problem). *Publications of the Marine Biological Statation, Al- Ghardaqa*
748 11:3–39.
- 749 Graham WM, Bayha KM. 2008. Biological invasions by marine jellyfish. *Biological Invasions*:
750 Springer, 239–255.
- 751 Gueroun SK, Moura CJ, Almansa E, Escáñez A. 2024. *Cassiopea andromeda* (Cnidaria,
752 Scyphozoa) in the subtropical eastern Atlantic. *Journal of the Marine Biological*
753 *Association of the United Kingdom* 104:e92. DOI: 10.1017/S0025315424000882.
- 754 Haeckel E. 1880. Das System der Acraspeden. 2te Hälfte des Systems der Medusen. Acht
755 Nachträge zur Vervollständigung des Systems. *enschriften der Medicinisch-*
756 *Naturwissenschaftlichen Gesellschaft zu Jena* 2:361–672.
- 757 Holland BS, Dawson MN, Crow GL, Hofmann DK. 2004. Global phylogeography of *Cassiopea*
758 (Scyphozoa: Rhizostomeae): molecular evidence for cryptic species and multiple
759 invasions of the Hawaiian Islands. *Marine Biology* 145:1119–1128.
- 760 Hopf J, Kingsford MJ. 2013. The utility of statoliths and bell size to elucidate age and condition
761 of a scyphomedusa (*Cassiopea* sp.). *Marine Biology* 160:951–960. DOI:
762 10.1007/s00227-012-2146-8.
- 763 Hummelinck WP. 1968. Caribbean scyphomedusae of the genus *Cassiopea*. *Studies on the*
764 *Fauna of Curaçao and Other Caribbean Islands* 25:1–57.

- 765 Jarms G, Morandini AC, Schmidt-Rhaesa A, Giere O, Straehler-Pohl I. 2019. *World atlas of*
766 *jellyfish: Scyphozoa except Stauromedusae*. Dölling und Galitz Verlag: Hamburg.
- 767 Keable SJ, Ah Yong ST. 2016. First records of the invasive “upsidedown jellyfish”, *Cassiopea*
768 (Cnidaria: Scyphozoa: Rhizostomeae: Cassiopeidae), from coastal lakes of New South
769 Wales, Australia. *Records of the Australian Museum* 68:23–30. DOI: 10.3853/j.2201-
770 4349.68.2016.1656.
- 771 Knowlton N. 2000. Molecular genetic analyses of species boundaries in the sea. *Hydrobiologia*
772 420: 73–90.
- 773 Kramp P. 1961. *Synopsis of the medusae of the world*. Journal of the Marine Biological
774 Association of the United Kingdom. Cambridge University Press Cambridge, UK.
- 775 Kramp P. 1965. Some medusae (mainly scyphomedusae) from Australian coastal waters.
776 *Transactions of the Royal Society of South Australia* 89:257–278.
- 777 Larson R. 1997. Feeding behaviour of Caribbean scyphomedusae: *Cassiopea frondosa* (Pallas)
778 and *Cassiopea xamachana* Bigelow. *Studies of the Natural History of the Caribbean*
779 *Region* 73:43–54.
- 780 Lawley JW, Gamero-Mora E, Maronna MM, Chiaverano LM, Stampar SN, Hopcroft RR,
781 Collins AG, Morandini AC. 2021. The importance of molecular characters when
782 morphological variability hinders diagnosability: systematics of the moon jellyfish genus
783 *Aurelia* (Cnidaria: Scyphozoa). *PeerJ* 9:e11954. DOI: 10.7717/peerj.11954.

- 784 Lessios HA. 2008. The great American schism: divergence of marine organisms after the rise of
785 the Central American Isthmus. *The Annual Review of Ecology, Evolution, and*
786 *Systematics* 39:63–91. DOI: 10.1146/annurev.ecolsys.38.091206.095815.
- 787 Light S. 1914. Some Philippine Scyphomedusae, including two new genera, five new species
788 and one new variety. *Philippine Journal of Science* 9:195–231.
- 789 Linnaeus C. 1758. Systema Naturae per regna tria naturae, secundum classes, ordines, genera,
790 species, cum characteribus, differentiis, synonymis, locis. *Editio decima, reformata [10th*
791 *revised edition]* 1:824.
- 792 Lobo J, Costa PM, Teixeira MA, Ferreira MS, Costa MH, Costa FO. 2013. Enhanced primers for
793 amplification of DNA barcodes from a broad range of marine metazoans. *BMC Ecol*
794 13:1–8.
- 795 Maggio T, Allegra A, Bosch-Belmar M, Cillari T, Cuttitta A, Falautano M, Milisenda G, Nicosia
796 A, Perzia P, Sinopoli M. 2019. Molecular identity of the non-indigenous *Cassiopea* sp.
797 from Palermo Harbour (central Mediterranean Sea). *Journal of the Marine Biological*
798 *Association of the United Kingdom* 99:1765–1773.
- 799 Mayer AG. 1910. *Medusae of the world, vol. 3. The Scyphomedusae* (Washington, DC: Carnegie
800 Institute).

- 801 McKenzie MR, Templeman MA, Kingsford MJ. 2020. Detecting effects of herbicide runoff: the
802 use of *Cassiopea maretensis* as a biomonitor to hexazinone. *Aquatic Toxicology*
803 221:105442. DOI: 10.1016/j.aquatox.2020.105442.
- 804 Morandini AC, Schiariti A, Stampar SN, Maronna MM, Straehler-Pohl I, Marques AC. 2016.
805 Succession of generations is still the general paradigm for scyphozoan life cycles.
806 *Bulletin of Marine Science* 92:343–351. DOI: 10.5343/bms.2016.1018.
- 807 Morandini AC, Stampar SN, Maronna MM, Da Silveira FL. 2017. All non-indigenous species
808 were introduced recently? The case study of *Cassiopea* (Cnidaria: Scyphozoa) in
809 Brazilian waters. *Journal of the Marine Biological Association of the United Kingdom*
810 97:321–328. DOI: doi:10.1017/S0025315416000400.
- 811 Muffett K, Miglietta MP. 2023. Demystifying *Cassiopea* species identity in the Florida Keys:
812 *Cassiopea xamachana* and *Cassiopea andromeda* coexist in shallow waters. *PloS one*
813 18:e0283441. DOI: 10.1371/journal.pone.0283441.
- 814 Ohdera AH, Abrams MJ, Ames CL, Baker DM, Suescún-Bolívar LP, Collins AG, Freeman CJ,
815 Gamero-Mora E, Goulet TL, Hofmann DK, Jaimes-Becerra A, Long PF, Marques AC,
816 Miller LA, Mydlarz LD, Morandini AC, Newkirk CR, Putri SP, Samson JE, Stampar SN,
817 Steinworth B, Templeman MA, Thomé PE, Vlok M, Woodley CM, Wong JC, Martindale
818 MQ, Fitt WK, and Medina M. 2018. Upside-down but headed in the right direction:

- 819 review of the highly versatile *Cassiopea xamachana* system. *Frontiers in Ecology and*
- 820 *Evolution* 6:35. DOI: 10.3389/fevo.2018.00035.
- 821 Pallas P. 1774. *Spicilegia Zoologica: Quibus Novae Imprimis Et Obscurae Animalium Species*
- 822 *Iconibus, Descriptionibus Atque Commentariis Illustrantur*. Fasciculus Berolini.
- 823 Richardson AJ, Bakun A, Hays GC, Gibbons MJ. 2009. The jellyfish joyride: causes,
- 824 consequences and management responses to a more gelatinous future. *Trends in Ecology*
- 825 *and Evolution* 24:312–322. DOI: 10.1016/j.tree.2009.01.010.
- 826 Rowe CE, Figueira W, Keable SJ, Ah Yong ST. 2022a. Physiological responses of the upside-
- 827 down jellyfish, *Cassiopea* (Cnidaria: Scyphozoa: Cassiopeidae) to temperature and
- 828 implications for *Cassiopea*’s range expansion along the east coast of Australia. *Journal of*
- 829 *Experimental Marine Biology and Ecology* 554:151765. DOI:
- 830 10.1016/j.jembe.2022.151765.
- 831 Rowe CE, Figueira WF, Kelaher BP, Giles A, Mamo LT, Ah Yong ST, Keable SJ. 2022b.
- 832 Evaluating the effectiveness of drones for quantifying invasive upside-down jellyfish
- 833 (*Cassiopea* sp.) in Lake Macquarie, Australia. *PloS one* 17:e0262721. DOI:
- 834 10.1371/journal.pone.0262721.
- 835 Rowen DJ, Templeman MA, Kingsford MJ. 2017. Herbicide effects on the growth and
- 836 photosynthetic efficiency of *Cassiopea maremetens*. *Chemosphere* 182:143–148. DOI:
- 837 10.1016/j.chemosphere.2017.05.001.

- 838 Schiariti A, Morandini AC, Jarms G, von Glehn Paes R, Franke S, Mianzan H. 2014. Asexual
839 reproduction strategies and blooming potential in Scyphozoa. *Marine Ecology Progress*
840 *Series* 510:241–253. DOI: 10.3354/meps10798.
- 841 Southcott R. 1982. Jellyfishes (Classes Scyphozoa and Hydrozoa). *Marine Invertebrates of South*
842 *Australia Part I*:115–159.
- 843 Stampar SN, Gamero-Mora E, Maronna MM, Fritscher JM, Oliveira BS, Sampaio CL,
844 Morandini AC. 2020. The puzzling occurrence of the upside-down jellyfish *Cassiopea*
845 (Cnidaria: Scyphozoa) along the Brazilian coast: a result of several invasion events?
846 *Zoologia (Curitiba)* 37:e50834. DOI: 10.3897/zoologia.37.e50834.
- 847 Stecher G, Tamura K, Kumar S.2020. Molecular Evolutionary Genetics Analysis (MEGA) for
848 macOS. *Molecular Biology and Evolution* 37:1237–1239. DOI: 10.1093/molbev/msz312.
- 849 Stephenson W. 1962. A peculiar jellyfish from Moreton Bay. *The Queensland Naturalist* 16:94.
- 850 Stiasny G. 1924. Über einige von Dr. C. J. van der Horst bei Curaçao gesammelte Medusen.
851 Bijdragen tot de Kenntnis der Fauna van Curaçao. Resultaten eener Reis van C. J. van der
852 Horst in 1920. *Bijdragen to de Dierkunde* 23:83–92.
- 853 Stiasny G. 1931. Die rhizostomeen-sammlung des British Museum (natural history) in London.
854 *Zoologische Mededelingen* 14:137–178.
- 855 Stoner EW, Yeager LA, Sweatman JL, Sebilian SS, Layman CA. 2014. Modification of a
856 seagrass community by benthic jellyfish blooms and nutrient enrichment. *Journal of*

- 857 *Experimental Marine Biology and Ecology* 461:185–192. DOI:
- 858 10.1016/j.jembe.2014.08.005.
- 859 Team RC. 2021. R: A language and environment for statistical computing. V2021.09.01ed: R
- 860 *Foundation for Statistical Computing, Vienna, Austria.*
- 861 Templeman MA, Kingsford MJ. 2015. Predicting aqueous copper and zinc accumulation in the
- 862 upside-down jellyfish *Cassiopea maremetens* through the use of biokinetic models.
- 863 *Environmental monitoring and assessment* 187:1–8. DOI: 10.1007/s10661-015-4657-5.
- 864 Templeman MA, McKenzie MR, Kingsford MJ. 2021. The utility of jellyfish as marine
- 865 biomonitors. *Marine Pollution Bulletin* 173:113056. DOI:
- 866 10.1016/j.marpolbul.2021.113056.
- 867 Thé J, Gamero-Mora E, da Silva MVC, Morandini AC, Rossi S, de Oliveira Soares M. 2020.
- 868 Non-indigenous upside-down jellyfish *Cassiopea andromeda* in shrimp farms (Brazil).
- 869 *Aquaculture* 532:735999.
- 870 Verde EA, McCloskey L. 1998. Production, respiration, and photophysiology of the mangrove
- 871 jellyfish *Cassiopea xamachana* symbiotic with zooxanthellae: effect of jellyfish size and
- 872 season. *Marine Ecology Progress Series* 168:147–162.
- 873 Zhang J, Kapli P, Pavlidis P, Stamatakis A. 2013. A general species delimitation method with
- 874 applications to phylogenetic placements. *Bioinformatics* 29:2869–2876. DOI:
- 875 10.1093/bioinformatics/btt499.

876

Figure 1

Localities for *Cassiopea* compared in this study.

Specimens sourced from the field and from museum collections. Map created in R (v2021.09.01).

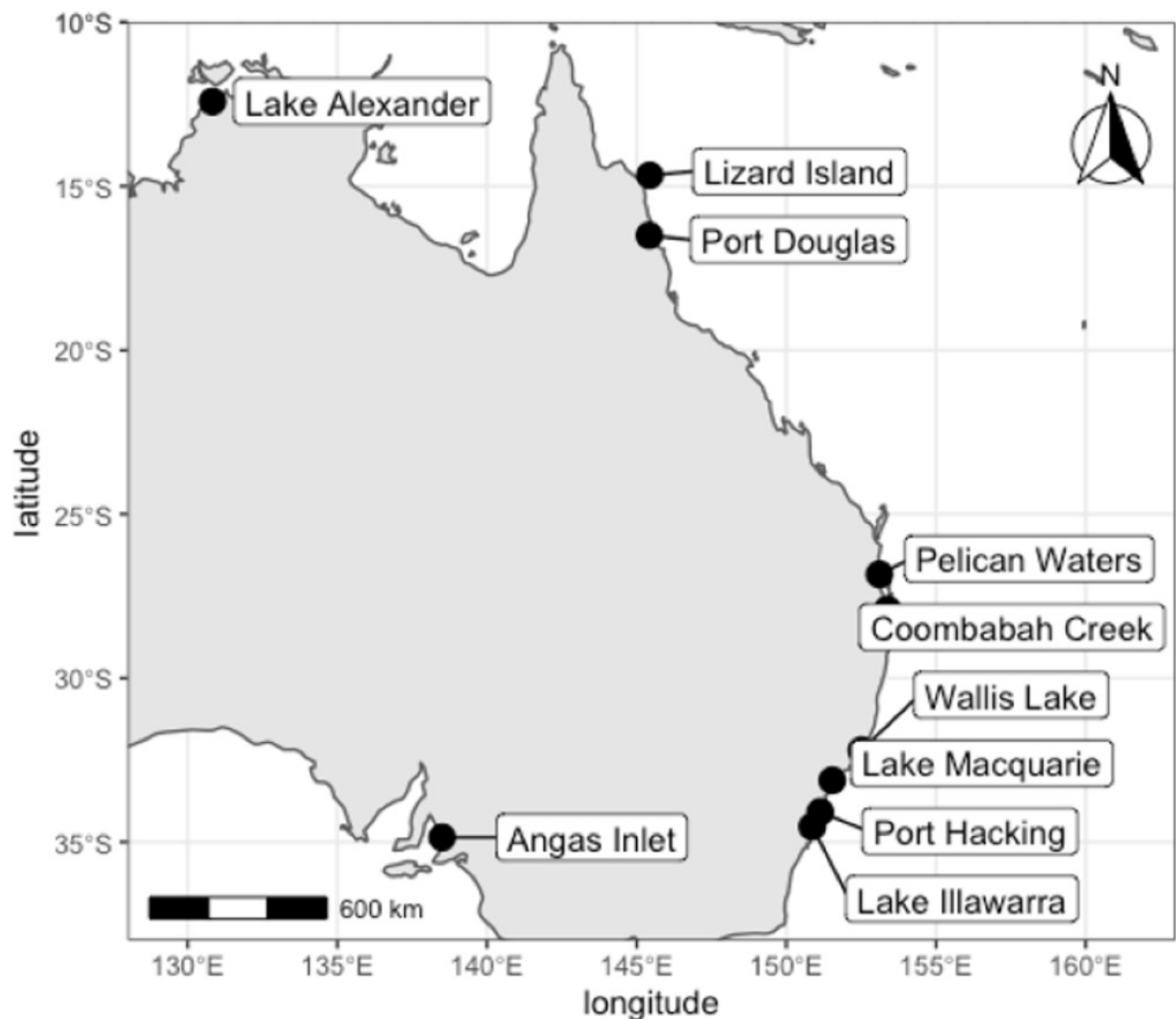


Figure 2

Schematic diagram of *Cassiopea* indicating key morphological features examined.

Created with BioRender.com.

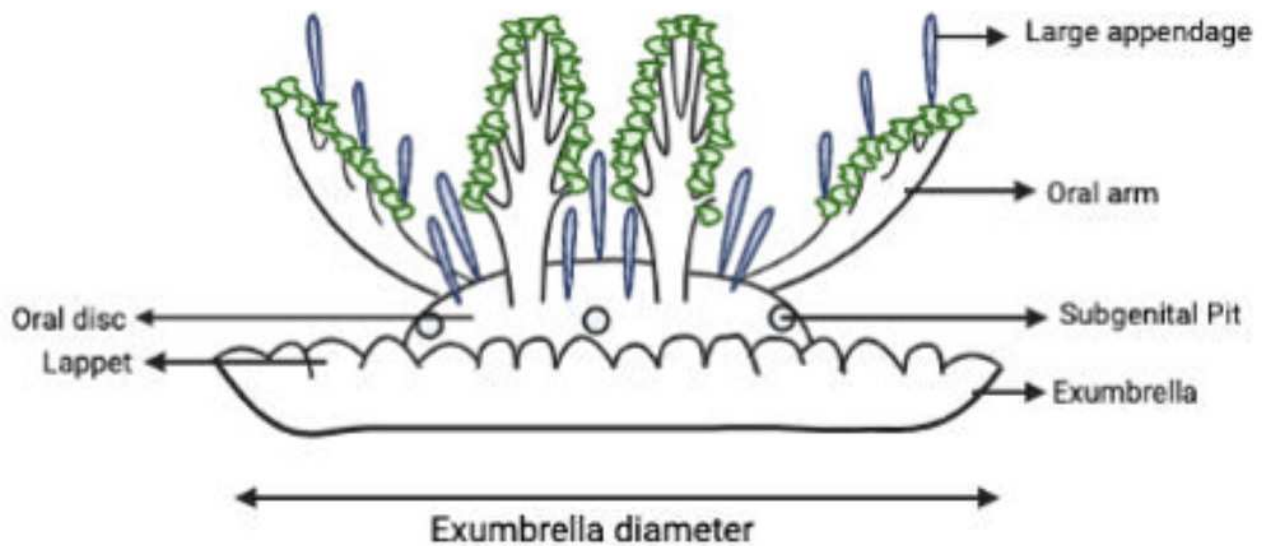


Figure 3

Maximum likelihood tree of Australian *Cassiopea* based on COI gene .

Bootstrap values (1000 pseudoreplicates) displayed at each node and scale bar indicates length of each branch. Blue indicates sequences from Lake Macquarie specimens. * material from the type locality of *Cassiopea maremetans* Gershwin, Zeidler & Davie, 2010.

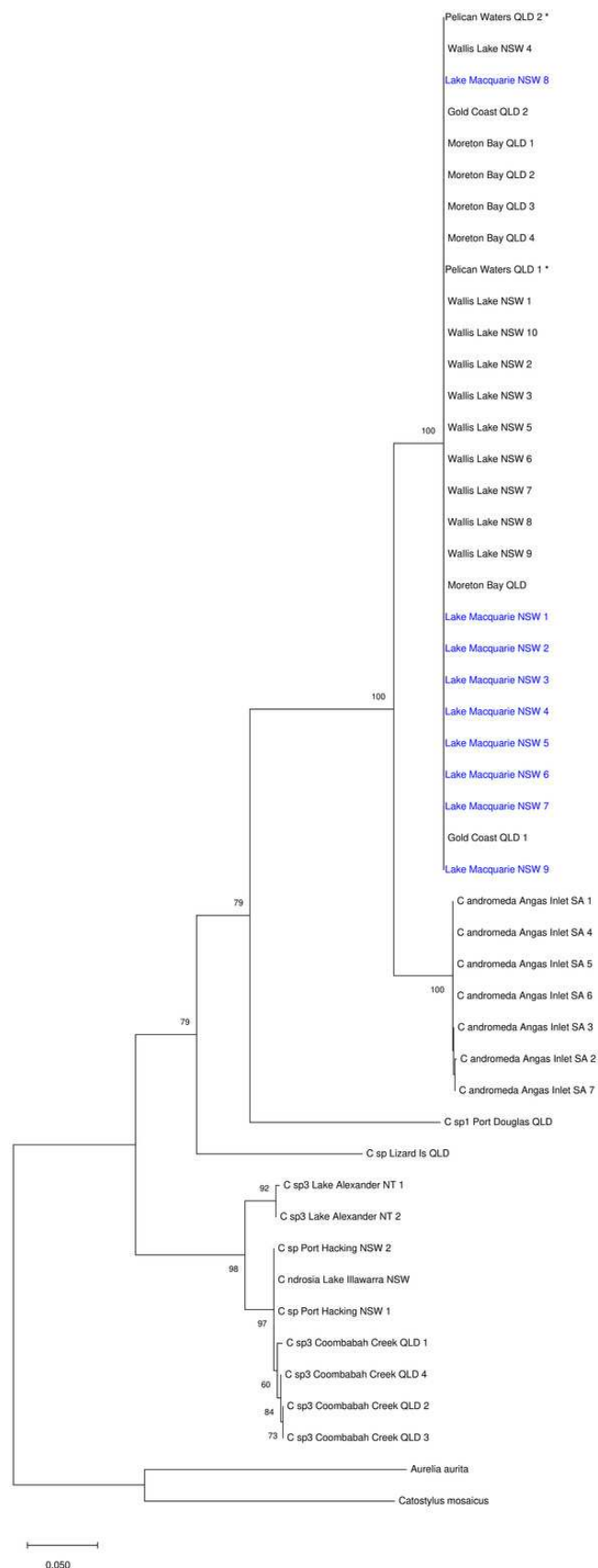


Figure 4

Global phylogeny of *Cassiopea* based on COI gene.

Bootstrap values (maximum likelihood, 1000 reiterations) displayed at each node and scale bar indicated branch length. Blue indicates Lake Macquarie specimen sequences. *material from the type locality of *Cassiopea maremetans* Gershwin, Zeidler & Davie, 2010.

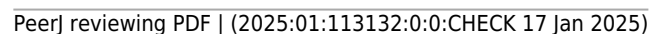


Figure 5

NMDS plot indicating the clustering between the morphological characters across specimens.

Material examined includes putative *Cassiopea* sp.3 from Coombabah Creek, Lake Illawarra, Port Hacking and Lake Alexander, *Cassiopea* sp.2 from PNG and *Cassiopea xamachana* Bigelow, 1892 from Lake Macquarie, Wallis Lake and Pelican Waters. Stress level of NMDS is 0.176.

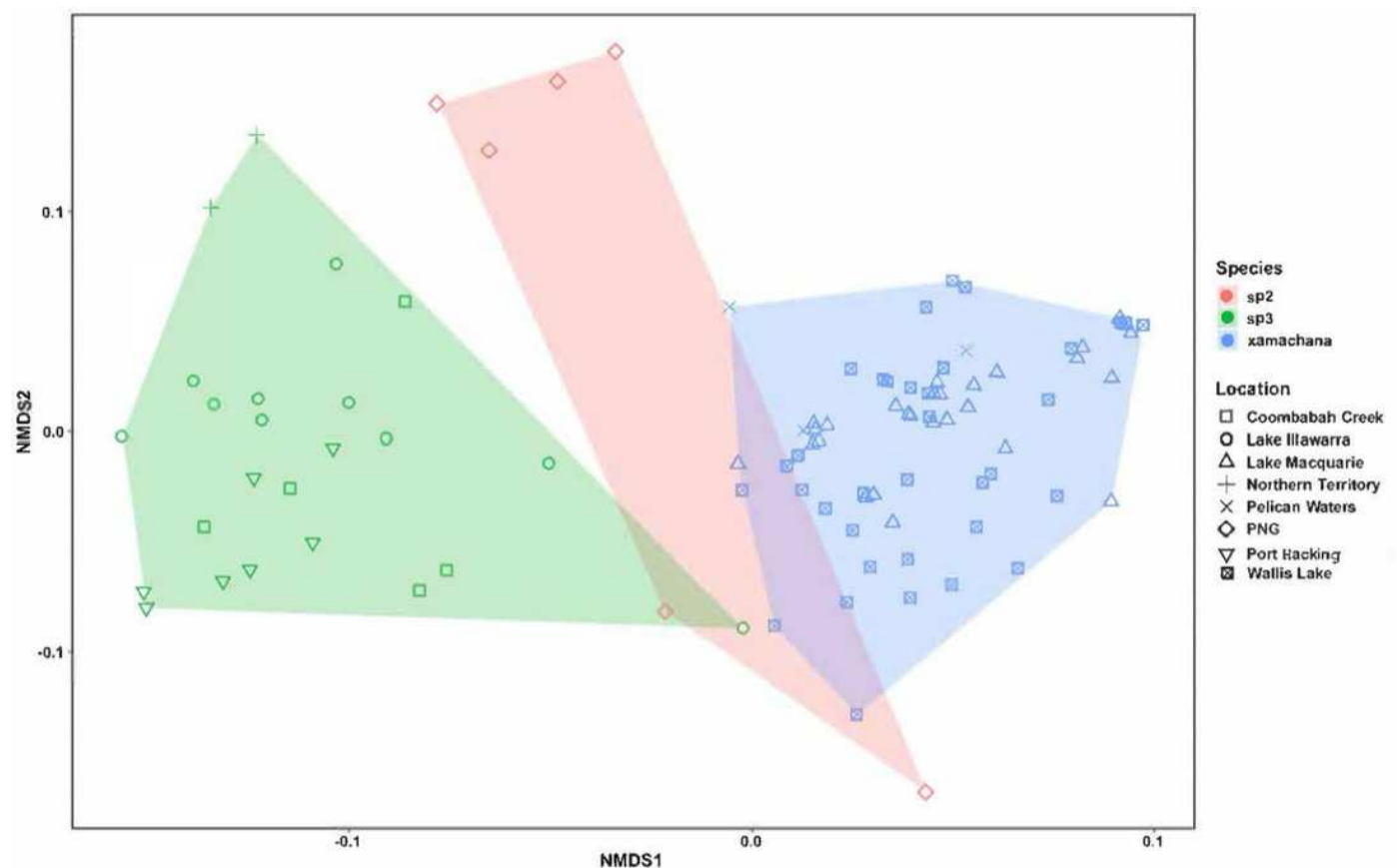


Figure 6

Cluster dendrogram of all specimens of *Cassiopea* examined.

Red lines indicate morphologically homogeneous clusters detected by Ward Hierarchical Clustering and the k-means of the specimens. Labels indicate species and their registration number. All specimens from the AM, except for the Holotype (QM G.326486).

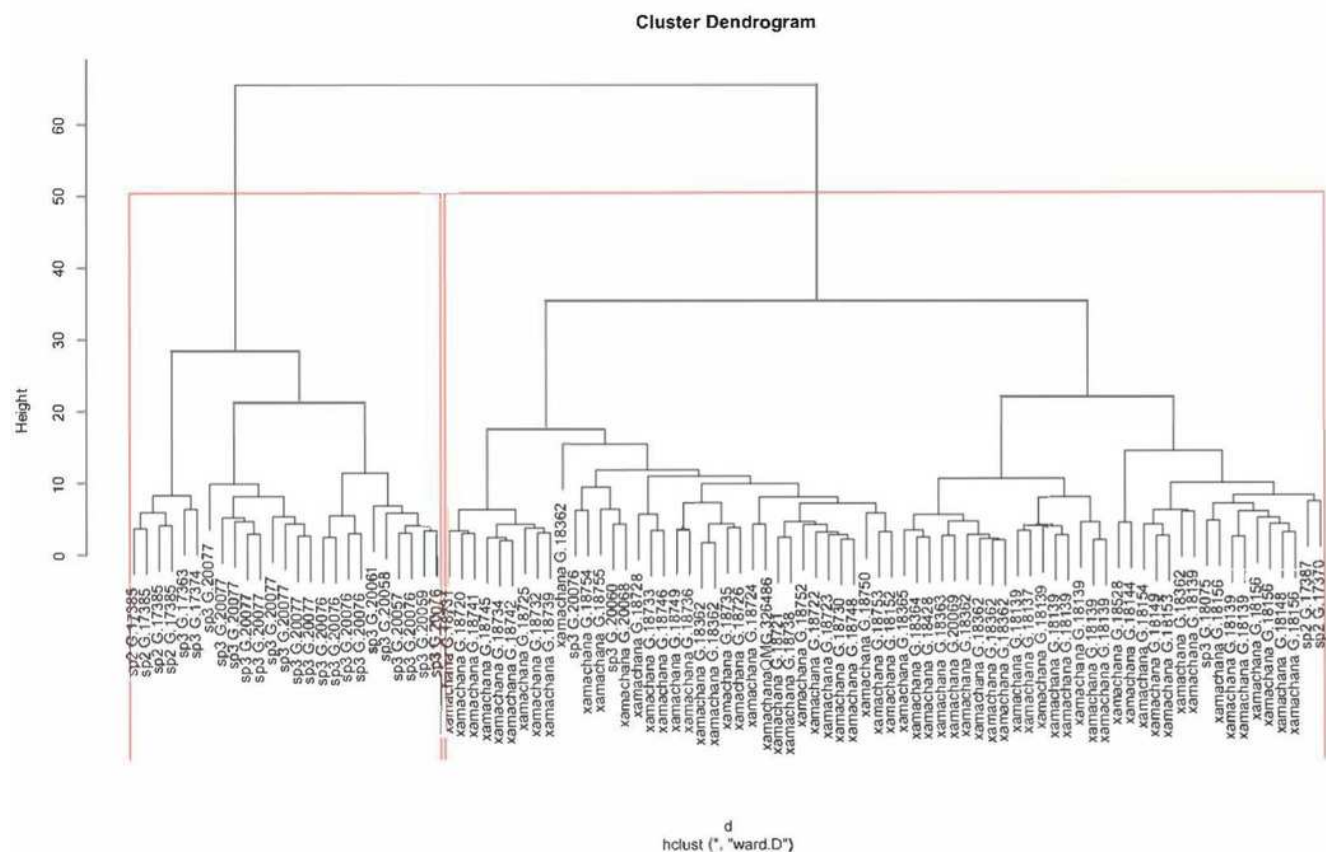


Figure 7

Cluster dendograms of the putative populations of *Cassiopea*.

A) Cluster  *Cassiopea xamachana* Bigelow, 1892. B) *Cassiopea* sp.3. Red lines indicate morphologically homogeneous clusters detected by Ward Hierarchical Clustering and the k-means of the specimens. Labels indicate the population and their AM registration number.

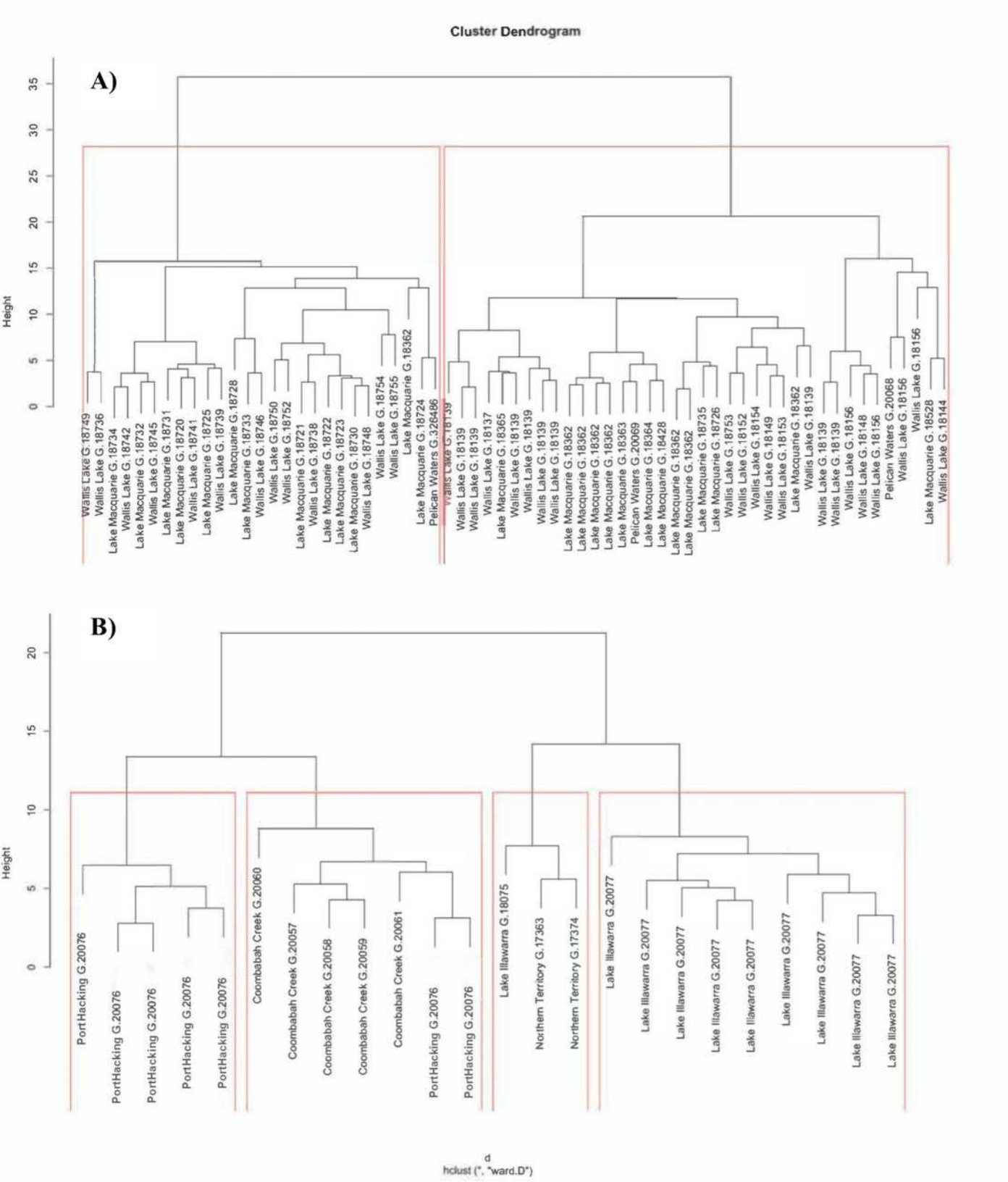


Figure 8

Number and relative length of large appendages on sampled specimens of *Cassiopea*.

A) Number of large appendages on oral disc between. B) Length (cm) of large appendages on oral arm divided by bell diameter (cm).

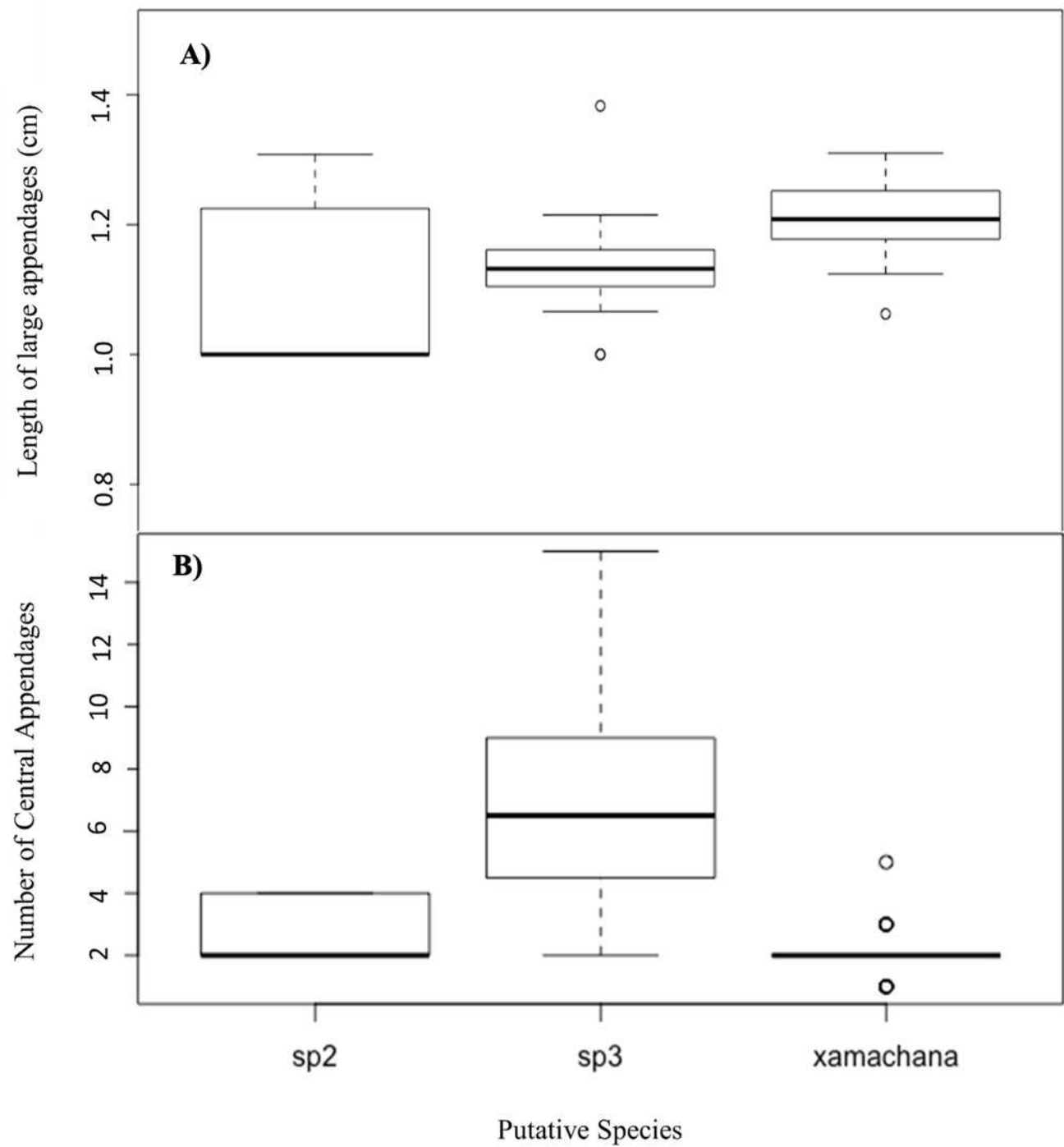


Figure 9

Branching pattern of lateral branches along oral arm among sampled specimens of *Cassiopea*.

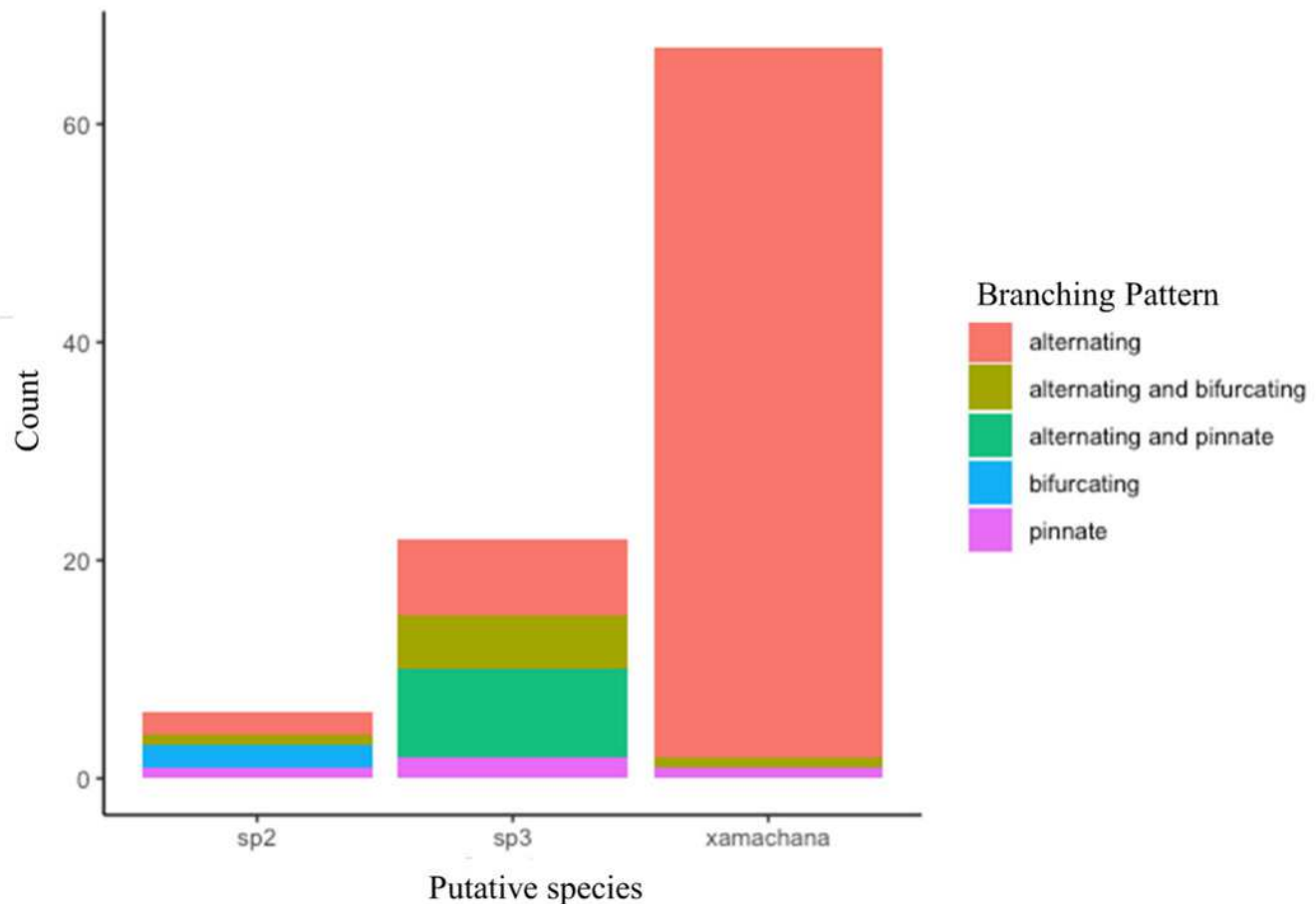


Figure 10

Cassiopea xamachana Bigelow, 1892 (holotype of *Cassiopea maremetens* Gershwin, Zeidler & Davie, 2010), QM-G326486, 17.5 cm diameter).

A) aboral view of preserved medusa. B) aboral view of oral disc, red arrow points to large oral appendage located in the centre of the disc. C) oral view of branching pattern on oral arm, red arrow point to large oral appendage. D) aboral view of the detail of the number and shape of lappets in a paramere. E) arrow pointing to rhopalium, aboral view.

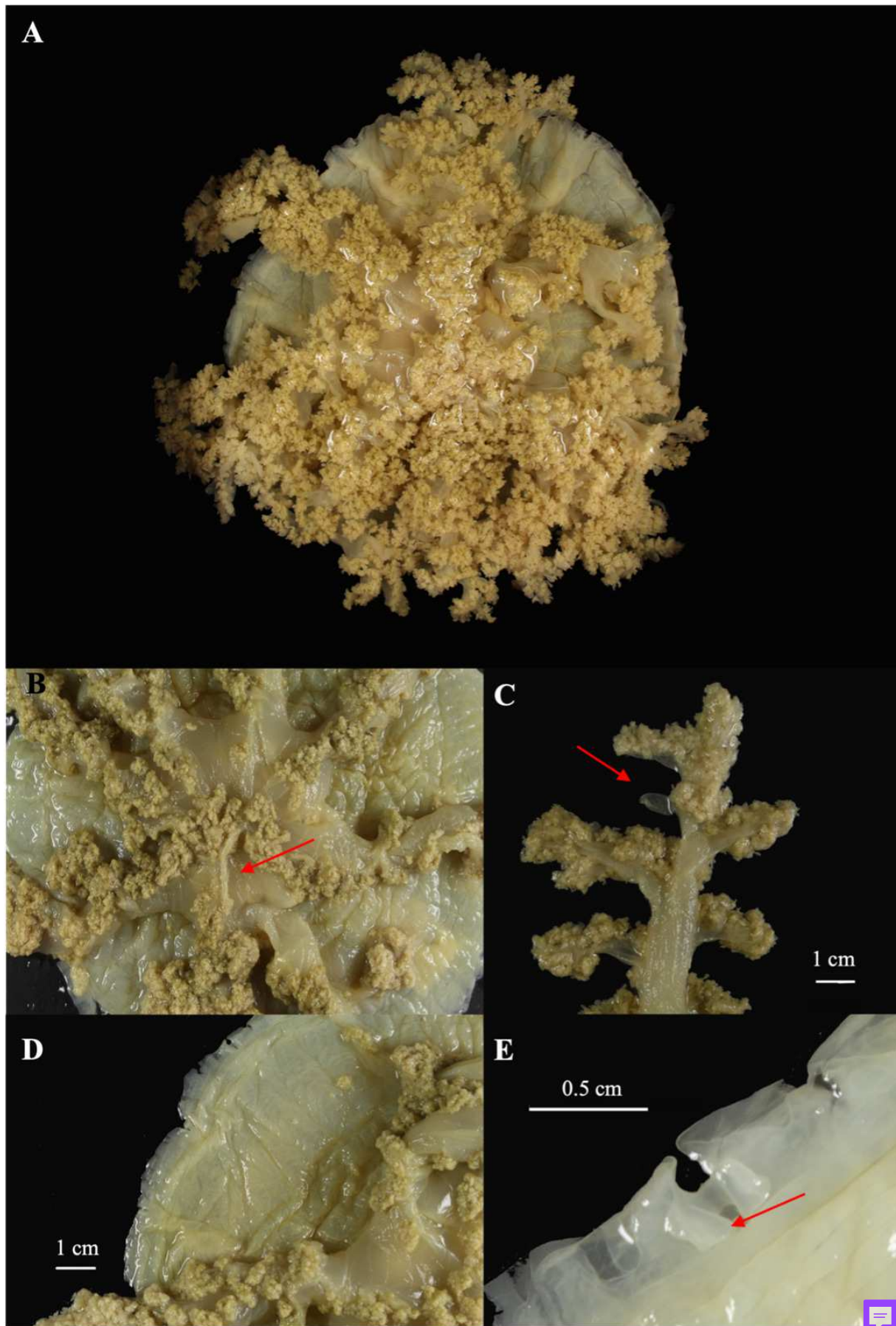


Figure 11

Cassiopea xamachana Bigelow, 1892, Lake Macquarie, New South Wales, AM-G18732, 19.7 cm diameter, female.

A) aboral view of preserved medusa. B) red arrow points to large oral appendage located in the centre of the oral disc, aboral view. C) branching pattern on oral arm, red arrow points to large oral appendage, oral view. D) number and shape of marginal lappets. E) branching pattern of the oral arm and subgenital pit, oral view.

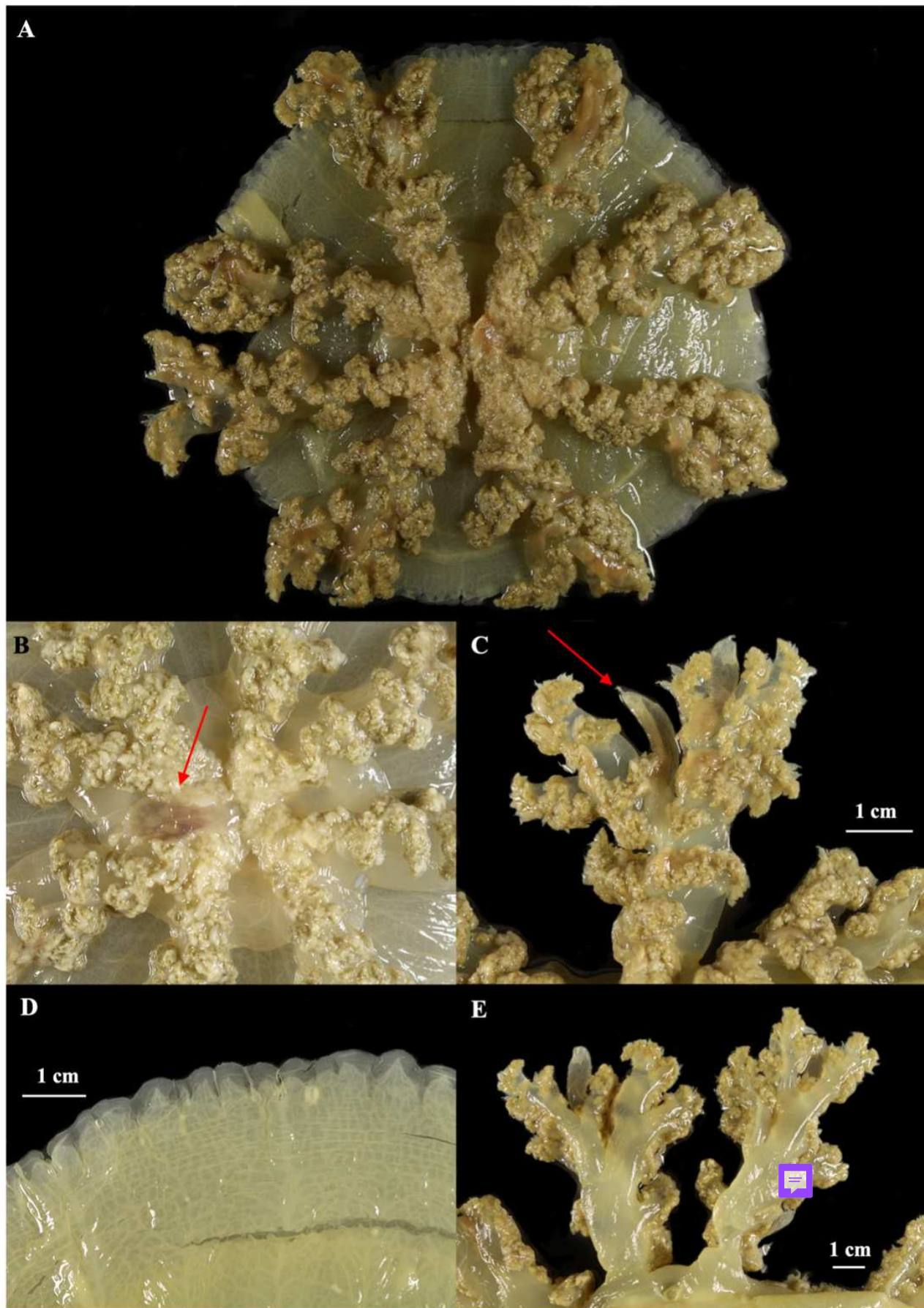


Table 1(on next page)

SIMPER results showing top three cumulative contributions of morphological characters that distinguish specimens.

Average is the contribution to dissimilarity, Standard deviation of dissimilarity, and Cumulative Sum ordered cumulative contribution.

Comparison of putative species	Morphological Feature	Average	Standard Deviation	Cumulative Sum	Figure
<i>C. xamachana</i> – <i>C. sp.3</i>	Number of large appendages on oral disc	0.0070329	0.0054158	0.06399	8A
	Oral arm branching pattern	0.0065736	0.0053396	0.12380	9
	Length of large appendages on oral arm	0.0058753	0.0036507	0.17726	8B
<i>C. xamachana</i> – <i>C. sp.2</i>	Ring canal presence	0.0109182	0.012637	0.08762	
	Distribution of large appendages on oral arm	0.0097679	0.006158	0.16601	
	Oral arm branching pattern	0.0091168	0.006856	0.23918	9
<i>C. sp.3</i> – <i>C. sp.2</i>	Ring canal presence	0.011149	0.012803	0.09511	
	Distribution of large appendages on oral arm	0.008733	0.005651	0.16960	
	Oral arm branching pattern	0.007707	0.005118	0.23534	9