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)

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

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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*.

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Keywords: invasive, jellyfish, taxonomy

Abstract

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*.

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. maremetens,* 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 $MgCl_2$ (1.5 µl), dNTPs (1.5 µl), Invitrogen Taq DNA Polymerase (0.1 µl), Milli-Q H_2O (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 millimetre 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:

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

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

Australian results

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

Global results

The global ML tree (Figure 4) recovered the Lake Macquarie, Wallis Lake and Pelican Waters specimens in the same clade and genetically invariant, with low divergence from populations in Hawaii, Brazil, Panama and the Florida Keys (mean within species K2P ± 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

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

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

Cassiopea xamachana Bigelow, 1892: 212–221.—Bigelow, 1893: 301.—Bigelow, 1900: 191, figs A–L, pl. 31–38. —Mayer, 1910: 499–735, pl. 56–76.— Kramp, 1961.—Hummelinck, 1968: 1–57. —Holland et al., 2004: 1119.—Morandini et al., 2017: 321. —Ohdera et al., 2018: 1.— Gamero-Mora et al., 2019: 1.—Jarms & Morandini, 2019: 504.

Cassiopea maremetens Gershwin, Zeidler & Davie, 2010: 91, fig. 6C–F.—Templeman & Kingsford, 2015:1–8. —Epstein et al. 2016: 340–346.—~~James~~ & Morandini, 2019: 492. —Gamero-Mora, et al., 2020: 113, fig 43. .—Rowen et al., 2017:143–148.—McKenzie et al., 2020:1–7.—Templeman et al., 2021:1. [New synonymy]

Cassiopea cf. *maremetens*. — Keable & Ahyong, 2016: 26, figs 2E–H, 3C–D, 4D–F.

Cassiopea sp. —Rowe et al., 2022a: 2.—Rowe et al., 2022b: 1.

Cassiopea medusa. — Durieux et al., 2023: 9 [not *C. medusa* Light, 1914, erroneous citation of Rowe et al. (2022a) who indicate *Cassiopea* medusae, i.e. medusae of *Cassiopea* sp., subsequently identified here as *C. xamachana*].

?*Cassiopea andromeda*. — Stephensen, 1962: 94 [doubtfully *C. andromeda* Forsskål, 1775].

Type material of *C. maremetens* examined. Holotype: QM G.326486, female (17.5 cm diameter), off Lamerough Canal, Lake Magellan, Pelican Waters, Queensland, Australia, 26°49’47”S, 153°6’36”E, D. Potter and G. Cranitch, 24 May 2007. Paratypes: QM G.6645, 8 specimens (9.8, 10, 10.9, 10.3, 11, 11.5, 11.5, 11.5 cm), Mud Island, Moreton Bay, Queensland, Australia, 27°19’47”S, 153°15’0”E, C. Wallace, 4 August 1972. QM G.327932, 2 females (8.8, 17.2 cm), off Lamerough Canal, Lake Magellan, Pelican Waters, Queensland, Australia, 26°49’47”S, 153°6’36”E, D. Potter and G. Cranitch, 24 May 2007. QM G.327969, 2 females (11.9, 14.5 cm), estuary on NW side of Bentick Island, Queensland, Australia, 17°3’35”S, 139°29’24”E, P. Davie, 20 November 2002.

Additional material examined (all from Australia). <u>Pelican Waters, Queensland:</u> AM G.20068-20069, 2 females (15.5, 16.1 cm), 26°50’01”S, 153°06’44”E, C.E. Rowe, 10 March 2021. <u>Gold Coast, Queensland:</u> QM G.339123–339125, 3 specimens (5.9, 7.7, 12.1 cm), 28°3’S, 153°24’26”E, M. Ekins and I. Jamieson, 8 August 2019. QM G.339126–339130, 5 females (6.7, 7.2, 8, 10.3, 17.3 cm), 28°10’S, 153°24’37”E, M. Ekins and I. Jamieson, 8 August 2019. <u>SeaWorld Culture, Queensland:</u> AM G.18699−18701, 3 specimens (6.1, 6.5, 10.4 cm), most probably originating from a small inlet on southern end of South Stradbroke Island, Gold Coast Council Region, 25°55’3”S, 153°25’15”E, 22 January 2019. <u>Wallis Lake, New South Wales</u>: 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), in channel splitting Godwin Island approximately one-third distance from southern shore, 32°11’45”S, 152°29’55”E, R. Pearce, 15 August 2014. AM G.18143−18156, 12 specimens (2.9, 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, Pipers Creek, “The Keys”, 32°12’0”S, 152°30’39”E, R. Pearce, 18 September 2014. AM G.18181−18183, 4 specimens, inlet on south-east side of Mather Island, 32°11’26”S, 152°29’36”E, S.J. Keable and A.D. Hegedus, 28 April 2015. AM G.18184, 1 specimen, Pipers Creek within Smugglers Cove Caravan Park, 32°11’58”S, 152°30’39”E, S.J. Keable and A. Murray, 30 April 2015. AM G.18736–18755, 20 females (8.9, 11, 12, 12.5, 12.8, 13.1, 13.3, 15, 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 Smugglers Cove Caravan Park, 32°11’58”S, 152°30’39”E, S.J. Keable and C.E. Rowe, 10 May 2019. Lake Macquarie, New South Wales: AM G.18362–18365, 12 specimens (7.1, 10, 12.6, 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. Keable, D.J. Keable, S. Jones, D. Jones, and E.M. Keable, 4 June 2017. AM G.18428, 1 specimen (13.4 cm), Lake Petite, 33°07’00”S, 151°31’58”E, S.J. Keable and A. Hay, 9 February 2018. AM G.18528, 1 specimen (6.1 cm), Karignan Creek, 33°10’36”S, 151°34’03”E, C.E. Rowe and S.J. Keable, 16 May 2018. AM G.18711–18712, 2 specimens (11.9, 12.4 cm), Mannering Bay, 33°09’28”S, 151°31’41”E, C.E. Rowe and S.J. Keable, 7 March 2019. AM G.18716–18723, 8 females (10.3, 14.3, 12, 15.5, 16.4, 17, 19.6, 20 cm), Karignan Creek, 33°10’32”S, 151°34’00”E, S.J. Keable and C.E. Rowe, 6 May 2019. AM G.18724–18728, 5 females (11.3, 12, 12.2, 12.9, 14.4 cm), creek north of Kilaben Creek, 33°01’43”S, 151°35’01”E, S.J. Keable and C.E. Rowe, 9 May 2019. AM G.18729–18735, 7 females (14.2, 16.1, 17.2, 19.2, 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 2019.

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

Cassiopea sp.2: Papua New Guinea: AM G.17370 and G.17385, 6 specimens (4.6, 7.3, 7.7 8.9, 9,10.5 cm), outer slope, west side of Mascot Channel mouth, 2°40'04"S, 150°25'58"E, P.L. Colin, 3 July 2003. AM G.17387, 1 specimen (5.2 cm), atoll ~70 nautical miles south-south-east of Manus Island, Sherburne Reef, 3°19’59”S, 148°01’03”E, D. de Mara, 20 June 2002. Queensland, Australia: AM G.18344, 1 specimen (6.3 cm), north east coast, Lizard Island, 14°40’01”S, 145°27’37”E, A. Hoggett, 21 February 2016.

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

Description. (Where variation occurs, value for holotype of *C. maremetans* given in brackets). Exumbrella: marginal outline circular; aboral surface mostly flat with small concavity; maximum diameter 24 cm, mean 11.5 cm (holotype: 17 cm); height 0.2–2.7 cm (0.4 cm), mean 0.9 cm. *Colour pattern* brown, yellow, green (greenish yellow). *Markings* include small white spots around bell margin, triangle facing towards each ocellus on live specimens (markings lost in preservation). *Rhopalia* 13–23 (19), mean 15, located in incised notches; ocelli present. *Marginal lappets* 32–120 (104), mean 83 depending on the condition of the specimen, with 3–7 per paramere (4–6), not always distinct, distal edge with rounded low lobes; 1–5 (3) velar lappets located between 2 ocular lappets. *Ring canals* 1 in total, raised.

Oral disc 0.2–2 cm height, with a mean of 0.94 cm, 0.5–8.2 cm width, and with a mean of 3.88 cm, typically 1.6-5.4 × the bell diameter, with a mean of 0.4 × bell diameter. *Subgenital* *pit* circular or rhomboid, 0.1–0.9 cm width, with 0.65 cm mean, 0.09–0.58 × oral disc height, with 0.3 mean. *Large appendages* present on specimens > 8 cm; 1 or 2 in centre of oral disc (1), fusiform, 0.7–3.4 cm in length (2.5 cm), averaging 1.3 cm, 0.17–0.67 × oral disc width, but typically 0.25 × oral disc width, width of large appendage ranges from 0.06 to 0.4 × length of large appendage (0.4), typically 0.27 length of large appendage.

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

<u>Gonads:</u> 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. maremet~~a~~ns* and *C. xamachana* are indistinguishable and we recognise the former as a junior synonym of the latter.

<u>Variation in Australian *C. xamachana*:</u>

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 × 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/biogeo.19.133

Bigelow RP. 1892. On a new species of *Cassiopea* from Jamaica. *Zoologischer Anzeiger* 15:212–214.

Bigelow RP. 1893. *Some observations on* Polyclonia frondosa. Baltimore, MD: Johns Hopkins University Circulars, 106.

Bigelow RP. 1900. The anatomy and development of *Cassiopea xamachana*. *Boston Society of Natural History*:191–236.

Bolton TF, Graham WM. 2006. Jellyfish on the rocks: bioinvasion threat of the international trade in aquarium live rock. *Biological Invasions* 8:651–653.

Brandt JF. 1838. Ausführliche Beschreibung der von C. H. Mertens auf seiner Weltumsegelung beobachteten Schwimmquallen. *Mémoires de l'Académie impériale des sciences de St-Pétersbourg* 6:237–412.

Brotz L, Pauly D. 2016. Studying jellyfish fisheries: toward accurate national catch reports and appropriate methods for stock assessments. In: Mariottini GL, ed. *Jellyfish: ecology, distribution patterns and human interactions Nova Publishers, New York*, 313–329.

Chiaverano LM, Bayha KW, Graham WM. 2016. Local versus generalized phenotypes in two sympatric *Aurelia* species: understanding jellyfish ecology using genetics and morphometrics. *PloS one* 11:e0156588. DOI: 10.1371/journal.pone.0156588.

Collins AG, Jarms G, Morandini AC. 2022. World List of Scyphozoa. *Cassiopea* Péron & 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, Scrogham 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 Buletin* 107:340–346. DOI: 10.1016/j.marpolbul.2016.03.027.

Fitt WK, Hofmann DK, Kemp DW, Ohdera AH. 2021. Different Physiology in the Jellyfish *Cassiopea xamachana* and *C. frondosa* in Florida Bay. *Oceans* 2:811–821. DOI: 10.3390/oceans2040046.

Forskål P. 1775. Descriptiones animalium, avium, amphibiorum, piscium, insectorum, vermium; quae in itinere orientali observavit Petrus Forskål. *Hauniae*:1–64.

Gamero-Mora E, Collins A, Boco S, Geson III S, Morandini A. 2022. Revealing hidden diversity among upside-down jellyfishes (Cnidaria: Scyphozoa: Rhizostomeae: *Cassiopea*): distinct evidence allows the change of status of a neglected variety and the description of a new species. *Invertebrate Systematics* 36:63–89. DOI: 10.1071/IS21002.

Gamero-Mora E, Halbauer R, Bartsch V, Stampar SN, Morandini AC. 2019. Regenerative capacity of the upside-down jellyfish *Cassiopea xamachana*. *Zoological Studies* 58:e37. DOI: 10.6620/ZS.2019.58-37.

Gamero-Mora E, Maronna MM, Lawley JW, Collins AG, Dawson MN, Morandini AC. 2020. Hidden diversity and insights into the origin and biogeogrpahy of the upside-down jellyfishes (Cnidaria: Scyphozoa). *PhD Thesis*.

Gershwin L, Zeidler W, Davie PJ. 2010. Medusae (Cnidaria) of Moreton Bay, Queensland, Australia. *Memoirs of the Queensland Museum* 54:47–108.

Gohar H, Eisawy A. 1960. The biology of *Cassiopea andromeda* (from the Red Sea)(with a note on the species problem). *Publications of the Marine Biological Statation, Al- Ghardaqa* 11:3–39.

Graham WM, Bayha KM. 2008. Biological invasions by marine jellyfish. *Biological Invasions*: Springer, 239–255.

Gueroun SK, Moura CJ, Almansa E, Escánez A. 2024. *Cassiopea andromeda* (Cnidaria, Scyphozoa) in the subtropical eastern Atlantic. *Journal of the Marine Biological Association of the United Kingdom* 104:e92. DOI: 10.1017/S0025315424000882.

Haeckel E. 1880. Das System der Acraspeden. 2te Hälfte des Systems der Medusen. Acht Nachträge zur Vervollständigung des Systems. *enkschriften der Medicinisch-Naturwissenschaftlichen Gesellschaft zu Jena* 2:361–672.

Holland BS, Dawson MN, Crow GL, Hofmann DK. 2004. Global phylogeography of Cassiopea (Scyphozoa: Rhizostomeae): molecular evidence for cryptic species and multiple invasions of the Hawaiian Islands. *Marine Biology* 145:1119–1128.

Hopf J, Kingsford MJ. 2013. The utility of statoliths and bell size to elucidate age and condition of a scyphomedusa (*Cassiopea* sp.). *Marine Biology* 160:951–960. DOI: 10.1007/s00227-012-2146-8.

Hummelinck WP. 1968. Caribbean scyphomedusae of the genus *Cassiopea*. *Studies on the Fauna of Curaçao and Other Caribbean Islands* 25:1–57.

Jarms G, Morandini AC, Schmidt-Rhaesa A, Giere O, Straehler-Pohl I. 2019. *World atlas of jellyfish: Scyphozoa except Stauromedusae.* Dölling und Galitz Verlag: Hamburg.

Keable SJ, Ahyong ST. 2016. First records of the invasive “upsidedown jellyfish”, *Cassiopea* (Cnidaria: Scyphozoa: Rhizostomeae: Cassiopeidae), from coastal lakes of New South Wales, Australia. *Records of the Australian Museum* 68:23–30. DOI: 10.3853/j.2201-4349.68.2016.1656.

Knowlton N. 2000. Molecular genetic analyses of species boundaries in the sea. *Hydrobiol*ogia 420: 73–90.

Kramp P. 1961. *Synopsis of the medusae of the world. Journal of the Marine Biological Association of the United Kingdom.* Cambridge University Press Cambridge, UK.

Kramp P. 1965. Some medusae (mainly scyphomedusae) from Australian coastal waters. *Transactions of the Royal Society of South Ausrtralia* 89:257–278.

Larson R. 1997. Feeding behaviour of Caribbean scyphomedusae: *Cassiopea frondosa* (Pallas) and *Cassiopea xamachana* Bigelow. *Studies of the Natural History of the Caribbean Region* 73:43–54.

Lawley JW, Gamero-Mora E, Maronna MM, Chiaverano LM, Stampar SN, Hopcroft RR, Collins AG, Morandini AC. 2021. The importance of molecular characters when morphological variability hinders diagnosability: systematics of the moon jellyfish genus *Aurelia* (Cnidaria: Scyphozoa). *PeerJ* 9:e11954. DOI: 10.7717/peerj.11954.

Lessios HA. 2008. The great American schism: divergence of marine organisms after the rise of the Central American Isthmus. *The Annual Review of Ecology, Evolution, and Systematics* 39:63–91. DOI: 10.1146/annurev.ecolsys.38.091206.095815.

Light S. 1914. Some Philippine Scyphomedusae, including two new genera, five new species and one new variety. *Philippine Joural of Science* 9:195–231.

Linnaeus C. 1758. Systema Naturae per regna tria naturae, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis. *Editio decima, reformata [10th revised edition]* 1:824.

Lobo J, Costa PM, Teixeira MA, Ferreira MS, Costa MH, Costa FO. 2013. Enhanced primers for amplification of DNA barcodes from a broad range of marine metazoans. *BMC Ecol* 13:1–8.

Maggio T, Allegra A, Bosch-Belmar M, Cillari T, Cuttitta A, Falautano M, Milisenda G, Nicosia A, Perzia P, Sinopoli M. 2019. Molecular identity of the non-indigenous *Cassiopea* sp. from Palermo Harbour (central Mediterranean Sea). ~~*Joural*~~ *of the Marine Biological Association of the United Kingdom* 99:1765–1773.

Mayer AG. 1910. *Medusae of the world, vol. 3.* The Scyphomedusae (Washington, DC: Carnegie Institute).

McKenzie MR, Templeman MA, Kingsford MJ. 2020. Detecting effects of herbicide runoff: the use of *Cassiopea maremetens* as a biomonitor to hexazinone. *Aquatic Toxicol*ogy 221:105442. DOI: 10.1016/j.aquatox.2020.105442.

Morandini AC, Schiariti A, Stampar SN, Maronna MM, Straehler-Pohl I, Marques AC. 2016. Succession of generations is still the general paradigm for scyphozoan life cycles. *Bulletin of Marine Science* 92:343–351. DOI: 10.5343/bms.2016.1018.

Morandini AC, Stampar SN, Maronna MM, Da Silveira FL. 2017. All non-indigenous species were introduced recently? The case study of *Cassiopea* (Cnidaria: Scyphozoa) in Brazilian waters. *Journal of the Marine Biological Association of the United Kingdom* 97:321–328. DOI: doi:10.1017/S0025315416000400.

Muffett K, Miglietta MP. 2023. Demystifying *Cassiopea* species identity in the Florida Keys: *Cassiopea xamachana* and *Cassiopea andromeda* coexist in shallow waters. *PloS one* 18:e0283441. DOI: 10.1371/journal.pone.0283441.

Ohdera AH, Abrams MJ, Ames CL, Baker DM, Suescún-Bolívar LP, Collins AG, Freeman CJ, Gamero-Mora E, Goulet TL, Hofmann DK, Jaimes-Becerra A, Long PF, Marques AC, Miller LA, Mydlarz LD, Morandini AC, Newkirk CR, Putri SP, Samson JE, Stampar SN, Steinworth B, Templeman MA, Thomé PE, Vlok M, Woodley CM, Wong JC, Martindale MQ, Fitt WK, and Medina M. 2018. Upside-down but headed in the right direction:

review of the highly versatile *Cassiopea xamachana* system. *Frontiers in Ecology and Evolution* 6:35. DOI: 10.3389/fevo.2018.00035.

Pallas P. 1774. *Spicilegia Zoologica: Quibus Novae Imprimis Et Obscurae Animalium Species Iconibus, Descriptionibus Atque Commentariis Illustrantur.* Fasciculus Berolini.

Richardson AJ, Bakun A, Hays GC, Gibbons MJ. 2009. The jellyfish joyride: causes, consequences and management responses to a more gelatinous future. *Trends in Ecology and Evolution* 24:312–322. DOI: 10.1016/j.tree.2009.01.010.

Rowe CE, Figueira W, Keable SJ, Ahyong ST. 2022a. Physiological responses of the upside-down jellyfish, *Cassiopea* (Cnidaria: Scyphozoa: Cassiopeidae) to temperature and implications for *Cassiopea's* range expansion along the east coast of Australia. *Journal of Experimental Marine Biology and Ecology* 554:151765. DOI: 10.1016/j.jembe.2022.151765.

Rowe CE, Figueira WF, Kelaher BP, Giles A, Mamo LT, Ahyong ST, Keable SJ. 2022b. Evaluating the effectiveness of drones for quantifying invasive upside-down jellyfish (*Cassiopea* sp.) in Lake Macquarie, Australia. *PloS one* 17:e0262721. DOI: 10.1371/journal.pone.0262721.

Rowen DJ, Templeman MA, Kingsford MJ. 2017. Herbicide effects on the growth and photosynthetic efficiency of *Cassiopea maremetens*. *Chemosphere* 182:143−148. DOI: 10.1016/j.chemosphere.2017.05.001.

Schiariti A, Morandini AC, Jarms G, von Glehn Paes R, Franke S, Mianzan H. 2014. Asexual reproduction strategies and blooming potential in Scyphozoa. *Marine Ecology Progress Series* 510:241–253. DOI: 10.3354/meps10798.

Southcott R. 1982. Jellyfishes (Classes Scyphozoa and Hydrozoa). *Marine Invertebrates of South Australia Part I*:115–159.

Stampar SN, Gamero-Mora E, Maronna MM, Fritscher JM, Oliveira BS, Sampaio CL, Morandini AC. 2020. The puzzling occurrence of the upside-down jellyfish *Cassiopea* (Cnidaria: Scyphozoa) along the Brazilian coast: a result of several invasion events? *Zoologia (Curitiba)* 37:e50834. DOI: 10.3897/zoologia.37.e50834.

Stecher G, Tamura K, Kumar S.2020. Molecular Evolutionary Genetics Analysis (MEGA) for macOS. *Molecular Biology and Evolution* 37:1237–1239. DOI: 10.1093/molbev/msz312.

Stephenson W. 1962. A peculiar jellyfish from Moreton Bay. *The Queensland Naturalist* 16:94.

Stiasny G. 1924. Über einige von Dr. C. J. van der Horst bei Curaçao gesammelte Medusen. Bijdragen tot de Kenntnis der Fauna van Curaçao. Resultaten eener Reis van C. J. van der Horst in 1920. *Bijdragen to de Dierkunde* 23:83–92.

Stiasny G. 1931. Die rhizostomeen-sammlung des British Museum (natural history) in London. *Zoologische Mededelingen* 14:137–178.

Stoner EW, Yeager LA, Sweatman JL, Sebilian SS, Layman CA. 2014. Modification of a seagrass community by benthic jellyfish blooms and nutrient enrichment. *Journal of*

Experimental Marine Biology and Ecology 461:185–192. DOI: 10.1016/j.jembe.2014.08.005.

Team RC. 2021. R: A language and environment for statistical computing. V2021.09.0*1ed: R Foundation for Statistical Computing, Vienna, Austria.*

Templeman MA, Kingsford MJ. 2015. Predicting aqueous copper and zinc accumulation in the upside-down jellyfish *Cassiopea maremetens* through the use of biokinetic models. *Environmental monitoring and assessment* 187:1–8. DOI: 10.1007/s10661-015-4657-5.

Templeman MA, McKenzie MR, Kingsford MJ. 2021. The utility of jellyfish as marine biomonitors. *Marine Pollution Bulletin* 173:113056. DOI: 10.1016/j.marpolbul.2021.113056.

Thé J, Gamero-Mora E, da Silva MVC, Morandini AC, Rossi S, de Oliveira Soares M. 2020. Non-indigenous upside-down jellyfish *Cassiopea andromeda* in shrimp farms (Brazil). *Aquaculture* 532:735999.

Verde EA, McCloskey L. 1998. Production, respiration, and photophysiology of the mangrove jellyfish *Cassiopea xamachana* symbiotic with zooxanthellae: effect of jellyfish size and season. *Marine Ecology Progress Series* 168:147–162.

Zhang J, Kapli P, Pavlidis P, Stamatakis A. 2013. A general species delimitation method with applications to phylogenetic placements. *Bioinformatics* 29:2869–2876. DOI: 10.1093/bioinformatics/btt499.

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).

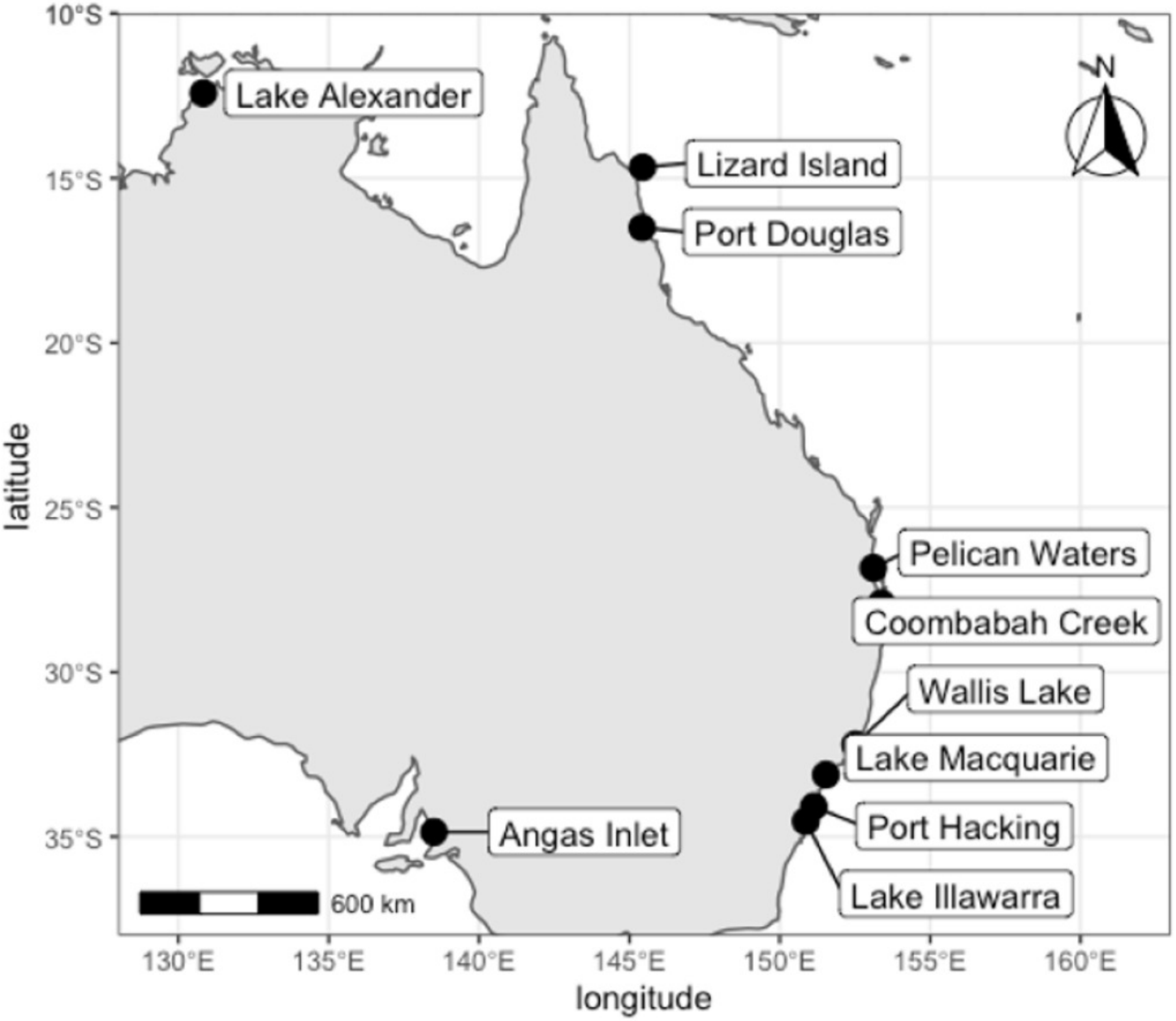

Figure 2

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

Created with BioRender.com.

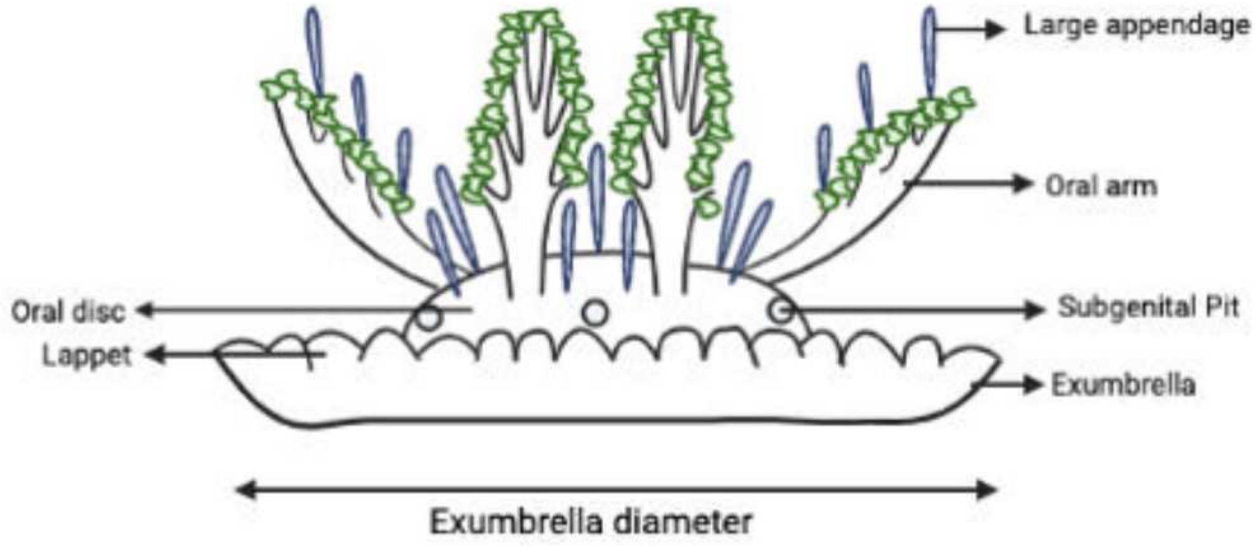

Figure 3

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.

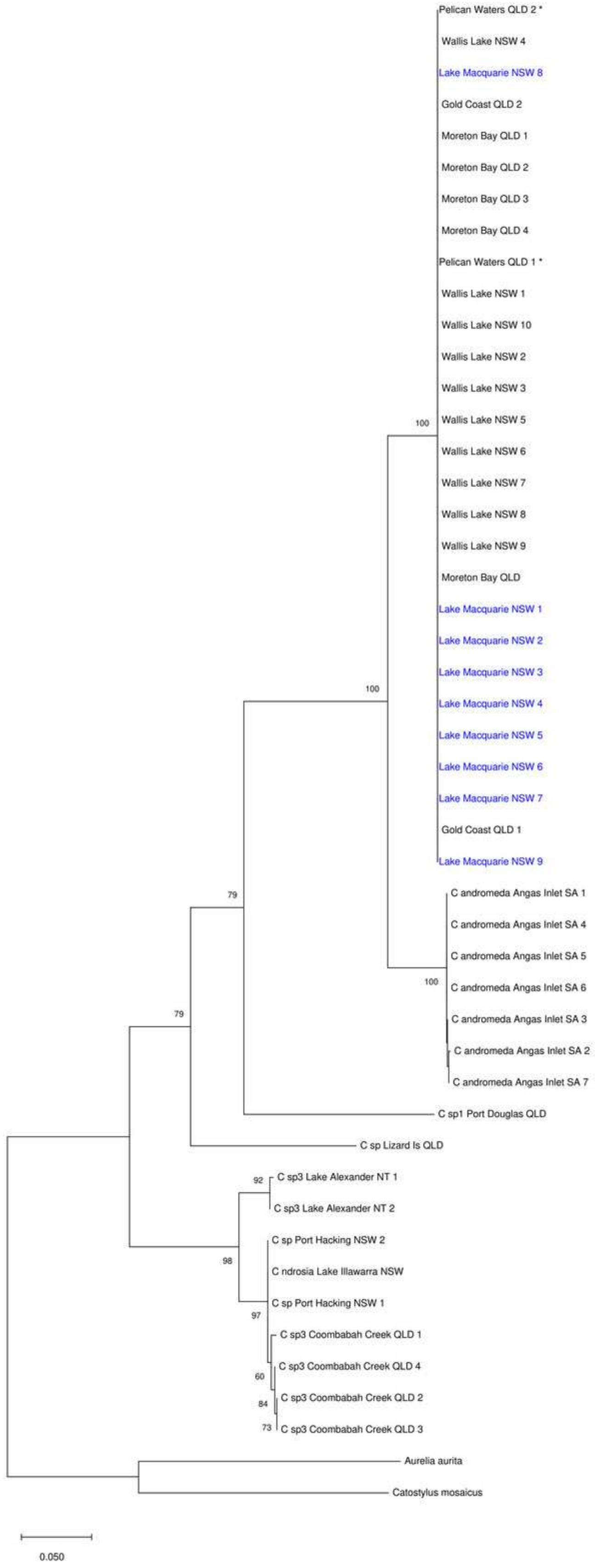
Pelican Waters QLD 2 *
Wallis Lake NSW 4
Lake Macquarie NSW 8
Gold Coast QLD 2
Moreton Bay QLD 1
Moreton Bay QLD 2
Moreton Bay QLD 3
Moreton Bay QLD 4
Pelican Waters QLD 1 *
Wallis Lake NSW 1
Wallis Lake NSW 10
Wallis Lake NSW 2
Wallis Lake NSW 3
100
Wallis Lake NSW 5
Wallis Lake NSW 6
Wallis Lake NSW 7
Wallis Lake NSW 8
Wallis Lake NSW 9
Moreton Bay QLD
Lake Macquarie NSW 1
Lake Macquarie NSW 2
Lake Macquarie NSW 3
100
Lake Macquarie NSW 4
Lake Macquarie NSW 5
Lake Macquarie NSW 6
Lake Macquarie NSW 7
Gold Coast QLD 1
Lake Macquarie NSW 9
79
C andromeda Angas Inlet SA 1
C andromeda Angas Inlet SA 4
C andromeda Angas Inlet SA 5
100
C andromeda Angas Inlet SA 6
79
C andromeda Angas Inlet SA 3
C andromeda Angas Inlet SA 2
C andromeda Angas Inlet SA 7
C sp1 Port Douglas QLD
C sp Lizard Is QLD
92
C sp3 Lake Alexander NT 1
C sp3 Lake Alexander NT 2
C sp Port Hacking NSW 2
98
C ndrosia Lake Illawarra NSW
C sp Port Hacking NSW 1
97
C sp3 Coombabah Creek QLD 1
C sp3 Coombabah Creek QLD 4
60
C sp3 Coombabah Creek QLD 2
84
73
C sp3 Coombabah Creek QLD 3
Aurelia aurita
Catostylus mosaicus
0.050

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.


C andromeda Molokai Hawaii 1
C andromeda Walsingham Pond Bermuda 2
C andromeda Molokai Hawaii 2
C andromeda Brazil 1
C andromeda Florida Keys
C andromeda Richardson Bay Bermuda 1
C andromeda Angas Inlet Australia
C andromeda Israel
C andromeda Walsingham Pond Bermuda 1
C andromeda Oahu Hawaii
C andromeda Egypt
C andromeda Richardson Bay Bermuda 2
C andromeda Mexico
C sp French Polynesia
C andromeda Brazil 2
C andromeda Brazil 3
C andromeda Brazil 4
C andromeda Brazil 5
C andromeda Western Indian Ocean
C frondosa Panama 3
C xamachana unknown location
C sp Florida Keys
C xamachana Northwest Pacific Ocean
C xamachana Brazil 2
C andromeda Palau
C xamachana Brazil 1
C xamachana Oahu Hawaii 2
C xamachana Oahu Hawaii 3
C xamachana Oahu Hawaii 1
Lake Macquarie Australia
Wallis Lake Australia
Pelican Waters Australia *
C sp Papua
C andromeda Japan
C sp6 Palau
C sp2 Observation Point PNG 1
C sp Lizard Island Australia
C sp2 Observation Point PNG 2
C ornata Palau 2
C ornata Palau 10
C ornata Palau 1
C ornata Fiji
C ornata Palau 9
C ornata Palau 11
C ornata Palau 6
C ornata Palau 5
C ornata Palau 4
C ornata Palau 3
C ornata Palau 7
C ornata Palau 8
C ornata Indonesia
C ornata Singapore
C sp1 Port Douglas Australia
C sp Solomon Islands
C ornata California
C sp Indonesia
C sp4 Palau 15
C sp4 Palau 1
C sp4 Palau 2
C sp4 Palau 3
C sp4 Palau 5
C sp4 Palau 10
C sp4 Palau 4
C sp4 Palau 11
C sp4 Palau 12
C sp4 Palau 6
C sp4 Palau 8
C sp4 Palau 9
C sp4 Palau 7
C sp4 Palau 13
C sp4 Palau 14
C frondosa Panama 1
C frondosa Panama 2
100
C frondosa Florida Keys
C xamachana Panama 1
C xamachana Panama 2
C xamachana Panama 3
C sp3 Coombabah Creek Australia 2
C sp3 Coombabah Creek Australia 3
C sp3 Coombabah Creek Australia 4
C sp3 Coombabah Creek Australia 1
C ndrosia Lake Illawarra Australia
C sp Port Hacking Australia
C sp3 Lake Alexander Australia 2
C sp3 Lake Alexander Australia 1
C sp3 Lake Alexander Australia 3
C sp3 Emona PNG 1
C sp 3 Emona PNG 2
C sp3 Oahu Hawaii 3
C sp3 Japan 3
C sp3 Japan 1
C sp3 Japan 2
C sp 3 Oahu Hawaii 1
C sp 3 Oahu Hawaii 2
C sp3 Oahu Hawaii 4
Catostylus mosaicus
Aurelia aurita
0.1

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.

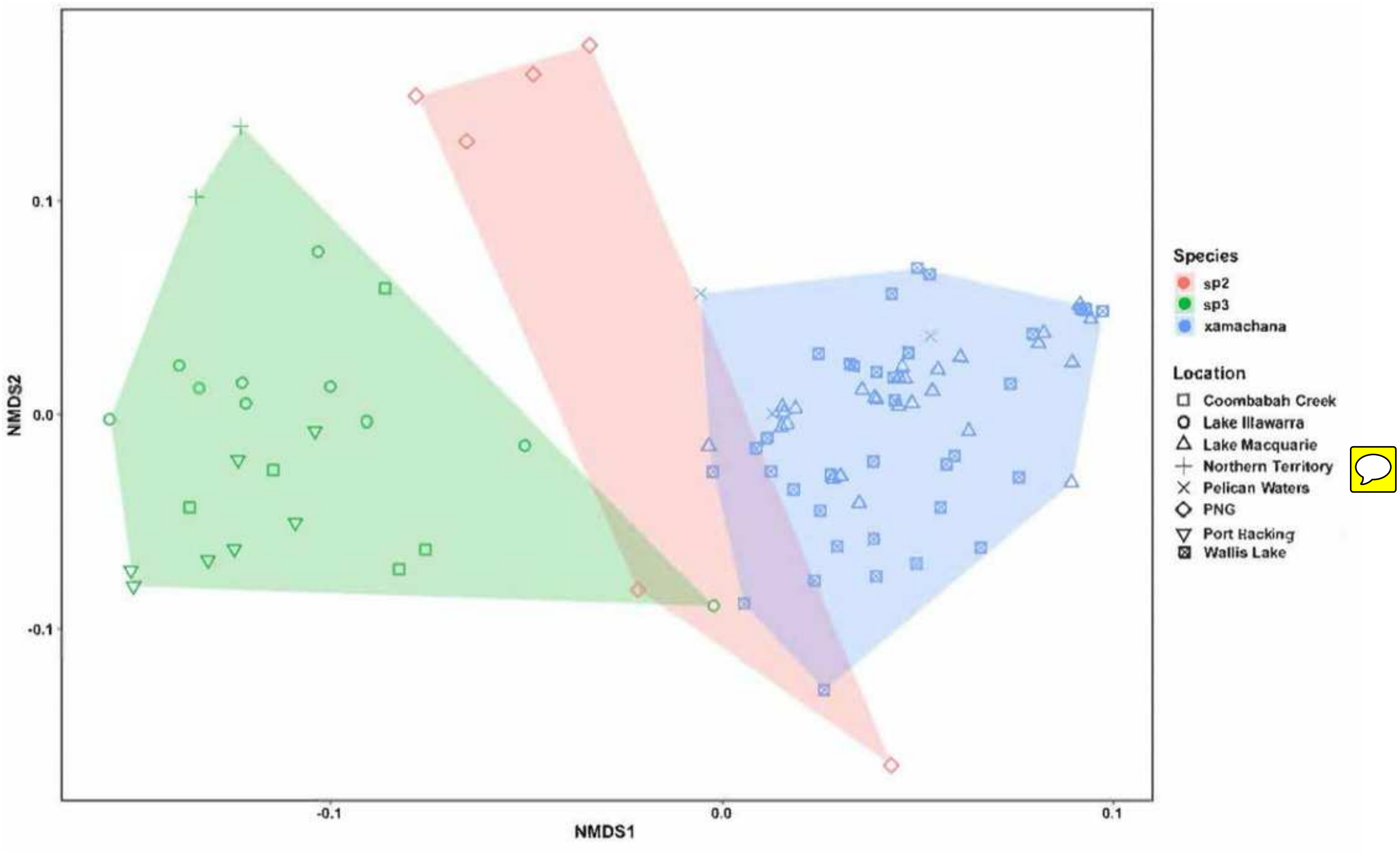

Figure 6

Cluster dendogram 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).

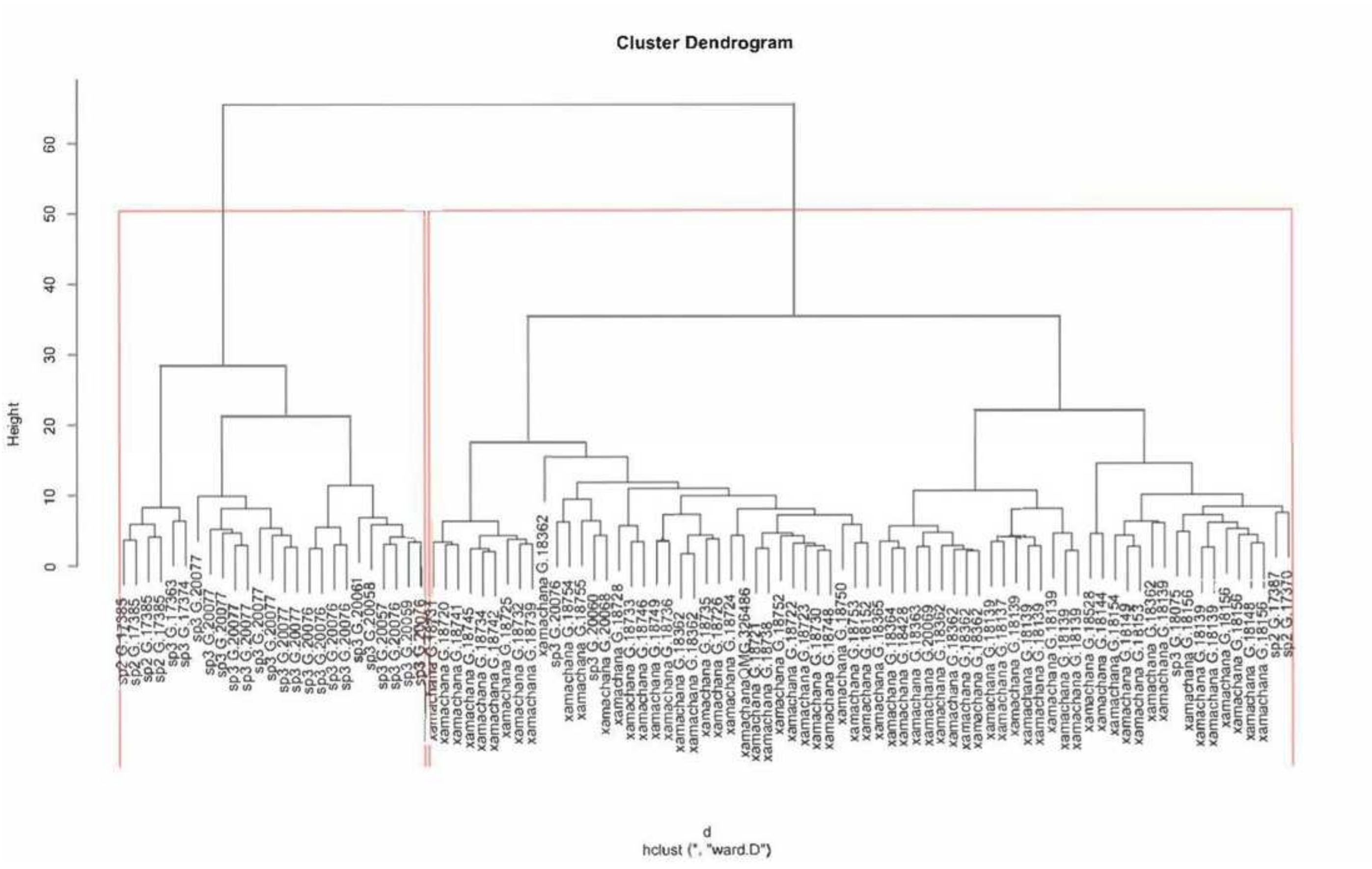

Figure 7

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.

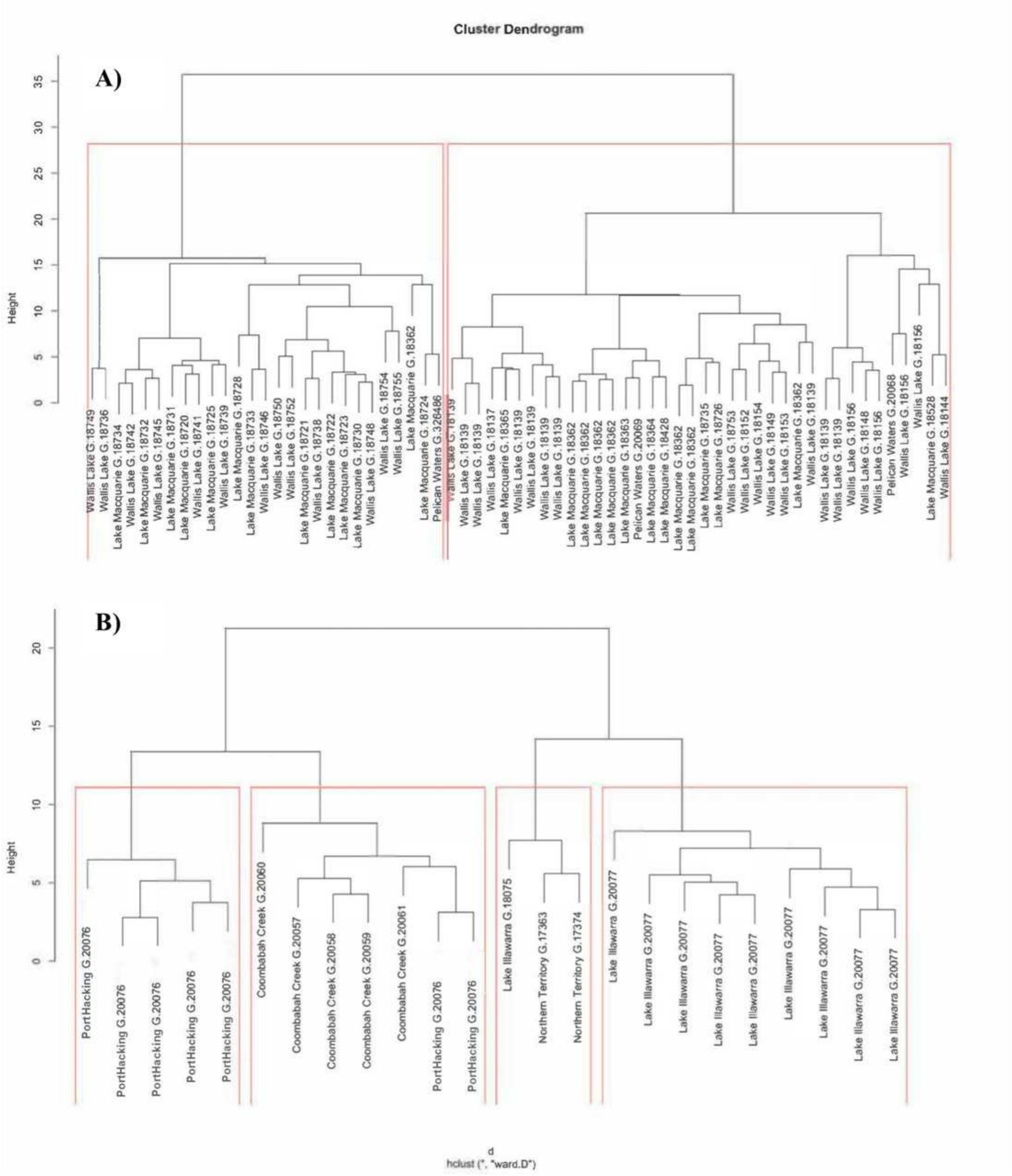
Cluster Dendrogram
A)
Height
0
5
10
15
20
25
30
35
Wallis Lake G.18749
Wallis Lake G.18736
Lake Macquarie G.18734
Wallis Lake G.18742
Lake Macquarie G.18732
Wallis Lake G.18745
Lake Macquarie G.18731
Lake Macquarie G.18720
Wallis Lake G.18741
Lake Macquarie G.18725
Wallis Lake G.18739
Lake Macquarie G.18728
Lake Macquarie G.18733
Wallis Lake G.18746
Wallis Lake G.18750
Wallis Lake G.18752
Lake Macquarie G.18721
Wallis Lake G.18738
Lake Macquarie G.18722
Lake Macquarie G.18723
Lake Macquarie G.18730
Wallis Lake G.18748
Wallis Lake G.18754
Wallis Lake G.18755
Lake Macquarie G.18362
Lake Macquarie G.18724
Pelican Waters G.326486
Wallis Lake G.18139
Wallis Lake G.18139
Wallis Lake G.18139
Wallis Lake G.18137
Lake Macquarie G.18365
Wallis Lake G.18139
Wallis Lake G.18139
Wallis Lake G.18139
Wallis Lake G.18139
Lake Macquarie G.18362
Lake Macquarie G.18362
Lake Macquarie G.18362
Lake Macquarie G.18362
Lake Macquarie G.18363
Pelican Waters G.20069
Lake Macquarie G.18364
Lake Macquarie G.18428
Lake Macquarie G.18362
Lake Macquarie G.18362
Lake Macquarie G.18735
Lake Macquarie G.18726
Wallis Lake G.18753
Wallis Lake G.18152
Wallis Lake G.18154
Wallis Lake G.18149
Wallis Lake G.18153
Lake Macquarie G.18362
Wallis Lake G.18139
Wallis Lake G.18139
Wallis Lake G.18139
Wallis Lake G.18156
Wallis Lake G.18148
Wallis Lake G.18156
Pelican Waters G.20068
Wallis Lake G.18156
Wallis Lake G.18156
Lake Macquarie G.18528
Wallis Lake G.18144
B)
Height
0
5
10
15
20
PortHacking G.20076
PortHacking G.20076
PortHacking G.20076
PortHacking G.20076
PortHacking G.20076
Coombabah Creek G.20060
Coombabah Creek G.20057
Coombabah Creek G.20058
Coombabah Creek G.20059
Coombabah Creek G.20061
PortHacking G.20076
PortHacking G.20076
Lake Illawarra G.18075
Northern Territory G.17363
Northern Territory G.17374
Lake Illawarra G.20077
Lake Illawarra G.20077
Lake Illawarra G.20077
Lake Illawarra G.20077
Lake Illawarra G.20077
Lake Illawarra G.20077
Lake Illawarra G.20077
Lake Illawarra G.20077
Lake Illawarra G.20077
d
hclust (*, "ward.D")

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).

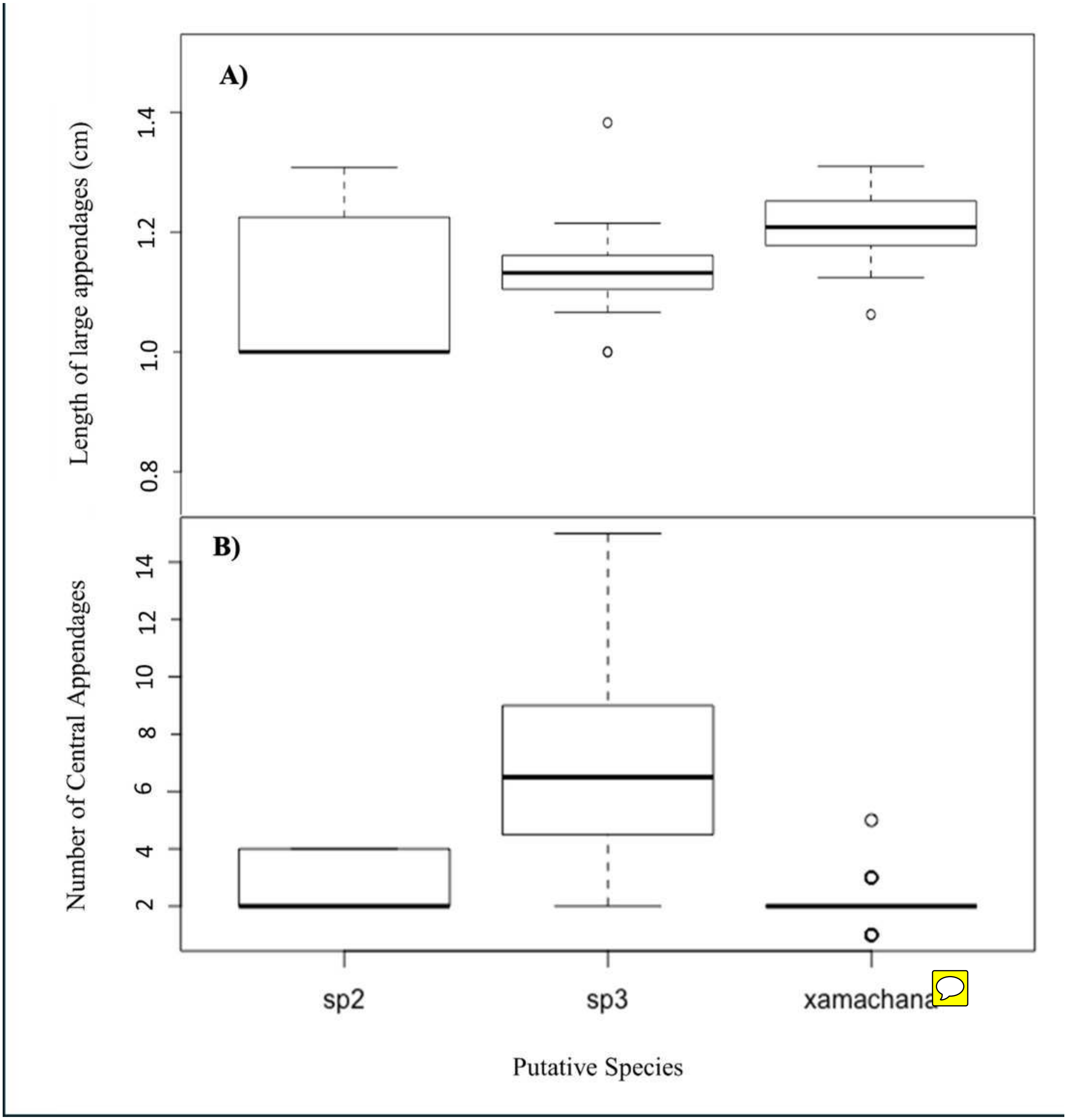
A)
Length of large appendages (cm)
0.8
1.0
1.2
1.4
B)
Number of Central Appendages
2
4
6
8
10
12
14
sp2
sp3
xamachana
Putative Species

Figure 9

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

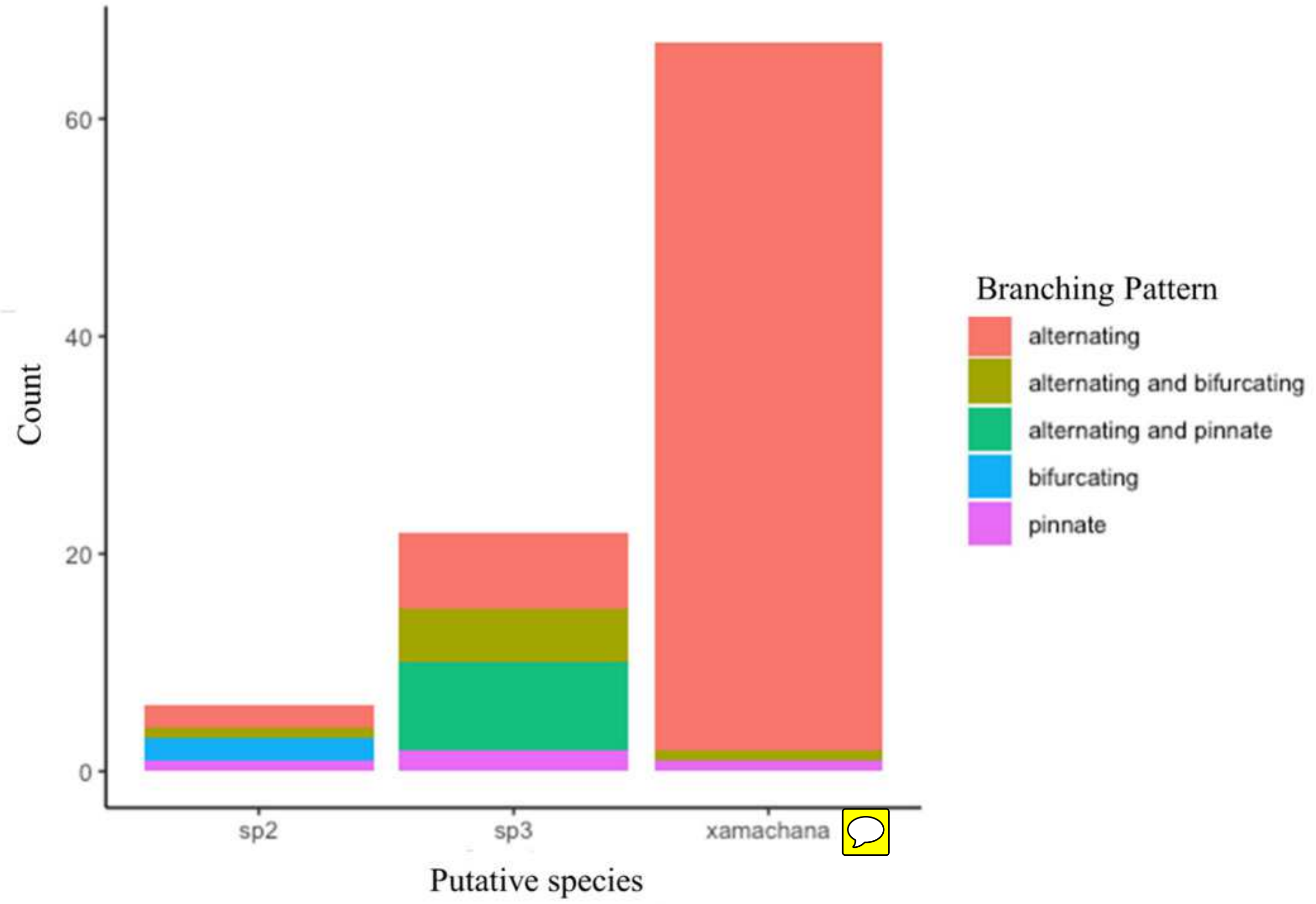

Figure 10

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

A) ~~ab~~oral view of preserved medusa. B) ~~ab~~oral 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) ~~ab~~oral view of the detail of the number and shape of lappets in a paramere. E) arrow pointing to rhopalium, aboral view.

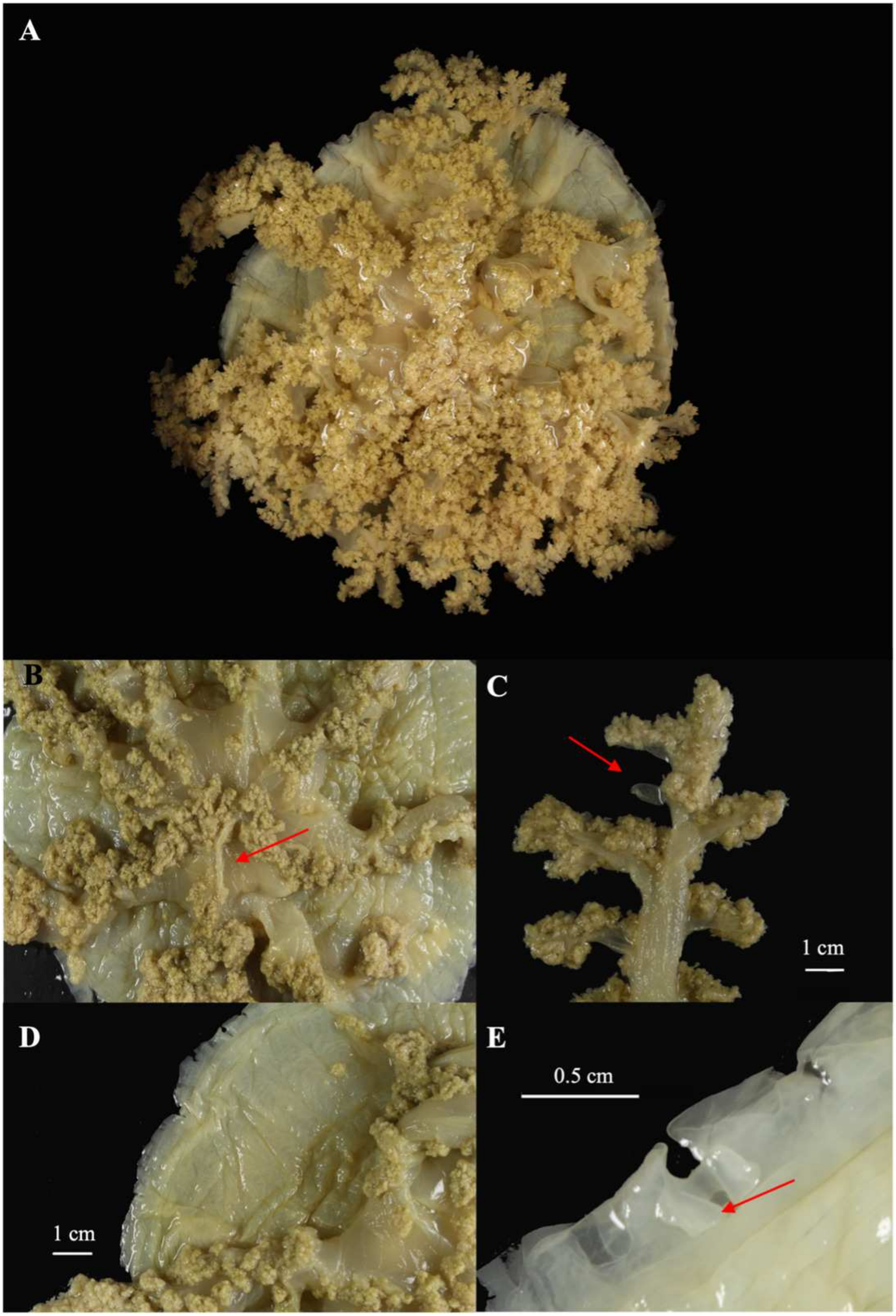
A
B
C
1 cm
D
1 cm
E
0.5 cm

Figure 11

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

A) ~~ab~~oral view of preserved medusa. B) red arrow points to large oral appendage located in the centre of the oral disc, ~~ab~~oral 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.

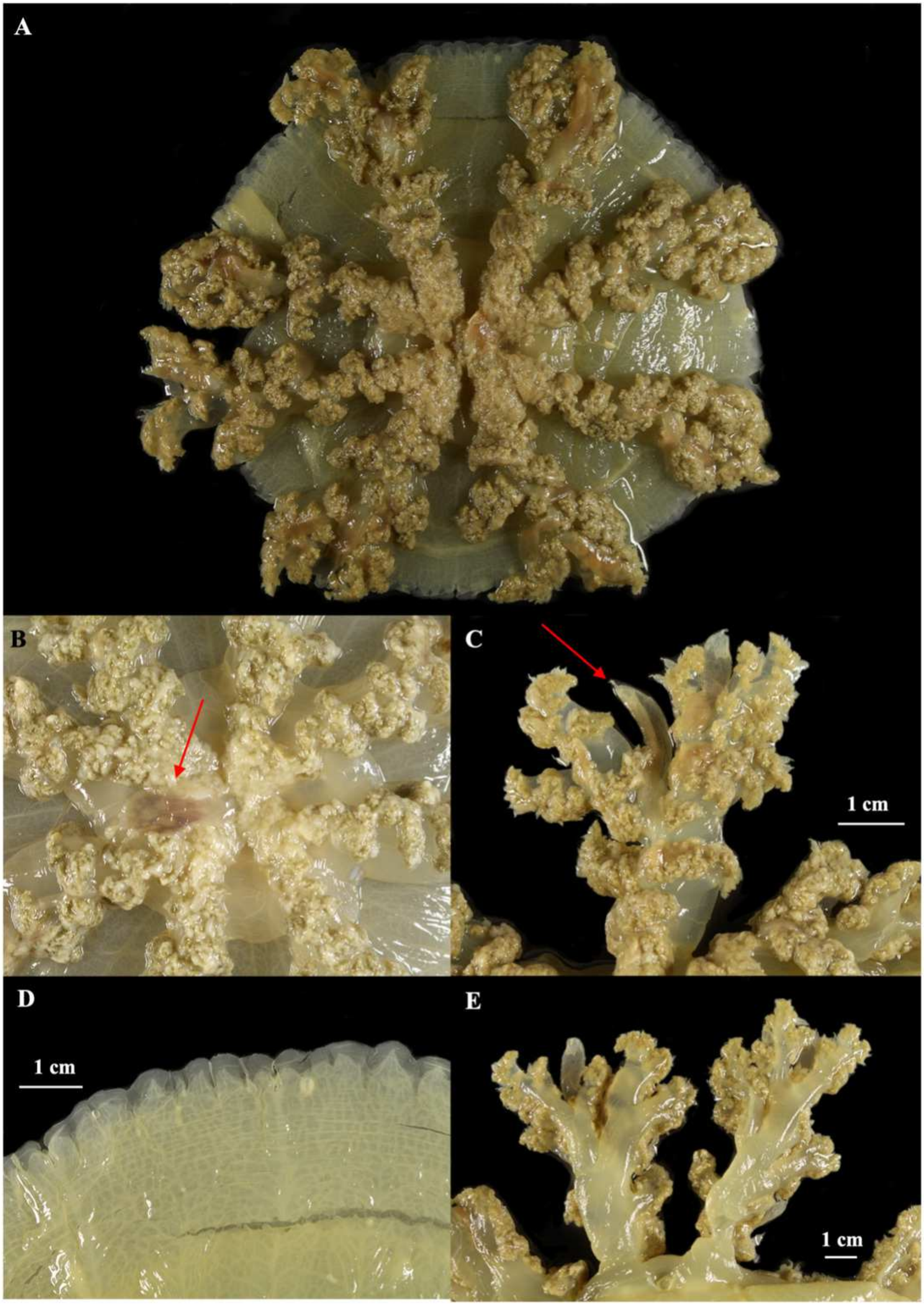
A
B
C
1 cm
D
1 cm
E
1 cm

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**
C. xamachana – *C.* sp.3	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
C. xamachana – *C.* sp.2	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
C. sp.3 – *C.* sp.2	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