

## RESEARCH ARTICLE

# The puzzling occurrence of the upside-down jellyfish *Cassiopea* (Cnidaria: Scyphozoa) along the Brazilian coast: a result of several invasion events?

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<http://zoobank.org/B879CA8D-F6EA-4312-B050-9A19115DB099>

**ABSTRACT.** The massive occurrence of jellyfish in several areas of the world is reported annually, but most of the data come from the northern hemisphere and often refer to a restricted group of species that are not in the genus *Cassiopea*. This study records a massive, clonal and non-native population of *Cassiopea* and discusses the possible scenarios that resulted in the invasion of the Brazilian coast by these organisms. The results indicate that this jellyfish might have invaded the Brazilian coast multiple times.

**KEY WORDS.** Invasive species, jellyfish bloom, medusa, non-native species

## INTRODUCTION

*Cassiopea* Péron & Lesueur, 1810 is a unique genus of large jellyfishes that occur in several shallow water environments around the world (Holland et al. 2004). Members of the genus are getting more attention as a model system to study different aspects of host-symbiont association, besides other topics (Ohdera et al. 2018). The distribution of *Cassiopea* is partly the result of a number of introduction events (Carlton and Geller 1993, Morandini et al. 2017). *Cassiopea* medusae cover large areas in shallow environments. They can be easily recognized because they lay on the substrate with mouth arms facing upward, to which they owe the popular name "upside-down jellyfish". Species of this genus are often found in mangrove areas, mainly because their polyps use the leaves of these plants as a substrate (Fleck and Fitt 1999). Their life cycle is metagenetic, as usual for many Rhizostomeae (planula larva, benthic solitary polyp, juvenile ephyra, and adult medusae; most species with internal fertilization and dioic individuals), with monodisc strobilation (Gohar and Eisawy 1960). Polyp populations may multiply

when the temperature increases, producing a large amount of free-swimming buds (Hofmann et al. 1978). This reproductive pattern allows new polyps form rapidly from buds, and colonize other areas quickly (Curtis and Cowden 1971, Fitt and Costley 1998, Hofmann et al. 2003).

Populations of upside-down jellyfish have been found in several parts of the world where they had never been found before, and several of these records have been regarded as the products of invasion events (Holland et al. 2004, Graham and Bayha 2007, Morandini et al. 2017). Often in these cases, *Cassiopea andromeda* (Forskål, 1775) has been the culprit. These records, however, are tied to an outdated taxonomic knowledge (species diversity) of the genus where there is no clear consensus about which species are invaders (Holland et al. 2004, Graham and Bayha 2007, Morandini et al. 2017, Ohdera et al. 2018, Jarms and Morandini 2019). Since a reliable identification of the species in this genus is only possible with the use of molecular data, owing to the phenotypic plasticity of these jellyfish (Holland et al. 2004, Morandini et al. 2017, Maggio et al. 2019), most of the records that are not based on this marker cannot be trusted

(e.g., Çevik et al. 2006, Keable and Ahyong 2016, Prasade et al. 2016, Cillari et al. 2018).

It is argued that the population found in most of the Brazilian coast was originated from an old invasion, probably during the Age of Discovery (Great Navigations) approximately 500 years ago (Morandini et al. 2017). Here we report new data providing evidence that the Brazilian coast was invaded by more than one *Cassiopea* lineage. The present study aims to refine the discussion about invasion events, mainly from a large population found in the tropical coast of Brazil (Maceió, state of Alagoas, NE Brazil).

## MATERIAL AND METHODS

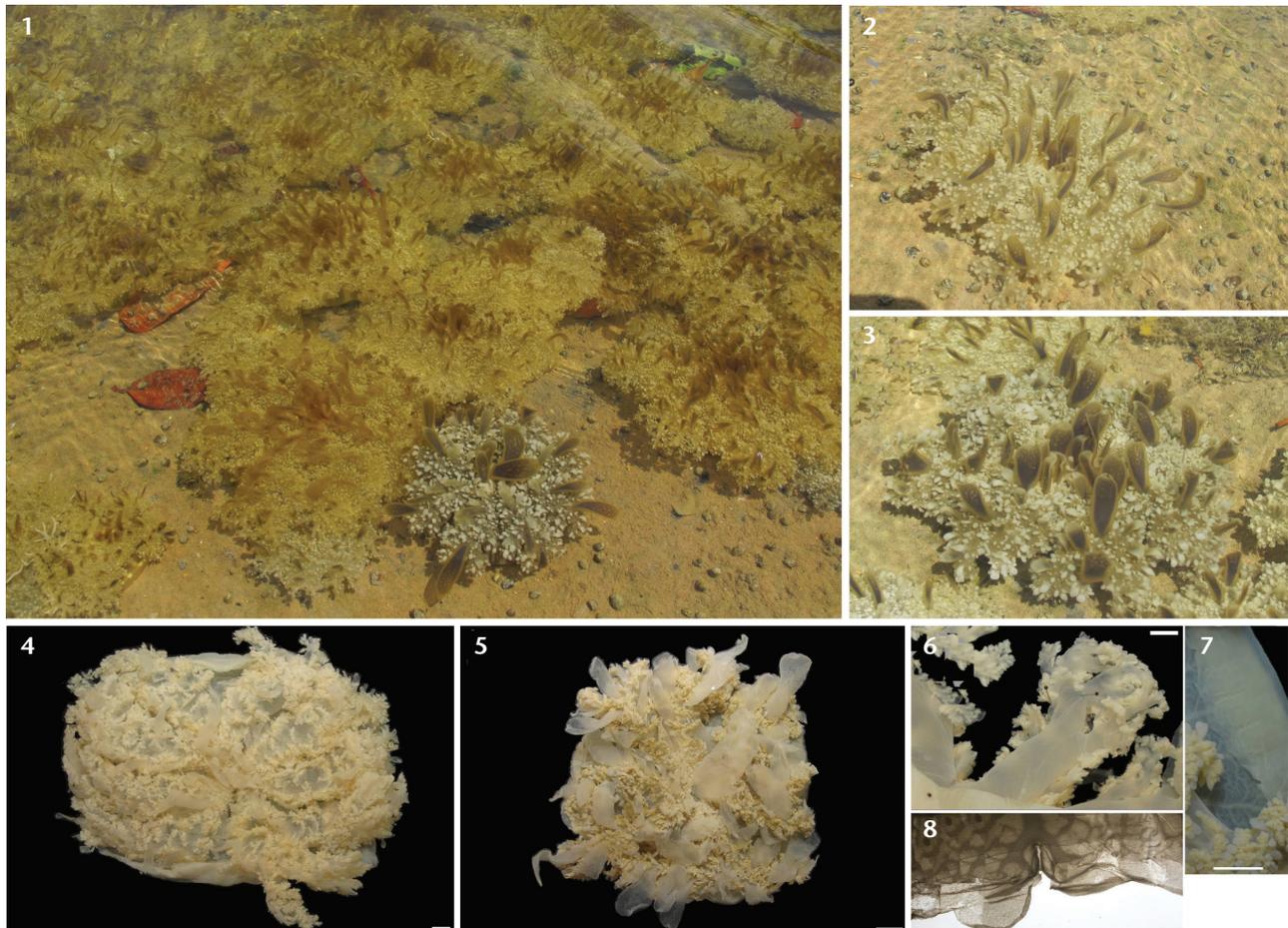
Dozens of *Cassiopea* jellyfish (Figs 1–8) were observed and collected on October 09–12<sup>th</sup> 2016 at the mouth of the Meirim River, Maceió, Alagoas, Brazil (09°32'53"S, 35°37'19"W) close to a beach resort (Fig. 9). These jellyfish were collected haphazardly

and immediately preserved in a 4% formaldehyde-seawater solution or 99% ethanol and then kept refrigerated. Snorkeling and digital photographs were undertaken to document the upside-down jellyfish and to provide further observations of the local fauna.

Observations on living specimens were mostly restricted to the color pattern and size and shape of the mouth-arms appendages. Preserved individuals were measured, checked for morphological features (mouth-arms ramification, number of rhopalia and lappets), and sexed (small piece of gonad tissue observed under the microscope).

Since the mouth of the Meirim River was inside a touristic area of a private beach resort, we questioned the managers about anecdotal effects of jellyfish on tourism.

Data sampling for jellyfish coverage was carried out in January 2017. We performed 11 underwater visual censuses (UVC) in two stretches of the Meirim River, in a region where the upside-down jellyfish had invaded (7 UVCs). Up that stretch (4



Figures 1–8. Specimens of *Cassiopea* from Meirim River estuary: (1) general view of the touristic area with dozens of specimens; (2, 3) details of some large specimens, around 17 cm; (4, 5) details of formalin preserved large specimens; (6–8) details of oral arms, marginal lappets and a rhopalial niche, respectively. Scale bars: 4–7 = 1 cm, 8 = 1000  $\mu$ m.

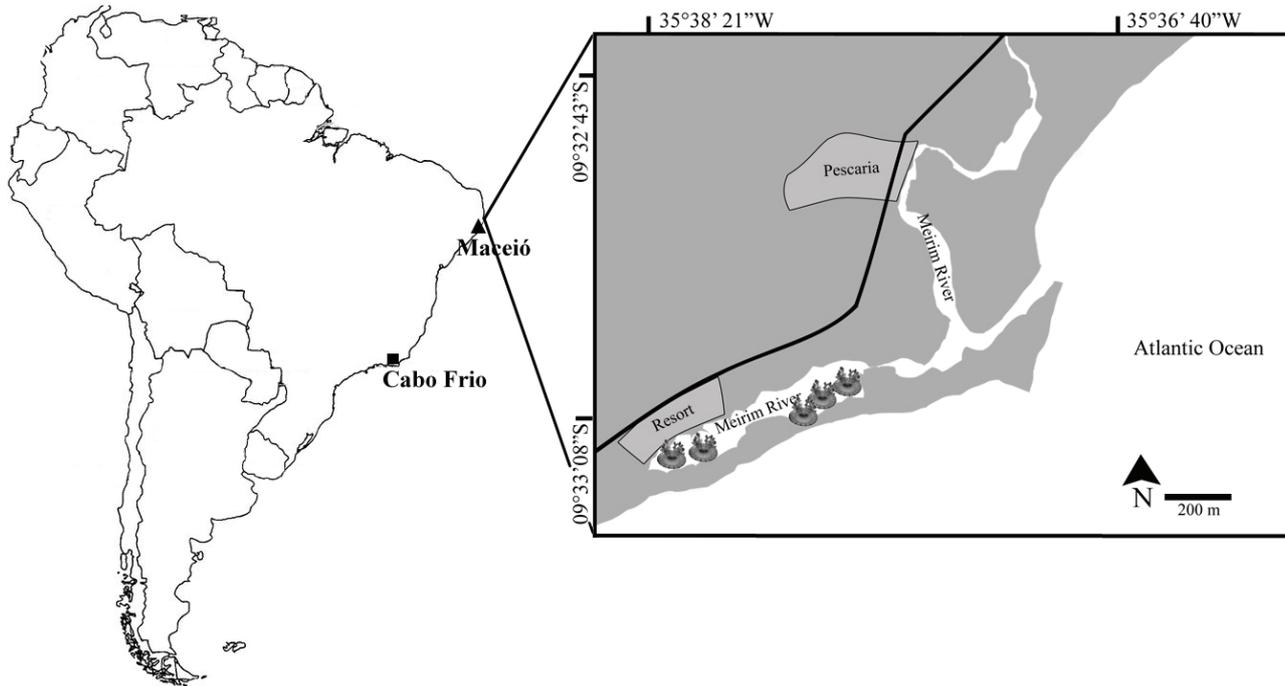


Figure 9. South America map indicating populations of *Cassiopea* from Cabo Frio, Rio de Janeiro and Maceió, Alagoas. In detail map of the area of occurrence of *Cassiopea andromeda* in Alagoas (Meirim River).

UVCs) there is a region where it is not present. In both stretches, the depth of the water ranged from 0.5 to 1.5. Each visual census was 40 m<sup>2</sup> (20 x 2 m), totaling 280 m<sup>2</sup> in the invaded region and 160 m<sup>2</sup> in the region without *Cassiopea*. The occurrence of other organisms at censuses such as fish, crustaceans, mollusks, seagrass and algae was also registered. Additionally, we asked the fishermen and the tourists questions about recreational and fishing activities in the region, even in the presence of *Cassiopea*, at the same time we collected other data.

DNA was extracted using two alternative techniques: a Chelex-based (InstaGene Matrix/Bio-Rad #732-6030) and Salt-precipitation protocol (ammonium acetate). Total DNA extractions were checked with NanoDrop 2000c and 2% Agarose gels stained with GelRed (Biotium #41001 – 41003-T). The Cytochrome Oxidase I (COI) was the molecular/genetic marker of choice: a partial sequence of this gene was amplified using PCR for a total of four individuals (universal primers: LCO1490 and HCO2198; using the original PCR program) based on the protocol defined by Morandini et al. (2017). The PCR results were confirmed with Agarose gels with GelRed and those positives purified with Agencourt AMPure XP system (B37419AB). After sequencing with BigDye reaction, final precipitations were obtained with sodium acetate and ethanol. Sequencing was carried out on an ABI PRISM@3100 genetic analyzer (Hitachi) and sequences were assembled and edited using Geneious™ 9 (Kearse et al. 2012), and new sequences were deposited in GenBank (Tab. 1). COI sequences were compared to those of the other *Cassiopea* specimens

available on GenBank (Numbers MT806178-MT806181). Only sequences of four specimens were included in the study because we suspected that the specimens were clonal, and the available data from other species is based on the same number or less (see Table S1 and Fig. S1). Sequence alignment was made with MAFFT v.7.271 (Katoh and Standley 2013) using the L-INS-i method. After the sequences were aligned, the alignment was visualized to check for potential errors; two gaps were found considering two sequences retrieved from GenBank, AY319460 and AY319465.1 (positions in our alignment: 382 and 547, respectively). Because COI is a protein-coding gene, an alignment devoid of indels breaking the reading codon-framing was expected. Since there is a low likelihood that indels would be present, both gaps were replaced with N.

Uncorrected pairwise distances were retrieved from the software Geneious™ 9 (Kearse et al. 2012). The Maximum Likelihood analysis was conducted via IQ-TREE v1.6.10 (Nguyen et al. 2015). ModelFinder (Kalyaanamoorthy et al. 2017), implemented in IQ-TREE, was used to select the optimal partition scheme and substitution models; partitions corresponding to 1st, 2nd and 3rd codon positions were specified. Trying to circumvent limitations from different clade stability techniques (Anisimova et al. 2011), we calculated two parametric (approximate likelihood ratio test – aLRT and approximate transformation Bayes test – aBAYES) and two non-parametric (standard bootstrap – BS and SH-aLRT) methods. We considered that a clade had high stability if at least three of four methods presented high values, where high values

Table 1. List of nucleotide sequences included in the phylogenetic analysis.

Terminal ID	Molecular species ID	Locality	GenBank accession number	Reference
CandroHaw1	<i>C. andromeda</i>	Hilton Lagoon, Waikiki Beach, Oahu, Hawaii, USA	AF231109.1	Holland et al. (2004)
CxamaUSAF1	<i>C. andromeda</i>	Key Largo, Florida Keys	AY319468.1	Holland et al. (2004)
CfrondUSAF1	<i>C. andromeda</i>	Key Largo, Florida Keys	AY319467.1	Holland et al. (2004)
CxamaBerm1	<i>C. andromeda</i>	Walsingham Pond, Bermuda	AY319466.1	Holland et al. (2004)
CxamaBerm2	<i>C. andromeda</i>	Richardson Bay, Bermuda	AY319465.1	Holland et al. (2004)
CxamaBerm3	<i>C. andromeda</i>	Richardson Bay, Bermuda	AY319464.1	Holland et al. (2004)
CxamaBerm4	<i>C. andromeda</i>	Walsingham Pond, Bermuda	AY319463.1	Holland et al. (2004)
CandroEgyRSea	<i>C. andromeda</i>	El Ghardaqa, Egypt, Red Sea	AY319458.1	Holland et al. (2004)
CandroHaw4	<i>C. andromeda</i>	Kainaone fish pond, Moloka'i, Hawaii, USA	AY319454.1	Holland et al. (2004)
CandroHaw5	<i>C. andromeda</i>	Kainaone fish pond, Moloka'i, Hawaii, USA	AY319453.1	Holland et al. (2004)
CandroHaw7	<i>C. andromeda</i>	Hilton leeward, Oahu, Hawaii, USA	AY319451.1	Holland et al. (2004)
CandroHaw8	<i>C. andromeda</i>	Hilton leeward, Oahu, Hawaii, USA	AY319450.1	Holland et al. (2004)
CandroHaw9	<i>C. andromeda</i>	Hilton leeward, Oahu, Hawaii, USA	AY319449.1	Holland et al. (2004)
CandroHaw10	<i>C. andromeda</i>	Hilton leeward, Oahu, Hawaii, USA	AY319448.1	Holland et al. (2004)
CandroBra1	<i>C. andromeda</i>	Cabo Frio, Rio de Janeiro, Brazil	KC464458.1	Morandini et al. (2017)
Cassiopea_Alagoas_4	<i>C. andromeda</i>	Pratagy, Maceió, Alagoas, Brazil	MT806178	This study
Cassiopea_Alagoas_3	<i>C. andromeda</i>	Pratagy, Maceió, Alagoas, Brazil	MT806179	This study
Cassiopea_Alagoas_2	<i>C. andromeda</i>	Pratagy, Maceió, Alagoas, Brazil	MT806180	This study
Cassiopea_Alagoas_1	<i>C. andromeda</i>	Pratagy, Maceió, Alagoas, Brazil	MT806181	This study
CfrondPan1	<i>C. frondosa</i>	San Blas Islands, Panama	AY319470.1	Holland et al. (2004)
CfrondPan2	<i>C. frondosa</i>	San Blas Islands, Panama	AY319469.1	Holland et al. (2004)
CandroInd1	<i>C. ornata</i>	Kakaban, Kalimantan, Indonesia	AY319473.1	Holland et al. (2004)
CandroInd2	<i>C. ornata</i>	Kakaban, Kalimantan, Indonesia	AY319472.1	Holland et al. (2004)
CandroFiji	<i>C. ornata</i>	Dravuni, Fiji	AY319457.1	Holland et al. (2004)
CandroPal1	<i>C. ornata</i>	Short Drop Off, Palau	AY319456.1	Holland et al. (2004)
CandroPal2	<i>C. ornata</i>	Short Drop Off, Palau	AY319455.1	Holland et al. (2004)
CxamaPan1	<i>C. xamachana</i>	Bocas del Toro, Panama	JN700936.1	Holland et al. (2004)
CxamaBra1	<i>C. xamachana</i>	São Sebastião, São Paulo, Brazil	MN539722.1	This study
CxamaBra2	<i>C. xamachana</i>	Imbé, Rio Grande do Sul, Brazil	MN602311.1	Gamero-Mora et al. (2019)
CandroAus	<i>Cassiopea</i> sp. 1	Port Douglas, Queensland, Australia	AY319471.1	Holland et al. (2004)
CandroPNG1	<i>Cassiopea</i> sp. 2	Observation Point, Papua New Guinea	AY319459.2	Holland et al. (2004)
CandroPNG4	<i>Cassiopea</i> sp. 2	Observation Point, Papua New Guinea	AY319460.1	Holland et al. (2004)
CandroPNG2	<i>Cassiopea</i> sp. 3	Emona, Papua New Guinea	AY319461.1	Holland et al. (2004)
CandroHaw6	<i>Cassiopea</i> sp. 3	Wedding Chapel, windward O'ahu, Hawaii, USA	AY331594.1	Holland et al. (2004)
CandroHaw11	<i>Cassiopea</i> sp. 3	Mid Pacific Golf Course pond, windward O'ahu, Hawaii, USA	AY331593.1	Holland et al. (2004)
CandroJap1	<i>Cassiopea</i> sp. 5	Unknown	AB563740.1	Ojimi and Hidaka (2010)
CandroJap2	<i>Cassiopea</i> sp. 5	Unknown	AB563739.1	Ojimi and Hidaka (2010)
Catostylus_mosaicus	<i>Catostylus mosaicus</i>	Lake Illawara, New South Wales, Australia	AY319476.1	Holland et al. (2004)

are aLRT  $\geq 0.9$ , aBAYES  $\geq 0.95$ , BS  $\geq 75$ , SH-aLRT  $\geq 0.85$  (Anisimova et al. 2011, Maronna et al. 2016).

## RESULTS

### Morphological study

The 26 specimens collected resemble those previously identified as *C. andromeda* from Cabo Frio, SE Brazil (Morandini et al. 2017). The umbrella is flat, and varies from 34–173 mm in diameter (from field observations we had a variation of 20–300 mm). The umbrellar margin is brownish-amber, whereas the

exumbrella color is olive-brownish with 10 to 24 white rhopalial streaks, starting at the inner edge of the marginal lappets; over the ocular lappets there is also one white spot over each velar lappet. There are five lappets in each octant; 10–24 rhopalium (mean = 17) each set in a triangular niche partially covered by a flap. The mouth-arms are pinnately branched, slightly longer than the bell margin; brownish with white spots.

The oral appendages display two patterns: specimens with broadly large ovate green appendages; and animals with elliptic elongated brownish appendages. In both morphotypes, there are no more than eight appendages on each mouth arm. The

appendages vary in size according to the diameter of the specimen (namely, the bigger the jellyfish, the bigger the appendage). Usually, the longest appendage is at the center of the mouth-arm disk (either elliptic elongated or broadly ovate). It can be isolated or surrounded by smaller appendages. Throughout the mouth arms there are many small club-shaped beige-white appendages. In general, both morphotypes resemble one from Cabo Frio with large appendages because of the color (brownish-greenish), distribution (at the center, at the base of each pair of oral arms, and at the axil of the terminal bifurcation of oral arms) and shape (oval-elliptic elongated) of the appendages; however, there are difference in the number of appendages at the center of the disc, which is associated to sexual dimorphism.

From the 26 examined jellyfish, 13 had gonads and are females; but no clusters of eggs were found among the oral arms – which suggests no sexual reproduction is taking place in the area. Regardless of the morphotype, abnormalities were observed. Two specimens presented an abnormal number of gonads (3 and 5 instead of 4). Also, an abnormal number of oral arms was observed in 1/3 of the inspected organisms: 7, 9 or 10 instead of 8.

**Molecular study**

The phylogenetic relationship based on sequences of COI using Maximum Likelihood analysis (Fig. 10) reveals a group of *Cassiopea* from Alagoas (numbered 1–4) within the

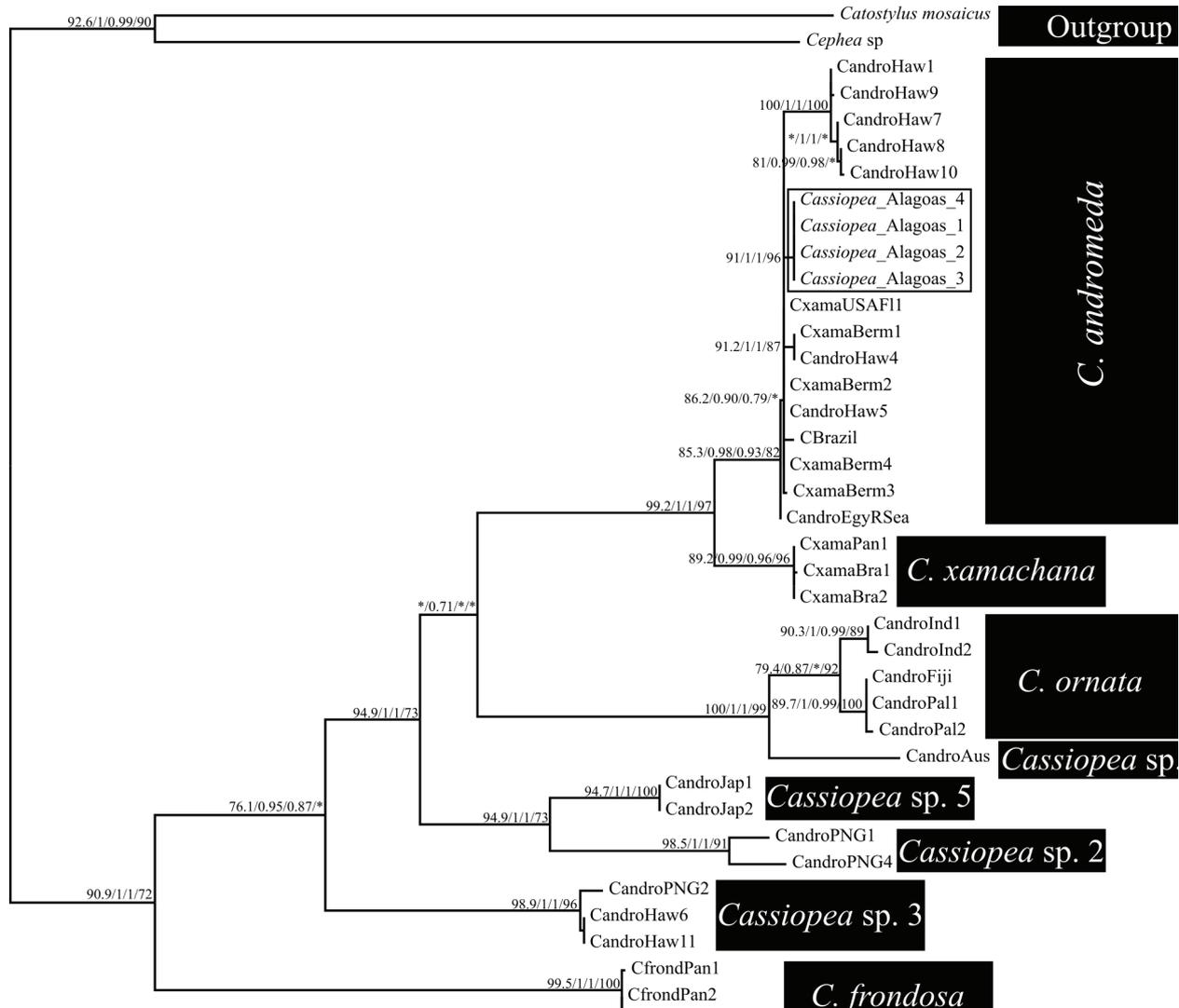


Figure 10. Molecular reconstruction inferred under Maximum Likelihood (score: -lnL 3124) based on COI with Morandini et al. (2017) dataset and newly sequenced specimens (see Table 1 for details on the sequences used in the analysis). Numbers in nodes represent SH-aLRT support (%)/parametric aLRT support/aBayes support/standard bootstrap support (%); \* less than 70/0.70.

cluster of other specimens previously recorded as *C. andromeda*. Therefore, it is possible to conclude that the specimens found in the northeast of Brazil can be identified as the same species: *C. andromeda*. Although these new specimens from Alagoas cluster with others from Cabo Frio (Rio de Janeiro, SE Brazil), there is a consistent difference (1.1% of bases which are not identical) between specimens from the two Brazilian populations (detailed data in supplemental material Table 1).

#### Ecological and societal implications

An 80% coverage of *Cassiopea* specimens was estimated on the invaded shallow and sheltered part of the Meirim River estuary, Maceió, Alagoas. In this area macroalgae and seagrass were not registered. Besides that, fish species typical of the region, such as mullet, mojarra and snapper, were seldom seen. Demersal fish, like flounder and gobies, besides sea slugs were not registered. In the upper stretch of the Meirim River *Cassiopea* was not recorded, with seagrass and algae covering 40% of the bottom.

During October 2016 sampling, the managers of the beach resort (final part of Meirim River) noted on the impact noticed on touristic activities. People were avoiding the riverine waters for recreation because they were concerned about the presence of jellyfish in the water. The few people who had the courage to entered the water complained about stings. On January 2017 only two fishermen and six tourists were present at the study area at the time of sampling.

## DISCUSSION

#### Morphological study

Invasive species populations usually derive from a small number of founders, which may face a different environment from where they occur naturally. To survive in the new environment, morphological plasticity is often important (Davidson et al. 2011). As a response to challenging environments, we observe that in some scyphozoan genera there are certain species that have phenotypes with high relative fitness within particular environments, or species that have phenotypes with high fitness across environments (Chiaverano et al. 2016). Independently of which ability we are focusing (high fitness to a specific environment or across different environments), the result is that the species have a greater invasive potential (adaptability). In fact, members of the genus *Cassiopea* have a huge adaptability. Some biological features of members of the genus also enhance the potential to invasiveness (Bayha and Graham 2014): metagenetic life cycle with asexually reproducing polyps; high environmental tolerance; and a myxotrophic nutrition.

The main morphological variation seen in the collected specimens was the shape and colour of the oral appendages, resulting in two distinct sympatric morphotypes that are genetically identical (see molecular section). Larger appendages may provide more light to the zooxanthellae housed in their oral ap-

pendages. Also, it improves feeding ability by helping to capture the meiofauna that is flushed to the oral appendages (Jantzen et al. 2010). Larger appendages are able to store and release more nematocysts. Those morphological intrapopulation differences have ecological implications: e.g., differences in habitat use may lead to niche partitioning which, among other factors, could help to facilitate adaptation and long-term invasive potential. In the population from Cabo Frio (SE Brazil) (Figs 11–13), there were also two morphotypes (one with large appendages and dark greenish color, and another with smaller appendages and whitish to cream-colored) (Morandini et al. 2017). Even though the authors found such morphological differences, those morphotypes were genetically identical (Morandini et al. 2017), reinforcing the suggestion of the authors that there was a single invasive event at Cabo Frio. Additionally, different phenotypes of clones illustrate the wide morphological variation among members of the genus *Cassiopea* as published in the literature by so many authors (e.g. Mayer 1910, Hummelinck 1968).

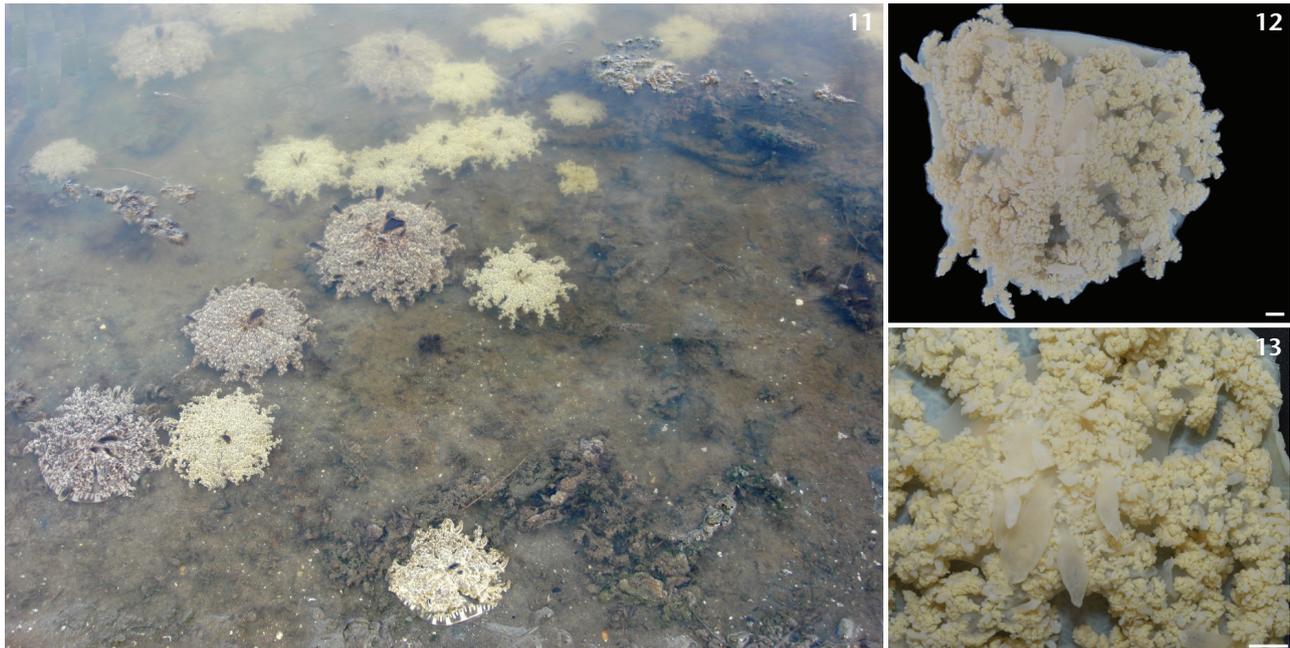
Such morphological variation in color and appendage shape in clonal jellyfish individuals has not been discussed in the literature. There are records of morphological abnormalities in medusae (aquarium conditions) because of abnormalities found in polyp symmetry (Gershwin 1999). Weather the anomalies influence the fitness of these jellyfish is still unknown.

#### Ecological and societal implications

In the invaded area of the Meirim River, macroalgae and seagrass seem to be affected by physical contact and/or shading caused by large jellyfish.

Another impact noticed was the absence of artisanal fishermen and tourists. They sought other fishing and recreational areas, fearing the jellyfish stings. According to the resort manager, tourists, and artisanal fishermen, the jellyfish bloom is a reason to avoid the area. The stinging ability of several jellyfish species is widely known (Haddad Jr 2016). In the case of *Cassiopea*, although the species have an epibenthic habit and do not possess tentacles, they may cause a phenomenon known as “stinging water”, which can cause some irritating sensation in bathers (Ames et al. 2020).

This is a different situation from the *Cassiopea* bloom recorded in the Araruama lagoon (Cabo Frio, Rio de Janeiro, SE Brazil), where no ecological or economic consequences were observed or reported (Morandini et al. 2017). In the case of the Meirim River in the Brazilian NE, in addition to the ecological impact of causing a reduction of the marine vegetation, invertebrates and fish, the jellyfish bloom also caused an economic impact in the local area through the reduction of tourists and local artisanal fishermen. Possibly these differences are related to the size and uses of these two coastal areas. Contrasting with the Araruama lagoon, a much larger, deeper and more polluted waterbody, the Meirim River estuary is a smaller, shallower area often used by tourists (Souza et al. 2003) – in the case of Cabo Frio the bloom occurred is a small branch of the lagoon only



Figures 11–13. Specimens of *Cassiopea andromeda* from Cabo Frio, Rio de Janeiro, Brazil: (11) general view of the area with dozens of specimens, largest one ~25 cm; (12) oral view of a preserved specimen in formalin; (13) details of oral appendages of a formalin fixed specimen. Scale bars: 12, 13 = 1 cm.

used by fishermen to repair and dock the fishing boats. This population of *Cassiopea* found in the mouth of the Meirim River is apparently established and goes through cycles of disappearance and reappearance (December/2019) (Dr Victor Cedro – personal communication).

#### Molecular study: a different invasion event?

Data retrieved from molecular phylogenetic reconstruction indicates that there are two different *C. andromeda* populations on the coast of Brazil. As both populations are composed of clonal individuals, probably originating from a single initial polyp in each case, the assumption that they are not from the same introduction event is reasonable. There are two possible hypotheses explaining those two populations. In the first scenario there was a unique invasion followed by broad dispersal, and a subsequent process of genetical isolation between two populations. This scenario is possible if we accept the proposal of Morandini et al. (2017) which indicates an ancient invasion of the coast of Brazil by this species. However, the level of accumulated divergence indicates that it is more likely that the two populations have been isolated for longer than the 500 years previously suggested by Morandini et al. (2017). The second scenario is based on independent invasions of the Brazilian coast. This pattern is better supported by new genetic data. Taking into account COI sequences from *C. andromeda* it is clear that each Brazilian population has a unique haplotype (Cabo Frio, Rio de Janeiro and Maceió, Alagoas); in fact, the Cabo

Frio COI sequence is more similar to GenBank's sequences from Bermuda than the Alagoas samples (6 mutations vs 3 mutations). Identification of *Cassiopea* is a difficult task, so future species confirmation of these North Atlantic samples or at least, new samples from the localities will be useful. A similar situation to this second scenario has been described for Hawaii by Holland et al. (2004). The difference between the Brazilian situation and the one described for Hawaii (Holland et al. 2004) is that in the second case the time of divergence between lineages is very long, probably indicating invasions from different areas and time periods. For Brazil the evidence of multiple invasions by proximal populations cannot be discarded.

Current knowledge of the genetic variation at inter and intra specific levels in *C. andromeda* is the result of molecular data on polymorphisms at a single locus (Bridge et al. 1995, Holland et al. 2004, Bayha et al. 2010). Therefore, it is not sufficient to establish robust hypotheses about historical patterns and processes of diversification and speciation of *Cassiopea*, which could be developed in multilocus analyses. New approaches are being proposed in systematics and population genetics considering hundreds or even thousands of genetic markers thanks to Next Generation Sequencing technologies. Techniques such as RADseq data, Exon capture, RNAseq or even whole genome sequencing can provide a large amount of data from orthologous loci to the fields of phylogeography and population genomic inference (Andrews and Luikart 2014), and consequently employed to establish the route of a certain invasion. So, in the context

of *Cassiopea* research (and other zooplanktonic species), a solid genetic database will lead to a better understanding of invasive jellyfish adaptation and evolution. From this technology and information, it will subsequently be possible to develop methods to monitor or avoid conditions favorable to human-based biological invasions (Darling et al. 2017). These approaches are recommended for future study of *Cassiopea* distributions and associated taxonomic work.

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### Supplementary material 1

Figure S1. Map showing the collection localities of *Cassiopea* sequences used in the phylogenetic analysis. The green square refers to the site of collection of the population of *C. andromeda* from Alagoas, Brazil. Each color indicates a species. *Cassiopea* sp. 5 does not appear because of its unknown locality.

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### Supplementary material 2

Table S1. Genetic distance matrix over sequence pairs within *Cassiopea andromeda*. Values shown below diagonal are uncorrected pairwise distances. Above the diagonal are the number of bases that are not identical. 1. *Cassiopea\_Alagoas\_1*; 2. *Cassiopea\_Alagoas\_2*; 3. *Cassiopea\_Alagoas\_3*; 4. *Cassiopea\_Alagoas\_4*; 5. *CandroHaw8*; 6. *CandroHaw10*; 7. *CandroHaw7*; 8. *CandroHaw9*; 9. *CandroHaw1*; 10. *CandroEgyRSea*; 11. *Cbrazil*; 12. *CxamaBerm3* 13. *CxamaBerm4* 14. *CandroHaw4* 15. *CxamaBerm1* 16. *CandroHaw5* 17. *CxamaBerm2* 18. *CxamaUSAF11*.

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