

Original Article

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Molecular identity of the non-indigenous *Cassiopea* sp. from Palermo Harbour (central Mediterranean Sea)

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Abstract

The upside-down jellyfish *Cassiopea* is a benthic scyphozoan, considered a non-indigenous invasive species in the Mediterranean, forming large blooms in eutrophic areas. Taxonomy of the genus *Cassiopea* is extremely difficult because morphological/meristic characters used are variable within the same species, overlapping among different species, and cryptic species have been identified by molecular markers; nine *Cassiopea* species are recognized on the basis of molecular study. Mediterranean records of *Cassiopea* have been ascribed to *andromeda* species on the basis of a hypothesized invasion pathway from the Suez Canal. In the current study, an analysis of the main morphological characters of the sampled *Cassiopea* jellyfish from Palermo (Tyrrhenian Sea) was carried out and subsequently, molecular analyses were performed by using COI barcode in order to identify the species. Molecular data were compared with published information in GenBank. Morphological characters were highly variable, but molecular analyses confirmed that Mediterranean *Cassiopea* specimens belong to *andromeda* species. Moreover, high values of sequence divergence were found between Mediterranean *Cassiopea* and the other *C. andromeda* from the Red Sea, Hawaii and Florida. These results lead to a discussion of possible explanations linked to life history features of the species. Two different explanations are proposed; the first is that Mediterranean *C. andromeda*, finding a suitable ecological niche good for colonization and proliferation, could have been isolated in Palermo Harbour. The second considers the possibility of multiple introduction events by human transport as demonstrated for other non-indigenous jellyfish; in this case *Cassiopea* genetic differences increased in the invaded area.

Introduction

The presence of non-indigenous species, and in particular invasive ones, is globally considered as one of the most important causes of biodiversity loss at ecosystem, habitat and species level, and may also entail a significant social and economic impact favoured by climate change and anthropogenic disturbance. Particularly, in the case of the Mediterranean Sea, invasive species can enter the Basin from the Atlantic Ocean or the Red Sea through natural and anthropogenic dispersion. Natural dispersal would be facilitated by water warming, that allows tropical and subtropical species to widen their distribution (Bianchi, 2007; Lasram *et al.*, 2008; Parravicini *et al.*, 2015); while anthropogenic activities can act as vectors of introduction through maritime traffic (ballast waters and hull fouling), aquarium trade, aquaculture facilities and even voluntary introduction. Thorough knowledge of vectors and pathways of introduction and dispersion of invasive species, as well as knowledge of their impacts on biodiversity at species, habitat and ecosystem levels are necessary to elaborate adequate management strategies. Several of these species are clearly identifiable because of their recognizable diagnostic characters but the invasion process also involves cryptic species which still need in-depth study. One of these is the upside-down jellyfish *Cassiopea* Péron & Lesueur, 1810 (Cnidaria, Rhizostomeae), a benthic scyphozoan, commonly found in tropical and subtropical shallow coastal ecosystems. The genus belongs to the Cassiopeidae family and includes nine recognized species by molecular analysis (Holland *et al.*, 2004; Morandini *et al.*, 2016; Arai *et al.*, 2017) although many other species have been identified using morphological data.

Cassiopea spp. shows a unique posture among the scyphomedusae, the exumbrella adheres to the seafloor while the oral arms and the convex subumbrella are turned upwards. They live in a symbiotic relationship with photosynthetic dinoflagellate microalgae of the genus *Symbiodinium* which determine the highly variable typical colouration of the umbrella and



share nutrient exchange; further it has been demonstrated that *Symbiodinium* represents a key factor in strobilation induction of *Cassiopea* polyps (Gohar & Eisawy, 1960; Hofmann *et al.*, 1996).

In the last few years, research on these gelatinous species has increased and *Cassiopea* has been considered a model species in studying symbiotic relationships due to the ease of culturing and maintaining polyps and medusae in the laboratory, but also because the *Cassiopea/Symbiodinium* interaction is a clear example of a successful cooperation under stressful environmental conditions (shallow waters, high temperatures, high levels of irradiation and potentially large changes in salinity) and therefore it is also interesting in a climate change context (Lampert, 2016). Moreover, *Cassiopea* spp. may make an informative bioindicator/biomonitoring species since it can accumulate heavy metals and other chemical compounds and it could supply useful information for the application of coastal management actions (Templeman & Kingsford, 2012). Recently sleep behaviour was demonstrated (Nath *et al.*, 2017) (unusual in cnidarians due to the lack of a centralized nervous system) and so *Cassiopea* became a model organism for the study of behavioural biology.

The taxonomy and systematics of the genus *Cassiopea* are extremely difficult, mainly because the morphology of the species varies greatly in different habitats and at different stages of growth (Hopf & Kingsford, 2013). Moreover, in an attempt to solve phylogeography and systematics of the genus at a global scale through molecular analyses, Holland *et al.* (2004) demonstrated the presence of cryptic species supporting the hypothesis that six nominal species belong to *Cassiopea*. These are *C. frondosa* from the western Atlantic, which is the only species morphologically distinguishable; (2) *C. andromeda* from the Red Sea, western Atlantic and Hawaiian Islands; (3) *C. ornata*, from the area around Indonesia, Palau and Fiji Islands; and three other species not yet named as distinct taxonomic units from (i) eastern Australia, (ii) Papua New Guinea and (iii) Hawaiian Islands and Papua New Guinea. In addition Arai *et al.* (2017), using molecular markers, found two new different lineages in the Palau Islands (*C. sp4* and *C. sp6*) which are different from the other species analysed by Holland *et al.* (2004) and another distinct unit (*C. sp 5*) from GenBank (AB563740 and AB563739).

Although *Cassiopea* genus is widely distributed in tropical and sub-tropical waters, it is considered a non-indigenous species in Brazil (Morandini *et al.*, 2016), Indo-Pacific Papua New Guinea, Hawaii (Holland *et al.*, 2004), Australia (Keable & Ahyong, 2016) and the Mediterranean Sea (Maas, 1903).

In the Mediterranean, after records of *Cassiopea andromeda* in the Suez Canal in 1886 and 1887, Maas (1903) reported its subsequent migration to the waters of Cyprus in 1903; in 1955 the species appeared in the Aegean Sea near Santorini (Schäfer, 1955); Goy *et al.* (1988) and Spanier (1989) reported it in Lebanon and Israel respectively, and later, Çevik *et al.* (2006) found it along the Levantine coast of Turkey; Schembri *et al.* (2010) recorded it in Maltese waters and Ounifi-Ben Amor *et al.* (2015) in northern Tunisia. In Italian waters, the presence of *Cassiopea* was reported from the 2010s in online magazines, in the Tyrrhenian Sea (Livesicilia, 2014). Piraino *et al.* (2016) reported the occasional occurrence of *C. andromeda* in the Gulf of Palermo (South Tyrrhenian Sea) and Servello *et al.* (2019) reported it in the Baia di Augusta (Ionian Sea), but both records have no details or specimen vouchers. The first documented report of *Cassiopea* cfr *andromeda* in Italian waters is from Cillari *et al.* (2018) who found the medusae in Palermo Cala Harbour in 2014.

More recently, *Cassiopea* sp. reached western Mediterranean coasts in Spain where it was reported in 2017 in the Mar Menor (Murcia) (Rubio, 2017). Reports of *Cassiopea* in the

Mediterranean Sea indicate the occurrence of the jellyfish mainly in semi-enclosed eutrophic waters characterized by low hydrodynamism. Biological features such as high tolerance to variation of environmental parameters and prolific asexual reproduction, as demonstrated by high rate of planuloid production (Schariti *et al.*, 2014), make it a potentially successful invader in a wide variety of coastal ecosystems.

To date, Mediterranean records of *Cassiopea* have been ascribed to *C. andromeda* on the basis of its hypothesized invasion pathway starting from the Red Sea through the Suez Canal, reaching the Eastern and Central Mediterranean; Galil *et al.* (1990) referred it as the first Lessepsian jellyfish in Mediterranean sea. Here, we report a study of specimens of *Cassiopea* sp. from the South Tyrrhenian Sea (Palermo, Italy) using molecular analyses in order to identify which species was introduced to the Mediterranean Sea, to compare our results with published information and lastly, to hypothesize a pattern of introduction in the Mediterranean Sea.

Materials and methods

Sixteen specimens of *Cassiopea* sp. were collected in Palermo Cala Harbour (38°07.22'N 13°22.09'E) using a hand net on 29 November 2017 and 8 February 2018. Palermo Cala Harbour is a recently reclaimed part of the commercial harbour, which hosts small- and medium-sized pleasure craft (Figure 1). Mean water depth in this small marina is about 7 metres, ranging from 0.5 m to about 12 m. The marina hosts several artificial structures and floating wharfs which could favour the settlement of sessile organisms, including *Cassiopea* polyps. The first documented record of *Cassiopea* in this area dates back to 2014 (Cillari *et al.*, 2018) and has since resulted in an abundant population until winter 2018 when medusae numbers decreased until they disappeared in February 2019.

During sampling, measurements of salinity and water temperature were recorded. Salinity varied between 35.2–35.6‰ and water temperature ranged from 14.1°C (in February) to 17.6°C (in November).

Cassiopea sp. individuals collected were transported to the laboratory where morphological analysis was immediately performed by stereo microscope observation of different characters and photographic sampling.

Molecular analyses

Specimens of *Cassiopea* were stored at –20°C or analysed immediately after collection. Genomic DNA was purified using PureLink Genomic DNA Kits (Invitrogen Corporation, Carlsbad, CA, USA) from the umbrella and tentacles, according to the manufacturer's instructions. The DNA concentrations were verified by spectrophotometry at 260 nm and stored at –20°C for future use.

Universal PCR primers (LCO1490 and HCO2198; Folmer *et al.*, 1994) for the cytochrome oxidase I gene failed in the amplification of the COI barcode region. Thus, specific primers were generated aligning all *Cassiopea* spp. sequences from GenBank (Table 1) and searching them in the flanking COI region avoiding dimerization capability, significant hairpin formation, secondary priming sites in the template and mispriming.

Sequences of the designed primers were the following: CasF 5'-GGTTCTTCTCCACCAACCACAARGAYATHGG 3' and CasR 5'-ATTTCTATCHGTTARYAACATTGTRAT 3'. 50 ng of purified DNA was used as the template for PCR amplification. Reactions were carried out in a total volume of 25 µl using Platinum Taq DNA Polymerase (Thermo Fisher Scientific Inc., Carlsbad, USA) in 1× buffer, 0.2 mM dNTPs (Euroclone), BSA (0.5 mg ml⁻¹;



Fig. 1. Palermo Cala Harbour. Square 38° 07.22'N 13° 22.09'E. Triangles are sampling sites.

New England BioLabs), 1 μ M primers. PCR were performed following these conditions: hot start of 2 min at 95°C, 30 cycles of 94°C for 30 s, 48°C for 30 s and 72°C for 45 s, with a final 72°C extension for 7 min. PCR fragments were visualized on 1% (w/v) TAE agarose gel, purified and sequenced using an ABI Prism 373 automated sequencer. These primers successfully amplified the COI region for all the specimens analysed. Sequences were aligned using the MUSCLE plugin in MEGA 6 software (Tamura *et al.*, 2013). Furthermore sequences were compared to those of the other *Cassiopea* specimens available in GenBank (Holland *et al.*, 2004; Arai *et al.*, 2017) (Table 1). Genetic distances were calculated using the Kimura 2-parameter model. Phylogenetic reconstructions were performed based on the Neighbour-joining and Maximum likelihood method generated in MEGA version 6. To estimate support for the nodes, 1000 bootstrap replicates were performed and we retained only the values supporting the nodes accounting for more than 50% of the bootstrap replicates.

Results

Cassiopea specimens showed an exumbrella disc-shaped, sometimes convex, concave or flat with a central dome; the marginal lappets were short, blunt, and variable in number from 4 to 6. They bore eight oral arms which were dichotomous, wide and flat, from shorter to longer than bell radius; each oral arm bore 4–6 flat, short side branches arising from each arm in a tree-like manner. The colour of the umbrella was mostly brown and varied from yellowish white to dirty blue, sometimes exhibiting a circular white band; arm colouration varied from beige to brown, and vesicles exhibited the largest array of colours being of different shades of brown, green, violet and blue. Morphological analyses revealed 16 rhopalia in the intact specimens. The number of inter rhopalia lappets ranged from 4 to 6 and the number of mouth arms branches (from 4 to 6), were

variable, both within the same specimen and among different ones (Figure 2).

The COI gene was successfully amplified from all *Cassiopea* sp. individuals. Some specimens showed identical sequences, resulting in six haplotypes that were used for the subsequent analyses. Alignment of the haplotypes built with GenBank sequences resulted in 498 base pairs; of the total 498 base pairs aligned, 274 were conserved and 224 were polymorphic with 186 informative and 38 singleton substitutions. The nucleotide frequencies were 0.287 (A), 0.349 (T), 0.173 (C) and 0.191 (G), revealing a thymine bias. The overall transition/transversion bias ($R = 1.24$) showed that the greater part of nucleotide variation was due to transitions, as is common in protein coding genes.

Phylogenetic analyses conducted with different approaches resulted in phylogenetic trees with similar topology, so we report the NJ in Figure 3. This tree shows the presence of seven monophyletic groups: *C. ornata*; *Cassiopea* sp.1 from Australia; *Cassiopea* sp.2 from Papua New Guinea, as defined by Holland *et al.* (2004); *Cassiopea* sp.4 from Palau (Tlake, Kamo and Ongel Lakes); *Cassiopea* sp.6 from Palau (Milki Way Lake and NGE Lake) as defined by Arai *et al.* (2017) and *C. andromeda* from Bermuda, Florida Keys, Hawaii, Red Sea and Indian Ocean. The last group includes the Mediterranean samples. Moreover, *Cassiopea frondosa* from Holland *et al.* (2004) split from all the other groups.

The seven units obtained in the NJ tree were compared, calculating sequence divergence as Kimura 2-parameter method. These values ranged from 7% (found in *C. ornata* vs *Cassiopea* sp.1) to 21.9% (found in *C. andromeda* vs *C. frondosa* and in *C. sp.6* vs *C. frondosa*) (Table 2). Sequence divergence intragroups was always lower than between groups.

Within groups molecular divergence calculated with the Kimura 2-parameter model within *C. andromeda* lineage ranged from 2.2% (*C. andromeda* from Mediterranean vs *C. andromeda* from Brazil) to 0% (*C. andromeda* from Florida vs *C. andromeda* Bermuda) with an average value of 1.6% (Table 3).

Table 1. List of *Cassiopea* spp. sequences from GenBank used for primer design, molecular and phylogenetic analysis in the present work

Species identified as	Sampling site	Accession no.	Reference
<i>C. ornata</i>	South Cassiopea lake, Palau	LC198724	Arai & Hanzawa (unpubl.)
<i>C. ornata</i>	NGE lake 2, Palau	LC198725	
<i>C. ornata</i>	NGE lake 1, Palau	LC198726	
<i>C. ornata</i>	NGE lake 1, Palau	LC198727	
<i>C. ornata</i>	Milky Way lake 2, Palau	LC198728	
<i>C. ornata</i>	Milky Way lake 2, Palau	LC198729	
<i>C. ornata</i>	Milky Way lake 2, Palau	LC198730	
<i>C. ornata</i>	Milky Way lake 2, Palau	LC198731	
<i>C. ornata</i> / <i>C. sp.1</i>	Palau	LC198735	
<i>C. ornata</i> / <i>C. sp.1</i>	Palau	LC198736	
<i>C. ornata</i> / <i>C. sp.1</i>	Palau	LC198737	
<i>C. sp.1</i>	Palau	LC198738	
<i>C. sp.1</i>	Palau	LC198739	
<i>C. sp.1</i>	Palau	LC198740	
<i>C. sp.1</i>	Palau	LC198741	
<i>C. sp.1</i>	Palau	LC198742	
<i>C. sp.1</i>	Palau	LC198743	
<i>C. sp.1</i>	Palau	LC198744	
<i>C. sp.1</i>	T Lake, Palau	LC198745	Arai <i>et al.</i> (2017)
<i>C. sp.1</i>	T Lake, Palau	LC198746	
<i>C. sp.4</i>	T Lake, Palau	LC198747	
<i>C. sp.4</i>	T Lake, Palau	LC198748	
<i>C. sp.4</i>	T Lake, Palau	LC198749	
<i>C. sp.4</i>	T Lake, Palau	LC198750	
<i>C. sp.4</i>	T Lake, Palau	LC198751	
<i>C. sp.4</i>	T Lake, Palau	LC198752	
<i>C. sp.4</i>	T Lake, Palau	LC198754	
<i>C. sp.2</i>	Palau	LC198755	
<i>C. sp.2</i>	Palau	LC198756	
<i>C. sp.2</i>	Palau	LC198757	
<i>C. sp.2</i>	Palau	LC198758	
<i>C. sp.2</i>	Palau	LC198759	
<i>C. sp.2</i>	Palau	LC198760	
<i>C. sp.2</i>	Palau	LC198761	
<i>C. sp.2</i>	Palau	LC198762	
<i>C. sp.2</i>	Palau	LC198763	
<i>C. sp.2</i>	Palau	LC198766	
<i>C. sp.2</i>	Palau	LC198767	
<i>C. sp.2</i>	Palau	LC198768	
<i>C. sp.2</i>	Palau	LC198769	
<i>C. andromeda</i>	Oahu, Hawaii	AY319448	Holland <i>et al.</i> (2004)
<i>C. andromeda</i>	Oahu, Hawaii	AY319449	
<i>C. andromeda</i>	Oahu, Hawaii	AY319450	
<i>C. andromeda</i>	Oahu, Hawaii	AY319451	
<i>C. andromeda</i>	Molokai, Hawaii	AY319453	
<i>C. andromeda</i>	Molokai, Hawaii	AY319454	

(Continued)

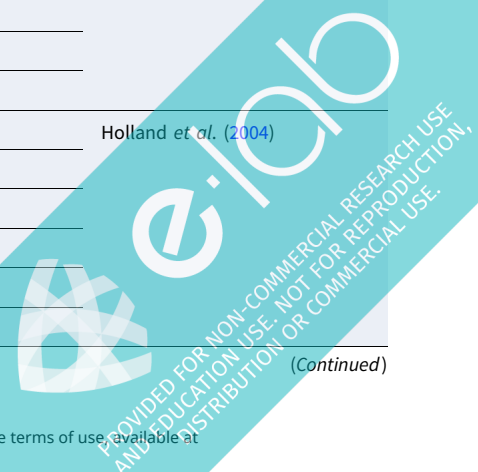


Table 1. (Continued.)

Species identified as	Sampling site	Accession no.	Reference
<i>C. andromeda</i>	Palau	AY319455	
<i>C. andromeda</i>	Palau	AY319456	
<i>C. andromeda</i>	Fiji	AY319457	
<i>C. andromeda</i>	Egypt, Red Sea	AY319458	
<i>C. andromeda</i>	Papua New Guinea	AY319459	
<i>C. andromeda</i>	Papua New Guinea	AY319460	
<i>C. andromeda</i>	Papua New Guinea	AY319461	
<i>C. andromeda</i>	Papua New Guinea	AY319462	
<i>C. xamachana</i>	Bermuda: Walsingham Pond	AY319463	
<i>C. xamachana</i>	Bermuda: Richardson Bay	AY319464	
<i>C. xamachana</i>	Bermuda: Richardson Bay	AY319465	
<i>C. frondosa</i>	USA: Florida Keys	AY319467	
<i>C. xamachana</i>	USA: Florida Keys	AY319468	
<i>C. frondosa</i>	Panama: San Blas Islands	AY319469	
<i>C. frondosa</i>	Panama: San Blas Islands	AY319470	
<i>C. andromeda</i>	Australia: Port Douglas, Queensland	AY319471	
<i>C. andromeda</i>	Indonesia: Kakaban, Kalimantan	AY319472	
<i>C. andromeda</i>	Hawaii	AY331594	
<i>C. andromeda</i>	Oahu, Hawaii	AF231109	
<i>C. andromeda</i>	Brasil	KC464458	Morandini <i>et al.</i> (2016)
<i>C. andromeda</i>	Monterey, USA	KF683387	
<i>C. andromeda</i>	Baja California Sur, Mexico	KY610551	Gómez Daglio & Dawson (2017)
<i>C. andromeda</i>	Bocas del Toro, Panama	KY610560	
<i>C. andromeda</i>	Western Indian Ocean	HF930521	Armani <i>et al.</i> (2013)

Discussion

Accurate taxonomic identification of *Cassiopea* species other than *C. frondosa*, based on morphological characters is not possible due to the high plasticity of characters that are not always systematically informative. Most of the diagnostic characters considered actually vary within a species and may be shared with other

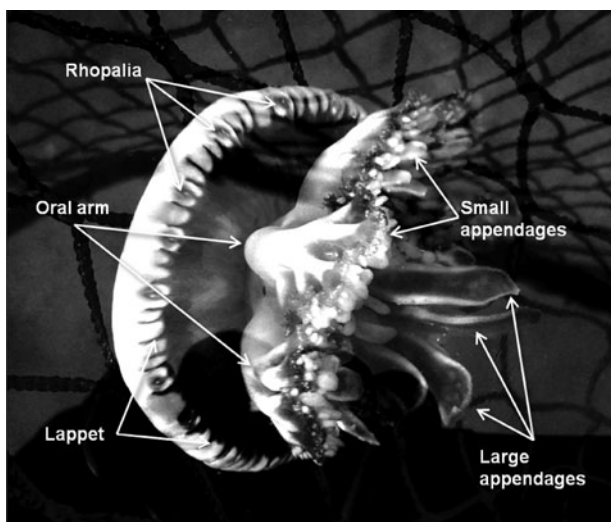


Fig. 2. Adult specimen of *Cassiopea* sp. jellyfish from Palermo Cala Harbour.

species. This has generated confusion in the past, leading to the introduction of presumed new species that were subsequently disclaimed by molecular analyses (Holland *et al.*, 2004; Ohdera *et al.*, 2018). Up to now, the systematic issues of the genus *Cassiopea* are far from being solved and species within this genus are considered cryptic. Jellyfish species identification based only on analysis of morphological characters has always been hard and controversial. Morphological and meristic characters are often variable within the same species and in some cases they overlap between different species. In recent decades, molecular tools have helped systematics, solving species classification and sometimes identifying cryptic species complexes (Dawson *et al.*, 2005; Scorrano *et al.*, 2017); Consequently, several authors have underlined the need to link morphological and molecular approaches to reach an integrative taxonomy and to solve patterns of marine biodiversity (Dawson, 2005; Dayrat, 2005; Wiens, 2007; Scorrano *et al.*, 2017).

Therefore, in the present study molecular tools were used to identify *Cassiopea* specimens belonging to a dense population settled in Palermo Cala Harbour (South Tyrrhenian Sea). This harbour comprises parts with very shallow water (i.e. 0.5 m) or very transparent, which are suitable for the survival of *Cassiopea*, allowing photosynthetic activity of the symbiotic algae. Molecular analyses were essential to identify the Mediterranean species as *Cassiopea andromeda*. In particular, Mediterranean specimens grouped with *C. andromeda* from Red Sea–Hawaii–Florida as identified by Holland *et al.* (2004).

Dawson & Jacobs (2001) studying the *Aurelia* species complex, considering the taxonomic problems in jellyfish species

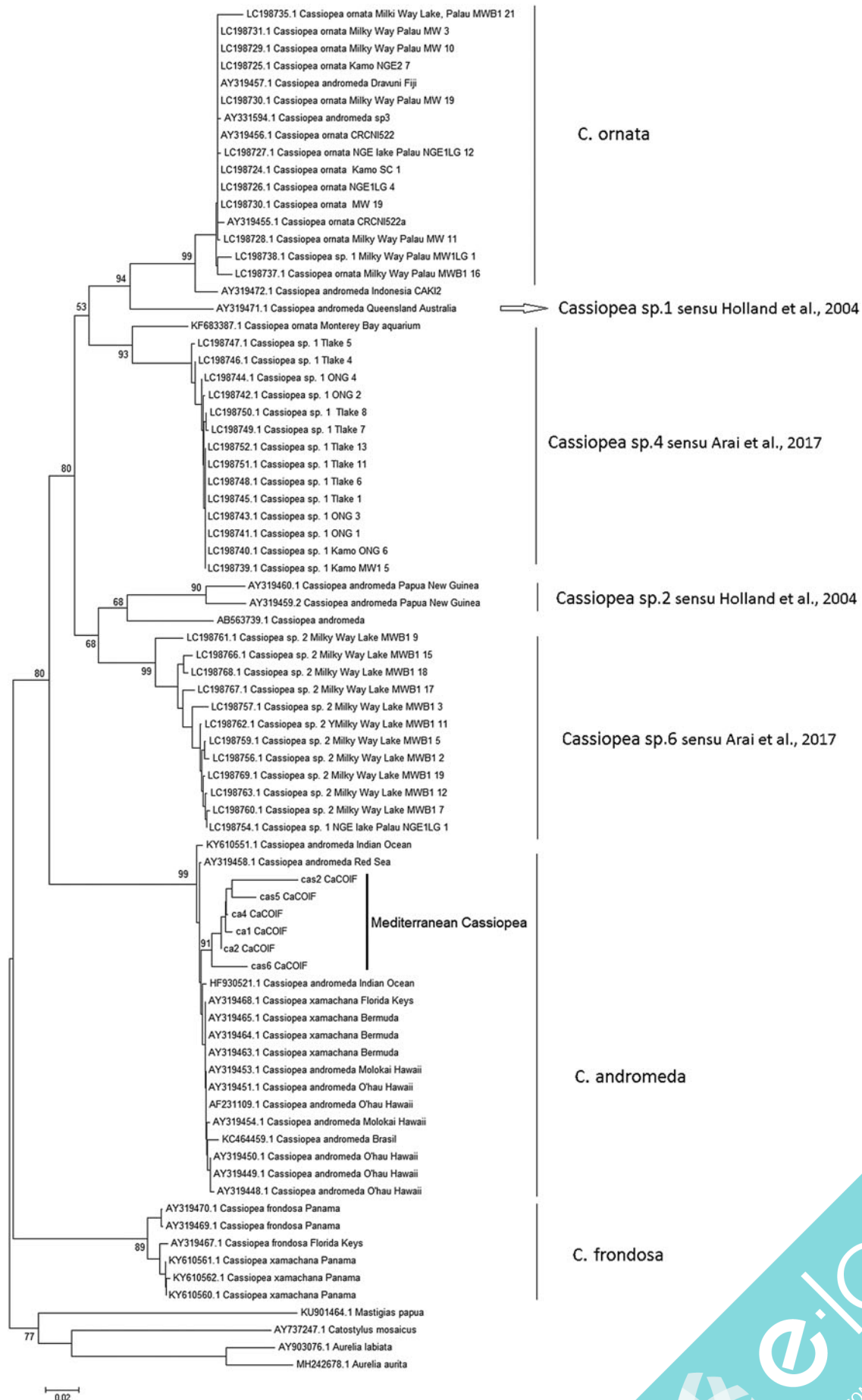


Fig. 3. Phylogenetic reconstruction using Neighbour-joining method among *Cassiopea* spp. Evolutionary distances were computed using the Kimura 2-parameter method and are in the units of the number of base substitutions per site for Cytochrome Oxidase I. Bootstrap values on 1000 replicates are next to the branches.

Table 2. Pairwise genetic distance among the identified groups computed using Kimura 2-parameter substitution model

	<i>C. andromeda</i>	<i>Cassiopea</i> sp.1	<i>Cassiopea</i> sp.2	<i>Cassiopea</i> sp.4	<i>Cassiopea</i> sp.6	<i>C. frondosa</i>	<i>C. ornata</i>
<i>C. andromeda</i>							
<i>Cassiopea</i> sp.1	0.202						
<i>Cassiopea</i> sp.2	0.196	0.186					
<i>Cassiopea</i> sp.4	0.173	0.139	0.156				
<i>Cassiopea</i> sp.6	0.209	0.155	0.131	0.156			
<i>C. frondosa</i>	0.219	0.212	0.206	0.190	0.219		
<i>C. ornata</i>	0.205	0.070	0.195	0.152	0.161	0.213	

Table 3. Pairwise genetic distance computed using Kimura 2-parameter substitution model among *C. andromeda* units

	<i>C. andromeda</i> Mediterranean	<i>C. andromeda</i> Brasil	<i>C. andromeda</i> Florida	<i>C. andromeda</i> Bermuda	<i>C. andromeda</i> Red Sea	<i>C. andromeda</i> Hawaii
<i>C. andromeda</i> Mediterranean						
<i>C. andromeda</i> Brasil	0.0225					
<i>C. andromeda</i> Florida	0.0163	0.0062				
<i>C. andromeda</i> Bermuda	0.0163	0.0062	0.0000			
<i>C. andromeda</i> Red Sea	0.0172	0.0069	0.0007	0.0007		
<i>C. andromeda</i> Hawaii	0.0167	0.0065	0.0003	0.0003	0.0010	

delimitation and identification arising from their high variability, accepted a species limit of 10% for COI. Given this assumption, our results support the assignation of Mediterranean *Cassiopea* specimens to *andromeda* species. Moreover, looking at the *andromeda* lineage obtained with phylogenetic reconstruction, the mean sequence divergence of Mediterranean *Cassiopea* with the other conspecific from Hawaii–Red Sea–Florida is 1.8%. This value, although lower than inter-specific values, is much higher than intra-specific ones. To estimate the divergence time using the molecular clock hypothesis between Mediterranean *Cassiopea* and the other conspecific we use the formula $T = d/2\lambda$ (where d is the nucleotide distance between populations and the mutation rate $\lambda = 4.87 \times 10^{-6}$) (Dawson, 2005). The resulting value indicates that Mediterranean *Cassiopea* specimens diverged from the conspecific from Hawaii–Red Sea–Florida more or less 1600 years ago; this corresponds to the period of the early Middle Ages and so does not fit with the Mediterranean first record of the species. Two different scenarios can be proposed to justify the high value of genetic divergence in the Mediterranean *C. andromeda* population: the first would imply that the species found a suitable ecological niche in the partially isolated Palermo Cala Harbour with good conditions to settle, grow and possibly to reproduce, with potential to become permanently established in this area. This could subsequently lead to local adaptation phenomenon although no examples of this are known. *Cassiopea* requires relatively clear shallow waters to allow symbionts to photosynthesize and it can find these conditions in the shallow parts of Palermo Cala Harbour; but local conditions outside of this enclosed area may not be suitable due to higher water column depth and turbidity. During recent years, in this site we have observed periods of *Cassiopea* blooms alternating with periods with occurrence of very few or no specimens, probably due to the interaction of the changing environmental conditions and the natural life cycle of the species. When jellyfish disappear, polyp populations probably remain active (colonizing new substrates or even performing asexual reproduction), waiting for an environmental trigger that

induces the strobilation process and a new *Cassiopea* medusae bloom.

Another more plausible scenario would assume that genetic divergence estimated in Mediterranean *C. andromeda* could be inflated by multiple introduction events in the Basin. Vessels visiting different ports could collect and transport different organisms. This would imply a new introduction event and if multiple introductions come from multiple genetically differentiated geographic sources or if transported individuals come from a single genetically diverse source population, an introduction of new genotypes and increasing of genetic diversity of the species in the invaded range would occur. Dawson *et al.* (2005), studying the distribution of the *Aurelia* spp. at global scale, concluded that the assumption of low level of genetic diversity in the introduced species is questionable, mainly because multiple human-mediated introductions can rapidly increase genetic diversity in the non-indigenous population. *Cassiopea* spp. jellyfish have been characterized 'as prime candidates for accidental introduction as a non-native species in places where they do not belong' mainly because of their biological traits (Widmer, 2008); further *C. andromeda* was considered an invasive species (Katsanevakis, 2011) probably due to its ability to grow rapidly producing large blooms in a very short time. The high value of sequence divergence between Mediterranean specimens of *C. andromeda* and those from the Red Sea–Hawaii–Florida could indeed be the result of multiple introduction events mediated by human transport. Since the presence of stable populations in the eastern Mediterranean Sea has been confirmed (Özgür & Öztürk, 2008), it could be useful to analyse their genetic diversity in order to verify multiple introduction events and then reconstruct the invasion pattern.

In recent decades, jellyfish bloom events have been increasing in frequency in many coastal areas, altering ecosystem functions and consequently ecosystem services. Several factors are identified as some of the main drivers responsible for jellyfish bloom increase: overfishing activity; eutrophication and hypoxia; warming of

seawater temperature; and the increase in artificial substrates expanding the potential attachment sites for polyps (Condon et al., 2013). All these factors occurring together act synergistically, increasing their singular effect. Undoubtedly *Cassiopea*, among jellyfish, is a successful invader mainly because of its life history features such as prolific asexual reproduction (Schariti et al., 2014) as well as high tolerance to environmental variation (Morandini et al., 2016). Its presence has been empirically linked to human impacts, either by transport through maritime traffic or by eutrophication favouring blooms. Recently, Stoner et al. (2011) demonstrated that populations of *Cassiopea* spp. are larger and more abundant in human-impacted coastal systems in the Bahamas, suggesting that human activity may trigger or facilitate blooms of this species, producing cascade effects on ecosystems. Further the high potential of *Cassiopea* to become established and then spread seems to be increased by the presence of an exogenous chemical compound acting as a natural inducer of metamorphosis (Hofmann et al., 1996).

The *C. andromeda* population detected in Palermo Cala Harbour (present study) seems to be confined to this artificial habitat, with optimal environmental features for its growth and reproduction, as confirmed by rapid surveys of the surrounding coastal areas that did not reveal any occurrence of this species. It is possible that this environment could act as a rearing site from which *Cassiopea* could be dispersed by vessels in other more or less distant places. For this reason, monitoring and research of this species should be strengthened in order to define management strategies for limiting its invasion, impact on ecosystems and spread elsewhere.

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