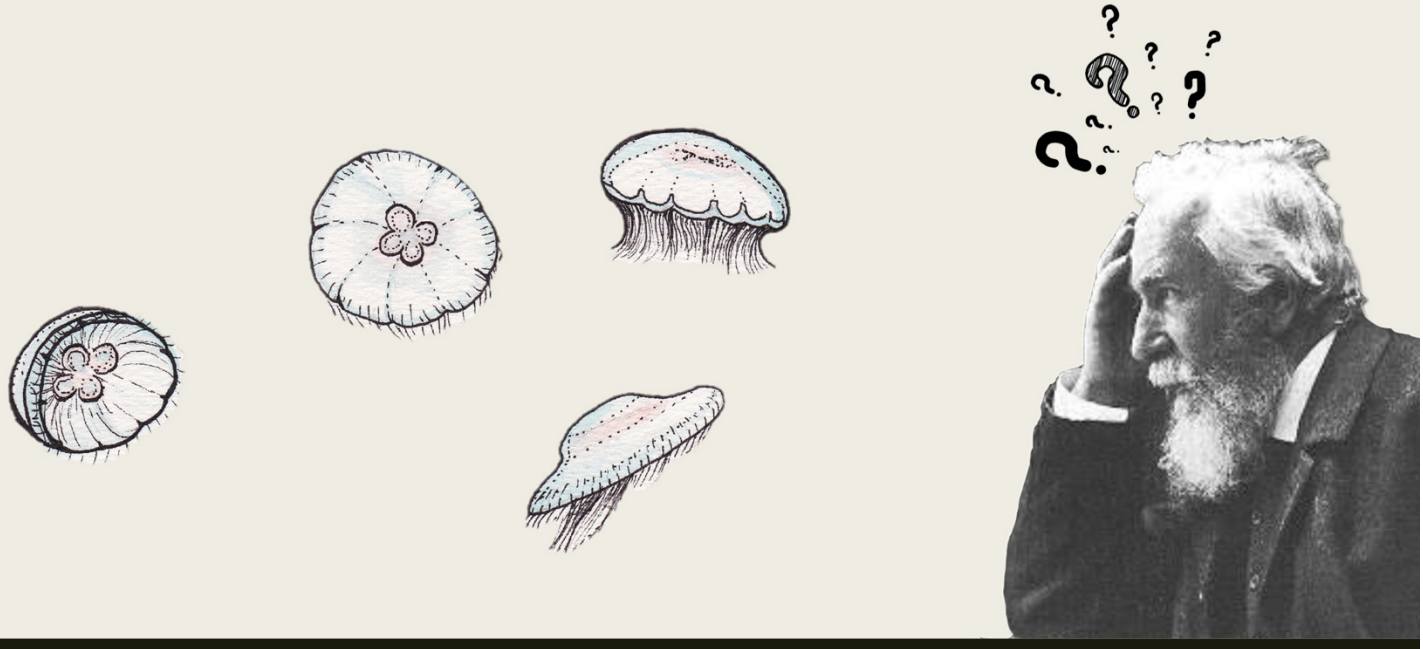
Jonathan Wanderley Lawley

Delimitação e descrição de espécies crípticas: lições da sistemática de *Aurelia* (Cnidaria, Scyphozoa)

Delimitation and description of cryptic species: lessons from the systematics of *Aurelia* (Cnidaria, Scyphozoa)



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Dissertação apresentada ao Instituto de Biociências da Universidade de São Paulo, para a obtenção de Título de Mestre em Ciências, na Área de Zoologia.

Orientador: Prof. Dr. André Carrara Morandini

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Prof(a). Dr(a).

Prof(a). Dr(a).

Prof. Dr. André Carrara Morandini

Me decís qué espera la ascidia en su campana transparente?
qué espera?
Yo os digo,
espera como vosotros el tiempo.

You ask what the luminous bell of the tunicate awaits in the water:
what does it hope for?
I tell you,
it waits for the fullness of time,
like yourself.

Pablo Neruda, *Los enigmas*

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Disclaimer

None of the zoological names and nomenclatural acts in this thesis are published for purpose of zoological nomenclature. This is a disclaimer with reference to Article 8.2 of the International Code for Zoological Nomenclature (ICZN, 1999).

General Introduction

"All systematists today, whether they like it or not, are Hennigian cladists" (Wheeler, 2012: 19). This sentence highlights a consensus that appears to exist across the scientific community, that a phylogenetic relationship means a genealogical relationship, and that evidence of these relationships comes from features that are shared and derived (Hennig, 1966). These relationships have been traditionally reconstructed based on morphology, but with the technological and methodological advances that accompanied the end of the 20th century, molecular data started to be included in the framework, and is nowadays an important component of systematic studies (Wheeler, 2012). The integration of genetic data brought not only new findings, but also new questions and issues.

One of these issues arise from the idea of DNA barcodes, that was proposed as the sole mean to overcome taxonomic impediment as we go through a global diversity crisis (Hebert *et al.*, 2003). Many studies since then have adopted barcoding into their studies, but as a neo-phenetic approach, its issues soon started to be acknowledged, at least for species delimitation (Valdecasas *et al.*, 2008; Collins & Cruickshank, 2013). Coalescent theory also brings new possibilities (Knowles & Carstens, 2007), but we are yet to understand how to operationally distinguish the fine-line between population and species level divergences (Sukumaran & Knowles, 2017), and so coalescent-based methods should be used with caution. In this sense, phylogenetic systematics, even though with its caveats, still compose a major component in species delimitation (Grant *et al.*, 2006; Gómez-Daglio & Dawson, 2017).

To some extent, the scientific community seems to agree in a broader species concept, or at least in the separation of the abstract concept of species and the operation of delimiting these units (de Queiroz, 2007). Nevertheless, it seems there is still some confusion. Congruence across methods, or even operational criteria, have been suggested as evidence for species delimitation, and therefore each of the methods and criteria considered would be different lines or sources of evidence (de Queiroz, 2007; Carstens *et al.*, 2013). The use of congruence and the discovery operations it represents, has already been discussed in the context of evolutionary biology (Kluge, 1998; Grant, 2002), yet epistemological issues remain.

Alongside species delimitation, challenges also arise for species descriptions. Molecular data have revealed previously undetected diverging lineages, which could not be readily told apart by morphology (Struck *et al.*, 2017). This hidden diversity, namely

‘cryptic’, has now been uncovered in many metazoan groups (Bickford *et al.*, 2007), and it sometimes creates parallel worlds populated by candidate species without formal descriptions (Jörger & Schrödl, 2013). The transition from species delimitation to description is a first necessary step in recognizing cryptic diversity and reducing nomenclatural confusion, which in turn is important not only for conservation but for many other studies, such as understanding ecological interactions, pathogen spread and disease, and even for chemical and pharmaceutical studies (Bickford et al., 2007).

Recent efforts have attempted to provide genetic data in taxonomic descriptions, although not in a standardized way (Goldstein & DeSalle, 2010). Yet, a consensus that seems to remain is that descriptions should be character-based (Bauer *et al.*, 2011), regardless if these characters are derived from morphology or molecules. Even though there may be no reason to believe that a diagnostic character underlies the nature of species, these provide a comparable framework for species hypothesis, as well as facilitates species discovery and identification (Grant *et al.*, 2006; Bauer *et al.*, 2011). Recent efforts have accepted the undertaking and have described cryptic species based on molecular diagnostic characters, opening a new window in taxonomy (e.g., Jörger and Schrödl, 2013).

In this thesis, I attempted to further discussions on issues involving species delimitation, as well as perform delimitation and formal descriptions. The scyphozoan jellyfish genus *Aurelia*, long known to potentially encompass cryptic species (Dawson & Jacobs, 2001), was used in this context. In **Chapter 1**, a discussion is presented on the misconceptions and the epistemology that encircles species delimitation, currently applied methods, and the nature of evidence and discovery operations. In **Chapter 2**, initially, morphological analyses were performed, in order to better understand morphological variation that can occur in *Aurelia* medusae. With this data, alongside molecular analyses and recorded distributions, species were delimited and described for the genus. With this study, I expect to encourage and improve the framework for cryptic species delimitation and description, which I believe will help forward not only discussions on systematics and taxonomy, but also on an important second step to understand morphological plasticity and the patterns and processes involved in generating crypsis.

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Chapter 1

Formatted for submission as Opinion Piece, to Trends in Ecology and Evolution

How to provide evidence-based species delimitation: on discovery operations, methods and congruence

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Keywords

species boundaries, coalescent, method congruence, evidence, phylogenetics, population genetics

Highlights

The increasing accessibility to molecular data and their potential to aid on the reconstruction of evolutionary relationships has led to many exciting discoveries, but has been also accompanied by inconsistencies within systematics framework.

Here we demonstrate the epistemological issues of method congruence as evidence for species delimitation, as these are merely exclusive discovery operations and have no value as multiple lines of evidence.

Some methods are discussed, as well as their issues, but our main goal is to advise the scientific community of caution on the choice of methods, especially when considering molecular data, so that they comply with the foundations of phylogenetic systematics, which if fallible, is still a useful tool for species delimitation.

Glossary

Coalescence: a model that estimates genealogies backward in time to a common ancestor.

Datum (singular of data): result of an observation.

Discovery operations: the subset of methods that aim to generate and test scientific hypotheses. If the differences between discovery operations lie entirely in their treatment of the data they are exclusive, and if otherwise they measure different properties of the same putative thing they are complementary.

Evidence: a datum that is related to a hypothesis.

Lineage: an ancestor-descendent series.

Metapopulation: an inclusive population made up of connected subpopulations.

Observation: result of a direct and selected perception.

Outstanding Questions

How much evidence is necessary to untangle the boundaries between species?

How do we operationally distinguish between population structure and species boundaries?

How can molecular diagnostic characters impact molecular species identification? Can be a more useful tool than traditional similarity-based barcoding?

Abstract

Species are vital units for research in ecology and evolution, both in terms of the concept but even further in the operation of identifying boundaries and diversity, called species delimitation. Many methods for delimitation have been devised, more traditionally based on morphology, but more recently on molecular data, especially with the associated recent technological advances and increasing accessibility. These molecular-based methods tend to be devised, and even applied, more rapidly then they are critically analyzed, and should therefore drive caution in the scientific community. Nevertheless, recent proposals have advised that congruence across these methods can encompass their shortcomings, which should be used as a more robust approach for species delimitation. We herein discuss that this framework is problematic, as the congruence across exclusive discovery operations (methods) based on a datum cannot provide further evidence for delimitation. For a robust species delimitation, we argue the

importance of multiple lines of evidence, which can only be achieved by analyzing different datasets. Furthermore, we advise the careful choice of methods for the analysis of each datum, placed with an operational and epistemic justification for their use.

Taking a Step Back on Species Delimitation and the Species Concept

Species are fundamental units in ecological and evolutionary studies, and their objective documentation is one of the main goals of systematic research (Frost & Kluge, 1994; Sites & Marshall, 2004; de Queiroz, 2007). As we face biodiversity loss incurred from climate change and direct human influences (Pimm *et al*., 1995), this documentation becomes an increasingly important task. Traditionally, identifying boundaries between species and their diversity, herein treated as species delimitation, is based on morphological data. With increasing technological advances, the accessibility to molecular data has grown and many methods have been devised to analyze these data for species delimitation, increasing the repertoire of possible observations (Sites & Marshall, 2003; Carstens *et al.*, 2013). Nevertheless, the multitude of methods devised in a short period of time makes it difficult for the scientific community to clearly and critically assess them (Sukumaran & Knowles, 2017), which should lead researchers to be cautious, and seek for both operational and epistemological justifications for their use.

For a long time, the debate on the concept of species, also known as the "species problem", was confused with species delimitation (for a review of some contemporary concepts see de Queiroz, 2007). In an attempt to solve that problem, many authors had been discussing the benefits in separating the abstract concept of species from the underlying operations used to delimit species (Kluge, 1990; Frost and Kluge, 1994; Brooks & McLennan, 1999; Grant, 2002; Sites & Marshall, 2003, 2004). In that sense, de Queiroz (2007) proposed a unified species concept that emphasized the fundamental similarities between previously proposed definitions. This concept, which equates species to separately evolving metapopulation lineages (see Glossary), according to the author, would be separated from the continuum of operational criteria that could be used as lines of evidence in the empirical application of species delimitation. These criteria would be derived from the secondary properties of the alternative species concepts previously used, such as phenetic distinguishability, reciprocal monophyly, reproductive isolation, exclusive coalescence, diagnosability, and so forth. Still, even though unifying many previous species concepts, a possible shortcoming for the definition by de Queiroz appears when we consider uniparental species (asexual or unisexual; see Frost & Wright,

1988). In this case, since there is no gene flow between subpopulations, the use of metapopulation to define species becomes blurred. Due to this scenario, the concept of species as the smallest historical individuals with a parental pattern of ancestry and descent seems more clear and comprehensive (Frost & Wright, 1988; Kluge 1990; Grant, 2002), even though it shares similarities with the proposal by de Queiroz (2007). The species concept problem is a centennial one, and even though these discussions should be furthered, our intention here is to focus on the debate on the methods and philosophy behind species delimitation.

A Brief Commented History on Species Delimitation Methods

Sites & Marshall (2003, 2004) provide one of the first overviews on species delimitation methods. These were mainly divided into non-tree based methods, which focus on delimiting species based on indirect inferences of the presence or absence of gene flow, and tree-based methods, which base species delimitation on the phylogenetic tree topology (hierarchical). Interestingly, even though these authors recognize the separation between species delimitation and species concept, they review delimitation methods in light of their concepts, not their operational criteria (e.g., monophyly is one of the operational criteria, or properties, derived from the phylogenetic species concept).

Despite these reviews, most of the methods described that involved DNA data are currently seldom used. This is likely due to the fact that they were conceived to understand the distribution of allele frequencies, mostly from allozymes (alleles of an enzyme identified through electrophoresis), which were quickly replaced by acquisition of DNA sequence data (i.e., nucleotides) that in turn yielded more fine-scale information on polymorphisms. Since then, other methods have been proposed and are more utilized, many of which are likely based on the ideas underlying the previous methods. One of the main ideas that has gained much appeal over the years is DNA barcoding (Herbert *et al.*, 2003) and its plea to free species discovery from the "frustrating grasp" of traditional taxonomy (Janzen, 2004).

As suggested by Hebert *et al.* (2003), DNA "barcodes" could be developed for species-level assignment of organisms, and they recommended the mitochondrial gene cytochrome c oxidase I (COI) as a good candidate for barcoding all animal life. They further emphasize the limitations of morphology-based taxonomy, such as incorrect identification of specimens due to phenotypic plasticity, presence of cryptic taxa in many groups, and the expertise required for morphological keys and their limitation to a certain

life stage or gender. These all seem valid points, which is what has likely burst biologists into barcoding life. As for all scientific theories and endeavors, the limitations of DNA barcoding soon started to be discussed, concurrent with the arousal of new methods and ideas, one of which came from coalescent theory.

From a population genetics standpoint, Knowles & Carstens (2007) foremost present a discussion on the limitations of exclusivity criteria for species delimitation, such as the property of reciprocal monophyly and also the 10x rule used in DNA barcoding. They argue that exclusivity criteria, even though may present a very utilitarian approach, require a substantial amount of time after initial divergence of species to be observed (see further references and simulation studies cited within Knowles & Carstens, 2007). Therefore, the overlooked discordance of gene genealogies, mainly due to the species-gene-tree discordance problem (Maddison, 1997), could provide important information on the history of species splitting, which can occur before reciprocal monophyly is detected. The authors then suggest, with the results of their simulation study, that a coalescent-based approach that models species history probabilistically, would be able to evaluate the likelihood of lineage splitting when there hasn't been sufficient time for full sorting of ancestral polymorphism. However, one of the limitations pointed out regards sampling, both in terms of loci and individuals, which even highlights further complications for species delimitation with DNA barcodes.

As the popularity and feasibility for sequencing DNA grew, many studies were basing their delimitations solely on molecular data, especially after the development of coalescent-based tools (Olave *et al.*, 2014). Concern on these delimitations increased, as related methodological issues started to gain notice. For example, Olave *et al.* (2014) discuss that coalescent-based species delimitation may be influenced by the prior putative species number and assignment of individuals, which if done solely under coalescent theory, will not be accurate unless large datasets are used (approaching 100 independent loci). They suggest that a more efficient approach would be to gather different data types, such as from morphology and distribution, especially for these prior assignments.

Further criticism to the multispecies coalescent has arisen in that it is not capable to discern population- from species-level lineages, and therefore can only detect genetic structure (Sukumaran & Knowles, 2017). Even when the simulations presented by the authors treated speciation as a continuous process instead of an instantaneous event, which differs from what most coalescent-based methods do, not all lineages that arise can be distinguished as population- or species-level structure. This observation is particularly

interesting in light of the many coalescent-based methods that have been devised and their incongruences (Carstens *et al.*, 2013). As before, Sukumaran & Knowles (2017) recommend that these methods can only be used for species delimitation if there is other data or information that can further corroborate or reject the hypothesis.

Some Issues Regarding Operational Criteria, Method Congruence and Evidence

Foremost, as discussed by de Queiroz (2007) and previous authors, it is both useful and beneficial to separate the abstract concept of species from the operational criteria related to species delimitation. Nevertheless, a confusing issue that arises within the author's discussion is the equivalence of operational criteria (phenetic distinguishability, reciprocal monophyly and others) to *lines of evidence*, which was mentioned herein in the first section. Mahner & Bunge (1997) refer to evidence as the result of an observation (i.e., a datum) relevant to a hypothesis. This observation is based on a fact or thing, and the resulting datum should be acquired by empirical operations (Mahner & Bunge, 1997). For example (as provided by these authors), a barometer reading could be evidence for or against a hypothesis regarding some weather process related to atmospheric pressure (for further discussions see sections on perception, observation, datum and evidence within Mahner & Bunge, 1997). Other authors have also highlighted this relationship between evidence and observation, which associated to background knowledge, compose the premises of a hypothesis (Salmon, 1984; Fetzer & Almeder, 1993; Scheiner, 2004). Therefore, as operational criteria are not data, but otherwise a statement based on data, there seems to be no logical basis to accept them as evidence. Operational criteria can nevertheless be satisfied by evidence. Consider, for example, that a single dataset from hypothetical sibling species, perhaps a mitochondrial marker such as COI, returns reciprocal monophyly from phylogenetic analysis. The reciprocal monophyly operational criteria was satisfied by COI evidence. If another discovery operation (*sensu* Grant, 2002) was performed, such as multivariate statistics from uncorrected pairwise genetic distances, this could satisfy the phenetic distinguishability criteria. However, the discovery operations mentioned above vary only in their treatment of the data and measure the same properties, and therefore *do not provide evidence* (Grant, 2002; see Box 1), which relates to the next issue on evidence for species delimitation that we will discuss.

Box 1. Evidence-Based Construction of Hypothesis in Species Delimitation

As we are always building on prior knowledge, we usually have a set of organisms on which to build species delimitation hypothesis. An observation, as a direct and selected perception, results in a datum, which refers to a fact or thing but it is not *per se* a fact. In the figure below, we present an example with jellyfish ephyrae, previously hypothesized as belonging to the genus *Aurelia*. Datum A is the result of the empirical operation of sequencing a molecular marker from the observed tissue of the ephyrae, and datum B is the result of morphological measurements from these same organisms.

After applying a discovery operation, a datum can become evidence for a hypothesis. In the example, for datum B (morphometric measurements from the organisms), if a multivariate analysis is performed, such as multidimensional scaling (MDS), we can detect three phenetic clusters, as in the figure below. Therefore, morphometric measurements can be evidence for a hypothesis of three species among the analyzed organisms, each represented by one of the detected morphotypes. In the case of datum A, with DNA sequences, spedeSTEM could be performed as a discovery operation, and we would also retrieve three groups, as shown in the figure. The DNA sequences obtained are therefore another line of evidence for our three species hypothesis, based on the specific discovery operation used.

The discovery operations mentioned above, spedeSTEM and MDS, are based on different data, different lines of evidence for the hypothesis, and therefore are complementary discovery operations (green arrows). If we consider another discovery operation used for the same datum, such as if BPP is also performed alongside spedeSTEM, these are recognized as exclusive discovery operations (red box), as they only vary in their treatment of the datum, and therefore not providing further evidence.

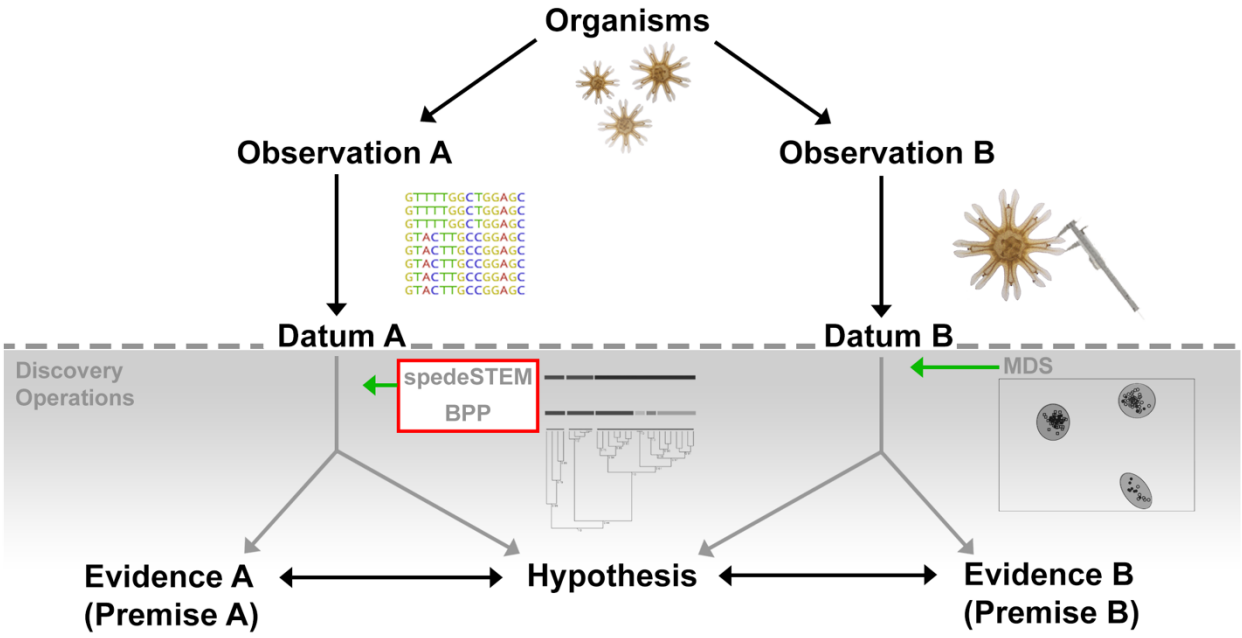

Figure I. Scheme for rationale underlying species delimitation hypothesis, with an example. The organisms represented are jellyfish ephyrae from the genus *Aurelia*. The grey shaded area represents the instance in which discovery operations are performed, the red box representing exclusive discovery operations, and green arrows complementary discovery operations. The image related to spedeSTEM and BPP is adapted from Satler *et al.* (2013) and the one related to the MDS is adapted from Chiaverano *et al.* (2016).

Carstens *et al.* (2013) provide a more recent overview of species delimitation methods, especially considering those more closely related to population genetics and phylogeography, such as genetic clustering or coalescent-based approaches. Within this review, they suggest that researchers should apply more than only a handful of available methods and trust the congruence across them, as this would compensate for violated assumptions in any of the methods. As Grant (2002: 100) mentions, "scientific studies that apply multiple exclusive discovery operations [operations that lie entirely in their treatment of the data] to the same dataset as a test of competing hypothesis have no evidentiary basis for the conclusions they draw and thus amount to nothing more than mere sophistry". Once again, evidence can only be provided by discovery operations that measure different properties of the same putative thing, and as the problem and the data are the same when considering method congruence, it is logically impossible for those operations to be measuring different properties (Grant, 2002). This would be the case if,

following the previously mentioned example, we included morphological data of the sibling species from which COI was being considered.

There seems to be no epistemological relevance for the argument of Carstens *et al.* (2013) on method congruence. Even though they point out that incongruence in results indicates individual shortcomings of the methods used, it is neither an empirical test nor a heuristic procedure, as it does not evaluate the amount of evidential support and is therefore unable to identify hypotheses that are weakly corroborated (Grant & Kluge, 2003). Method congruence reveals only the degree to which the different analyses lead to the same or different conclusions (Grant, 2002). Nevertheless, many recent publications still seem to seek for methodological congruencies from a datum (*sensu* Mahner & Bunge, 1997) as evidence, even though some of them do not base their species delimitations solely on it (Berbel-Filho *et al.*, 2018; Cheng *et al.*, 2018; Hawlitschek *et al.*, 2018; Hsieh *et al.*, 2018; Leliaert *et al.*, 2018). As it is logical to expect that accurate methods converge, it might seem counterintuitive to acknowledge that this does not necessarily mean that convergent methods are accurate (Grant & Kluge, 2003).

Congruence aside, the concern that coalescent-based methods, reviewed in Carsterns *et al.* (2013), delimits structure and not species has already been discussed (Sukumaran & Knowles, 2017). In this same line, DNA barcoding has been criticized for its use in species delimitation (Collins & Cruickshank, 2013). These authors discuss that genetic dissimilarity thresholds are arbitrary and instead, should be optimized based on the data. If there is overlap between intra- and interspecific distances, which the authors refer as likely more common than expected, the barcodes would be uninformative. Also, genetic distances do not differentiate between symplesiomorphy and synapomorphy and so fail to explain observed variation (Grant *et al.*, 2006). Nevertheless, as pairwise distances do not require detailed analysis, they could be used as a good proxy for species identity, but not for delimitation (Grant *et al.*, 2006; Collins & Cruickshank, 2013).

Phylogenetic Systematics and Concluding Remarks

Phylogenetic analyses, if fallible, is still a valid method for species discovery (Frost *et al.*, 1998). It has its shortcomings for the delimitation purpose, such as imposing hierarchy on lineages that might be related tokogenetically, and the subjectivity of species limits when terminals are specimens (Grant *et al.*, 2006). Ultimately, individuation of species requires diagnostic characters, and their discovery is facilitated by phylogenetic analyses (Grant *et al.*, 2006). Regardless, of all the methods herein discussed, the goal for

corroborating a species delimitation hypothesis should be to seek for *evidence* (e.g., different data types, such as morphology, DNA, geographical distribution and so forth). Incongruence between the analyses of different evidence would only indicate need for further study. Also, further development of coalescent-based methods and simulation studies, aside recent developments and increasing affordability for high-throughput sequencing, will certainly forward the field. With a growing body of evidence, we recommend the thorough and justified choice of methods, instead of seeking for congruency.

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Chapter 2

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Morphological plasticity hinders diagnosability: recognizing cryptic diversity in *Aurelia* (Cnidaria, Scyphozoa)

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Keywords
species delimitation, jellyfish, taxonomy, systematics, synapomorphy, DNA barcoding

Abstract
Increased ease to obtain molecular data has brought not only exciting possibilities and discoveries but also challenges. Integrating all useful evidence, not only from molecules, seems paramount as one of these challenges, which are posed on the construction of species hypothesis. Yet, discriminating among useful evidence and how to interpret them can be a daunting task, which is further complicated by cryptic species. These have now been detected across metazoan groups, and while there may be no morphological features to distinguish them, this should not impede taxonomists from formal descriptions. Some studies have accepted this challenge, which involves the employment of taxonomic requirements, such as the presentation of diagnostic characters. We also embraced this challenge for the jellyfish genus *Aurelia*, which has a long and confusing taxonomic history, with recent studies delimiting at least another 16 species from the previously

recognized based on morphology. We demonstrate that morphological plasticity in medusae overlaps across very distinct geographic localities. Even though some morphological features seem responsible for most of the variation across specimens, there is neither a regional geographic structure on dissimilarities, which could be useful for local species distinctions. This is further emphasized by the morphological differences found in the comparison of lab-cultured *Aurelia coerulea* medusae with the diagnostic features in the species' description. Previous studies have also highlighted the difficulties in distinguishing polyps and ephyrae across *Aurelia* species, as well as the potential for morphological plasticity in these other life cycle stages. Therefore, mostly based on molecular data, we recognize 28 species in this genus, of which 6 were already described, 17 are newly described and 4 are resurrected. We present diagnostic molecular characters for mitochondrial and nuclear markers for all species, as well as type material for the newly described, filling the current taxonomic requirements. Recognizing this diversity is paramount as a taxonomic service not only for conservation efforts, but for further studies. These could seek to understand the practical implications and uses of molecular diagnostic characters, as well as focus on the patterns and processes that generate crypsis.

1. Introduction

The accessibility and usage of molecular data for species delimitation has grown, and there have been proposals of a new integrative taxonomy that accounts for it (Dayrat et al., 2005). Yet it seems that taxonomy has always been integrative with the resources it had at hand, and with the advent of new technologies, it poses no surprise that the derived information should also be included (Valdecasas et al., 2008). This framework should therefore integrate different lines of evidence, such as morphology, distribution, behavior as well as molecular data (for more on evidence-based species delimitation see Lawley et al., unpublished manuscript, chapter 1 herein). But more importantly, integration should be exercised with caution, in order to discriminate among useful evidence and their interpretation to construct species hypotheses (Valdecasas et al., 2008). For example, coalescent theory, even though may bring exciting developments to systematics (Wiens, 2008), can present an issue in that it delimits structure, but not necessarily species (Sukumaran and Knowles, 2017). Further studies have also drawn attention to issues with method congruence (Lawley et al., unpublished manuscript, chapter 1 herein), as well as DNA barcoding (Goldstein and DeSalle, 2010; Collins and Cruickshank, 2013) for species delimitation.

Usage of DNA barcodes has been proposed as a tool for rapid species identification, with the promise of relieving taxonomists of this burden in the imminence of a biodiversity crisis (Hebert et al., 2003, 2004; Webb et al., 2006). Issues already begin with how the term 'species identification' has been used in the barcoding literature, in some cases referring to specimen identification, while in others to species discovery or delimitation (Collins and Cruickshank, 2013). There are also issues in assuming a distance threshold for either specimen identification or delimitation, as intraspecific distance for one species may exceed interspecific distances for others (Wiemers and Fiedler, 2007). There are other issues with barcoding, such as when reference databases are incomplete, when there are misidentified sequences and more (Collins and Cruickshank, 2013). In spite of these caveats, improvements have been made, for example, to optimize thresholds for specific datasets and assess their viability for certain groups (Virgilio et al., 2012). From barcoding and beyond, molecular data are vital to the identification of cryptic species, which are two or more distinct species previously unrecognized due to at least apparent morphological resemblance (Bickford et al., 2007).

Cryptic species seem to occur across all metazoan taxa and biogeographic zones, and some studies have suggested phylogenetic and ecological patterns on the distribution of this phenomenon (Bickford et al., 2007; Pfenninger and Schwenk, 2007). However, molecular data have only recently been applied to species discovery, and it is highly questionable whether it has been studied thoroughly and randomly across taxa to confidently assume any patterns (Trontelj and Fišer, 2009). It may also seem overly simplistic to generalize cryptic species diversity to phyla, as there is an astounding variety of speciation-related processes that occur at the genus level (Trontelj and Fišer, 2009; see Coyne and Orr, 2004). The lack of morphological characters to distinguish species should further research to deepen understanding of morphological variation and acknowledgement of cryptic diversity. The challenge is posed on the shift from species delimitation to species descriptions. Recognizing this diversity is essential not only for conservation efforts to define priorities and avoid local extinctions, but also for understanding patterns and processes that generate crypsis, an essential next step (see review in Bickford et al., 2007 and further discussions in Struck et al., 2017).

Taxonomy remains incomplete if discovered entities are not formally described, and species hypothesis are flagged as merely putative, creating parallel worlds populated by numbered candidate taxa (Jörger and Schrödl, 2013). The urgency set on the collapse of taxonomic expertise and the use of molecular data as the only solution for sustainable

identification (Hebert et al., 2003), should lead us to reconcile the precise mechanics of these data with the empirical and philosophical rigor of systematics and taxonomy (Goldstein and DeSalle, 2010). Without formal descriptions and testable hypotheses, the discovered species may not be properly documented nor associated to vouchered specimens deposited at museums, and there can be confusion in the different naming or numbering of detected lineages, thus refusing a taxonomic service (Jörger and Schrödl, 2013; Pleijel et al., 2008). Many attempts have been made to incorporate DNA sequence information in taxonomic descriptions, such as including the GenBank accession numbers, the DNA barcode sequence, raw distance measures and phenetic or phylogenetic trees, but rarely including diagnostic characters (see review in Goldstein and DeSalle, 2010). Nevertheless, a consensus that seems to remain is that species descriptions should be character-based (Bauer et al., 2011).

Even though it may be artificial to assume that the biological reality of a species depends on a number of diagnostic characters, it provides a fallible and comparable framework in which to build species hypothesis (Grant et al., 2006; Bauer et al., 2011), as well as it is a requirement for new species names in the International Code of Zoological Nomenclature (further treated as ICZN; 1999, Article 13.1.1., also see definition for 'character' in its Glossary). There are now computational tools that can provide diagnostic molecular characters, such as CAOS and YBYRÁ (Sarkar, 2008; Machado, 2015). While CAOS identifies diagnostic character states with reference to the species hypothesis, informed by the user through a guide tree, YBYRÁ categorizes transformation events considering all possible optimization schemes in the input trees (Sarkar, 2008; Machado, 2015). Even though these programs compile and evaluate diagnostic characters under different strategies, which are yet to be assessed, they provide a basis for the description of cryptic species.

In the moon jellyfish genus *Aurelia*, the subject of the present study, taxonomic history dates back to the 18th century, starting with the description of the type species *Aurelia aurita* (Linnaeus, 1758). Since then, this genus has encompassed as many as 8 (Haeckel, 1880), 13 (Mayer, 1910, considering varieties), or 7 species (Kramp, 1961). More recently, only 2 species were considered, *Aurelia limbata* Brandt, 1835, which has a brown bell margin and is primarily from temperate regions, and a cosmopolitan near shore inhabitant *A. aurita*, which included as synonyms most of the previously used names (Larson, 1990; Arai, 1997). In the 2000s, two species (*Aurelia labiata* Chamisso and Eysenhardt, 1821 and *Aurelia marginalis* Agassiz, 1862) were resurrected based on

morphological and geographical differences (Gershwin, 2001; Calder, 2009), and with the incorporation of molecular data, there were indications of at least another 16 species, some of which are hypothesized to have been introduced (Dawson and Jacobs, 2001; Schroth et al., 2002; Dawson et al., 2005; Goméz-Daglio and Dawson, 2017). Later studies sought to reassess morphological features, taking into consideration morphometric data and not only in the medusa stage (Dawson, 2003), but also in other life cycle stages, such as polyps and ephyrae (Gambill and Jarms, 2014). A recent result of the integration of these morphological reassessments with molecular data described three species in the Mediterranean, linking previous candidate species to valid names (Scorrano et al., 2016). However, some of the reported diagnostic morphological features seemed to overlap across these species, and there seemed to be considerable morphological plasticity, especially in the resurrected *Aurelia coerulea* von Lendenfeld, 1884 (see Fig. 6 in Scorrano et al., 2016). Other findings further demonstrate the potential for ecophenotypic plasticity in *Aurelia* medusae (Chiaverano et al., 2016), as well as in the other stages of the life cycle (Chiaverano and Graham, 2017).

In the present study, we re-examine the use of morphological data in *Aurelia* medusae, the most conspicuous and collected of the life cycle stages, as well as present a molecular phylogeny for the genus, based on mitochondrial and nuclear markers. Also, we evaluated previous morphological diagnosis proposed for species, based on lab-cultured individuals. Alongside recorded geographic distributions, this provided a framework to delimit and describe species, as well as identify new geographical occurrences and potential introductions. With this study, we hope to encourage the transition from species delimitation to description, advance discussions on practical applications and improvements to molecular taxonomy, and expand perspectives for morphological studies to address questions regarding morphological plasticity and the evolution of cryptic diversity.

2. Material and Methods

2.1. Morphological data collection

Observations were made on living medusae from aquariums in the USA, and on preserved medusae from museums and universities from the USA, Brazil and Denmark, totalizing 173 specimens (Table 1; for more detailed information, see Supplementary Material S1). Two live medusa from the cultures of the laboratory at the University of São Paulo, identified from molecular analyses (presented further) as *Aurelia coerulea*, were also

used, but only for the purpose of a direct comparison with the species' description in Scorrano et al. (2016). Specimen observations included scaled photographs and measurements of features that involved "depth" or "thickness", which could not be acquired later from the photographs. When necessary, a stereomicroscope was also used for observations. Morphological measurements were acquired from scaled photographs with the program Fiji (Schindelin et al., 2012).

Table 1. Institutions from which specimens were observed and included in this study. N = number of specimens. Acronyms of other institutions cited, mostly in the species descriptions are as follows: CAS/CASIZ = California Academy of Sciences, Invertebrate Zoology, USA; M0D = University of California, Merced, USA; MCZ = Museum of Comparative Zoology, Harvard, USA; UNIPD = Museum of Adriatic Zoology Giuseppe Olivi, Italy; UNIS_SCY = Laboratory of Zoology and Marine Biology in the University of Salento, Italy.

Institution	**City, Province**	**Country**	**Live/ Preserved**	**N**
Zoological Museum of the University of Copenhagen (ZMUC)	Copenhagen	Denmark	Preserved	25
Yale Peabody Museum of Natural History (YPM)	New Haven, CT	USA	Preserved	24
Smithsonian Institution's National Museum of Natural History (USNM)	Washington, DC	USA	Preserved	81
Federal University of Ceará (UFC)	Fortaleza, CE	Brazil	Preserved	1
Federal University of Bahia (UFBA/MZUFBA)	Salvador, BA	Brazil	Preserved	3
National Aquarium (NA)	Baltimore, MD	USA	Live	2
Museum of Zoology of the University of São Paulo (MZUSP)	São Paulo, SP	Brazil	Preserved	3
Laboratory for Cnidarian Studies and Cultivation of the University of São Paulo (LAB)	São Paulo, SP	Brazil	Preserved	20

Florida Museum of Natural History (FLMNH)	Gainesville, FL	USA	Preserved	1
American Museum of Natural History (AMNH)	New York, NY	USA	Preserved	5
Discovery Place (DP)	Charlotte, NC	USA	Live	8

Characteristics observed from medusae mainly followed Chiaverano et al. (2016), which included 26 characters, comprising continuous, meristic, and categorical features (Supplementary Material S2, characters *f* + number; Fig. 1, in white and black). Twenty extra characters were added (Supplementary Material S2, characters *f* + letter; Fig. 1, in green), novel or from previous studies (Gershwin, 2001; Dawson, 2003), mainly in an

attempt to unambiguously characterize categorical features, after observing their variation.

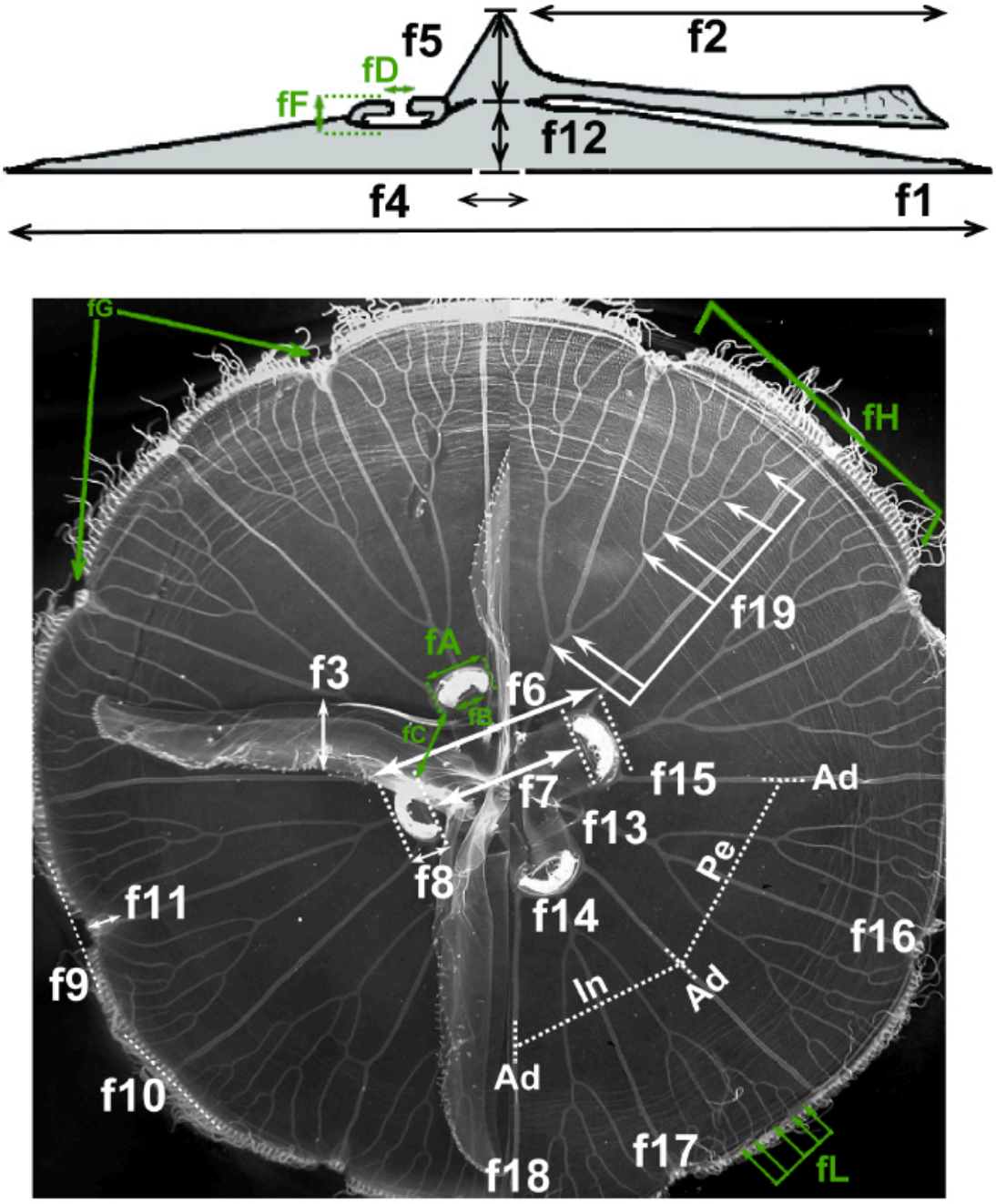


Figure 1. Cross-sectional (top) and subumbrellar (bottom) views of an *Aurelia* medusa, illustrating most of the continuous and meristic morphological features measured in this study. Characters from Chiaverano et al. (2016) appear in white and black, while novel or from previous studies appear in green. For more details and all features measured see Supplementary Material S2. (Modified from Chiaverano et al., 2016).

2.2. Morphological data analyses

To account for differences in shape, morphology must be characterized regardless of size. As continuous and meristic features in our dataset may vary with size (i.e., bell diameter - *f*1), we scaled all individuals to the same *f*1 by adapting the method of Lleonart et al. (2000), which considers potential allometric differences that can occur between species or even within species across geographic localities. Specimens analyzed were therefore separated into geographic localities defined by countries, and usually also by region within the country (e.g., southeast – SE). Size corrections followed the formula $Y^{*}=Y_i (f1_m/f1_i)^b$, in which the desired size-corrected feature (Y^{*}) equals its measurement in a specimen (Y_i) times the ratio between the average bell diameter in the locality group ($f1_m$) and the bell diameter of the specimen ($f1_i$), this raised to the power of the slope of the relationship between both log-transformed variables *Y* and *f*1 from the entire dataset (*b*), as we did not have enough samples per locality group to obtain significant relationships.

Some morphological characters in 104 of the observed specimens were damaged, missing, or could not be measured by photographs. In this case, prior to the size correction mentioned above, we adapted Lleonart's method to perform estimations of these missing data, using the same formula as before, but considering Y_i as the missing variable to be estimated and Y^{*} as the average of the given variable in the locality group. When data for the variable were not present within the locality group, we used data from the closest locality, also accounting for morphological similarity when possible (see Supplementary Table S1).

Features that were mostly invariable or that lacked a significant relationship with *f*1 were removed from further analyses, as they could bias the resulting dataset. Lastly, we normalized all variables to scale to a minimum of 0 and maximum of 1. Categorical features were excluded from analyses, as they may not be reliable due to the ambiguity seen in the specimens observed. Number of rhopalia (*f*G) and number of lobes (*f*H) were also removed as not to bias the results due to potential asymmetric development that may occur in some specimens.

In order to compare observed specimens based on the size corrected continuous and meristic morphological characters described above, we performed multidimensional scaling (MDS, Gower distance), with weighted average scores of variable contributions also mapped within. These analyses were separated into two sets, one that included all specimens with estimated missing data and subsequent size correction of variables, and another that excluded specimens with missing data, and therefore only size corrections

were performed, to check for potential biases in estimations. Regarding these analyses sets we also computed the relationship between geographic distance, in km, and morphological differences (Gower distance), excluding aquarium specimens. The comparison of morphological measurements of *Aurelia coerulea* from lab cultures and the description was performed by Welch's t-test. All of the corrections, estimations and further analyses mentioned above were performed using the software R version 3 (R Core Team, 2016) and the codes used are available on GitLab (gitlab.com/jonathan.lawley01, pending upload).

2.3. Molecular data collection

DNA was extracted from oral arms of medusae, entire polyps, or entire ephyrae from specimens collected in the field or cultured in the laboratory at the University of São Paulo, using a protocol based on ammonium acetate, adapted from Fetzner (1999) (see Supplementary Material S3 for details on samples used for molecular analyses). From the mitochondria, we amplified and sequenced two markers: a ~650-bp fragment of the mitochondrial large ribosomal RNA subunit (16S) and a ~650-bp fragment of the cytochrome c oxidase subunit I (COI) (primers derived from Lawley et al., 2016). From the nuclear genome, we obtained the internal transcribed spacer 1 (ITS1) with ~300-bp in length (primers jfITS1-5F, from Dawson and Jacobs, 2001; and ITS-R-28S-15, from Cunha et al., 2015), and a ~650-bp fragment from the large ribosomal RNA subunit (28S) (primers Aa_L28S_260 and Aa_H28S_1078 from Bayha et al., 2010). Polymerase chain reactions (PCR) followed standard procedures. Thermocycler profiles were conducted with initialization at 95 °C for 3 min, followed by 36-40 cycles of denaturation at 95 °C for 30 s, annealing at 46-58 °C (16S - 46 °C; COI - 52 °C; ITS1 - 57 °C; 28S - 58 °C) for 30-45 s, and extension at 72 °C for 1-2 min. Final extension was further conducted at 72 °C for 10 min. PCR products were purified using Agencourt AMPure XP DNA Purification and Cleanup kit (Beckman Coulter Inc.), and subsequently cycle-sequenced, with the same primers as before, to add fluorescently labeled dideoxy terminators. Chromatograms were generated on an Applied Biosystems 3730DNA at the Botany Department of the University of São Paulo.

2.4. Molecular analyses, species delimitation and descriptions

Sequenced chromatograms were assembled, trimmed and aligned in Geneious ver. 9.1.8 (Kearse et al., 2012), which also included most sequences available in GenBank for

Aurelia and some for *Drymonema dalmatinum* Haeckel, 1880 (Supplementary Material S3), the chosen outgroup taxa that had sequences for all markers herein studied. Alignments were performed using the software's implementation of MAFFT (Katoh and Standley, 2013), with the G-INS-i option and other default parameters, later visualized and edited manually to remove leading and trailing regions that varied in length. Because COI is a protein-coding region, the alignment is devoid of gaps if introns are absent, and therefore the static alignment (sensu Wheeler, 2001) generated with MAFFT was used for phylogenetic analyses. This alignment was submitted to TNT ver 1.5 (Goloboff and Catalano, 2016) and analyzed under parsimony as the optimality criterion, using its New Technology searches (Goloboff, 1999; Nixon, 1999) with the following parameters: consense 10, css, rss, xss, rep 10, ratchet 50, drift 50, fuse 10. Node support was assessed by Goodman-Bremer values (Goodman et al., 1982; Bremer, 1994; Grant and Kluge, 2008), calculated by running a modified version of the script BREMER.RUN distributed with TNT, which considered 1,000 replicates with 10 repetitions of ratchet and drift (Goloboff, 1999; Nixon, 1999) in constrained searches.

Opposed to COI, ribosomal RNA regions commonly present insertions and deletions, which makes multiple sequence alignment more challenging (Nagy et al., 2012). To deal with this, we submitted the resulting sequences from 16S, ITS1 and 28S to phylogenetic inference by direct optimization (Wheeler, 1996) using POY ver. 5.1.2 (Wheeler et al., 2014), under the parsimony optimality criterion. Tree search was performed by three independent 1, 3 and 6 hour searches assuming equal rates for character transformations. All unique trees compiled from the above searches were submitted to tree refinement by the tree-fusing algorithm (Goloboff, 1999) and re-diagnosed with the iterative pass algorithm (Wheeler, 2003a). The resulting implied alignment (sensu Wheeler, 2003b) was submitted to TNT to verify the results, under the same parameters as described before, including the Goodman-Bremer support. The analyses run with POY were conducted in an IBM x3850 X5 server with eight processors Intel Xeon CPU E7-8870 2.40 GHz, housed at the Genetics and Evolutionary Biology Department of the University of São Paulo.

Single-marker phylogenetic trees were used as basis for developing primary species hypothesis. In that sense, we considered cladogram topology and branch lengths, as well as previous mentions in the literature of the species' identity. Then, markers were combined for a total evidence phylogenetic analysis (Kluge, 1998). We imported the 16S, ITS1 and 28S implied alignments, and the COI static alignment, to Sequence Matrix ver

1.8 (Vaidya et al., 2011), and selected 3-5 sequences, or the amount available, of each marker for each of the hypothesized species. Within species, sequences selected were from geographic regions as diverse as possible, and for each terminal taxa, sequences of different markers were selected from the same specimen, the same locality, or the closest locality. The resulting file with combined alignments was analyzed in TNT as described previously. Alignments and trees retrieved in all molecular analyses were deposited in TreeBASE (treebase.org, pending upload). Supplementary Material S3 includes details on the sequences studied and used in each of the aforementioned analyses. All relevant codes used for molecular analyses are available in GitLab (gitlab.com/jonathan.lawley01, pending upload).

To delimit species, primary species hypotheses were reassessed based on criteria from two lines of evidence: (1) the species' monophyly and branch lengths on the combined-marker topology, and on (2) the species' distribution, based on collection localities. However, there are some caveats to this procedure. We recognize that phylogenetic analyses impose a hierarchy even on entities related tokogenetically (Davis and Nixon, 1992; Grant et al., 2006), and consequently species, which we herein consider as historical individuals, do not necessarily need to form a clade (Kluge, 1990; Frost and Kluge, 1994; Skinner, 2004). Therefore, branch lengths of the species' clades were also considered, as these are a measure of their differentiation. Nevertheless, due to variation in evolutionary rates and collection efforts, branch lengths may vary even across congeners (Grant et al., 2006). Considering species distributions can also be misleading, as there are likely multiple introductions in different *Aurelia* species (Dawson et al., 2005), as well as sympatry (Chiaverano et al., 2016). In spite of these caveats, these are clear and fallible criteria that can facilitate species discovery and diagnosability (Frost et al., 1998; Grant et al., 2006).

After species delimitation, diagnostic characters were identified for each marker using the program YBYRÁ (Machado, 2015), considering the implied alignments retrieved for 16S, ITS1 and 28S, the COI static alignment, and the parsimony phylogenies recovered for each. Reported diagnostic character-states for positions in the alignment are color-coded in the program's output, based on optimization of synapomorphies (sensu Grant and Kluge, 2004): white are ambiguous, and other colors are unambiguous; black are unique and non-homoplastic; red are unique and homoplastic; and blue are non-unique and homoplastic (see further details in Machado, 2015).

We also calculated uncorrected pairwise distances (number of base mismatches divided by total sequence length, also known as uncorrected p), which were retrieved from the software Geneious. We did not use this measure to delimit species, as (1) pairwise distances only discriminate among samples, and therefore cannot diagnose any particular entity (Frost, 2000); (2) they fail to explain observed variation, as they cannot distinguish between symplesiomorphy and synapomorphy; and (3) due to variation in evolutionary rates that could occur even among congeners, as previously mentioned, there seems to be no justification to set an arbitrary distance as threshold for granting species status (Grant et al., 2006). Nevertheless, we evaluated the use of this measure across molecular markers, as it can provide a rapid heuristic for species identification without the need of a complete phylogenetic analysis, in a similar way as dichotomous keys can be useful identification tools (Grant, 2002; Grant et al., 2006).

From the recent literature that incorporates *Aurelia* systematics, we noticed that authors did not name species, likely because the potential for morphological plasticity in the genus was still unclear. In this work, with further evidence, we describe species based mostly on molecular diagnosis, providing an important development for the taxonomy of the group.

3. Results

3.1. Morphological assessment

Before any measurements or statistical analyses, it was already possible to observe variation among specimens in the same lot, as illustrated in Fig. 2. Among these specimens we could see that even though they are similar in size, some morphological features vary. For example, the size of the gonads and sub-genital pores, as well as number of oral arm folds (curving points) (Fig. 2).

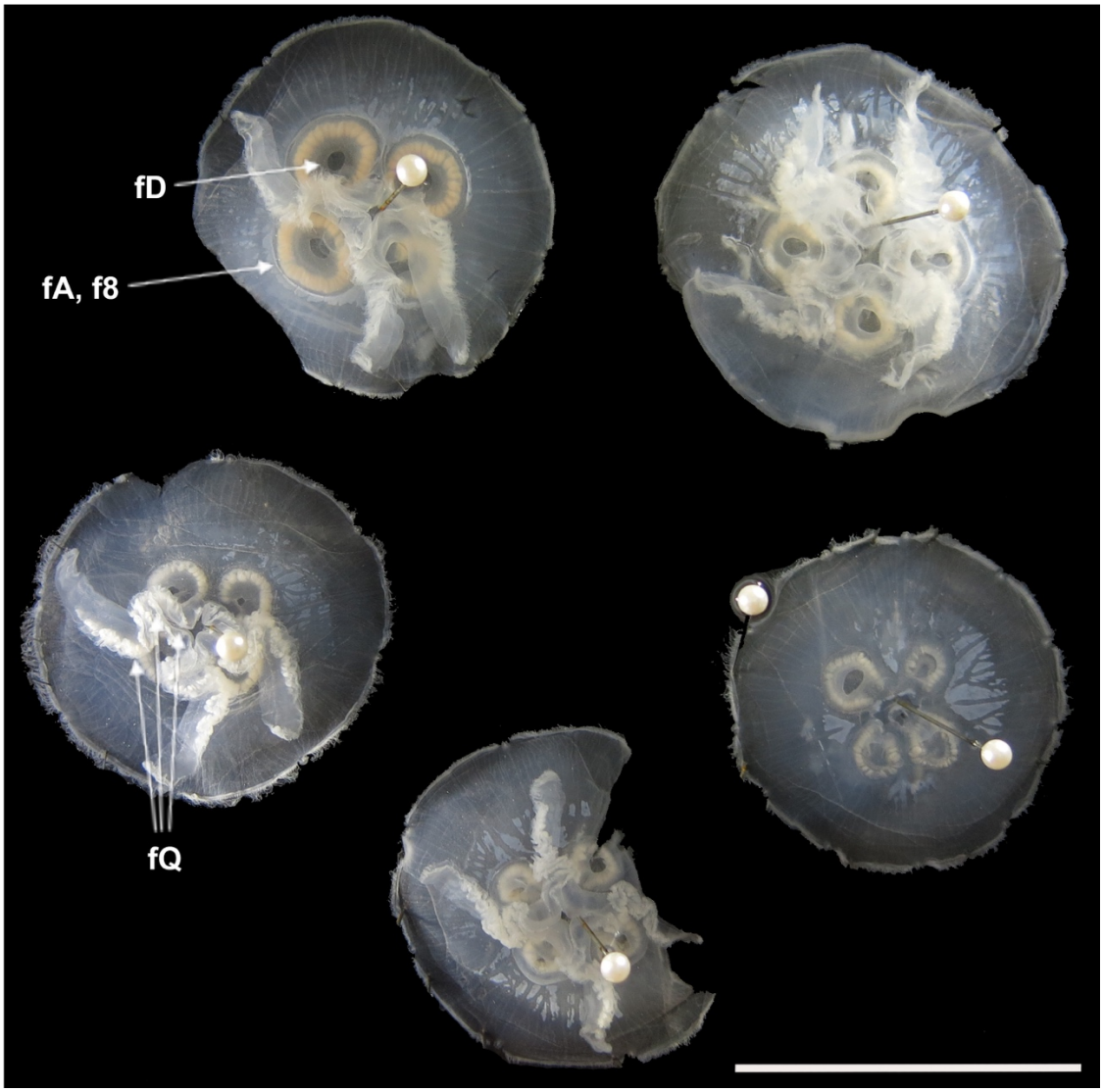


Figure 2. Medusae in lot YPM 29380, from Massachusetts, USA. *f*D = Lateral sub-genital pore diameter; *f*A = Lateral gonad diameter (furthest points); *f*8 = Size of gonads; *f*Q = Number of oral arm folds (curving points per arm). For more details, see Fig. 1 and Supplementary Material S2. Scale = 5 cm.

Neither of the MDS analyses (with or without estimation of missing data) presented a clear geographic structure on morphological dissimilarities (Figs. 3-4). Specimens from lot YPM29380 from the northeastern coast of the USA, shown in Fig. 2, for example, were sometimes more similar to specimens from very distant localities, such as the Maldives or the Marshall Islands, than to others in the same lot (highlighted in blue in Figs. 3-4). Another example of morphological resemblance among distant localities are the specimens from the northeastern coast of Canada and southwestern coast of the USA (highlighted in green in Figs. 3-4; Figs. 5A-D). It is possible to see the similarity in the oral arms (*f*2 and *f*3), the size of the sub-genital pores (*f*D), as well as in the branching

pattern of interradial canals (*f*O) (Figs. 5A-D; also see Fig. 1 and Supplementary Material S2 for references on morphological features). In that sense, even though some individuals within a locality or lot may seem closer in the MDS, morphological variation within these groups still seem to be variable enough to overlap across very distant localities (Figs. 3-4). The only example in which many specimens from the same locality group cluster closely together, is in the case of individuals analyzed from the aquarium at Discovery Place, USA (highlighted in orange in Figs. 3-4).

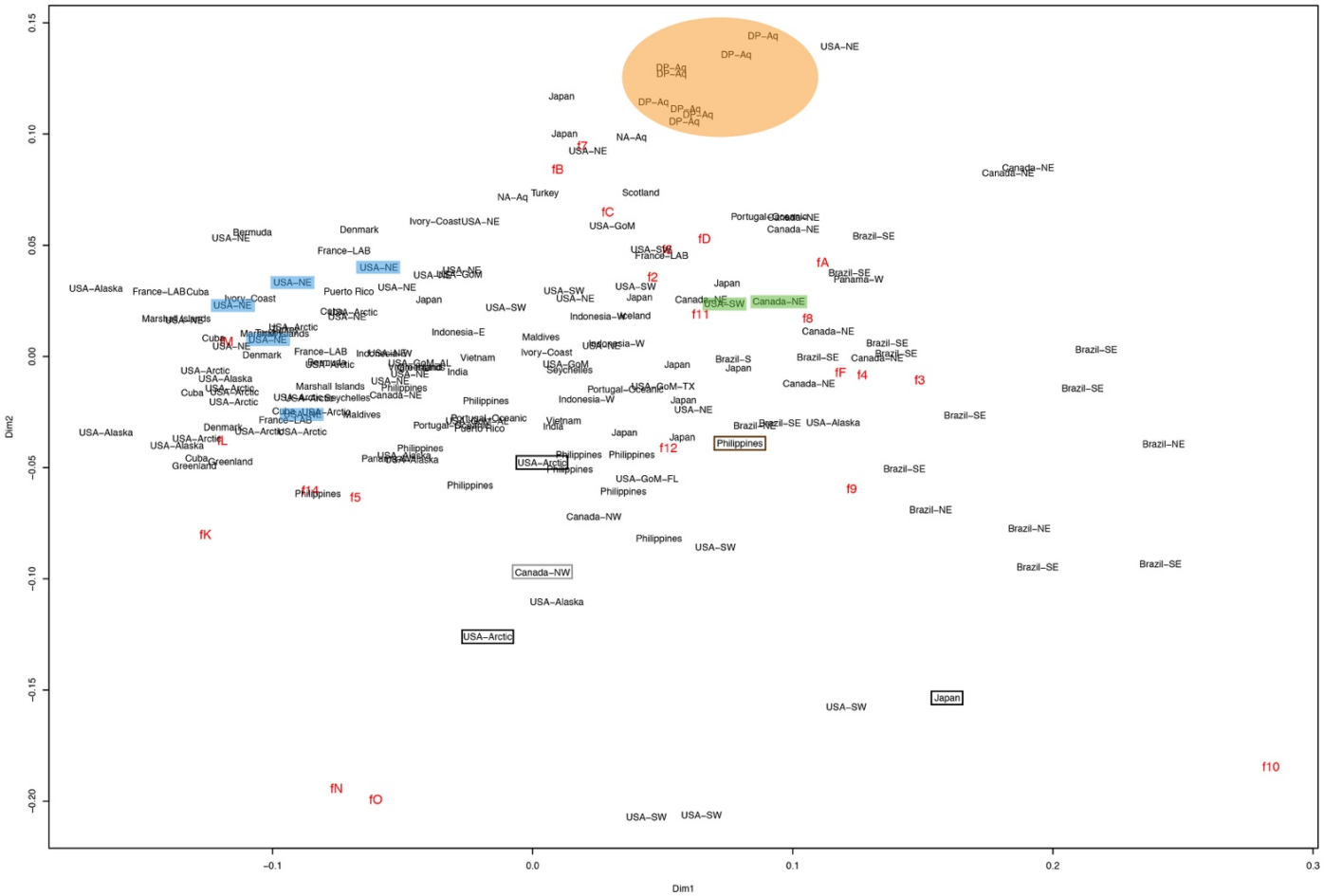


Figure 3. Multidimensional scaling (MDS) of morphological features *with estimation of missing data*. Specimens are depicted as locality groups, in black, and features appear in red, as weighted averages of their contributions. See Table 1 for details on some acronyms and 'Results' section for more details on specimens highlighted. Supplementary Material S1 and S2 contain more information on specimens measured and morphological features.

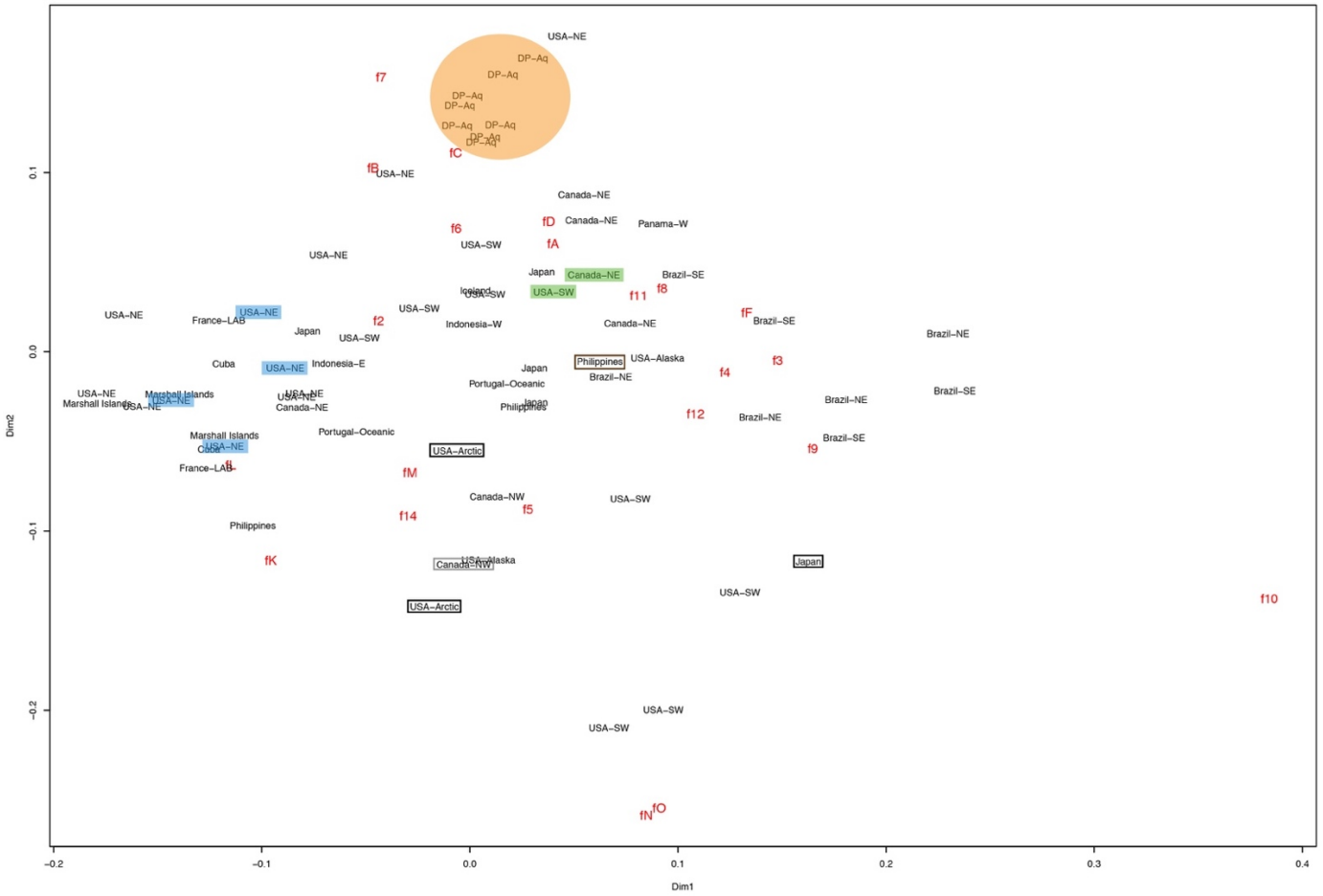


Figure 4. Multidimensional scaling (MDS) of morphological features *without estimation of missing data*. Specimens are depicted as locality groups, in black, and features appear in red, as weighted averages of their contributions. See Table 1 for details on some acronyms and 'Results' section for more details on specimens highlighted. Supplementary Material S1 and S2 contain more information on specimens measured and morphological features.

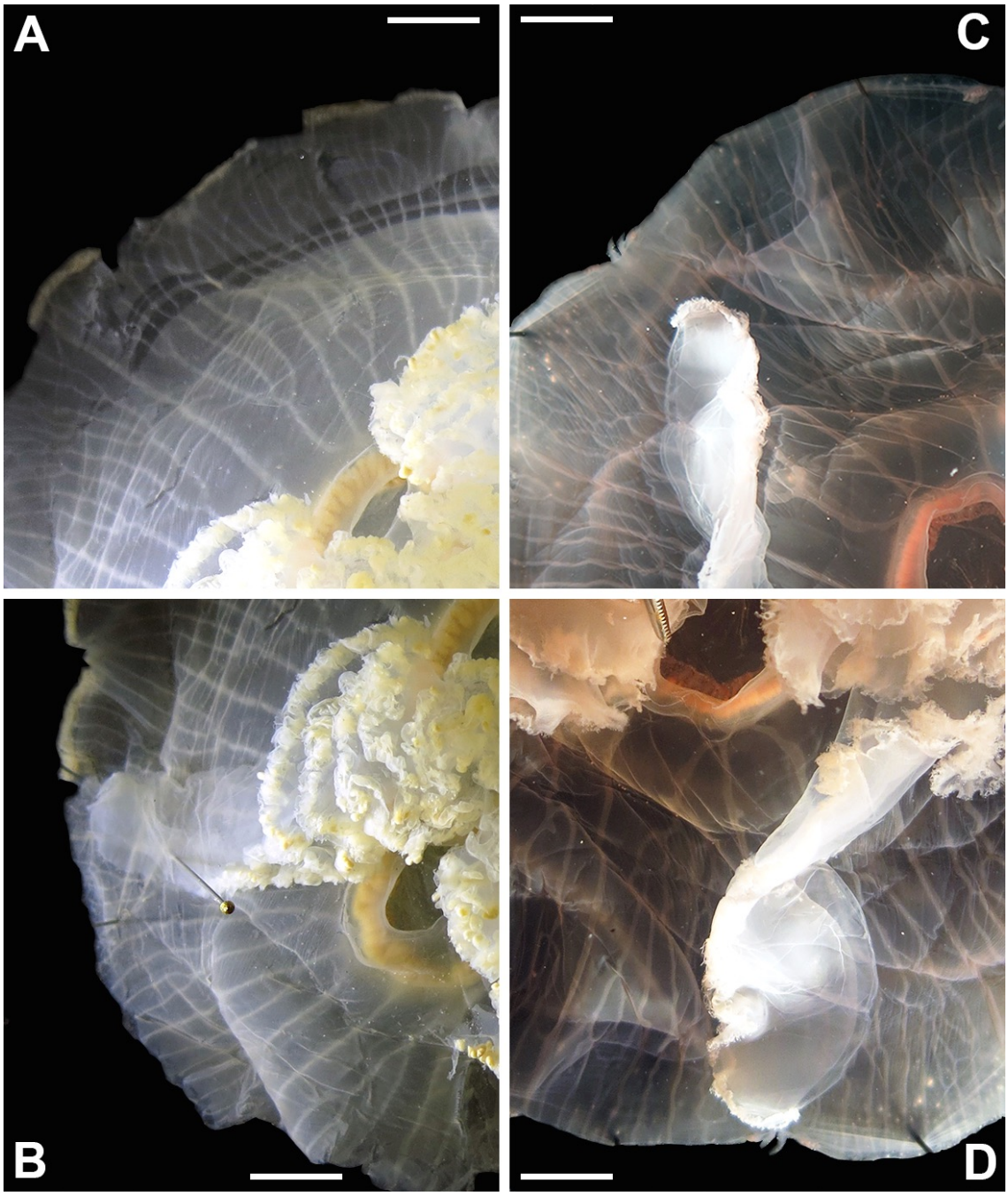


Figure 5. Medusae from northeastern Canada (USNM 30988) (A-B) and southwestern USA (USNM 92912-5) (C-D). The images show an interradial sector, from the gonad to the bell margin, and emphasize similarities on the oral arms, the size of the sub-genital pores, the margin indentations, as well as on the branching pattern of radial canals. For references on morphological features, see Fig. 1 and Supplementary Material S2. Scales = 1 cm.

Even though there is no apparent geographic structure in dissimilarities, some of the measured characters seem to be more variable across all analyzed specimens, which are represented closer to the edges of the morphological scape in the MDS (Figs. 3-4, characters in red). Specimens represented closer to these characters do not necessarily have the greatest values for it, but that character is the one that most contributed for the specimen's position in the MDS. Individuals from the aquarium at Discovery Place (DP-Aq), for example, seem to have the greatest distance between proximal edges of opposing gonads, as well as between proximal tips in each gonad (*f*7, *f*B; highlighted in orange in Figs. 3-4; Fig. 6A; Supplementary Material S1). Rhopaliar and non-rhopaliar indentations are the largest in some specimens from Brazil and one from the Philippines (*f*9, *f*10; brown square for Philippine specimen in Figs. 3-4; Fig. 6B; Supplementary Material S1). Some specimens from the Arctic and a specimen from Japan have the highest number of perradial and interradial branching points, although it is also high in some specimens from the southwestern coast of the USA, and seems to contribute greatly for their position (*f*N, *f*O; black squares for Arctic and Japanese specimens in Figs. 3-4; Fig. 6C; Supplementary Material S1). In the same way, a specimen from northwestern Canada has the highest number of interradial terminations, although it is also high in a specimen from Cuba, contributing greatly for its position (*f*L; grey square for Canadian specimen in Figs. 3-4; Fig. 6D; Supplementary Material S1). The number of perradial terminations, on the other hand, is higher in a specimen from the Philippines (*f*K; Figs. 3-4; Supplementary Material S1).

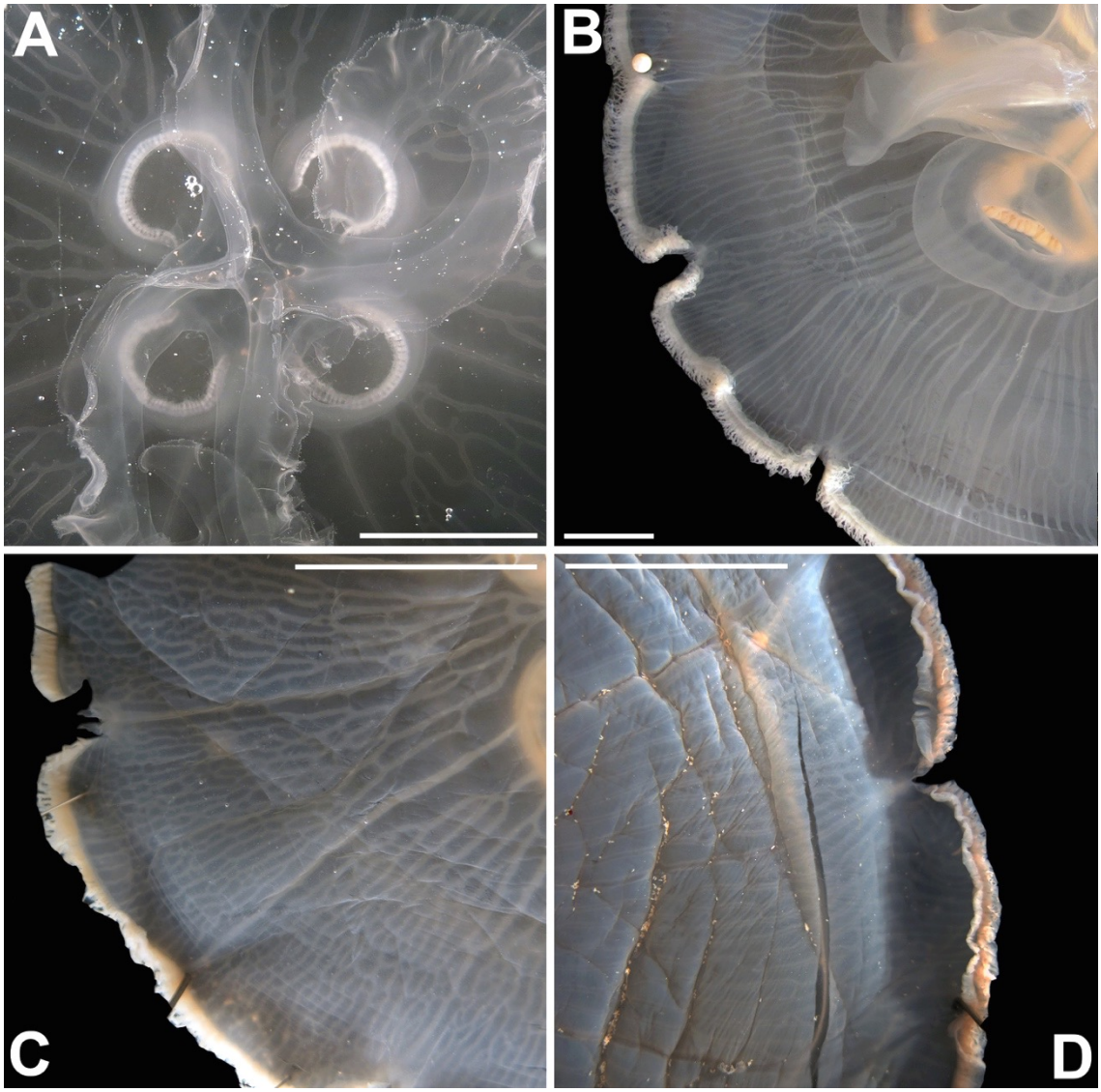


Figure 6. Medusae from the aquarium at Discovery Place, USA (DP3-4) (A), Brazil (LAB08) (B), the Arctic (USNM 44243-2) (C) and northwestern Canada (USNM 92913-1) (D). The images illustrate some morphological features that are distinguished in these specimens, such as *f*7 and *f*B (A), *f*9 and *f*10 (B), *f*N and *f*O (C), and *f*L (D). For references on morphological features, see Fig. 1 and Supplementary Material S1 and S2. Scales = 2 cm.

If specimens from neighboring localities had distinguishable morphological features, it could be argued that morphotypes could be identified regionally. Our analyses of the relationship between geographic distance and morphological differences presented the opposite pattern, with and without the estimation of missing morphological data (Fig. 7A-B). Specimens from nearby localities were significantly more similar to each other

than specimens from more distant localities, although there was a very weak relationship (Fig. 7A - R^2= 0.021, p<0.001; Fig. 7B - R^2= 0.026, p<0.001).

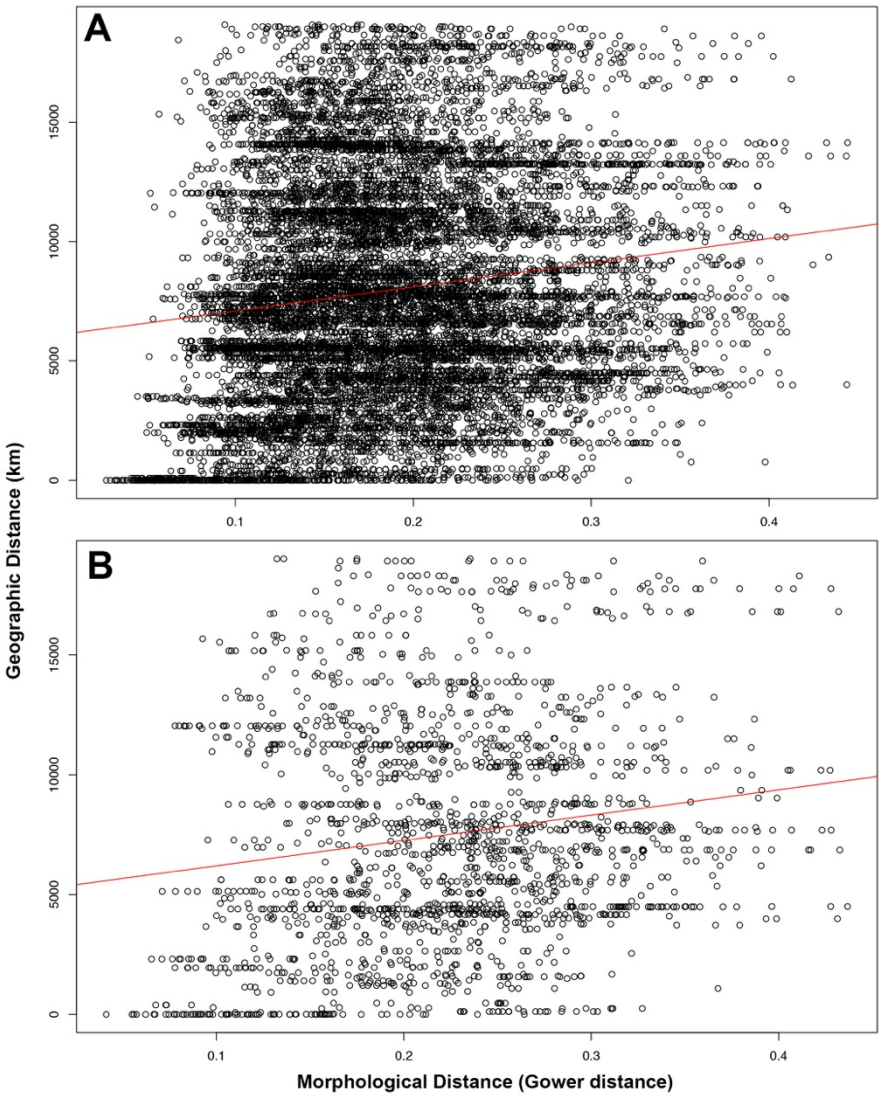


Figure 7. Relationship between morphological differences and geographic distances for both datasets (A) with and (B) without estimations of missing morphological features. There was only a slight but significant positive relationship, with higher morphological differences between specimens from more distant localities (A - R^2= 0.021, p<0.001; B - R^2= 0.026, p<0.001).

3.2. Morphological plasticity and diagnosis in A. coerulea

In view of the high morphological plasticity observed, we compared two medusae from lab cultures, that were identified from molecular sequences as *A. coerulea* (presented further), with the diagnostic features presented in the species' description based on

Mediterranean specimens (see Table 2 in Scorrano et al., 2016). By comparing Figs. 8A and C, some morphological differences can already be perceived, and this is further emphasized in Fig. 9, which highlights the significant differences in the continuous and meristic characters measured. Also, specimens cultured in the lab had a rounded hood covering the rhopalia, while Mediterranean specimens presented a triangular hood (Figs. 8B, D).

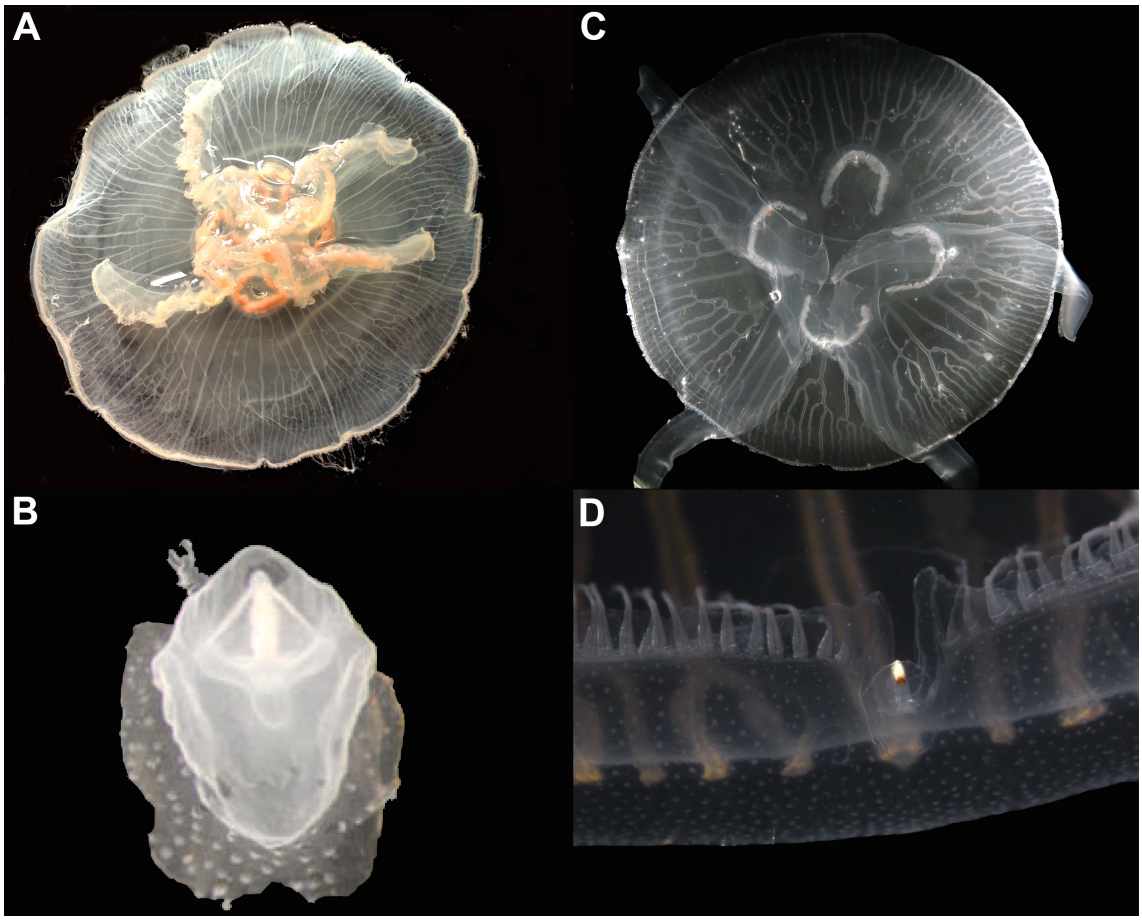


Figure 8. *Aurelia coerulea* from the Mediterranean (collected from the field) (A-B) and from lab cultures (C-D). Some morphological differences can be perceived when comparing the medusa's overall appearance (A, C), such as the oral arms and gonads. The hood that covers the rhopalia is also different, (B) triangular in Mediterranean specimens and (D) rounded in cultured specimens. A-B, bell diameter (*fl*) = 12.5 cm (image A provided by S. Scorrano and image B was adapted from Scorrano et al., 2016); C-D, *fl* = 7.5 cm.

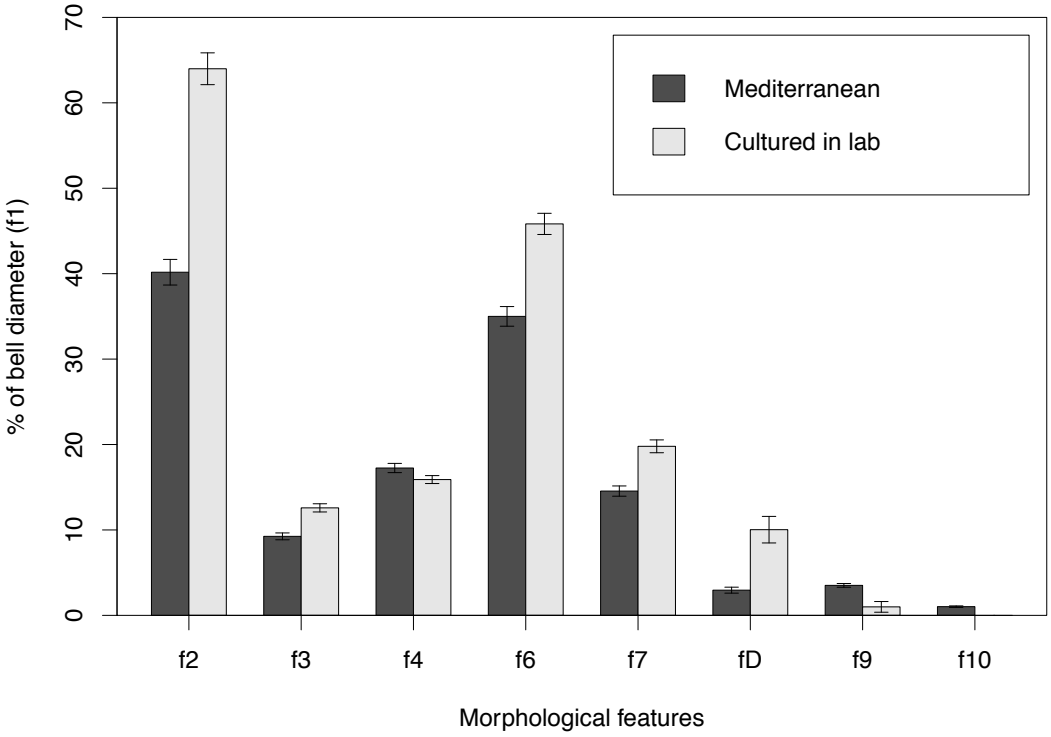


Figure 9. Comparison of morphological features measured from Mediterranean specimens (Table 2 from Scorrano et al., 2016) and from lab cultures. Averages and standard deviations are presented for each morphological feature, for which a Welch's t-test returned significant differences between specimens ($p<0.05$). For references on morphological features, see Fig. 1 and Supplementary Material S2.

3.3. Species delimitation

The total evidence phylogenetic analysis, which combined all markers herein studied (16S, COI, ITS1 and 28S), alongside other considered evidence (detailed in the remarks of the species descriptions herein), revealed 28 species hypothesis, of which 6 had already been recognized and described (Fig. 10). Of the 22 left, 2 were collected and sequenced in this study for the first time (*Aurelia ayla* sp. nov. and *Aurelia insularia* sp. nov.), and some of the others had been previously recognized as species hypothesis but not formally described (e.g., *Aurelia* sp. 2 sensu Dawson and Jacobs, 2001, herein described as *Aurelia cebimarensis* sp. nov.). An updated distribution map for *Aurelia* species is shown in Fig. 11, based on sequence data from all markers analyzed (see Supplementary Material S3 for more detailed information). Single-marker phylogenies, which were used to construct primary species hypothesis, are represented as cladograms in Figs. 12-15 (for more detailed phylogenies see Supplementary Material S4).

Uncorrected pairwise distances are reported for each marker, as well as their frequency histogram, in Figs. 12-16. 16S was the only marker in which there was no overlap between intra- and interspecific distances (Fig. 12), although COI presented an overall larger gap between these (Figs. 13, 16). Nevertheless, ITS1 had the greatest number of unique and non-homoplastic synapomorphies (in black, Fig. 14). The 'most similar species' presented for each species in Figs. 12-15, illustrates that similarity may not reflect evolutionary relationships, even for sister species, such as *Aurelia daglioi* sp. nov. for 16S (Fig. 12), that is more closely related to *Aurelia bajacaliforniana* sp. nov. but is more similar to *Aurelia mianzani* sp. nov..

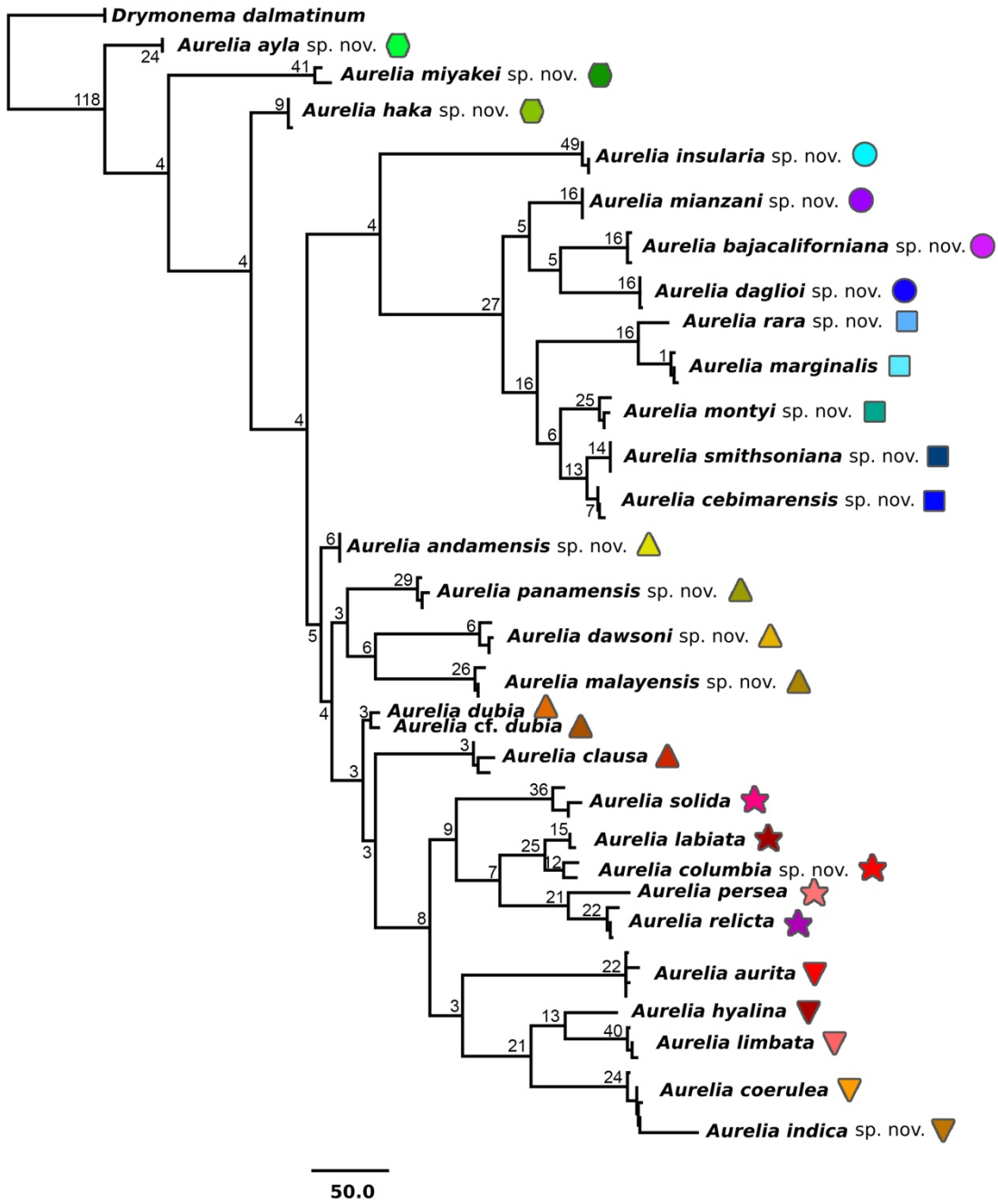


Figure 10. Total evidence phylogenetic analysis, indicating relationships between species hypotheses treated herein. This analysis combined markers 16S, COI, ITS1 and 28S, reconstructing relationships under parsimony as the optimality criterion. Numbers on nodes indicate Goodman-Bremer support values and symbols beside species names are equivalent to symbols in the distribution map (Fig. 11). Supplementary Material S3 contains further details on sequences used to reconstruct this phylogeny. Single-marker phylogenies are presented in Supplementary Material S4, and represented as cladograms in Figs. 12-15

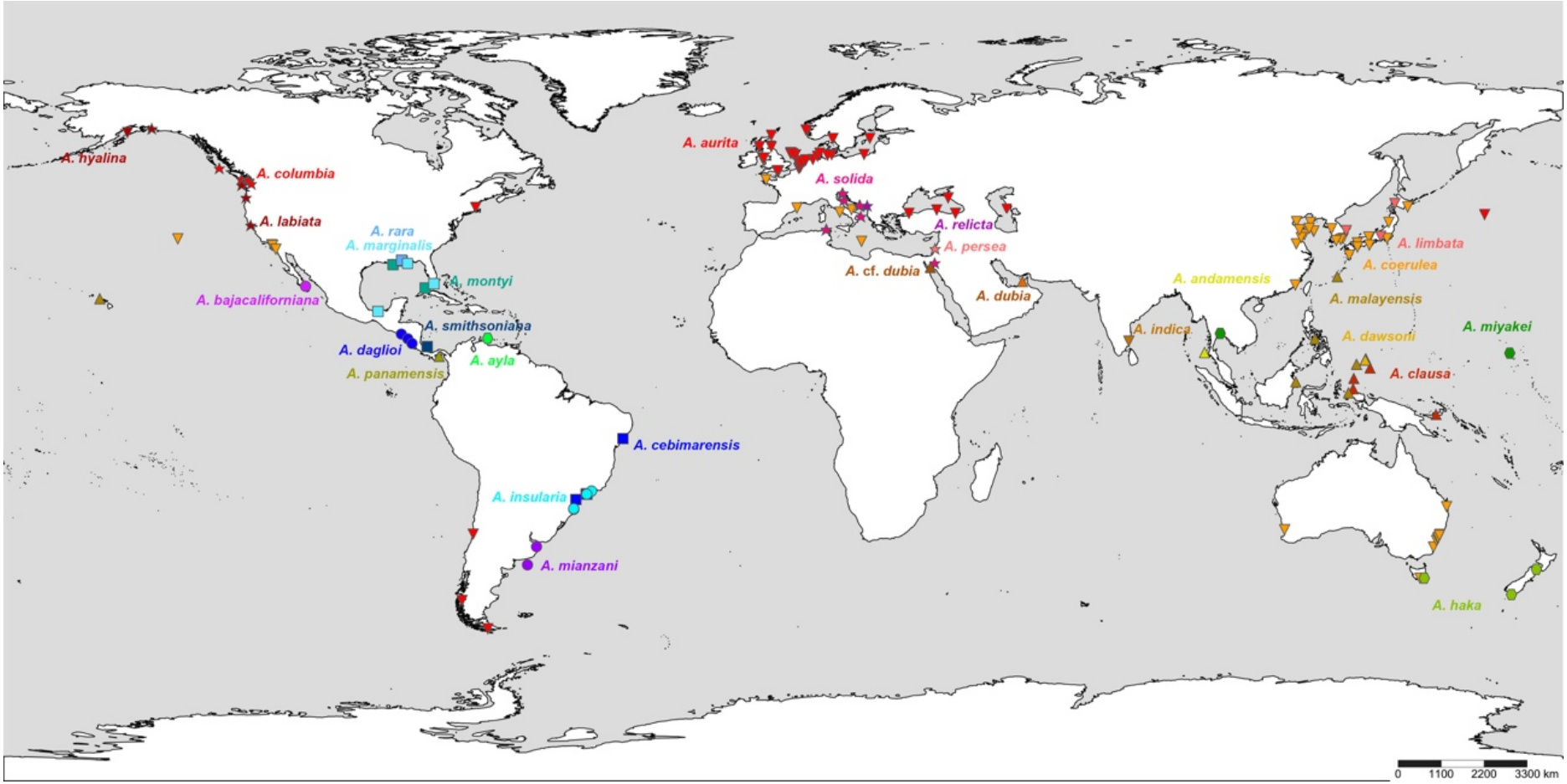
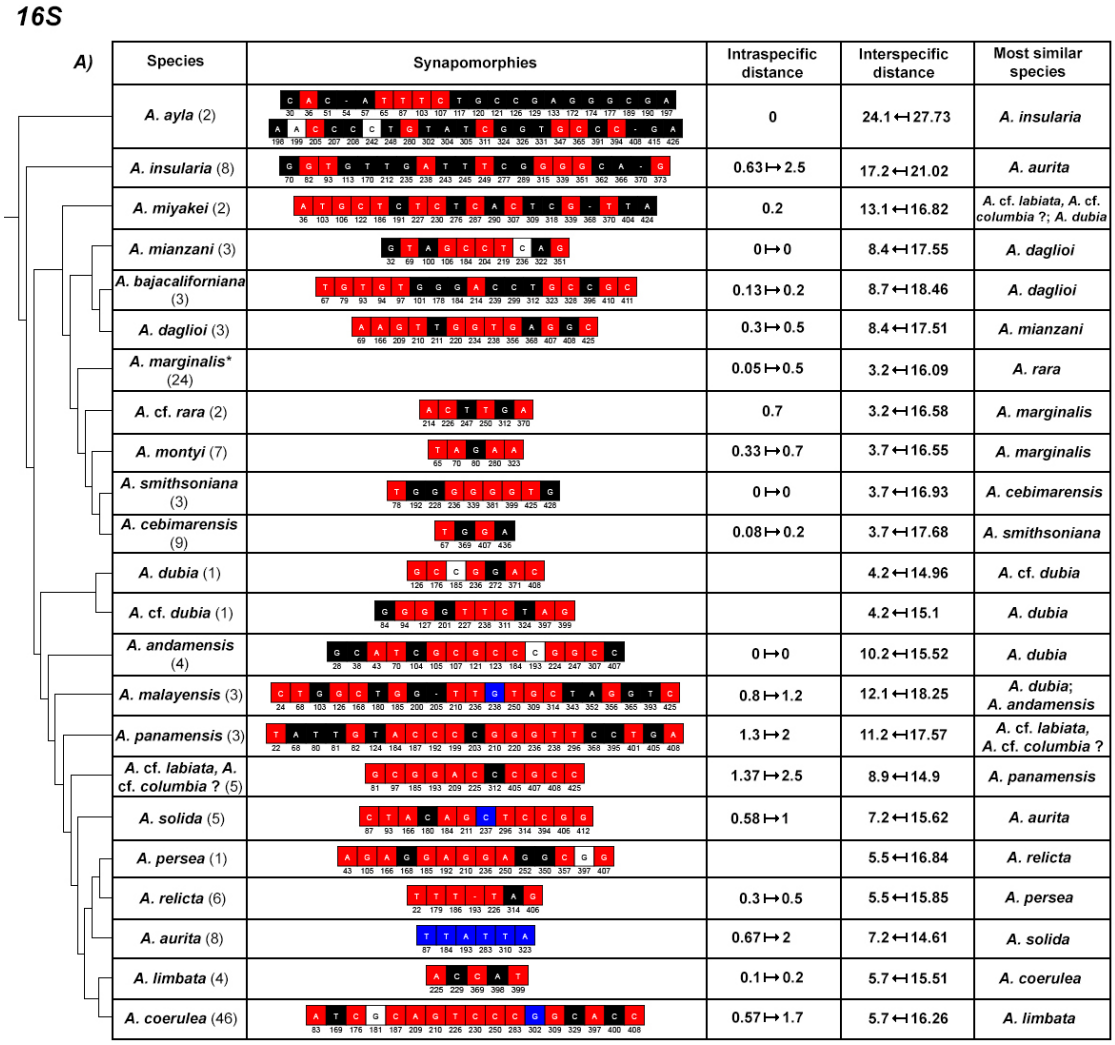


Figure 11. Distribution of *Aurelia* species treated herein, based on sequenced specimens. Symbols on the map are equivalent to the species names beside them, with the same color, as well as to symbols in the combined-marker phylogeny (Fig. 10). Supplementary Material S3 contains further details on the localities and their respective sequences.



16S

A) Species	Synapomorphies	Intraspecific distance	Interspecific distance	Most similar species
A. ayla (2)	C A C - A T T T C T G C C G A G G G C G A 30 36 51 54 57 65 87 103 107 117 120 121 126 129 133 172 174 177 189 190 197 A A C C C C T G T A T C G G T G C C G - G A 198 199 205 207 208 242 248 280 302 304 305 311 324 326 331 347 365 391 394 408 415 426	0	24.1↔27.73	*A. insularia*
A. insularia (8)	G G T G T T G A T T T C G G G G C A - G 70 82 93 113 170 212 235 238 243 245 249 277 289 315 339 351 362 366 370 373	0.63↦2.5	17.2↔21.02	*A. aurita*
A. miyakei (2)	A T G C T C T C T C A C T C G - T T A 36 103 106 122 186 191 227 230 276 287 290 307 309 318 339 368 370 404 424	0.2	13.1↔16.82	*A.* cf. *labiata*, *A.* cf. *columbia* ?; *A. dubia*
A. mianzani (3)	G T A G C C T C A G 32 69 100 106 184 204 219 236 322 351	0↦0	8.4↔17.55	*A. daglioi*
A. bajacaliforniana (3)	T G T G T G G G A C C T G C C G C 67 79 93 94 97 101 178 184 214 239 299 312 323 328 396 410 411	0.13↦0.2	8.7↔18.46	*A. daglioi*
A. daglioi (3)	A A G T T G G T G A G G C 69 166 209 210 211 220 234 238 356 368 407 408 425	0.3↦0.5	8.4↔17.51	*A. mianzani*
*A. marginalis** (24)		0.05↦0.5	3.2↔16.09	*A. rara*
A. cf. *rara* (2)	A C T T G A 214 226 247 250 312 370	0.7	3.2↔16.58	*A. marginalis*
A. montyi (7)	T A G A A 65 70 80 280 323	0.33↦0.7	3.7↔16.55	*A. marginalis*
A. smithsoniana (3)	T G G G G G G T G 78 192 228 236 339 361 399 425 426	0↦0	3.7↔16.93	*A. cebimarensis*
A. cebimarensis (9)	T G G A 67 369 407 436	0.08↦0.2	3.7↔17.68	*A. smithsoniana*
A. dubia (1)	G C C G G A C 126 176 185 236 272 371 408		4.2↔14.96	*A.* cf. *dubia*
A. cf. *dubia* (1)	G G G G T T C T A G 84 94 127 201 227 238 311 324 397 399		4.2↔15.1	*A. dubia*
A. andamensis (4)	G C A T C G C G C C C G G C C 28 38 43 70 104 105 107 121 123 184 193 224 247 307 407	0↦0	10.2↔15.52	*A. dubia*
A. malayensis (3)	C T G G C T G G - T T G T G C T A G G T C 24 68 103 126 168 180 185 200 205 210 236 238 250 309 314 343 352 356 365 393 425	0.8↦1.2	12.1↔18.25	*A. dubia*; *A. andamensis*
A. panamensis (3)	T A T T G T A C C C C G G G T T C C T G A 22 68 80 81 82 124 184 187 192 199 203 210 220 236 238 296 368 395 401 405 408	1.3↦2	11.2↔17.57	*A.* cf. *labiata*, *A.* cf. *columbia* ?
A. cf. *labiata*, *A.* cf. *columbia* ? (5)	G C G G A C C C G C C 81 97 185 193 209 225 312 405 407 408 425	1.37↦2.5	8.9↔14.9	*A. panamensis*
A. solida (5)	C T A C A G C T C C G G 87 93 166 180 184 211 237 296 314 394 406 412	0.58↦1	7.2↔15.62	*A. aurita*
A. persea (1)	A G A G G A G G A G G C G G 43 105 166 168 185 192 210 236 250 252 350 357 397 407		5.5↔16.84	*A. relicta*
A. relicta (6)	T T T - T A G 22 179 186 193 226 314 406	0.3↦0.5	5.5↔15.85	*A. persea*
A. aurita (8)	T T A T T A 87 184 193 283 310 323	0.67↦2	7.2↔14.61	*A. solida*
A. limbata (4)	A C C A T 225 229 369 398 399	0.1↦0.2	5.7↔15.51	*A. coerulea*
A. coerulea (46)	A T C G C A G T C C C G G C A C C 83 169 176 181 187 209 210 226 230 250 283 302 309 329 397 400 408	0.57↦1.7	5.7↔16.26	*A. limbata*

Figure 12. Diagnostic molecular characters for each species hypothesis, as well as related uncorrected pairwise distances (%), based on 16S. (A) Numbers beside species names represent number of sequences; intraspecific distances are average – maximum (missing when there was only one sequence), and interspecific distances are minimum – average;

cladogram demonstrates reconstructed relationships between species, under the parsimony optimality criterion (see Supplementary Material S4 for a more detailed phylogeny); synapomorphies appear with the alignment position below them and are color-coded: in white are ambiguous, and other colors are unambiguous, with black as unique and non-homoplastic, red as unique and homoplastic, and blue as non-unique and homoplastic (see further details in Machado, 2015); '*' represents species hypotheses that were not monophyletic for this marker, and therefore synapomorphies are absent. '?' indicates cases of sympatric species, that could not be told apart due to lack of sampling or reciprocal monophyly for this marker. (B) Frequency histogram of uncorrected pairwise sequence distances (%); intraspecific in light-grey and interspecific in dark-grey.

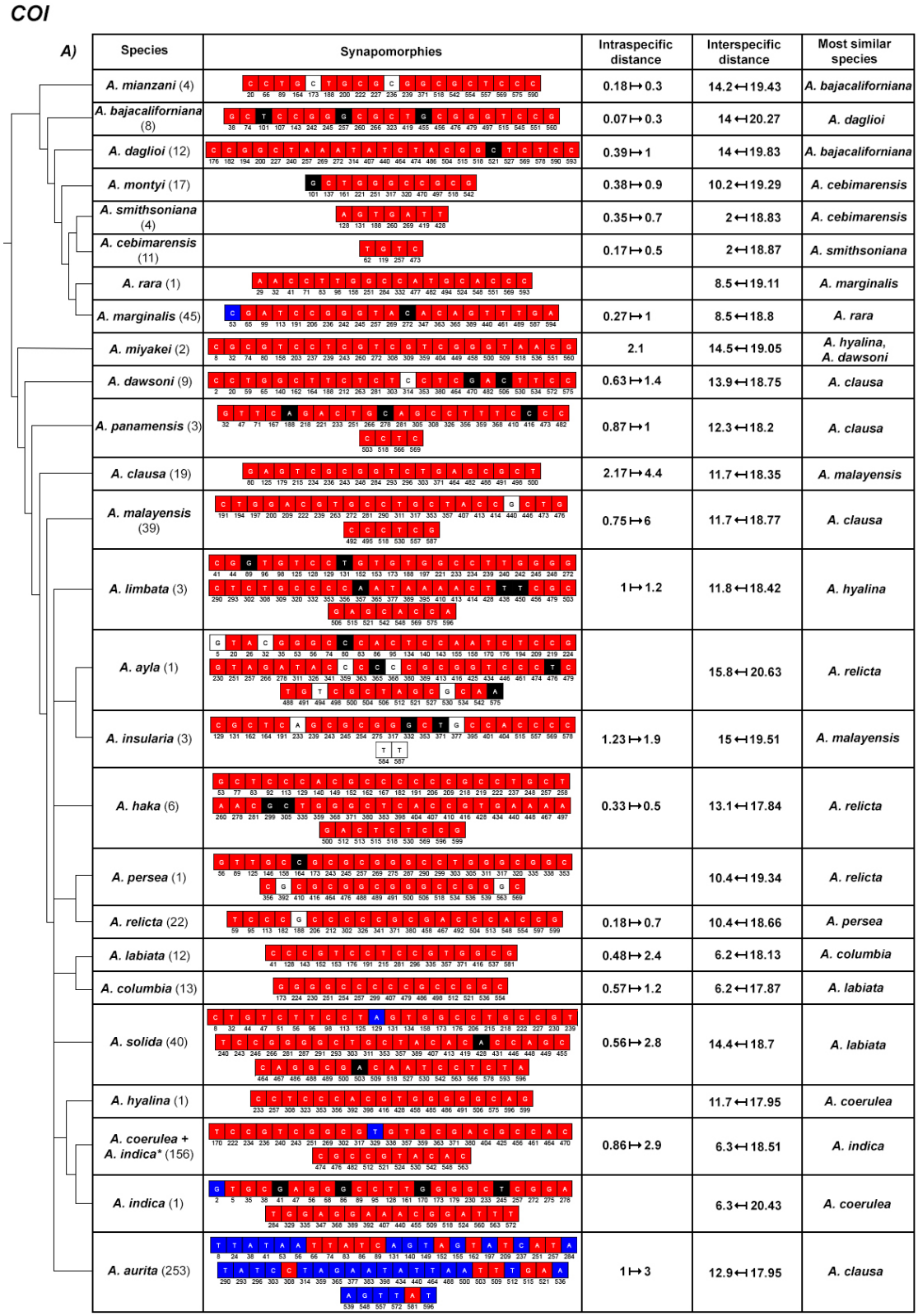


Figure 13. Diagnostic molecular characters for each species hypothesis, as well as related uncorrected pairwise distances (%), based on COI. (A) Numbers beside species names represent number of sequences; intraspecific distances are average – maximum (missing when there was only one sequence), and interspecific distances are minimum – average;

cladogram demonstrates reconstructed relationships between species, under the parsimony optimality criterion (see Supplementary Material S4 for a more detailed phylogeny); synapomorphies appear with the alignment position below them and are color-coded: in white are ambiguous, and other colors are unambiguous, with black as unique and non-homoplastic, red as unique and homoplastic, and blue as non-unique and homoplastic (see further details in Machado, 2015); '*' represents species hypotheses that were not monophyletic for this marker, and were therefore combined with the specimen or group missing for it to become monophyletic.

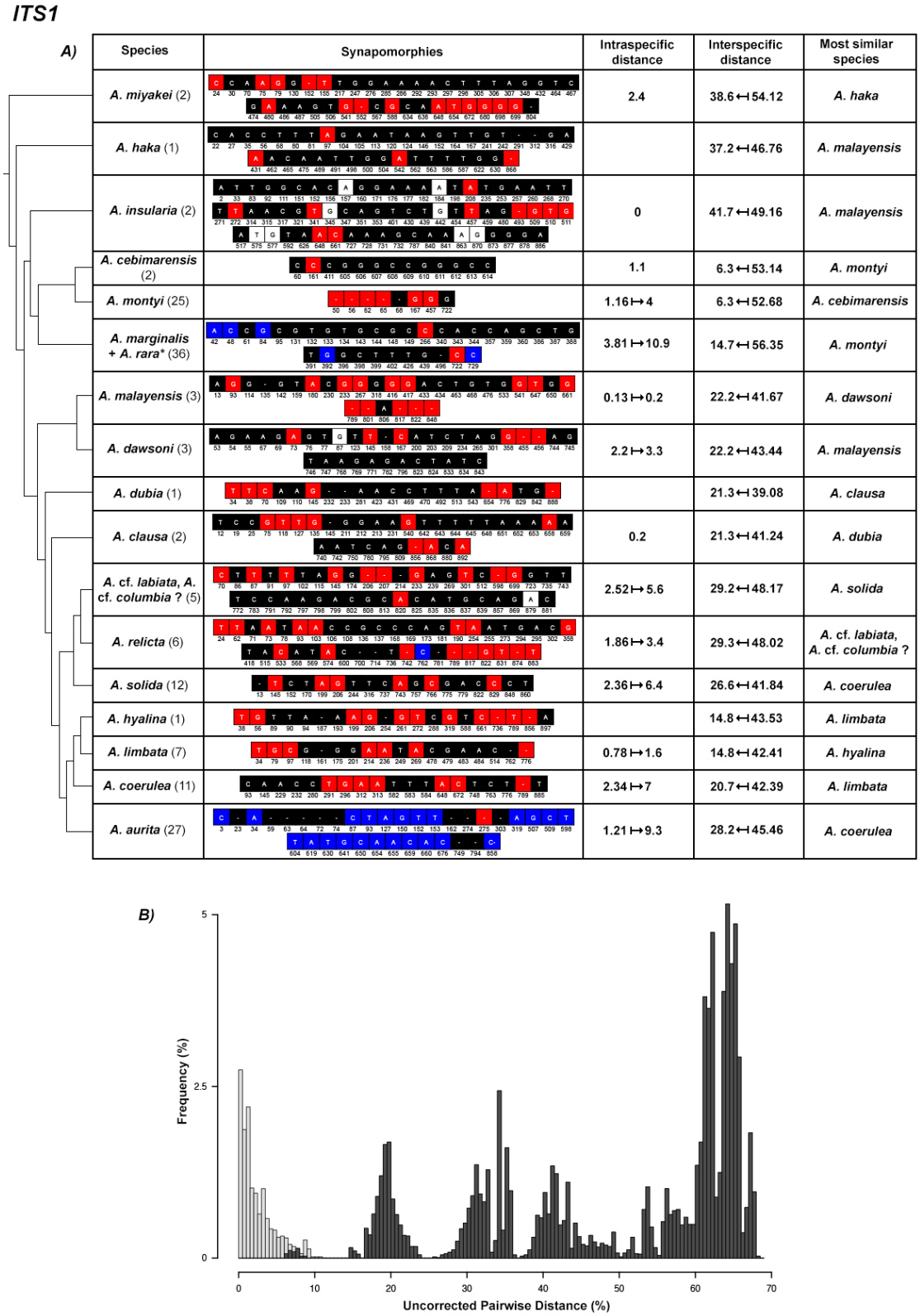


Figure 14. Diagnostic molecular characters for each species hypothesis, as well as related uncorrected pairwise distances (%), based on ITS1. (A) Numbers beside species names represent number of sequences; intraspecific distances are average – maximum (missing when there was only one sequence), and interspecific distances are minimum – average;

cladogram demonstrates reconstructed relationships between species, under the parsimony optimality criterion (see Supplementary Material S4 for a more detailed phylogeny); synapomorphies appear with the alignment position below them and are color-coded: in white are ambiguous, and other colors are unambiguous, with black as unique and non-homoplastic, red as unique and homoplastic, and blue as non-unique and homoplastic (see further details in Machado, 2015); '*' represents species hypotheses that were not monophyletic for this marker, and were therefore combined with the specimen or group missing for it to become monophyletic. '?' indicates cases of sympatric species, that could not be told apart due to lack of sampling or reciprocal monophyly for this marker. (B) Frequency histogram of uncorrected pairwise sequence distances (%); intraspecific in light-grey and interspecific in dark-grey.

28S

A)

Species	Synapomorphies	Intraspecific distance	Interspecific distance	Most similar species
*A. bajacaliforniana** (3)		0.8↦1	0.8↤6.67	*A. daglioi*
A. daglioi (3)	C G (79 296)	0.13↦0.2	0.8↤6.83	*A. bajacaliforniana*
A. mianzani (3)	A T A G C (81 201 292 293 336)	0↦0	1.8↤7.02	*A. bajacaliforniana, A. daglioi, A. marginalis*
A. marginalis (3)	T T C (29 62 197)	0.67↦0.8	1.8↤7.1	*A. mianzani*
*A. cebimarensis** (7)		0.13↦0.3	0↤7.24	*A. smithsoniana*
*A. smithsoniana** (2)		0.2	0↤7.24	*A. cebimarensis*
A. persea (1)	C - C (89 196 222)		1.4↤6.19	*A. relicta*
A. relicta (8)	C T (183 184)	0.88↦2.8	1.4↤6.71	*A. persea*
A. miyakei (1)	T T C G G C C T T A G C A T C C G (7 69 86 89 100 123 142 143 144 191 202 222 223 254 280 389 437)		5.3↤7.6	*A. panamensis*
A. malayensis (3)	G G G A (150 199 242 268)	0.23↦0.3	2.1↤6.05	*A. panamensis*
A. insularia (7)	C C C C G G C G G C T (1 29 86 118 130 135 147 150 202 242 254)	0↦0	3.1↤6.6	*A. panamensis*
A. aurita (10)	C G G G T C C G G G C C (12 88 92 119 120 132 136 141 243 263 266 399)	0.12↦0.6	3.7↤7.61	*A. panamensis, A. solida*
A. panamensis (3)	A G (69 130)	0↦0	1.4↤5.36	*A. solida*
A. solida (21)	G A T (89 90 251)	0.13↦0.8	1.4↤5.93	*A. panamensis*
A. limbata (1)	A C C C A T (49 120 123 242 272 399)		1.4↤7.4	*A. coerulea*
A. coerulea (26)	A (88)	0.14↦1	1.4↤6.66	*A. limbata*

B)

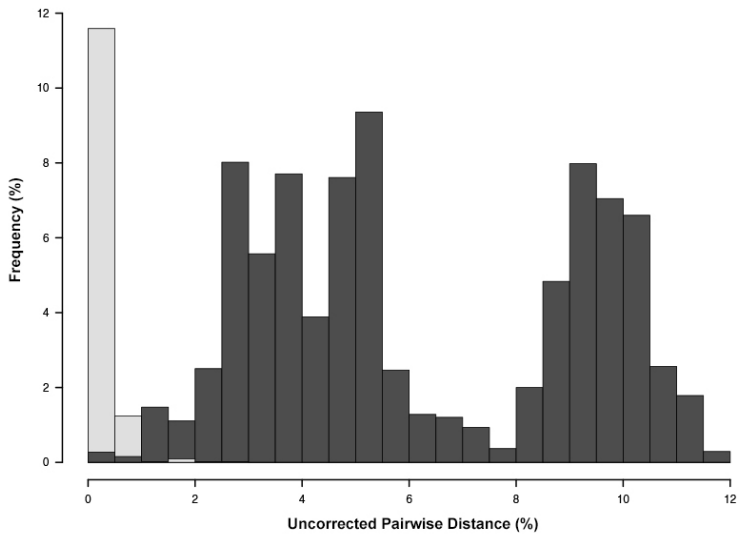


Figure 15. Diagnostic molecular characters for each species hypothesis, as well as related uncorrected pairwise distances (%), based on 28S. (A) Numbers beside species names represent number of sequences; intraspecific distances are average – maximum (missing when there was only one sequence), and interspecific distances are minimum – average; cladogram demonstrates reconstructed relationships between species, under the parsimony optimality criterion (see Supplementary Material S4 for a more detailed phylogeny); synapomorphies appear with the alignment position below them and are color-coded: in white are ambiguous, and other colors are unambiguous, with black as unique and non-homoplastic, red as unique and homoplastic, and blue as non-unique and

homoplastic (see further details in Machado, 2015); '*' represents species hypotheses that were not monophyletic for this marker, and therefore synapomorphies are absent. (B) Frequency histogram of uncorrected pairwise sequence distances (%); intraspecific in light-grey and interspecific in dark-grey.

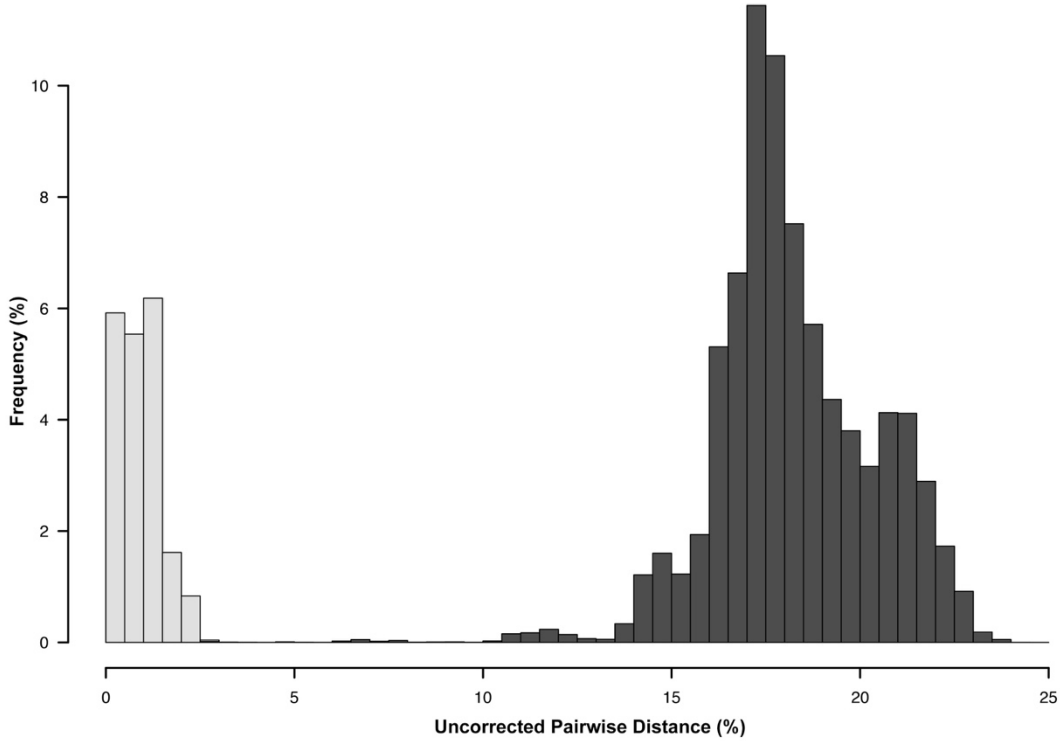


Figure 16. Frequency histogram of COI uncorrected pairwise sequence distances (%). Intraspecific distances are represented in light-grey, while interspecific in dark-grey. Fig. 13 shows these differences for each of the species hypotheses.

3.4. Systematic account

Genus *Aurelia* Lamarck, 1816

Type material: *Aurelia aurita* (Linnaeus, 1758)

Diagnosis: Ulmaridae with unbranched oral arms surrounding the mouth; much folded interradial gonads unconnected from each other, usually ranging from a flat-U to a drop-shaped circumference; radial canals branching and sometimes anastomosing, extending outwards to margin from central stomach; ring canal present; numerous small tentacles and lappet-like structures arising from exumbrella just above the margin; marginal rhopalia usually on the center of the perimeter of each radius, resulting on a bell

indentation (marginal cleft); non-rhopaliar indentations can be present; usually presents tetramerous radial symmetry (compiled from Mayer, 1910; Kramp, 1961; Russell, 1970; Calder, 2009; Scorrano et al., 2016; and own observations).

Species hypotheses are presented below in the order they appear in the combined-marker phylogenetic analyses, from top to bottom (Fig. 10).

Aurelia ayla Lawley, Gamero-Mora, Maronna, Chiaverano, Collins and Morandini **sp. nov.**

Type material: Holotype: DNA extraction, USNM (pending deposit). Paratypes: Tissue (Medusa), USNM (pending deposit).

Type locality: 12º 12' N, 68º 18' 31" W; Oil slick leap, Kralendijk, Bonaire, the Netherlands.

Etymology: Derived from the Turkish word *ayla*, meaning "halo of light around the moon", in honor of the daughter of AGC (co-author in this study), who shares the same name.

Distribution: Currently known only from the type locality (see Fig. 11).

Diagnosis: Molecular diagnosis is given in Figs. 12-13. GenBank accession numbers of sequences that belong to this species and were used in analyses are presented in Supplementary Material S3.

Remarks: Interestingly, this species does not fall within the clade that includes all other western Atlantic species. Further increasing the dataset with more molecular markers and specimen collections, especially from the southeastern Atlantic and Indian oceans, could resolve this matter, as it could not only be an effect of undersampling but also a case of introduction from another locality, which is not unprecedented in this genus (Dawson et al., 2005).

Aurelia miyakei Lawley, Gamero-Mora, Maronna, Chiaverano, Collins and Morandini **sp. nov.**

Aurelia sp. 11 Dawson et al., 2005. He et al., 2015; Chiaverano et al., 2016; Dong, 2018.

Type material: Holotype: DNA extraction, MZUSP (pending deposit). Paratype: Polyps, MZUSP (pending deposit); Tissue (Polyps), MZUSP (pending deposit)/ USNM (pending deposit).

Type locality: Gulf of Thailand, near Saen Suk, Thailand.

Etymology: Named after Prof. Dr. Hiroshi Miyake (Kitasato University, Japan), for his prominent research on jellyfish and constant collaborative efforts, including providing polyps from this species.

Distribution: Gulf of Thailand and Kwajalein, Marshall Islands (see Fig. 11).

Diagnosis: Molecular diagnosis is given in Figs. 12-15. GenBank accession numbers of sequences that belong to this species and were used in analyses are presented in Supplementary Material S3.

Remarks: Polyps were present in collected material from nearby the Institute of Marine Science, Burapha University, Saen Suk, Thailand.

Aurelia haka Lawley, Gamero-Mora, Maronna, Chiaverano, Collins and Morandini **sp. nov.**

Aurelia sp. 7 Dawson et al., 2005. Ki et al., 2008; Dong et al., 2015; He et al., 2015; Chang et al., 2016; Chiaverano et al., 2016; Scorrano et al., 2016; Abboud et al., 2018; Dong, 2018.

Type material: M0D (pending confirmation).

Type locality: 41º 17' S, 174º 47' E; Wellington, New Zealand.

Etymology: Derived from *haka*, the traditional war dance of the Maori, the indigenous Polynesian people of New Zealand.

Distribution: Tasmania and New Zealand (see Fig. 11; for specific localities see Supplementary Material S3).

Diagnosis: Molecular diagnosis is given in Figs. 13-14. GenBank accession numbers of sequences that belong to this species and were used in analyses are presented in Supplementary Material S3.

Remarks: Some of the sequences updated to GenBank that belong to this species' clade were identified as *Aurelia* aff. *clausa* (see Supplementary Material S3). However, *A. clausa* was described by Lesson (1830) from New Ireland, Papua New Guinea, which is much closer to the distribution range of another *Aurelia* species previously identified

and further treated herein (*Aurelia* sp. 6). *Aurelia vitiana* Agassiz and Mayer, 1899, on the other hand, was described from Suva, Fiji, and specimens from this locality could be connected to populations in Australia and New Zealand by the South Pacific Gyre. Nevertheless, as we cannot confirm the identity of the observed type specimen of *A. vitiana* (MCZ 1346) to the species clade herein discussed, and morphological descriptions do not seem useful to differentially diagnose species (further discussed), we refrained from resurrecting this epithet, and portrayed this species hypothesis under a new name.

Aurelia insularia Lawley, Gamero-Mora, Maronna, Chiaverano, Collins and Morandini **sp. nov.**

Aurelia sp. 2 Gambill and Jarms, 2014.

Type material: Holotype: DNA extraction, MZUSP (pending deposit). Paratypes: Tissue (Polyps), MZUSP (pending deposit)/ USNM (pending deposit); Polyps, MZUSP (pending deposit)/ USNM (pending deposit).

Type locality: 23º 07' 04" S, 44º 16' 59" W; Pinguino Wreck, Ilha Grande, Rio de Janeiro, Brazil.

Etymology: Derived from the Latin word *insularis*, meaning "of islands", in reference to the recorded occurrence of polyps mostly on or near islands.

Distribution: Mostly on or near islands in the south and southeastern coasts of Brazil (see Fig. 11; for specific localities see Supplementary Material S3).

Diagnosis: Polyps present 27-32 tentacles (Gambill and Jarms, 2014; see remarks below). Molecular diagnosis is given in Figs. 12-15. GenBank accession numbers of sequences that belong to this species and were used in analyses are presented in Supplementary Material S3.

Remarks: Polyps of this species were first collected in Ilha Grande, Rio de Janeiro, in 2000, by Prof. Dr. A. E. Migotto (CEBIMar-USP, Brazil). After Dawson and Jacobs (2001) identified sequences of medusae from the coast of São Paulo state as *Aurelia* sp. 2 (herein described under *Aurelia cebimarensis* sp. nov.), polyps from Ilha Grande were also assigned to this species. Therefore, Gambill and Jarms (2014), in their study of *Aurelia* scyphistomae and ephyrae, also recognized the polyps from Ilha Grande as *A.* sp. 2, and it was the only population that had 27-32 tentacles, while all others in the study presented ~16 tentacles. This remains as the only morphological diagnostic

character for this species, even though morphological plasticity has also been reported in *Aurelia* polyps and ephyrae (Chiaverano and Graham, 2017). Nevertheless, there are unambiguous molecular characters to support this species' diagnosis.

Aurelia mianzani Lawley, Gamero-Mora, Maronna, Chiaverano, Collins and Morandini **sp. nov.**

Aurelia sp. AA2501 South West Atlantic Ramšak et al., 2012.

Aurelia sp. 16 Gómez-Daglio and Dawson, 2017. Abboud et al., 2018; Dong, 2018.

Type material: Holotype: DNA extraction, USNM (pending deposit).

Type locality: 35º 56' 08" S, 56º 59' 08" W; Bahía Samborombón, Buenos Aires, Argentina.

Etymology: *In memoriam* of Dr. Hermes W. Mianzan (INIDEP, Argentina), who collected all sequenced specimens of this species, and for his lifelong contributions and dedication to understanding jellyfish biology and ecology in the Southwestern Atlantic.

Distribution: Currently known only from the type locality (see Fig. 11).

Diagnosis: Molecular diagnosis is given in Figs. 12-13, 15. GenBank accession numbers of sequences that belong to this species and were used in analyses are presented in Supplementary Material S3.

Remarks: In Ramšak et al. (2012), the specimen collected in the Southwestern Atlantic appeared as sister taxa to a specimen from the Mljet lakes, Croatia (currently known as *A. relicta*), in their combined-marker phylogeny. In our single-marker phylogenies, we observed that the COI sequence from that study fell within a clade alongside the other sequences from Argentina (Supplementary Material S4), while the ITS1 sequence from that same specimen fell within the *A. relicta* clade (Supplementary Material S4). This could be explained by contamination in sequencing the ITS1, as in the aforementioned study, *A. relicta* specimens from the Mljet lakes were also being processed. As other sequences from Argentina were available, the specimen from Ramšak et al. (2012) were disregarded for our combined-marker phylogenetic analysis.

Aurelia bajacaliforniana Lawley, Gamero-Mora, Maronna, Chiaverano, Collins and Morandini **sp. nov.**

Aurelia sp. 12 Gómez-Daglio and Dawson, 2017. Abboud et al., 2018; Dong, 2018.

Type material: Holotype: Medusa, M0D006054V (pending confirmation). Paratypes: Medusae, M0D (pending confirmation).

Type locality: 24º 10' 24" N, 110º 18' 56"; Bahía de La Paz, Baja California Sur, Mexico.

Etymology: Named after the type locality.

Distribution: Currently known only from the type locality (see Fig. 11).

Diagnosis: Molecular diagnosis is given in Figs. 12-13. GenBank accession numbers of sequences that belong to this species and were used in analyses are presented in Supplementary Material S3.

Remarks: Gómez-Aguirre (1991) recorded specimens of *Aurelia aurita* in the Gulf of California. As we cannot confirm the identity of these specimens to this species' clade, we refrain from synonymizing it to *Aurelia bajacaliforniana* sp. nov. This record was acknowledged by Gómez-Daglio and Dawson (2017), although under *Aurelia* sp. 13 (herein described as *Aurelia daglioi* sp. nov.).

Aurelia daglioi Lawley, Gamero-Mora, Maronna, Chiaverano, Collins and Morandini **sp. nov.**

Aurelia sp. 13 Gómez-Daglio and Dawson, 2017. Abboud et al., 2018; Dong, 2018.

Type material: Holotype: Medusa, M0D020163M (pending confirmation). Paratypes: Medusae, M0D (pending confirmation).

Type locality: 12º 3' 11" N, 86º 42' 15" W; El Tránsito, Nicaragua.

Etymology: Named after Dr. Liza Gómez-Daglio (University of California, USA), for her work on jellyfish biodiversity, including the collected specimens of this species.

Distribution: In the Tropical Eastern Pacific, from the coast of El Salvador to Costa Rica (see Fig. 11; for specific localities see Supplementary Material S3).

Diagnosis: Molecular diagnosis is given in Figs. 12-13, 15. GenBank accession numbers of sequences that belong to this species and were used in analyses are presented in Supplementary Material S3.

Remarks: This species, alongside *Aurelia bajacaliforniana* sp. nov., form a clade, which is sister to *Aurelia mianzani* sp. nov. and is within the clade shared by most western Atlantic *Aurelia* species (Fig. 10). This diversification across the Isthmus of Panama has been reported for other cnidarians (Stampar et al., 2012). Further biogeographical studies and increased sampling can verify this matter, as well as the curious position of the neighbor *Aurelia panamensis* sp. nov. on the combined-marker phylogeny (Fig. 10).

Previous mentions in the literature of this species are acknowledged in Gómez-Daglio and Dawson (2017), although the included records of Cortés (1996) and Gómez-Aguirre (1991), reported *Aurelia* from localities that do not match the sequenced specimens herein (see also remarks for *Aurelia bajacalifoniana* sp. nov.). The type locality chosen for this species was based on the oldest collected material among the sequenced specimens (see Gómez-Daglio and Dawson, 2017).

Aurelia rara Lawley, Gamero-Mora, Maronna, Chiaverano, Collins and Morandini **sp. nov.**

Aurelia sp. DI'03-4 Chiaverano et al., 2016.

Type material: Holotype: DNA extraction, USNM (pending deposit). Paratypes: Tissue (Medusa), USNM (pending deposit).

Type locality: 30º 09' 28" N, 88º 08' 22" W; Dauphin Island, Alabama, United States of America.

Etymology: Derived from the Latin word *rarus***,** meaning "rare" or "uncommon", due to its elusive occurrence among the other two species collected in the same locality (*Aurelia montyi* and *Aurelia marginalis*, herein considered).

Distribution: Currently known only from the type locality (see Fig. 11).

Diagnosis: Molecular diagnosis is given in Fig. 13. GenBank accession numbers of sequences that belong to this species and were used in analyses are presented in Supplementary Material S3.

Remarks: Chiaverano et al. (2016) sequenced one specimen from Dauphin Island (DI'03-4), which in their COI phylogeny did not group with any other species (also see Supplementary Material S4), while in the ITS1 phylogeny fell within *Aurelia marginalis* (previously recognized as *Aurelia* sp. 9, treated herein; also see Supplementary Material S4). In our combined-marker phylogeny however, as for COI, this species fell in a separate clade from *Aurelia marginalis*, and even though the Goodman-Bremer support value for this species is low, there seems to be a considerable amount of character-state transformations (i.e., branch length) separating these hypothetical species (Fig. 10). These two taxa considered as separate species should not come as a surprise, as previous studies also demonstrated the occurrence of another sympatric species in the area, *Aurelia montyi* (recognized as *Aurelia* cf. sp. 2 in Chiaverano et al., 2016; described herein).

In this study, we also obtained 16S sequences from other individuals collected in Dauphin Island that could also belong to *Aurelia rara* sp. nov., as they do not fall within species clades of the other sympatric species mentioned. Until we obtain more sequences from these individuals, COI for example, that can confirm if they fall within the same clade as individual DI'03-4, we identify them as *A.* cf. *rara* (Fig. 12; Supplementary Material S4).

Aurelia marginalis Agassiz, 1862

Aurelia sp. 9 Dawson et al., 2005. Ki et al., 2008; Dong et al., 2015; He et al., 2015; Chiaverano et al., 2016; Chiaverano and Graham, 2017; Gómez-Daglio and Dawson, 2017; Abboud et al., 2018; Dong, 2018.

Type material: Holotype: Medusa, MCZ 352.

Type locality: Key West, Florida, United States of America.

Distribution: Across the Gulf of Mexico (see Fig. 11; for specific localities see Supplementary Material S3).

Diagnosis: Molecular diagnosis is given in Figs. 13, 15. GenBank accession numbers of sequences that belong to this species and were used in analyses are presented in Supplementary Material S3.

Remarks: Specimens of both previously recognized *Aurelia* sp. 9 (here synonymized) and *Aurelia montyi* lineages (recognized as *Aurelia* cf. sp. 2 in Chiaverano et al., 2016; described herein) have been collected in the Florida Keys (Long Key, Florida,

USA), which is within the "reefs of Florida", locality cited in the description (Agassiz, 1862), and very near the type specimen's locality. Even though we portray in this study the unreliability of morphological data to species recognition (further discussed), we decided to synonymize *Aurelia* sp. 9 under *Aurelia marginalis*, as in this species' description, Agassiz (1862) mentions the distinct rose color of the gonads, which is also presented by Chiaverano et al. (2016) for *A.* sp. 9 when compared to *A. montyi* (see Fig. 1 in Chiaverano et al., 2016). Nevertheless, this should not be used as diagnostic, as color has been previously reported on holding no value for systematics in this genus Kramp (1968), and even in Medusozoa (Lampert et al., 2011; Holst and Laakmann, 2014).

Aurelia marginalis was recently resurrected by Calder (2009), due to differences with specimens from northeastern USA, which were reported as more similar to *Aurelia aurita* (treated herein) from northern Europe. These differences came mostly from polyps, on their free amino acid composition, nematocyst types, morphology, and asexual reproduction (Calder, 2009). The use of morphological characters in polyps to recognize different *Aurelia* species has been reported as problematic (Gambill and Jarms, 2014), which was further corroborated by the possibility of morphological plasticity due to environmental differences (Chiaverano and Graham, 2017). The use of nematocyst types for species recognition in Medusozoa can also be problematic (Gimenes et al., unpublished), as well as in other cnidarians (Francis, 2004; Acuña et al., 2011). Therefore, we do not report these as diagnostic for this species, but we corroborate the resurrection by Calder (2009) with a molecular diagnosis. Other synonyms for this species have been presented, but we refrain from maintaining them, as they could belong to other species present in the Gulf of Mexico, and there is no way to confirm it.

Aurelia montyi Lawley, Gamero-Mora, Maronna, Chiaverano, Collins and Morandini **sp. nov.**

Aurelia cf. sp. 2 Chiaverano et al., 2016.

Type material: Holotype: DNA extraction, USNM (pending deposit). Paratypes: Tissue (Medusa), USNM (pending deposit).

Type locality: 30º 09' 28" N, 88º 08' 22" W; Dauphin Island, Alabama, United States of America.

Etymology: Named after Dr. William "Monty" Graham (University of Southern Mississipi, USA), who was a pioneer in ecological studies with *Aurelia* in the Gulf of Mexico and former advisor of LMC (co-author in this study), both of which collected and sequenced most of the specimens that belong to this species.

Distribution: Eastern Gulf of Mexico (see Fig. 11; for specific localities see Supplementary Material S3).

Diagnosis: Molecular diagnosis is given in Figs. 12-14. GenBank accession numbers of sequences that belong to this species and were used in analyses are presented in Supplementary Material S3.

Remarks: This species was considered as *Aurelia* cf. sp. 2 because it was in the same clade as *Aurelia cebimarensis* sp. nov. (previously considered *Aurelia* sp. 2) in the ITS1 phylogeny (see Fig. 5 in Chiaverano et al., 2016), even though there were considerable branch lengths separating them and they were reciprocally monophyletic in the COI phylogeny (see Fig. 4 in Chiaverano et al., 2016). As we have presented previously, species do not necessarily need to form a clade (Frost and Kluge, 1994; Skinner, 2004; see 'Materials and Methods' section). Nevertheless, by including more molecular data, all of the phylogenies returned this species as a separate clade, which seems as enough evidence now to corroborate this species' hypothesis.

Aurelia smithsoniana Lawley, Gamero-Mora, Maronna, Chiaverano, Collins and Morandini **sp. nov.**

Aurelia sp. 15 Gómez-Daglio and Dawson, 2017. Abboud et al., 2018; Dong, 2018.

Type material: Holotype: DNA extraction, MZUSP (pending deposit). Paratypes: Medusa, M0D021370X (pending confirmation).

Type locality: 9º 14' 27" N, 82º 15' 08" W; Bocatorito Bay, Bocas del Toro, Panama.

Etymology: Named after the Smithsonian Tropical Research Institute, in Bocas del Toro, Panama, which has supported studies in marine science for decades, especially in the Bocas del Toro area, where this species is distributed.

Distribution: Bocas del Toro, Panama (see Fig. 11; for specific localities see Supplementary Material S3).

Diagnosis: Molecular diagnosis is given in Figs. 12-13. GenBank accession numbers of sequences that belong to this species and were used in analyses are presented in Supplementary Material S3.

Remarks: In our 28S phylogeny (Supplementary Material S4), this species appears in a single clade with *Aurelia cebimarensis* sp. nov., although they appear reciprocally monophyletic in the 16S and COI phylogenies (Supplementary Material S4), and more importantly in the combined-marker phylogeny (Fig. 10). In the latter, although branches for each species may appear short, there is considerable node support for each. Furthermore, even though there are reported cases of sympatric *Aurelia* species and multiple introductions (Dawson et al., 2005; Chiaverano et al., 2016), the disjunct distribution of these sister species in neighboring but different biogeographic realms (Costello et al., 2017), as well as different large marine ecosystems (Sherman, 1991), could be further evidence of lineage separation.

Aurelia cebimarensis Lawley, Gamero-Mora, Maronna, Chiaverano, Collins and Morandini **sp. nov.**

Aurelia sp. 2 Dawson and Jacobs, 2001. Dawson, 2003; Dawson et al., 2005; Morandini et al., 2005; Ki et al., 2008; Bayha et al., 2010; Ramšak et al., 2012; Dong et al., 2015; He et al., 2015; Chiaverano et al., 2016; Scorrano et al., 2016; Goméz-Daglio and Dawson, 2017; Dong, 2018.

Type material: Holotype: Medusae, MZUSP (pending deposit). Paratypes: Polyps, MZUSP (pending deposit)/ USNM (pending deposit); Tissue (Polyps), MZUSP (pending deposit)/ USNM (pending deposit).

Type locality: 23º 49’ 44” S, 45º 25’ 23” W; Baleeiro Rock at Cabelo Gordo Beach, São Sebastião, São Paulo, Brazil.

Etymology: Named after the Centro de Biologia Marinha (CEBIMar) of the University of São Paulo, situated exactly where the type specimen was collected. This center is an international reference in marine biology studies, and many of the authors in this study have depended heavily on these facilities for their education and research.

Distribution: Our records include specimens from across the São Paulo state and from Aracaju, Sergipe. Therefore, the distribution likely spans the Brazilian coast from southeast to northeast (see Fig. 11; for specific localities see Supplementary Material S3).

Diagnosis: Molecular diagnosis is given in Figs. 12-14. GenBank accession numbers of sequences that belong to this species and were used in analyses are presented in Supplementary Material S3.

Remarks: Mayer (1910) had identified specimens from the Brazilian coast as *Aurelia aurita* and further records in the literature followed this classification (Pantin & Dias, 1952; Vannucci, 1957; Goy, 1979; Mianzan & Cornelius, 1999). However, we cannot confirm their identity to *Aurelia cebimarensis* sp. nov. or others that occur or might occur in the country, and we therefore abstain from including as synonymous.

Gambill and Jarms (2014) had identified *Aurelia* polyps from the Brazilian coast (Ilha Grande, Rio de Janeiro), which had overall more tentacles than other populations, as *Aurelia* sp. 2. However, sequences retrieved from these polyps, which came from the same locality and same culture as in their study, were recognized as a different species, *Aurelia insularia* sp. nov. (see also remarks in this species' description).

Aurelia andamensis Lawley, Gamero-Mora, Maronna, Chiaverano, Collins and Morandini **sp. nov.**

Aurelia sp. Ruijuan et al., 2016.

Type material: Holotype: DNA extraction (pending confirmation).

Type locality: Nam Kem Village, Phangnga, Thailand.

Etymology: Named after the Andaman Sea, where the type locality is situated.

Distribution: Currently only known from the type locality (see Fig. 11).

Diagnosis: Molecular diagnosis is given in Fig. 12. GenBank accession numbers of sequences that belong to this species and were used in analyses are presented in Supplementary Material S3.

Remarks: In the 18S phylogeny in Ruijuan et al. (2016), the specimens that they collected in the western coast of Thailand fell within the same clade as an *Aurelia coerulea* (as *Aurelia* sp. EU276014) specimen from Korea. In their 16S phylogenetic tree, as in the 16S and combined-marker phylogenetic trees presented herein (Figs. 10, 12; also see Supplementary Material S4), these specimens appear in a distinct clade from other *Aurelia* sequences. Also, they appear in a distinct locality from any other specimen collected up to date. With these evidence, we recognize this lineage as a species hypothesis.

Aurelia panamensis Lawley, Gamero-Mora, Maronna, Chiaverano, Collins and Morandini **sp. nov.**

Aurelia sp. 14 Gómez-Daglio and Dawson, 2017; Dong, 2018.

Type material: Holotype: Tissue (Medusa), M0D014904F (pending confirmation). Paratypes: Tissue (Medusae), M0D (pending confirmation).

Type locality: 8º 59' 8" N, 79º 29' 32" W; Gulf of Panama, Panama.

Etymology: Named after the country where the type locality is situated.

Distribution: Currently only known from the type locality (see Fig. 11).

Diagnosis: Molecular diagnosis is given in Figs. 12-13, 15. GenBank accession numbers of sequences that belong to this species and were used in analyses are presented in Supplementary Material S3.

Remarks: This species does not form a clade with its congeners from the Tropical Eastern Pacific (*Aurelia bajacaliforniana* sp. nov. and *Aurelia daglioi* sp. nov.), which in turn fall within the clade that includes most of the western Atlantic species (similar to the case of *Aurelia ayla* sp. nov.; see Fig. 10). Further increasing the dataset with more molecular markers and specimen collections, especially from the southeastern Atlantic, Indian and southeastern Pacific oceans could resolve this matter. This could not only be an effect of undersampling, but also a case of introduction from another locality, which is not unprecedented for the genus (Dawson et al., 2005), and would not be a surprise due to the proximity to the Panama Canal, a region with intense naval traffic.

Aurelia dawsoni Lawley, Gamero-Mora, Maronna, Chiaverano, Collins and Morandini **sp. nov.**

Aurelia sp. 3 Dawson and Jacobs, 2001. Dawson, 2003; Dawson et al., 2005; Ki et al., 2008; Ramšak et al., 2012; Dong et al., 2015; He et al., 2015; Chang et al., 2016; Chiaverano et al., 2016; Scorrano et al., 2016; Abboud et al., 2018; Dong et al., 2017; Dong, 2018.

Type material: Holotype: M0D (pending confirmation).

Type locality: 7º 15' 52" N, 134º 26' 58" E; Tab Kukau Cove, Koror State, Palau.

Etymology: Named after Dr. Michael N. Dawson (University of California, USA), for his ongoing work on unveiling diversity in scyphozoan jellyfishes, especially in *Aurelia*, without which this work would not have been possible. His studies identified most of the diversity within the genus, but we name this species after him due to his research and collaborations in the Palau region.

Distribution: Palau coves (see Fig. 11; for specific localities see Supplementary Material S3).

Diagnosis: Molecular diagnosis is given in Figs. 13-14. GenBank accession numbers of sequences that belong to this species and were used in analyses are presented in Supplementary Material S3.

Remarks: Previous studies have shown that even though there are genetic differences distinguishing Palau specimens from those of other localities, they have similar rates of feeding, growth, respiration and swimming, if compared to *A. aurita* from the Black Sea (Dawson and Martin, 2001). Also, morphological variation between populations within a species sometimes exceeded variation between species within the Palau region (Dawson, 2003; *Aurelia* sp. 4, herein synonymized under *Aurelia malayensis* sp. nov., and *Aurelia* sp. 6, herein synonymized under *Aurelia clausa*), which makes morphological diagnosis unreliable, as we also present in this study (further discussed).

Aurelia malayensis Lawley, Gamero-Mora, Maronna, Chiaverano, Collins and Morandini **sp. nov.**

Aurelia sp. 4 Dawson and Jacobs, 2001. Dawson, 2003; Dawson et al., 2005; Ki et al., 2008; Ramšak et al., 2012; Bayha and Graham, 2014; Dong et al., 2015; He et al., 2015; Chang et al., 2016; Chiaverano et al., 2016; Scorrano et al., 2016; Dong et al., 2017; Abboud et al., 2018; Dong, 2018.

Type material: Holotype: M0D (pending confirmation).

Type locality: 7º 9’ N, 134º 23’ E; Ongeim’l Tketau, Koror, Palau.

Etymology: Named after the Malay Archipelago, situated between mainland Indo-China and Australia, which includes the type locality and the suggested endemic distribution for this species (Dawson et al., 2005).

Distribution: Across the Malay Archipelago to southern Japan, as well as in Hawaii (see Fig. 11; for specific localities see Supplementary Material S3).

Diagnosis: Molecular diagnosis is given in Figs. 12-15. GenBank accession numbers of sequences that belong to this species and were used in analyses are presented in Supplementary Material S3.

Remarks: This species has been hypothesized as endemic to eastern Borneo and Palau, with the possibility of natural dispersal across the Malay Archipelago, south to Australia and north to Japan (Dawson et al., 2005). Therefore, the occurrence in Hawaii would come from an anthropogenic introduction, likely after considerable WWII naval traffic (Dawson et al., 2005).

Mayer (1910) mentions the distribution of *Aurelia colpota* Brandt, 1835 across the Indo-Pacific. As the type specimen was described in South Africa and we cannot rely on morphology for further comparisons (further discussed), we refrain from resurrecting this name. For more information on previous studies regarding ecology and morphology of this species see remarks for *Aurelia dawsoni* sp. nov.

Aurelia dubia Vanhöffen, 1888

Aurelia ARAB lineage Schroth et al., 2002. Dawson, 2003; Dawson et al., 2005.

Type material: To our knowledge, no type material remains. Other material might remain in the private collection of Schroth et al. (2002), as they deposited the sequences for this species in GenBank.

Type locality: Persian (Arabian) Gulf.

Distribution: Arabian Peninsula, in the Red Sea and Persian Gulf (see Fig. 11; for specific localities see Supplementary Material S3).

Diagnosis: Molecular diagnosis is given in Figs. 12, 14. GenBank accession numbers of sequences that belong to this species and were used in analyses are presented in Supplementary Material S3.

Remarks: Schroth et al. (2002) defined the ARAB lineage with specimens from the Red Sea and from the Persian Gulf, the latter indicated as the type locality for *Aurelia dubia.* Nevertheless, they only deposited two sequences from this lineage in GenBank, one for 16S and one for ITS1, the former without any specification of the collection locality and the latter from a Persian Gulf specimen. In our single-marker phylogenies,

the ITS1 sequence appears separate from all other *Aurelia* (Supplementary Material S4), while for 16S, it forms a clade with a specimen from the Red Sea, although with considerable branch lengths separating them (Supplementary Material S4). In our combined-marker phylogeny, branch lengths separating these specimens seem less significant, although this can be related to the fact that these specimens only share the 16S marker (Fig. 10). Considering that the ARAB lineage was defined also based on samples from the Red Sea, it could be possible that these specimens belong to the same species. We herein resurrect *A. dubia* encompassing the distribution of the ARAB lineage, although we identify the specimen from the Red Sea as *A.* cf. *dubia*, until more markers are sequenced or further samples are collected that can ensure the identity of this specimen within the *A. dubia* species hypothesis.

Aurelia clausa Lesson, 1830

Aurelia sp. 6 Dawson and Jacobs, 2001. Dawson, 2003; Dawson et al., 2005; Ki et al., 2008; Häussermann et al., 2009; Ramšak et al., 2012; Dong et al., 2015; He et al., 2015; Chang et al., 2016; Chiaverano et al., 2016; Scorrano et al., 2016; Abboud et al., 2018; Dong, 2018.

Type material: To our knowledge, no type material remains. Other material from the type locality might remain in the private collection of Dawson et al. (2005), as they deposited sequences of a specimen from this locality in GenBank.

Type locality: New Ireland, Papua New Guinea.

Distribution: Palau lakes, Papua and Papua New Guinea (see Fig. 11; for specific localities see Supplementary Material S3).

Diagnosis: Molecular diagnosis is given in Figs. 13-14. GenBank accession numbers of sequences that belong to this species and were used in analyses are presented in Supplementary Material S3.

Remarks: Some sequences from New Zealand posted in GenBank were identified as *Aurelia* aff. *clausa* (see Supplementary Material S3). However, *A. clausa* was described from New Ireland, Papua New Guinea, where some specimens that belong to the previously considered *Aurelia* sp. 6 lineage were collected. Therefore, specimens in this lineage are here synonymized under *A. clausa,* while the lineage that contains specimens from New Zealand were given the new name *Aurelia haka* sp. nov. (also see

remarks in its description herein). For more information on previous studies regarding ecology and morphology of this species see remarks for *Aurelia dawsoni* sp. nov.

Aurelia solida Browne, 1905

Aurelia TET lineage Schroth et al., 2002.

Aurelia sp. 8 Dawson et al., 2005. Ramšak et al., 2012; Ki et al., 2008; Manzari et al., 2014; Dong et al., 2015; He et al., 2015; Marques et al., 2015; Chiaverano et al., 2016.

Aurelia sp. Tinta et al., 2010 (Bay of Piran).

Type material: Neotype: Medusae, UNIPD CN58CH. Paratype: Medusae, UNI_SCY_038.

Type locality: Gulf of Trieste, Italy.

Distribution: Sequences of specimens herein included derive from across the Mediterranean Sea and the Red Sea (see Fig. 11; for specific localities see Supplementary Material S3).

Diagnosis: Absence of an endodermal ocellus on the subumbrellar side of rhopalia (Scorrano et al., 2016; see remarks below). Molecular diagnosis is given in Figs. 12-15. GenBank accession numbers of sequences that belong to this species and were used in analyses are presented in Supplementary Material S3.

Remarks: The type locality is not concordant with that of the original description (Maldives), so resurrection of the name was based on the direction of the rhopalium, which pointed to the exumbrellar side (90º angle), as is noted in the recent description (Scorrano et al., 2016). However, we also observed this in specimens from very distinct localities, such as southwestern USA and the Atlantic Ocean off Portugal (Fig. 17A-D). Other observations have also indicated that morphology of rhopalia can vary even within species (Fig. 8B, D). Nevertheless, the presence or absence of an endodermal ocellus in specimens that also had an angled rhopalium could not be verified, as they can fade with preservation. This character may also vary, but until further specimens are analyzed, it is maintained as diagnostic. The molecular diagnosis is also present to support this species' hypothesis.

No sequences have been obtained from specimens of the Maldives to confirm the distribution of this species in this locality. Nevertheless, it has been hypothesized that this

species was introduced from the Indian Ocean into the Mediterranean through the Suez Canal (Dawson et al., 2005; Scorrano et al., 2016).

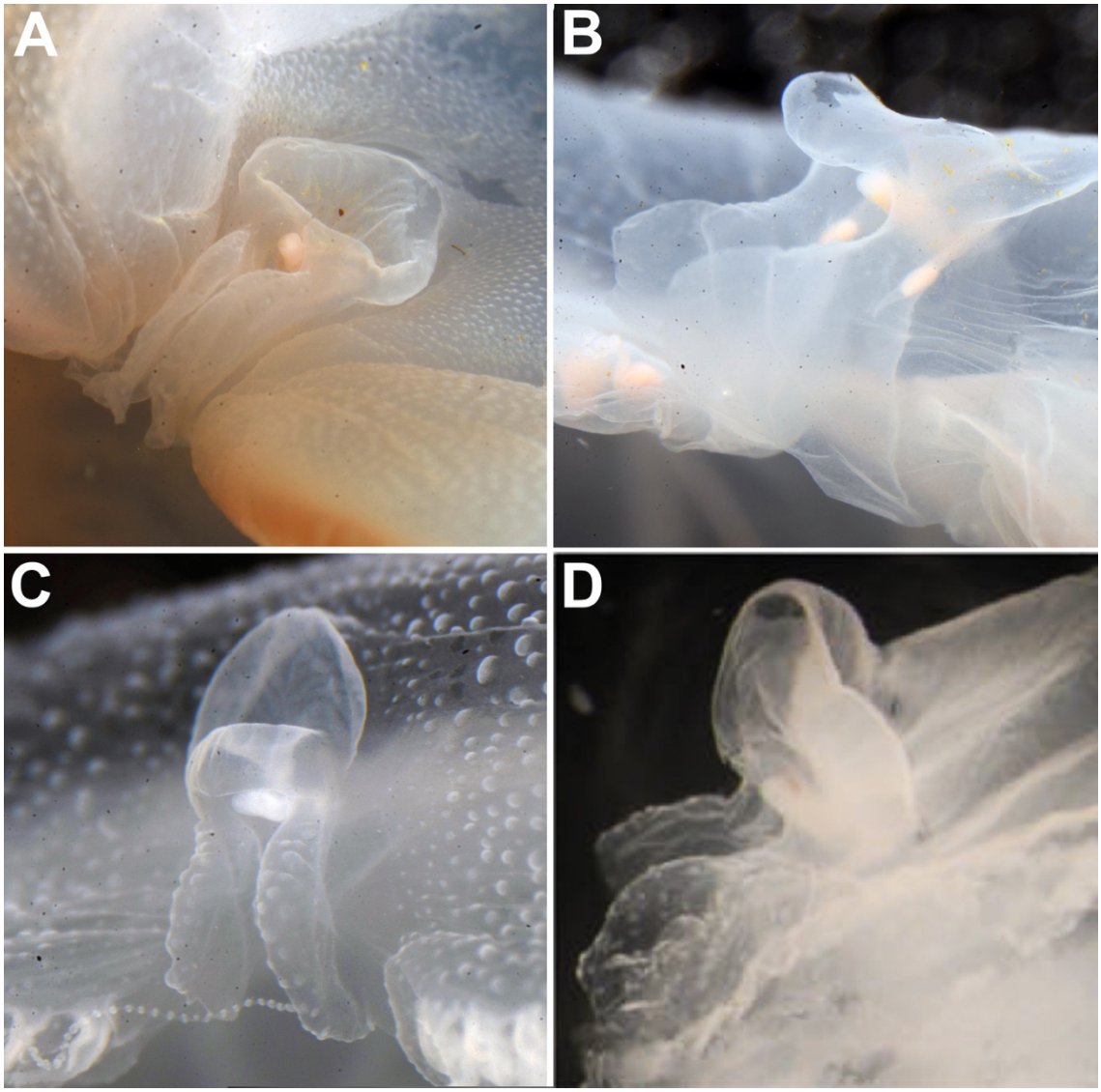


Figure 17. Comparison of rhopalia morphology observed in some *Aurelia* medusae. The 90º angled sense organ can be noticed in medusae from various localities, which includes (A, B) the southwestern coast of the USA (USNM 92911-1, 92912-4), (C) the Atlantic Ocean off Portugal (USNM 58263-1) and (D) *Aurelia solida*, from Scorrano et al., (2016). A, bell diameter (*f*1) = 13.45 cm; B, *f*1 = 10.6 cm; C, *f*1 = 5 cm; D, *f*1 = 14.4 cm (image D adapted from Scorrano et al., 2016).

Aurelia labiata Chamisso and Eysenhardt, 1821

Type material: Neotype: Medusa, CASIZ 111024.

Type locality: Monterey Bay, California, United States of America.

Distribution: Sequences of specimens herein included derive from the northern coast of California, USA, north to Canada and into Alaska, USA (see Fig. 11; for specific localities see Supplementary Material S3).

Diagnosis: Molecular diagnosis is given in Fig. 13. GenBank accession numbers of sequences that belong to this species and were used in analyses are presented in Supplementary Material S3.

Remarks: In the COI phylogeny, we were able to observe two distinct clades within what was considered as *Aurelia labiata* (Supplementary Material S4). These clades were separated by considerable branch lengths, even more than for other sister species, such as *Aurelia cebimarensis* sp. nov. and *Aurelia smithsoniana* sp. nov. Nevertheless, due to variation in evolutionary rates and collection efforts, branch lengths may vary even across congeners (Grant et al., 2006; see 'Material and Methods' section).

In the other single-marker phylogenies, due to less sampling or even to different evolutionary rates across markers, it was not possible to observe two very distinct reciprocally monophyletic clades as for COI (Supplementary Material S4). Furthermore, as these hypothetical species clades are sympatric (*A. labiata* and *Aurelia Columbia* sp. nov., herein described), without identifying these clades it is impossible to tell, for the other markers, which sequences belong to each species. For this same reason, we refrain from acknowledging any previous mentions as synonyms.

Only 4 specimens, two in each of the species' clades in COI, had at least one other sequenced marker, even though only COI was shared between these species (Supplementary Table S3). These specimens were used for the combined-marker phylogenetic analysis, which returned the same pattern as in COI. In only one of the COI clades was there a specimen from California, USA, where the type locality for *A. labiata* is situated, and is therefore described under this species' hypothesis. Also, additional preserved material in this species' redescription (Gershwin, 2001) is from Tomales Bay, California (CAS 111023), from where the Californian sequenced specimens included herein are.

A distinct character included as diagnostic in both the original description and redescription of *A. labiata*, is the prominent manubrium (from the latin *labium*, meaning "lip"; for images and illustrations see Gershwin, 2001). This feature has been previously reported for other localities in the Pacific and Indian oceans, in specimens identified as *A. labiata* or even as *A. maldivensis* (Mayer, 1910). We also made these observations in

some of the preserved specimens from the western coast of the USA, the Atlantic Ocean off Portugal and even from other localities, such as Japan and the western coast of Panama (Fig. 18A-B; also see *f*5 in Supplementary Material S1). Also, the number of marginal lobes (also called bell scalloping), considered previously as 16 for *A. labiata* and its variaties (Mayer, 1910), had already been disregarded as taxonomically significant in the species' redescription. This can be further emphasized in this study, as specimens from the Brazilian coast also seem to have more pronounced non-rhopaliar indentations (see *f*10 in Figs. 3-4), which defines the secondary scalloping.

None of the specimens sequenced from the western Pacific or Indian Oceans, which can present similar morphology to the previously considered *A. labiata*, clustered within any of the non-introduced northeastern Pacific species clades (which excludes *A. coerulea*). Until further studies can assess variability and plasticity of bell indentations and manubrium length, we refrain from using these characters in the diagnosis.

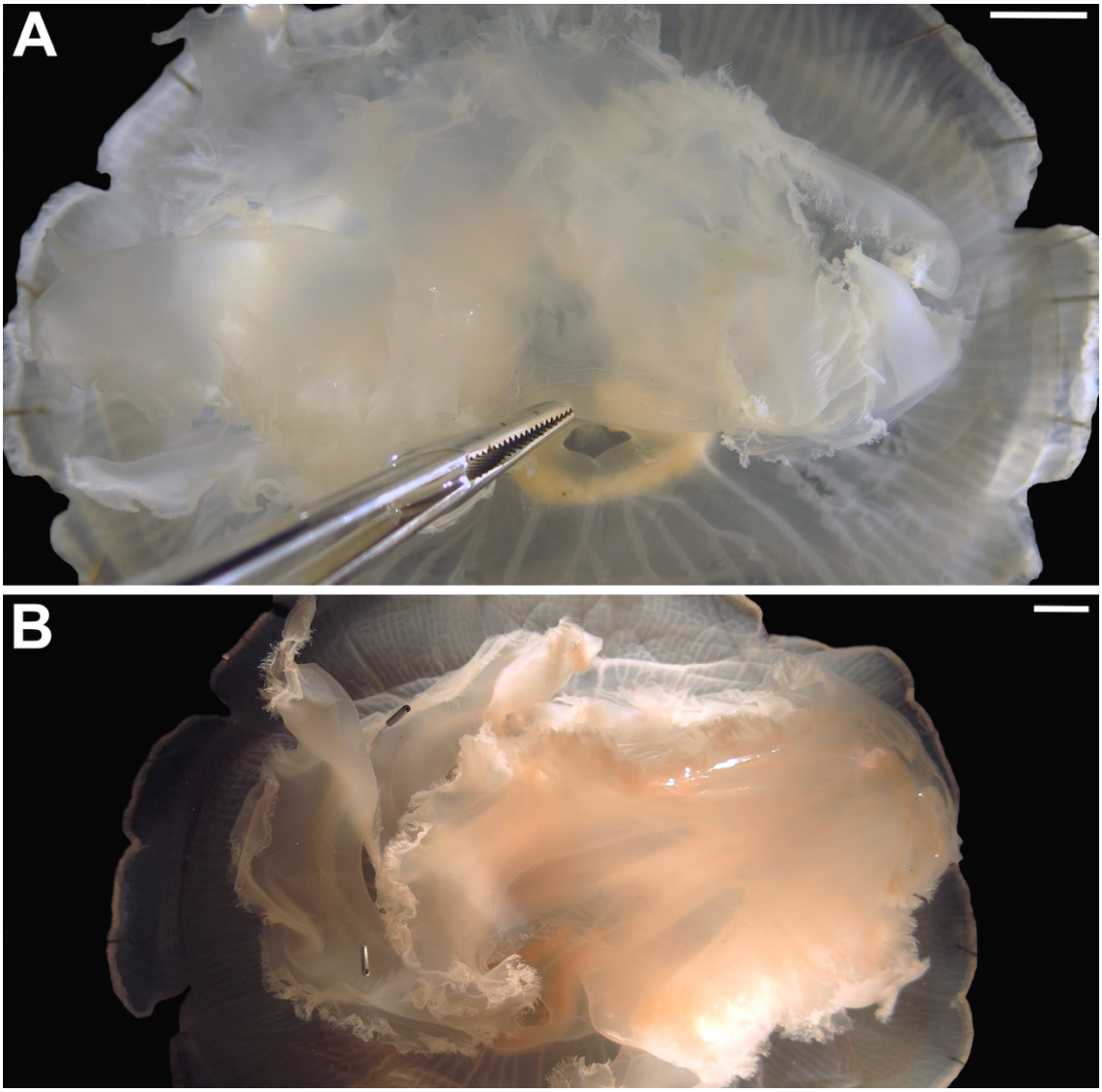


Figure 18. Comparison of manubrium morphology observed in two *Aurelia* medusae. The prominent manubrium, which sideways almost reached the margin of the umbrella, can be noticed in medusae from distinct localities, which includes (A) the Atlantic Ocean off Portugal (USNM 58284) and (B) the southwestern coast of the USA (USNM 92911-2). Scales = 1 cm.

Aurelia columbia Lawley, Gamero-Mora, Maronna, Chiaverano, Collins and Morandini **sp. nov.**

Type material: Holotype: M0D (pending confirmation).

Type locality: 48º 23' N, 123º 41' W; Sooke Basin, British Columbia, Canada.

Etymology: Named after the region where the type locality is situated, and where most of the sequenced specimens have been collected.

Distribution: Northwestern coast of the USA, north to Canada (see Fig. 11; for specific localities see Supplementary Material S3).

Diagnosis: Molecular diagnosis is given in Fig. 13. GenBank accession numbers of sequences that belong to this species and were used in analyses are presented in Supplementary Material S3.

Remarks: This species is sympatric with *A. labiata*, which was described to span from California to Alaska (USA) in the northeastern Pacific (see remarks for *A. labiata*, herein treated). Gershwin (2001), in the redescription of *A. labiata*, observed three morphotypes occurring in a latitudinal gradient. These morphotypes may not be species-specific, as we here describe another species that occurs across the range of *A. labiata*. Further studies integrating molecular phylogenetics and morphometrics may unravel morphological variation and plasticity within these species.

Aurelia persea (Forskål, 1775)

Aurelia sp. Mizrahi, 2014.

Type material: To our knowledge, no type material remains. Other material from the type locality region might remain in the private collection of Mizrahi (2014), as he deposited sequences of a specimen from this locality in GenBank.

Type locality: Mediterranean Sea.

Distribution: Sequences of specimens herein included derive only from Haifa Bay, Israel (see Fig. 11; for specific localities see Supplementary Material S3).

Diagnosis: Molecular diagnosis is given in Figs. 12-13, 15. GenBank accession numbers of sequences that belong to this species and were used in analyses are presented in Supplementary Material S3.

Remarks: The original description of this species is brief and simple, which therefore later rendered it as synonymous to *Aurelia aurita* (Agassiz, 1862). Even if the description were more informative, there is only one image of the sequenced specimen (see Fig. 17 in Mizrahi, 2014), from which hardly any information can be retrieved. Furthermore, as we portray in this study the unreliability of medusa morphology for species identification (further discussed), we resurrect *Aurelia persea* because it is the oldest available name that encompasses the locality of the sequenced specimen treated herein.

Aurelia relicta Scorrano, Aglieri, Boero, Dawson and Piraino, 2016

Aurelia sp. Benovic et al., 2000. Malej et. al., 2007; Turk et al., 2008; Tinta et al., 2010 (Big Lake);

Aurelia sp. 5 Dawson and Jacobs, 2001. Dawson et al., 2005; Ki et al., 2008; D'Ambra and Graham, 2009; Malej et al., 2009; Kogovšek et al., 2012; Korsun et al., 2012; Ramšak et al., 2012; D'Ambra et al., 2013; Manzari et al., 2014; Wang and Sun, 2014; Chiaverano et al., 2015; Dong et al., 2015; He et al., 2015; Marques et al., 2015; Chiaverano et al., 2016; Miloslavic et al., 2016.

Aurelia MS-MKL Schroth et al., 2002.

Type material: Holotype: Medusa, UNIPD CN57CH. Paratypes: Medusa, UNIS_SCY_028/29.

Type locality: Veliko Jezero, Mljet Island, Croatia.

Distribution: Mljet Island lakes, Croatia (see Fig. 11; for specific localities see Supplementary Material S3).

Diagnosis: Molecular diagnosis is given in Figs. 12-15. GenBank accession numbers of sequences that belong to this species and were used in analyses are presented, along with further details, in Supplementary Material S3.

Remarks: In Ramšak et al. (2012), one of the specimens collected in the Black Sea, in the Turkish coast, appeared as sister taxa to specimens from the West Atlantic, in their combined-marker phylogeny. In our single-marker phylogenies, we observed that the ITS1 sequence from that specimen fell within a clade alongside the other sequences from the same locality, within the *Aurelia aurita* clade (Supplementary Material S4), while the COI sequence from that same specimen fell within the *A. relicta* clade (Supplementary Material S4). This can potentially be due to contamination in sequencing the COI, as in the aforementioned study, *A. relicta* specimens from the Mljet lakes were also being sequenced. As other sequences from both *A. aurita* and *A. relicta* were available, this specimen from Ramšak et al. (2012) was disregarded for the combined-marker phylogenetic analysis herein.

Scorrano et al. (2016) presented a table with diagnostic characters for some of the Mediterranean species of *Aurelia*, from the polyp, ephyra and medusa stages. Nevertheless, based on the unreliability of medusa morphometric features for species

recognition shown herein (further discussed); and the potential confusion that can arise from polyp and ephyra morphology (Gambill and Jarms, 2014), especially considering the possibility of morphological plasticity in these life cycle stages (Chiaverano and Graham, 2017), we refrain from including them here. Furthermore, there seem to be no unambiguous categorical features, if compared to *A. coerulea* and *A. solida* (see Table 2 in Scorrano et al., 2016, and remarks of these species in this study).

Aurelia aurita (Linnaeus, 1758)

Aurelia BOR lineage Schroth et al., 2002.
Aurelia borealis Schroth et al., 2002.

Type material: To our knowledge, no type material remains. Other material from the type locality region might remain in the private collection of Ramšak et al. (2012), as they deposited most sequences of specimens from this locality in GenBank.

Type locality: Baltic Sea.

Distribution: Sequenced specimens studied herein were collected in the Northeast Atlantic, Black Sea, Caspian Sea, northeastern USA, Japan and South America (see Fig. 11; for specific localities see Supplementary Material S3).

Diagnosis: Molecular diagnosis is given in Figs. 12-15. GenBank accession numbers of sequences that belong to this species and were used in analyses are presented in Supplementary Material S3.

Remarks: In Ramšak et al. (2012), one of the specimens collected in the Mljet lakes, in Croatia, appeared as sister taxa to a specimen from the Southwest Atlantic, in their combined-marker phylogeny. In our single-marker phylogenies, we observed that the ITS1 sequence from that specimen fell within a clade alongside the other sequences from the same locality, within the *Aurelia relicta* clade (Supplementary Material S4), while the COI sequence from that same specimen fell within the *A. aurita* clade (Supplementary Material S4). This can be due to contamination in sequencing the COI, as in the aforementioned study, *A. aurita* specimens were also being sequenced. As other sequences from *A. relicta* were available, this specimen from Ramšak et al. (2012) was disregarded for the combined-marker phylogenetic analysis herein.

Previously, many species of *Aurelia* were synonymized under *A. aurita*, as no morphological distinction could be made, and this species was considered globally

distributed (Kramp, 1965, 1968; Russell, 1970; Larson, 1990; Arai, 1997). More recently, it has been recognized that, alongside *A. coerulea*, this species has one the widest distributions in the genus, but potentially due to multiple introductions from its endemic range in the Northeast Atlantic (potentially naturally dispersed to northeastern USA, although not so likely; see Dawson et al., 2005). Only one specimen of *A. aurita* is from the Northwest Pacific, reported from Armani et al. (2013), from a Japanese sample that is also present in Schroth et al. (2002). This could represent a new point of introduction of this species, and should be confirmed in the future with further collections in the area. Also, we recorded this species for the first time in Ushuaia, Argentina (Fig. 11), which could represent a new point of introduction, but also ongoing spread from a single introduction that has been recorded in other localities around that region of South America (Häussermann et al., 2009).

Aurelia hyalina Brandt, 1835

Aurelia limbata Dawson and Jacobs, 2001.

Aurelia sp. 10 Dawson et al., 2005. Ki et al., 2008; Häussermann et al., 2009; Ramšak et al., 2012; Dong et al., 2015; He et al., 2015; Scorrano et al., 2016; Dong, 2018.

Type material: To our knowledge, no type material remains. Other material might remain in the private collection of Dawson et al. (2005), as they deposited the sequences for this species in GenBank.

Type locality: Aleutian Islands, Alaska, United States of America.

Distribution: Southwestern Alaska, USA (see Fig. 11; for specific localities see Supplementary Material S3).

Diagnosis: Molecular diagnosis is given in Figs. 13-14. GenBank accession numbers of sequences that belong to this species and were used in analyses are presented in Supplementary Material S3.

Remarks: We resurrect this name based on the neighboring distribution with the sequenced specimen herein. This specimen was once considered to belong to *A. limbata* (Dawson and Jacobs, 2001), but later changed to its own species hypothesis once other sequences from Japan and South Korea were added, which derived from specimens that fit within the original description of *A. limbata* (Dawson et al., 2005; Chang et al., 2016; also see remarks for *A. limbata* in this study).

Aurelia limbata Brandt, 1835

Type material: To our knowledge, no type material remains. As the first sequences of this species were deposited in GenBank by Schroth et al. (2002), some material might remain in their private collection.

Type locality: Avacha Bay, Kamchatka, Russia.

Distribution: Sequenced specimens studied herein were collected in the northwestern Pacific (see Fig. 11; for specific localities see Table S3).

Diagnosis: Molecular diagnosis is given in Figs. 12-15. GenBank accession numbers of sequences that belong to this species and were used in analyses are presented in Supplementary Material S3.

Remarks: Brandt (1835) described this *Aurelia* from the northwestern Pacific as very distinct due to the dark-brownish color of its bell margin and the brown or yellowish coloration of radial canals, which were highly ramified. This is clearly represented in the illustration in his next publication (Brandt, 1838). This morphological pattern is also associated to records in the northeastern Pacific, including the cover photograph of the January 1974 issue of Audubon magazine, featuring a specimen from the Aleutian Islands (Larson, 1990; Gershwin, 2001). However, more recent accounts, including the sequences herein, are only from the northwestern Pacific (Miyake et al., 2002; Chang et al., 2016).

There are other *Aurelia* species that occur in the northeastern Pacific, in Alaska, USA, such as *A. hyalina* and *A. labiata*, the former even previously identified as *A. limbata* (see remarks of *A. hyalina* in this study). Gershwin (2001) even suggested that *A. limbata* could be a color morph, part of the *A. labiata* species complex. Whether the distribution of *A. limbata* actually extends across the North Pacific or the distinct coloration is not intraspecific, is still unclear. Considering this controversy and previous accounts on the unreliability of coloration for species recognition in this genus (Kramp, 1968) and in Medusozoa (Lampert et al., 2011; Holst and Laakmann, 2014), we refrain from including this as diagnostic.

Regarding the highly ramified radial canals in the original description (Brandt, 1835), we observed the highest number of branching points in specimens from Japan and Arctic Alaska, USA (black squares in Figs 3-4; Fig. 6C). This is concordant with the distribution of sequenced specimens of *A. limbata* (Japan), and likely with the distribution

of *A. hyalina* (A.G. Collins, pers. comm, based on newly acquired sequences from Arctic specimens). Therefore, as discussed previously for coloration, the ramification pattern of radial canals might not be intraspecific, and once more we refrain from including this in the diagnosis. This follows the conclusions of this study, that show the unreliability of morphology for species recognition due to morphological plasticity (further discussed), and we present a molecular diagnosis to support this species hypothesis.

In this potential confusion regarding distribution and morphology of *A. limbata* and *A. hyalina*, we abstain from reporting previous accounts as synonyms. Even with more recent studies that use molecular data, such as Schroth et al. (2002), there might be some issues. The 16S sequence they posted in GenBank from the LIM lineage, which they consider *A. limbata*, belongs to the Mljet lakes, Croatia, and therefore in our 16S phylogeny is part of the *A. relicta* clade (Supplementary Material S4; also see remarks for *A. relicta* in this study). Another issue is the LIM lineage ITS1 sequence posted in GenBank, which if submitted to NCBI's BLAST (http://blast.ncbi.nlm.nih.gov), returns *Cyanea capillata* (Linnaeus, 1758) as the most similar taxa, the chosen outgroup in that study. These issues are not uncommon, and can derive from contamination or even sample mislabeling. Still, within the LIM lineage there are specimens from Iceland, but they were not deposited in GenBank, and therefore we cannot confirm their identity to the species clades treated herein, likely either *A. limbata* or *A. hyalina*.

Aurelia coerulea von Lendenfeld, 1884

Aurelia japonica Kishinouye, 1891.

Aurelia sp. 1 Dawson and Jacobs, 2001. Dawson, 2003; Dawson et al., 2005; Ki et al., 2008; Häussermann et al., 2009; Ramšak et al., 2012; Wang and Sun, 2014; Dong et al., 2015; He et al., 2015; Marques et al., 2015; Chiaverano et al., 2016; Dong et al., 2017.

Aurelia UBI lineage Schroth et al., 2002.

Aurelia sp. Manzari et al., 2014.

Type material: Neotype: UNIPD CN56CH. Paratypes: UNIS_SCY_011/12.

Type locality: Varano Lagoon, Italy.

Distribution: Sequenced specimens studied herein were collected in the northwestern Pacific, Australia, west coast of the USA, Mediterranean and Atlantic coast of Europe (see Fig. 11; for specific localities see Supplementary Material S3).

Diagnosis: Molecular diagnosis is given in Figs. 12, 14-15. GenBank accession numbers of sequences that belong to this species and were used in analyses are presented in Supplementary Material S3.

Remarks: The type locality is not concordant with the inferred biogeographic origin in the coastal waters of the Western Pacific (Dawson et al., 2005). This species has one of the broadest distributions in the genus, with multiple introductions across the globe (Dawson et al., 2005). Anecdotal observations of polyps in cultivation in different temperatures (15-24°C), suggest that they strobilate more frequently than other *Aurelia* species, even though under the exact same conditions. This could enhance its potential for spread, a matter for future studies to test.

A potential distinct feature in this species is the dark-orange or brownish color of the recently released ephyrae, which is appointed as diagnostic (Scorrano et al., 2016) and that we also have observed in our lab cultures. However, until a further assessment of ephyrae coloration in more *Aurelia* species is undertaken, and due to past reports of the unreliability of coloration to species recognition in this genus (Kramp, 1968) and in Medusozoa (Lampert et al., 2011; Holst and Laakmann, 2014), we abstain from including this as diagnostic. Further characters also indicated as diagnostic for polyps and ephyrae can derive from morphological plasticity, which has been noticed in this species (Scorrano et al., 2016) and also in other species of the genus (Gambill and Jarms, 2014; Chiaverano and Graham, 2017). For more information on morphological plasticity in medusae of this species, see 'Morphological plasticity and diagnosis in *A. coerulea*' in the results of this study.

Aurelia indica Lawley, Gamero-Mora, Maronna, Chiaverano, Collins and Morandini **sp. nov.**

Aurelia aurita Arokiasundaram, 2015

Type material: Holotype: (pending confirmation).

Type locality: 11º 35' N, 79º 46' E; Parangipettai, Tamil Nadu, India.

Etymology: Named after the country where the type locality is situated.

Distribution: Currently known only from the type locality (see Fig. 11).

Diagnosis: Molecular diagnosis is given in Fig. 13. GenBank accession numbers of sequences that belong to this species and were used in analyses are presented in Supplementary Material S3.

Remarks: Within the Indian Ocean, *Aurelia maldivensis* is the available name with the closest type locality to the specimen herein treated. Nevetheless, there is a type specimen for this species for which we cannot confirm the identity to the species hypothesis presented herein, which we therefore designate as *Aurelia indica* sp. nov. This species was previously identified as *Aurelia aurita*, mostly based on genetic distances (Arokiasundaram, 2015), which may point to an issue on the use of these distances, and consequently genetic similarity, for species identification (further discussed).

In our combined-marker phylogeny, as for COI, even though this species falls within the *A. coerulea* clade, there seems to be a considerable amount of character-state transformations (represented by branch lengths) separating them (Fig. 10; Supplementary Material S4). As we have presented previously, species do not necessarily need to form a clade (Frost and Kluge, 1994; Skinner, 2004; see 'Material and Methods' section). Furthermore, even though there are reported cases of sympatric *Aurelia* species and multiple introductions (Dawson et al., 2005; Chiaverano et al., 2016), the disjunct distribution of these hypothetical species (*Aurelia coerulea* and *Aurelia indica* sp. nov.) in different biogeographic realms (Costello et al., 2017), as well as different large marine ecosystems (Sherman, 1991), could be further evidence of lineage separation. Including more collections and more molecular markers can help clarify these relationships in the future, but we consider the evidence herein as sufficient to consider it as a distinct species.

4. Discussion

4.1. Plasticity and the use of morphology as diagnostic

Most descriptions of *Aurelia* species were based on the medusa stage, which is the most conspicuous and easily collected of the life cycle stages (Mayer, 1910 reviews morphology of *Aurelia* species and their varieties that had been described). Overlaps in morphological differences across large spatial scales created much confusion for species identification, until the recent incorporation of molecular data propelled a re-evaluation of morphological characters in all life cycle stages (Dawson and Jacobs, 2001; Schroth et al., 2002; Dawson, 2003; Dawson et al., 2005; Gambill & Jarms, 2014). Recent

descriptions of species hypotheses based on molecular data acknowledge some morphological features of medusae as diagnostic (Scorrano et al., 2016).

In our evaluation of *Aurelia* specimens from across the globe, we found no geographic structure associated with morphological variation. On the contrary, morphological variation among specimens within regions, and even within collection lots, usually overlapped with that of specimens from very distinct localities (Figs 3-4). If, however, neighboring regions had structured morphological dissimilarities, this could mean that morphotypes might be distinguished in smaller spatial scales, and if related to species hypothesis, these could be useful to distinguish neighbor or even sympatric species (e.g., *Aurelia* in the Gulf of Mexico, as in Chiaverano et al., 2016). We did not observe this pattern, but the opposite, that specimens distributed closer to each other tended to be more similar, although this was presented as a weak relationship (Figs. 7A-B). The morphological variation discussed above also encompassed previously considered categorical features, which likely due to the higher sampling effort of this study, could not be unambiguously determined and were either removed from analyses or adapted to continuous or meristic features (such as *f*A-D reflecting continuous variation from previously used *f*25 and *f*S; see Table S2).

The comparison of cultured *A. coerulea* medusae with the species diagnosis, provided by specimens studied in the Mediterranean (Scorrano et al., 2016), further illustrates the potential for morphological plasticity, in both continuous and categorical features (illustrated in Fig. 8). Interestingly, the only specimens analyzed that were more morphologically similar to each other were from the cultures at the Discovery Place Aquarium (DP-Aq, Figs. 3-4). These are raised under roughly the same controlled conditions, such as temperature, water circulation, light intensity, and are fed the same amount at the same time. Controlled conditions that reflect a certain morphological pattern corroborates the hypothesis for environmentally determined morphological plasticity, which has already been demonstrated for medusae of an *Aurelia* species in the field (Chiaverano et al., 2016). All of the evidence mentioned above favor the argument that medusa morphology is likely uninformative for species diagnosis in this genus.

To further complicate matters, there are hypothesized multiple introductions of *Aurelia* species across the globe (Dawson et al., 2005; see examples in the remarks of *A. coerulea*, *A. aurita* and *A. solida* descriptions herein), and likely more still undetected. Even if species within neighboring regions could be distinguished by morphology, introduced specimens could confuse these distinctions. This could also have confused our

morphological analysis, as it is based on the geographic distribution of morphological dissimilarities that, in most cases, did not have direct equivalence to the molecular dataset, in which species hypotheses were based. Still, even considering potential confusions from that scenario, by relating the determined geographic regions with sampling sites of species hypothesis from molecular markers, no structure appears from morphological data (e.g., Japan and USA-SW, which could both belong to *A. coerulea*, see Figs. 3-4).

However uninformative medusae morphology may be for species distinction, it is interesting to ponder on the characters that account for most of the morphological variation across specimens, such as the branching pattern of radial canals and bell indentations, the latter which determines the number of lobes (scallops) on the umbrella margin. These characters were some of the previously used to recognize a few species: *A. labiata* and *A. limbata* were distinguished by the possession of 16 marginal scallops, while *A. aurita* only had 8 (Mayer, 1910; Gershwin, 2001); *A. limbata* was also reported to have highly branched radial canals in comparison to other species (Mayer, 1910; Gershwin, 2001). As more specimens were collected through time, these distinctions started to fade, and are further discussed for each species, when applicable, in the remarks of their systematic account in this study. Only one character from the medusa stage was maintained as potentially diagnostic, the absence of the endodermal ocellus in the rhopalia of *A. solida* (Scorrano et al., 2016). This character is usually faded in preserved material, and we could not observe it in the museum specimens analyzed.

Other candidates as diagnostic morphological characters derive from other stages of the life cycle, such as polyps and ephyrae (Gambill and Jarms, 2014; Scorrano et al., 2016), which were not the focus of the morphological assessment herein presented. Nevertheless, previous studies have compared them in *Aurelia*, and have shown the overlap in morphology of these stages in different hypothetical species (Gambill and Jarms, 2014), as well as morphological plasticity in different sets of controlled conditions (Chiaverano and Graham, 2017), following much of the trend discussed here for the medusae stage. Only one morphological character was here maintained as potentially diagnostic, the higher number of tentacles in polyps of *Aurelia insularia* sp. nov. (as *A.* sp. 2 from Gambill and Jarms, 2014), until further studies can re-address this more thoroughly across the recently recognized diversity. Further discussions on the morphology of polyps and ephyrae, when applicable, are present in the remarks of each species' description.

4.2. Species delimitation, cryptic diversity and the transition to species description

Acknowledging that morphology may not be informative for taxonomy, at least for some groups within metazoans, can be a daunting task. Morphology has been the basis of taxonomy for centuries, but with the recent increase in accessibility to genetic data, this has come to question (Dayrat, 2005). Many studies that embrace this new source of information have revealed a previously undetected diversity, mostly named as 'cryptic' (for a review see Bickford et al., 2007). In result, it has been suggested that molecular data could be the only solution to assess the planet's biodiversity in the midst of the extinction of both species and taxonomists (Hebert et al., 2003). Even though there is little consensus in that view (made clear by the reviews and comments in Goldstein and DeSalle, 2010 and Collins and Cruickshank, 2013), few studies have accepted the challenge of reconciling species delimitation and description, thus failing to provide both the scientific community and society of this taxonomic service (Jörger and Schrödl, 2013).

Prior to descriptions, we assessed the use of molecular markers herein studied as barcodes, in the sense of a potential tool for rapid identification. At first, COI seems the best candidate (as has been previously suggested for most metazoans, as well as medusozoans; Hebert et al., 2003; Ortman et al., 2010), as there appears to be a greater gap between most intra- and interspecific distances (Fig. 16). However, some of the hypothesized species have only ~6% of differences between them, and this divergence might be as low as 2% (Fig. 13). As evolutionary rates may vary across congeners, it is hard to set a threshold for species identification, and this gap could be merely an artifact of unknown diversity due to undersampling (see Gómez-Daglio and Dawson, 2017 for other examples in medusozoans; Wiemer and Fiedler, 2007 for butterflies). Also, species hypothesis may change with future studies, and this gap could become more or less pronounced depending on what species hypothesis are accepted and considered. This may be a useful tool for first assessments and the identification of potential cryptic species, but it might not be reliable for identification. Even less so should it be used for species delimitation, as neo-phenetic arbitrary constructs should not replace testable species hypothesis (Prendini, 2005; Valdecasas et al., 2008; see more in the 'Materials and Methods' section). For quite some time now has the scientific community accepted that similarity does not necessarily reflect kinship (i.e., evolutionary relationship), which is one of the basic principles of phylogenetic systematics (Hennig, 1966). The latter which remains as a key component for molecular species delimitation and taxonomy (Gómez-

Daglio and Dawson, 2017; for more discussions on species delimitation see Lawley et al., unpublished, chapter 1 herein).

With the results from past studies and those provided herein, we demonstrate that for the *Aurelia* genus, morphology is likely uninformative for distinguishing at least most of the species. Even though some characters might still reveal as useful, and as we are only beginning to understand morphological plasticity and diversity within the genus, providing formal descriptions with a character-based diagnosis seems paramount to develop a taxonomic basis for future studies. Character-based diagnosis, molecular or not, provide a fallible and comparable basis in which to build species hypothesis and descriptions (Grant et al., 2006; Bauer et al., 2011) and is required by the ICZN (1999; Article 13.1.1.). Also required to accompany newly described species are name-bearing types (ICZN, 1999; Article 72.3). Ideally, the type material that accompanies newly described species should be a specimen, from which a subsample is taken and DNA is extracted, if so the case. For most samples in this study that was not possible, so to comply with the ICZN, the type material is provided as tissues or DNA extractions, and further specimens from the same culture or collection (when no sympatry had been recorded), when available, were provided within the type series (for other examples of species descriptions with molecular diagnosis and tissues or DNA extractions as type material, see Jörger and Schrodl, 2013; Eitel et al., 2018).

Diagnostic molecular characters have been identified either as character attributes from sequence alignments, with sequences manually identified in groups of previously determined species hypotheses (Sarkar et al., 2008; as in Jörger and Schrödl, 2013), or as synapomorphies for the species clades observed in a phylogenetic tree (Machado, 2015; Eitel et al., 2018). We reported diagnostic characters as synapomorphies (sensu Grant and Kluge, 2004), as these rely directly on a phylogenetic inference and are portrayed in categories defined based on all possible optimization schemes for character-states (output from the program YBYRÁ; Machado, 2015). As a result, synapomorphies can be classified as ambiguously or unambiguously optimized, the latter which is further categorized into unique and non-homoplastic, unique and homoplastic or non-unique and homoplastic (Machado, 2015; see Figs. 12-15). The desired scenario regarding these categories would be to have unique and non-homoplastic synapomorphies (in black, Figs. 12-15) for each species hypothesis. With only four possible character-states, we observed that species with greater sampling had less or none of these synapomorphies (e.g., see COI diagnostic characters for *A. aurita* and *A. coerulea*, Fig. 13). Moreover, these species

seemed to have increasingly more non-unique and homoplastic synapomorphies (in blue, Figs. 12-15), which means that some terminals in a given species clade lost that character-state. In that sense, the combination of synapomorphies as diagnostic, regardless of the category, could be more reliable.

There seems to be great potential in synapomorphies not only to construct species hypothesis and provide descriptions, but also for species identification. A synapomorphy-based identification can be much more reliable than conventional barcoding or NCBI's BLAST (http://blast.ncbi.nlm.nih.gov), as it is not based on similarity but on specified characters that directly reflect species hypothesis. This has been somewhat attempted with CAOS's P-Elf program (Sarkar et al., 2008), but to our knowledge, none of the authors that report diagnostic characters from this program, such as Jörger and Schrödl (2013) (also Maggioni et al., 2017), have provided the output of the program's P-Gnome module, which would be used for classifying new sequences. These authors have otherwise suggested that diagnostic nucleotide positions from the alignment, retrieved from CAOS, should be mapped to a reference sequence and both positions reported in the description. Yet, if other researchers seek to manually map their newly acquired sequences with any of the suggested above, for species identification, insertions and deletions could highly confuse the process, especially in markers that commonly present them, such as those from ribosomal RNA regions (e.g., 16S, ITS1 and 28S). Furthermore, the algorithm used by P-Elf to classify new sequences is not clearly stated (Sarkar et al., 2008). A prospect for future studies would be to better evaluate and understand the possible issues involved in synapomorphy-based identifications and how to convert them in a computational pipeline that can be easily and widely used, such as the BLAST tool.

Our conclusion with this study is not that morphology should be left aside. On the contrary, we are just beginning to unravel how morphological variation can be environmentally induced (Chiaverano and Graham, 2017), as well as the evolutionary processes involved in morphological change and speciation (see Struck et al., 2017). For example, the morphological overlap we observed across species could be related to recent divergences, parallelism, convergence or even stasis, and most of these have already been demonstrated to occur in other medusozoans (Swift et al., 2016). A starting point for such studies in *Aurelia* could be investigating the characters that accounted for most of the morphological variation detected herein, such as bell indentations and ramification of radial canals. This next step is fundamental to understand mechanisms that generated biodiversity and how these could be impacted by future changes.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found temporarily through http://www.dropbox.com/sh/bj3rewbow52q4n9/AACg1yN7wlXSSZRf4rD4CgJba?dl=0

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General Discussion and Conclusion

Species delimitation and descriptions form the basis of biology (Frost & Kluge, 1994). We should take advantage of all the information at hand to perform these, but with careful scrutiny on what is useful and informative within the framework of systematics and taxonomy (Valdecasas *et al.*, 2008). Recent proposals have suggested congruency across methods, or even operational criteria, as evidence for species delimitation (de Queiroz, 2007; Carstens *et al.*, 2013). In this study, I discussed that both operational criteria and methods, if not derived from different data, represent exclusive discovery operations (Grant, 2002), and therefore have no epistemic value as evidence. They only represent the degree to which the different analyses lead to the same or different conclusions (Grant, 2002). In that sense, I advised the use of epistemological and operational justifications on the careful choice of methods, further highlighting issues with some of them, such as DNA barcoding, coaslescent theory, and even phylogenetic systematics.

DNA barcoding relies on a phenetic approach that can be of heuristic value for species identification in some groups, but it is unreliable for species delimitation (Collins & Cruickshank, 2013). This unreliability was here demonstrated, as well as its further limitations even for species identification. In the case of medusozoans (Gómez-Daglio & Dawson, 2017), which includes this study with *Aurelia*, COI seems as the best barcoding candidate, which can help first assessments and the detection of cryptic species. Nevertheless, there are still overlaps between some intra- and interspecific distances, likely due to variation on molecular evolution that can occur even across congeners (Grant *et al*., 2006; Gómez-Daglio & Dawson, 2017). This apparent overall 'gap' on distances can still be due to undersampling of diversity (Wiemers & Fiedler, 2007). Phylogenetic analysis, even though with its caveats, still has its place as an important tool for species delimitation (Frost *et al.*, 1998; Grant *et al*., 2006; Gómez-Daglio & Dawson, 2017).

We reassessed the use of medusae morphological characters for species delimitation and description, and observed an overlap in morphology across regions, with considerable variation within these, and even within collection lots and species hypotheses. Also, no regional structure in variation could be found, which could lead the distinction of species locally. This potential for morphological plasticity had been previously reported not only for the medusae stage (Chiaverano *et al.*, 2016), but also for other stages of the life cycle, such as polyps and ephyrae (Gambill & Jarms, 2014;

Chiaverano & Graham, 2017). Having detected this crypsis across *Aurelia* specimens, we based our species delimitation and descriptions mostly on multi-marker molecular analyses and species distributions. The description of biodiversity, whether cryptic or not, is a necessary step to accompany delimitation, especially in the current scenario of biodiversity crisis (Pimm *et al.*, 1995).

For species descriptions, I followed the current requirements and consensus in taxonomy of reporting character-based diagnosis (ICZN, 1999; Bauer *et al.*, 2011). Molecular diagnostic characters have been previously reported in descriptions, especially in the case of cryptic species (*e.g.*, Jörger & Schrödl, 2013; Maggioni *et al.*, 2017; see review in Goldstein & DeSalle, 2010). This scenario, allows a comparable outline for proposed species hypotheses (Bauer *et al.*, 2011; Jörger & Schrödl, 2013). Nevertheless, these diagnoses are not devoid of potential shortcomings. For example, with only four character-states possible, our study demonstrates that finding unique and non-homoplastic molecular synapomorphies might only be an artifact of low sampling. In that sense, a combination of the synapomorphies found can be more informative, and future studies can address the challenges of their practical applications for species identification. The use of diagnostic characters for identification can be more reliable than barcoding, as well as consistent with constructed species hypotheses.

This does not mean that morphology should be ignored, we should strive to better understand how speciation and morphology are related, what environmental factors may regulate morphological plasticity, and patterns and processes envolved in generating crypsis (Swift *et al.*, 2016; Struck *et al.*, 2017). With this study, I hope to encourage research on these questions, as well as the recognition of cryptic diversity with the due caution and rigor established in the epistemology of taxonomy, systematics, and biology.

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Resumo

A delimitação e descrição de espécies, as unidades fundamentais da biologia, tem intrigado cientistas por séculos. Suas identidades eram tradicionalmente reconhecidas com base na distribuição, ecologia e, acima de tudo, na morfologia. Dados moleculares recentemente entraram em cena, e hoje formam um importante componente na maioria dos estudos de sistemática. Muitos métodos foram rapidamente elaborados e aplicados para integrar esses dados no processo de delimitação e descrição de espécies, e foram acompanhados não só por fascinantes possibilidades e descobertas, mas também por novas questões e desafios. Algumas problemáticas epistemológicas aparecem a partir de proposições recentes que sugerem congruência entre métodos, ou mesmo critérios operacionais, como evidência para delimitação. Além disso, a descoberta de linhagens genéticas morfologicamente indistinguíveis, descritas como 'crípticas', tem dificultado o reconhecimento e avaliações formais da diversidade biológica. No Capítulo 1, abordei o raciocínio epistemológico que envolve as operações de descoberta, métodos e congruência para a delimitação de espécies baseada em evidências. Discutimos que a congruência entre métodos, ou mesmo critérios operacionais, se baseados nos mesmos dados, são operações de descoberta exclusivas e, portanto, não tem valor epistêmico como evidência. Questões relacionadas a alguns métodos também são destacadas, incluindo a teoria de coalescência, o código de barras de DNA, e até mesmo a sistemática filogenética. No Capítulo 2, passei para a aplicação da delimitação e descrição de espécies em *Aurelia* (Cnidaria, Scyphozoa). Uma reavaliação morfológica de medusas coletadas ao redor do globo, não revelou nenhuma estrutura geográfica nas dissimilaridades, com considerável variação morfológica entre indivíduos de um mesmo lote de coleta e até mesmo da mesma espécie hipotética. Esta plasticidade morfológica já havia sido relatada em medusas para algumas espécies de *Aurelia*, bem como nos estágios de pólipo e éfira. Considerado essa diversidade críptica, análises moleculares com múltiplos marcadores e dados da distribuição foram utilizados para delimitar e descrever espécies. Também discuti sobre a inconfiabilidade do código de barras de DNA para a delimitação de espécies, e suas limitações até mesmo para identificação. Os caracteres moleculares diagnósticos relatados não apenas preenchem os requisitos necessários para as descrições, mas também sugerem a possibilidade de seu uso prático para identificação, em lugar de utilizar o código de barras de DNA. Esperamos que este estudo encoraje futuras pesquisas não apenas na delimitação e descrição da diversidade críptica, que deve incluir uma cuidadosa avaliação dos métodos e dados utilizados, mas também sobre plasticidade morfológica e os padrões e processos envolvidos na geração dessa diversidade.

Palavras-chave: filogenética, evidência, água-viva, taxonomia, sinapomorfia.

Abstract

The delimitation and description of species, the fundamental units of biology, have puzzled scientists for centuries. Their identities were traditionally recognized based on distribution, ecology and most of all, morphology. Molecular data have recently come into play, and nowadays form an important component of most systematic studies. Many methods have been quickly devised and applied to integrate these data in the species delimitation and description process, and they have been accompanied not only by exciting possibilities and discoveries, but also by new questions and challenges. Some epistemological issues appear from recent proposals that suggest congruency across methods, or even operational criteria, as evidence for delimitation. Also, the discovery of morphologically indistinguishable genetic lineages, described as 'cryptic', has hindered recognition and formal assessments of biological diversity. In Chapter 1, I address epistemological reasoning that encircles discovery operations, methods and congruency for evidence-based species delimitation. We discuss that congruence across methods or operational criteria, if based on the same data, are exclusive discovery operations and therefore have no epistemic value as evidence. Issues regarding some methods are also highlighted, including coalescent theory, DNA barcoding, and even phylogenetic systematics. In Chapter 2, I move into the application of species delimitation and description in *Aurelia* (Cnidaria, Scyphozoa). A morphological reassessment of medusae specimens from across the globe revealed no geographic structure on dissimilarities, with considerable morphological variation within collection lots and even within hypothesized species. This morphological plasticity had already been reported for medusae in some *Aurelia*, as well as in the polyp and ephyra stages. Considering this crypsis, multi-marker molecular analyses and distribution records were used to delimit and describe species. I also address the unreliability of DNA barcoding for species delimitation and its limitations even for identification. The reported diagnostic molecular characters not only fill the requirements for descriptions, but also hint on the possibility of its practical uses for identification, rather than barcoding. This study should encourages future research not only on delimitation and description of cryptic diversity, which should include careful scrutiny of methods and data used, but also on morphological plasticity and the patterns and processes involved in generating crypsis.

Keywords: phylogenetics, evidence, jellyfish, taxonomy, synapomorphy.