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Systematics and phylogeography of shallow water jellyfish (Scyphozoa, Discomedusae) in the Tropical Eastern Pacific

A dissertation submitted in partial satisfaction of the requirements for the degree of Doctor of Philosophy

in

Quantitative and Systems Biology

by

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2016

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2016

DEDICATION

Ian, Morgan y Kylian

EPIGRAPH

"The highest possible stage in moral culture is when we recognize that we ought to control our thoughts"

Charles Darwin

"Seamos realistas y hagamos lo imposible. Podrán morir las personas, pero jamás sus ideas"

Che Guevara

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Gómez Daglio, L. (in prep). Integrative taxonomy: an unattainable need?

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ABSTRACT

Species diversity is declining, due to habitat loss, over-exploitation, pollution, and climate change. It is imperative that biodiversity and distributions be accounted for immediately, to understand the impacts of anthropogenic change, and to sustain natural resources. Biodiversity in the seas, and geographic variation, have been underestimated—due to challenges in (1) the delimitation of species, (2) a preponderance of cryptic species, (3) uneven sampling effort, and (4) limited systematic framework. As a consequence, the mechanisms that govern species richness in the seas are poorly understood. The magnitude of these issues varies by taxon and by region, leaving open questions such as: Are estimates of species richness accurate? What are the tempo and mode of evolution in marine species? What mechanisms determine species' distributions in the ocean?

Here, we tackle the first question, using the example of jellyfishes in the Tropical Eastern Pacific (TEP). The TEP is known as a 'hotspot' for its generally high biodiversity, but it harbors only five scyphozoan jellyfishes. To redress the four known challenges facing estimates of marine biodiversity, we increased sampling effort, combined molecular and morphological characters, and applied phylogenetic, barcoding, and morphospecies analyses to estimate species richness of scyphomedusae in the TEP. We found a total of 25 species; of which 22 are new to science, two are non-indigenous, and one is a previous record. Thus, by overcoming known challenges, we found that, as for other more well-known taxa, the TEP also is a hotspot for scyphozoans. To answer the second question, above, we test the hypotheses about the origins of the Discomedusae by synthesizing molecular and morphological phylogenies. We calibrate a scyphozoan molecular clock using geologic events and fossil records. We demonstrate that Coronatae is sister taxon to Discomedusae; we find evidence for geographic radiations in the genus *Stomolophus* and Family Pelagiidae, which are the most species rich taxa in the TEP. Their diversification rates confirm a rapid genetic radiation in the genera *Chysaora*, but the morphological characters mapped in the phylogeny did not show any shift in the rates of morphological evolution. To address the last question, we took advantage of a comparative phylogenetic approach. A multi-taxon comparison—including five species of *Stomolophus* and four *Chrysaora* species—demonstrates that biological factors play the more important role in shaping species' distributions and assemblages, compared to abiotic factors. The vicariance model of speciation is not the only process though which the biodiversity in the TEP could have originated. Peripatric and sympatric models of speciation also can define many of the diversification patterns in the TEP.

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Chapter 1: Integrative taxonomy: an unattainable need

Taxonomy is the scientific manifestation of humans' tendency — attributable to a basic need to reduce complexity by grouping like-things to understand the world (Powys, 1929) — to describe, name, and organize, with the sole purpose of giving meaning to our perceptions. Thus, the description, classification, and estimation of biodiversity has been one of civilization's most common endeavors for over 5000 years (Simonetta, 2003; Wheeler, 2004; Zhang, 2010; Maguire, 2012). We now recognize between 5.0–3.0 million eukaryotic species globally, of which ~226,000 are marine; although another ~275,000 marine eukaryotic species may exist but be undescribed (Appeltans et al., 2012; Costello et al., 2013a). But how much do we understand about each of these like-things — the species, and higher taxa — and how much more can we expect to learn? The imprecision of these estimates has been ascribed to the so-called *taxonomic impediment*, i.e. insufficient jobs and funding for taxonomists and therefore fewer taxonomists and publications (Wheeler, 2004; de Carvalho et al., 2007; Patterson, 2010), and to a penurious understanding of taxonomy as a scientific discipline and its foundations (McGregor Reid, 2010; Wheeler, 2010).

1. Misunderstanding taxonomy

The misunderstanding of taxonomy is not, for the most-part, a misunderstanding of the value of taxonomic findings or their implications for other scientific disciplines and society (Krupnick & Kress, 2003; Bouchet et al., 2009; Wheeler et al., 2012; Sluys, 2013). Rather, the misunderstanding is a *modern* under-appreciation of the conceptual and epistemological framework of taxonomy (Wheeler & Valdecasas, 2007; Wheeler, 2009; Roger, 2012; de Carvalho et al., 2013). For example, the terms classification, taxonomy, and systematics often are used as synonyms in a scientific context (McKelvey, 1982), yet biological *classification* is the hierarchical arrangement of living entities (Mayr, 1942; 1969; Simpson, 1961); *taxonomy* is the scientific discipline that provides the theory, principles, methods, and rules for naming, describing, identifying, and classifying living organisms (Simpson, 1961; Wheeler, 2009); *systematics* is "the study of the kinds and diversity of organisms and of any and all relationships among them" (Simpson, 1961).

The misunderstanding of taxonomy as a simply collection of names has led to its derogation as a scientific discipline. Contemporary taxonomic publications commonly lack clear hypotheses and structured conceptual frameworks (Wheeler & Valdecasas, 2007; Agnarsson & Kuntner, 2007; de Carvalho et al., 2007; 2013); examples of the latter include publications that state taxonomy's aim to generate a comprehensive biodiversity *census* through the identification of species (Godfray, 2002; Boero, 2010; Costello et al., 2013a; 2013b). Taxonomy has thus earned a reputation as non-experimental biology, as descriptive rather than hypothesis-driven science (de Carvalho et al., 2007; Wheeler, 2009; McGregor Reid, 2010), even though the products of taxonomy are rigorously testable hypotheses (Popper, 1959; Agnarsson & Kuntner, 2007; de Carvalho et al., 2007; Wheeler, 2009).

In addition, taxonomy has been confounded with other sciences. The "New Systematics" (Huxley, 1940) provides an intriguing example. In this book, the foundations for modern population biology are settled, emphasized experimental studies at the species

level and below are the reasonable scientific solution to find answers for the emerging evolutionary questions (e.g. speciation processes, mechanisms driving the changes in allele frequencies). The aims and methods for this new emerging science are quite different from those established by the taxonomic epistemology. On the other hand, the first thoughts about the need for a more integrative taxonomy and new methods are exposed in the book. Hennig (1966) placed taxonomy and its nomenclature and classification, into an evolutionary context through the use of cladograms. While rapid technological advancements have rejuvenated the fields of systematics and taxonomy with newer sources of data (e.g. short fragments of DNA, high-throughput sequencing), controversies and debates have been taking place for more than a decade trying to accommodate the use of new sources of data into the taxonomical framework. The intent to replace taxonomy with other sciences and methods exemplify a poor understanding of the taxonomic outcomes and aims (Wheeler, 2004; Pace et al., 2012). For example, phylogenies improve neither formal classifications nor the application of scientific names; phylogenies are necessary to understand the evolutionary patterns and relationships of a given taxon. Phylogenies need taxonomical background. Otherwise, its interpretation is futile (Wheeler, 2004). Another example is the implementation of DNA barcoding as a tool to delimit species (Wheeler, 2005; Pires & Marinoni, 2010; Boero, 2010; Schlick-Steiner et al., 2014), instead of a method for identifying species. Phylogenetic taxonomy (de Queiroz, 1992) promises to be the solution for the new emerging era of phylogenetics, the new approach includes the PhyloCode (Cantino & de Queiroz, 2004) which is proposed to replace the current Linnaean system and ICZN (Wheeler, 2004; Patterson et al., 2006; Patterson, 2010; Platnick, 2012).

2. The taxonomic impediment

Addressing the *taxonomic impediment* has been a focus of various agencies and grant programs [e.g. PEET (NSF-USA), Distributed European School of Taxonomy (DEST-EU), Global Taxonomy Initiative] for at least two decades, indicating the seriousness with which it is considered to impinge on the growth and progress of taxonomy (Wheeler, 2005; de Carvalho et al., 2007; Wheeler, 2009). However, currently, some authors claim the *taxonomic impediment* is nonexistent (Tancoigne & Dubois, 2013; Costello et al., 2013a; 2013b). For example, Costello et al. (2013b) emphasize the growing number of people describing species, and justify the small number of published species descriptions as a consequence of a limited number of species that remain to be discovered. Yet, these general trends, which are influenced strongly by well-known species-rich taxa such as birds and mammals (Joppa et al., 2011; Scheffers et al., 2012), are not universally true for all taxa. Likewise, reported trends in the number of taxonomists may overgeneralize the contributions of multiple authors.

A review of the taxonomic literature on scyphozoan jellyfishes, for example—itself one of the less-studied taxa (Appeltans et al., 2012)—demonstrates the trends found by Costello et al. (2013b) but also the heterogeneity among taxa and authors. Overall, the number of authors describing new scyphozoan taxa has increased while the number of new species being described has decreased (Fig. 1A, B). However, the ratio of 0.12 species/author during the last two decades, compared with 4.3 species/author during the decades of 1880 and 1910, can alternatively be interpreted as a symptom of changing publication norms

rather than a consequence of completeness of taxonomic inventories as inferred by Costello et al. (2013b). The actual ratio of number of authors/species and the number of published papers raises the question of the definition and meaning of a "taxonomist" used by Costello et al. (2013a, b). Being an author on a taxonomic publication does not necessary equate with being a highly active scientist working as a taxonomist. Other possibilities exist: authors may be bringing different expertise, sharing logistical or other costs generated by taxonomic research, and other plausible explanations. However, when 33 authors describe only five species in the last five years of the 21st century (Bayha & Dawson, 2010; Nishikawa et al., 2014; Piraino et al., 2014; Kolvasova et al., 2015), aspects of the International Code of Zoological Nomenclature are neglected — notably Recommendation 50A — gain added significance in light of bibliometric analyses. If "only … some of the authors … are directly responsible for the [species description, those] author(s) … should be identified explicitly" while "co-authors of the whole work who have not had such direct responsibility for the name should not automatically be included as authors of the name" (Anonymous, 2000). In the extreme, following Recommendation 50A might change the ration 1 species/author or less, and largely eradicate the apparently substantial gains in taxonomic expertise made during the last decade (Fig. 1).

3. Ghosts of taxonomies past

An historical review can illustrate the development of the precarious situation in modern taxonomy, at least in the taxonomy of a less-explored taxon. As for many other marine invertebrates, classification of scyphozoans was established using criteria designed with macro-morphological characters in mind. These morphological criteria resulted in five orders—Coronatae, Semaeostomeae, Rhizostomeae, Stauromedusae and Cubomedusae (Hyman, 1940; Mayer, 1910; Kramp, 1961). Later on, the cubomedusae and stauromedusae were elevated into the classes Cubozoa and Staurozoa (Werner, 1973; Marques & Collins, 2004; Collins et al., 2006). Within the class Scyphozoa, morphological phylogenies suggest the presence of two monophyletic groups: Coronatae and Discomedusae—the latter including semaeostomes and rhizostomes (Stiasny, 1921; Uchida, 1926; Marques & Collins, 2004; Van Iten et al., 2006), which was confirmed by molecular studies (Dawson, 2004; Collins et al., 2006; Zapata & Robertson, 2007; Bayha et al., 2010; Kayal et al., 2012). The most species rich within the class are the Discomedusae—60 coronates vs. 154 Discomedusae species (Mianzan & Cornelius, 1999; Daly et al., 2007).

Publication trends suggest that we might divide the taxonomic history of Discomedusae into three periods (Fig. 2). The first period—Taxonomic splendor (decades 1880s–1940s)—is a period in which taxonomists of Discomedusae flourished and ocean-wide expeditions resulted in prominent monographs and taxonomic publications (Péron & Lesueur, 1809; Agassiz, 1862; 1865; Haeckel, 1879; 1880; Fewkes, 1881; Lendenfeld, 1887; Agassiz & Mayer, 1898a; 1898b; 1902; Mayer, 1904; Bigelow, 1904; Maas, 1907). During this time, naturalists' endeavors discovered and described ~90% of the Discomedusae species known by 2010 (Appeltans et al., 2012). During this period, luminaries such as Haeckel (1879) described more than 35 new species, though 10% of these were made using a single specimen or damaged specimens; his illustrations were artistically incomparable (and perhaps largely useless for a taxonomical review).

Vanhöffen (1888; 1902; 1908) recorded 149 species worldwide; his detailed descriptions and artistic illustrations though clarified only some of the species. In addition, morphological nomenclature used to describe diagnostic characters varied by author, leading to inconsistency in the description of species. But, by the end of this period (1911–1940), a taxonomic revolution is recognizable in now classical publications and taxonomic reviews which included detailed descriptions, informative illustrations and diagrams, and improved standardization of diagnostic characters and nomenclature (e.g. Mayer, 1910; Stiasny, 1920; 1921; 1922; Uchida, 1926; Rao, 1931; Uchida, 1935; Stiasny, 1938; 1940). These taxonomic publications reflected the understanding of the taxonomic necessities of the time: the inclusion of more morphological characters, description of intraspecific morphological variation, and foundations for delimiting species (e.g. Bigelow, 1910; Mayer, 1910; Light, 1914; 1921; Uchida, 1926; 1933; Stiasny, 1933; Uchida, 1935; Stiasny, 1935; 1938; Uchida, 1947; Kramp, 1948). As a result of the standardization in the taxonomy of the group, the 149 species of Discomedusae described by Vanhöffen (1888) were reduced to 93 species plus 34 "varieties" by Mayer (1910). The vast amount of taxonomic knowledge generated during this period is unquestionable. Notably, also, the systematics of Discomedusae was being enriched with hypotheses regarding the evolution of macro-morphological characters.

The second period—Taxonomic recession (decades 1950s–1980s, Fig. 2) — saw a decline in the numbers of taxonomic publications, descriptions of species, and taxonomists (Fig. 1A, B). Few remarkable publications continued the taxonomic research (Kramp, 1952; 1955b; 1955a; Russell & Rees, 1960; Russell, 1962; 1967; Kramp, 1968; Russell, 1970; Segura-Puertas, 1984; Larson, 1986) and among these were oftentimes seen a separation of taxonomy (e.g. Kramp, 1955a, b; 1961; Larson, 1986) from other aspects of biology (but see Russell, 1970). The last major taxonomic revision (Kramp, 1961) eliminated all the *nomen dubium* species and synonymized all the described varieties, formalizing 140 described species of Discomedusae. Kramp's (1961) taxonomic classification is still the primary classification in use today (Mianzan & Cornelius, 1999; Daly et al., 2007), meanwhile researchers became primarily interested in other biological aspects of Discomedusae such as reproduction, life cycles, physiology, feeding behavior, and ecology (e.g. Calder, 1972; Hamner & Hauri, 1981; Calder, 1982; Larson, 1987; Strand & Hamner, 1988; Fig. 2).

The last period — Molecular taxonomy (1990s–present, Fig. 2) — began with resurgence in traditional morphological taxonomy in the 1990s (e.g. Galil et al., 1990; Larson, 1990; Martin et al., 1997), and has increasingly become linked with advances in molecular analyses. Although the first molecular analysis dates back to Zubkoff and Lin (1975), Greenberg et al. (1996) introduced morphometric and molecular analyses, both using the case study of *Aurelia*. Their results, which suggested multiple distinct lineages, were corroborated by DNA sequence-based phylogenetic evidence of at least six cryptic species (Dawson & Jacobs 2001). During the 2000's, molecular data became increasingly readily available and resulted in transitional publications addressing key taxonomic problems in Discomedusae, particularly the presence of cryptic species (Dawson & Jacobs, 2001; Holland et al., 2004; Dawson, 2005a, b; Holst & Laakmann, 2014). Studies also gave continuity to the unsolved questions regarding the systematics and taxonomy of Discomedusae (Gershwin & Collins, 2002; Marques & Collins, 2004; Dawson, 2004; 2005a, c; Morandini & Marques, 2010; Bayha et al., 2010; Straehler-Pohl et al., 2011).

Currently, 10 new valid species of Discomedusae have been published since 2000 (Table I), 50% of which exclusively used morphological characters to identify and delimit species (Galil et al., 1990; Martin et al., 1997; Matsumoto et al., 2003; Raskoff & Matsumoto, 2004; Gershwin & Zeidler, 2008a; 2008b; Gershwin & Davie, 2013).

Under this scenario — a period of taxonomic synonymization followed by a period applying new tools — it is perhaps unsurprising that the true species richness of Discomedusae is now estimated as twice the number described (155 spp.; Dawson, 2004; Appeltans et al., 2012). This estimation is founded, in part, on the recent discovery of new lineages using molecular data (e.g. ~17 molecular species in place of 5 morphospecies, Table I). But these species have not been described taxonomically, which is a commonality when non-morphological characters are used to delimit and identify species (Pante et al., 2014), which in turn underestimates recent taxonomic advances (Figs. 1, 2). Reciprocally, revision of deep arrangements in the classification and taxonomy of Discomedusae may be considered likely to be problematic when published without molecular evidence (Gershwin & Zeidler, 2008b; Straehler-Pohl et al., 2011; Gershwin & Davie, 2013). The instability in the systematics and taxonomy of the group and the poor knowledge about the variation and congruence between the different methods and types of data result in the incorrect assignation and identification of species (e.g. *Pelagia benovici* see Gómez Daglio and Dawson, in review).

Perhaps most importantly, therefore, the other 50% of valid species used both morphological and molecular criteria (Bayha & Dawson, 2010; Galil et al., 2010; Piraino et al., 2014; Nishikawa et al., 2014; Kolbasova et al., 2015). These publications meet the criteria for an integrative taxonomy and, perhaps for the first time since Huxley's (1940) 'new systematics'—notwithstanding Russell's (1970) tome on British scyphomedusae—offer a renaissance in scyphozoan taxonomy (Dawson 2005d).

4. The ghost of a taxonomy's future

At present, "integrative taxonomy" is the major progress in the theoretical framework of taxonomy (Dayrat, 2005) that aims to use multiple data types (behavioral, morphological, ecological, physiological, molecular, etc.) to delineate species boundaries, and to promote the integration of multiple disciplines. The necessary amendment in concepts, methods, and nomenclature have been the subject of multiple debates (Valdecasas et al., 2008; Padial & La Riva, 2010; Schlick-Steiner et al., 2010; Goldstein & DeSalle, 2010; Schlick-Steiner et al., 2014) (Schlick-Steiner et al., 2014). Several publications evince the efforts of scientists to follow this integrative approach, particularly, investigations that use molecular knowledge to address riddles that morphological approaches were unable to solve: "discovery and identification of cryptic species" (Beheregaray & Caccone, 2007; Schlick-Steiner et al., 2007; Jörger & Schrödl, 2013). These efforts are, at least in marine taxa, focused on economically and ecologically important, well-known taxa such as anthozoans (corals), mollusks, crustaceans, cetaceans, and fishes (Bouchet, 2006; Appeltans et al., 2012), although, many other taxa remain neglected, for example, the economic and ecologic importance of scyphozoan jellyfishes is increasingly understood in recent decades (Graham & Bayha, 2007; Purcell et al., 2007; Kitamura & Omori, 2010; Hamilton, 2016) but they are still dismissed taxonomically today.

In this context, building on the broader perspective hinted at by the 'new systematics'

almost 80 years ago (Huxley 1940) and ten years hence by 'integrative taxonomy' (Dayrat 2005; Schlick-Steiner et al., 2010) there is much to be gained by integrating different sources of data in Taxonomy. In this thesis, I attempt to demonstrate those benefits.

5. Outline of the thesis

In Chapter II, I address the kinds of advances in estimation of biodiversity that may become commonplace if the taxonomic impediment could be overcome. As a personal example, I undertook extensive surveys (34 localities, surveyed seasonal during 5 years) throughout Mexico and Central America to ask how many species are present there. I found numerous species, but it required an integrative taxonomic approach to classify all those new medusae found into 25 new lineages of Discomedusae in the Tropical Eastern Pacific. Morphology nor genetics alone provided the whole story.

In Chapter III, I explore the implications of the new understanding provided by correct species identification, delimitation, and description to contextualize the evolutionary patterns of Discomedusae. These provide the foundations for ecological and biogeographical studies that may change the common wisdom that evolution is a gradual and even process. I estimated the molecular clock and the diversification rates for scyphozoan jellyfish. In addition, we mapped morphological characters into the phylogeny. I found three main diversification shifts that occurred (~20-15 Ma) in the some of the tropical clades. The phylogenetic and distributional analyses suggest that the major functional groups arose 15 Ma ago. However, the morphological evidence suggest that the newer extant species may largely be functional equivalents, filling empty niches in space, rather than creating or filling new niches ecologically.

In Chapter IV, I further explore the origins of these geographic patterns by looking at the phylogeography of nine species in the TEP. I asked if the evolutionary patterns of Discomedusae follow the common patterns described for fishes and other benthic marine invertebrates in the TEP. The biogeographic patterns of Discomedusae couple, in part, the vicariant hypothesis proposed for the Gulf of California. However, in other areas of the TEP, the planktonic life style and ecology of the species play an important role to delimit the evolutionary patterns. Other phylogeographic filters and barriers exist in the TEP which vary in intensity, and might explain the species richness in the area.

In Chapter V, then, after this thorough exploration of discomedusan diversity in the TEP, I return to the matters established above, to what the ghosts of taxonomy's future hold in store. As discussed and demonstrated by the historical review, the taxonomic crisis exists (Wheeler, 2005; de Carvalho et al., 2007; Wheeler, 2009), and it is remarkable in marine taxa, such as Discomedusae. The expectations for the 21st Century is an increase of the taxonomic knowledge which should follow an integrative approach, taking advantages of novel technologies (e.g. large-scale sequencing) to reconcile the molecular and morphological outcomes and improves the theories and concepts regarding species limitations, boundaries and identifications. It is necessary to prioritize taxonomic and systematic studies, which should include the exploration of hotspot areas such as the Indo-Pacific Ocean.

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Zubkoff P. L. &Linn A. L. 1975. Isozymes of *Aurelia aurita* scyphistomae obtained from different geographical locations. *Isozymes IV, Genetics and Evolution* (ed. by C. L. Markert), pp. 915–930. Academic Press, New York.

Table 1. Summary of new taxa in Discomedusae that have been published since the beginning of the 21st Century, when molecular tools became broadly available for Scyphozoa. The criteria under the character source are based on the type of data; we did not assess the quality, quantity or analyses used to delimit the species. The molecular species considered are those referred to in taxonomic or systematic publications. Parenthetical numbers indicate the number of distinct 'species-level' lineages defined per genus.

Taxa	Character source		Taxonomic status	References
	Morphological	Molecular		
Semaeostomeae				
Pelagiidae				
Pelagia benovici	Yes	Yes	Valid	Piraino et al., 2014
Sanderia pampinosus	Yes	No	Valid	Gershwin & Zeidler, 2008a
Chrysaora kynthia	Yes	No	*Nomen dubium*	Gershwin & Zeidler, 2008b
Chrysaora wurlerra	Yes	No	*Nomen dubium*	Gershwin & Zeidler, 2008b
Chrysaora southcotti	Yes	No	*Nomen dubium*	Gershwin & Zeidler, 2008b
Cyaneidae				
Desmonema scoresbyanna	Yes	No	Valid	Gershwin & Zeidler, 2008b
Cyanea tzetlinii	Yes	Yes	Valid	Kolbasova et al., 2015
Desmonema sp.	No	Yes	No	Bayha et al., 2010
Drymonematidae				
Drymonema larsoni	Yes	Yes	Valid	Bayha & Dawson, 2010
Ulmaridae				
Stellamedusa ventana	Yes	No	Valid	Raskoff & Matsumoto, 2004
Tiburonia granrojo	Yes	No	Valid	Matsumoto et al., 2003
Aurelia spp. 11	In part	Yes	No	Dawson & Jacobs, 2001; Dawson, 2003; Dawson et al., 2005
Rhizostomeae				
Cepheidae				
Marivagia stellata	Yes	Yes	Valid	Galil et al., 2010
Netrostoma nuda	Yes	No	Valid	Gershwin & Zeidler, 2008a
Bazingidae				
Bazinga rieki	Yes	No	*Nomen dubium*	Gershwin & Davie, 2013
Cassiopeidae				
Cassiopea spp. 3	In part	Yes	No	Holland et al., 2004
Catostylidae				
Crambionella helmburi	Yes	Yes	Valid	Nishikawa et al., 2014
Acromitus sp.	No	Yes	No	Bayha et al., 2010
Lobonematidae				
Lobonematidae sp.	No	Yes	No	Bayha et al., 2010

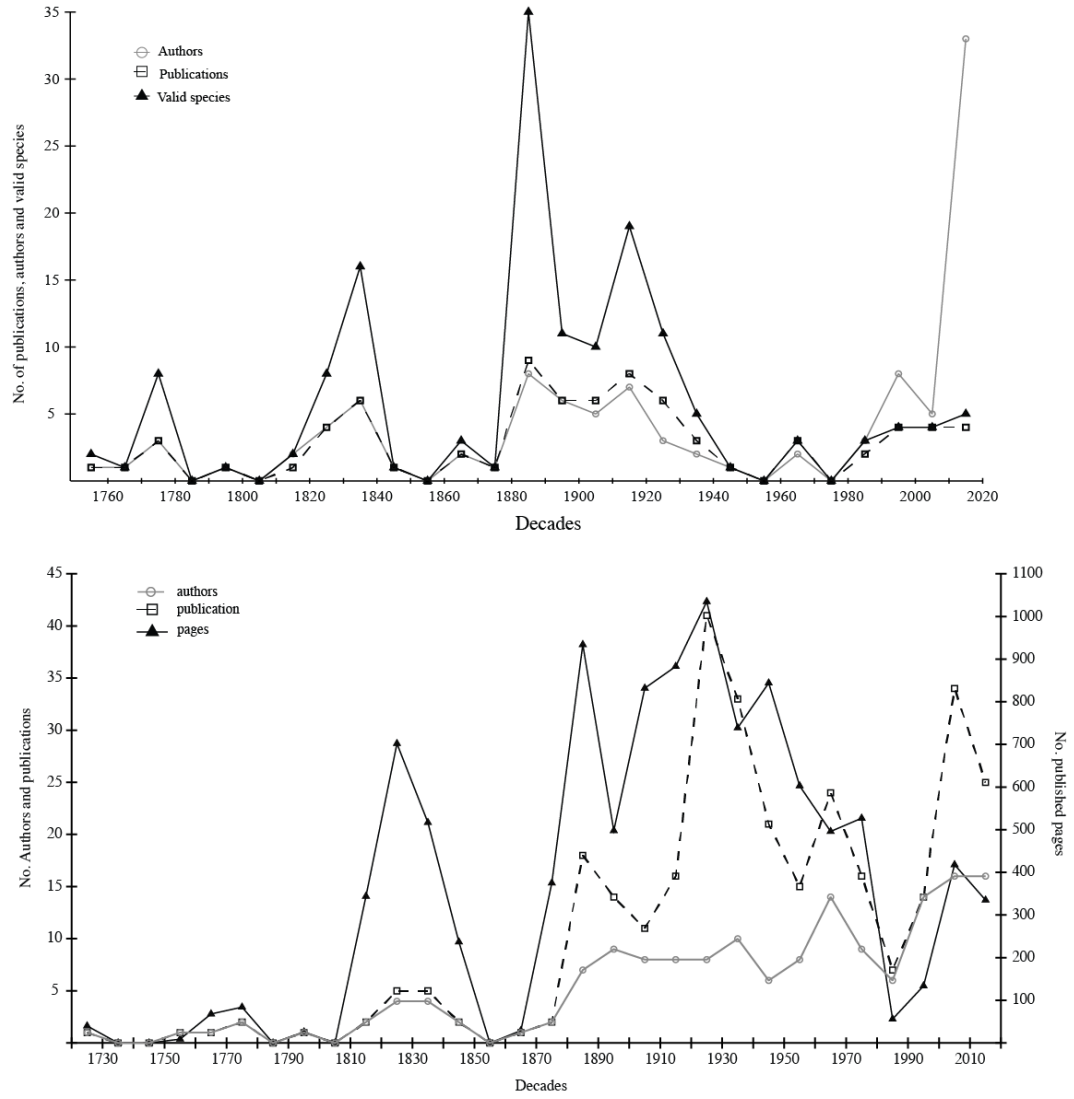

Figure 1. Graphical review of the history of Discomedusae taxonomy. **A.** Publications describing 155 valid species of Discomedusae from 1750 to 2015. The cumulative number of authors is 105 with 77 publications up to December 2015. The maximum number of authors occurs between 2010–2015 (33 authors), and the highest number of described species and publications (35 and nine, respectively) happens during the 1880s. Results based on taxonomic classification by Kramp (1961) and updated according to Daly et al. (2007) and Morandini & Marques (2010); references of species described between 2000–2015 are shown in Table I. **B.** Taxonomic publications from 1730–2015. The maximum number of publications and published pages (41 and 1035, respectively) is reached in the 1920 decade. The maximum number of authors (91) occurs between 2010–2015. A total of 313 taxonomic publications and 286 authors were retrieved from Zoological Records (Web of Science, Thomson Reuters), SCOPUS (Elsevier B.V.), and Biodiversity Heritage Library (Encyclopedia of Life) search engines using Topics searches for: Taxonomy + [Scyph* or Jellyfish* or Medus*], filtered: NOT topic: Hydro* + Cubo* + Ctenoph* + Fungi. Records from the 18th century (1720–1800) were added manually, using the references provided in Haeckel (1879), Vanhöffen (1888), and Mayer (1910). The resultant searches were concatenated into a single file and cleaned for duplicates. Publications focused exclusively on Coronatae jellyfish were excluded.

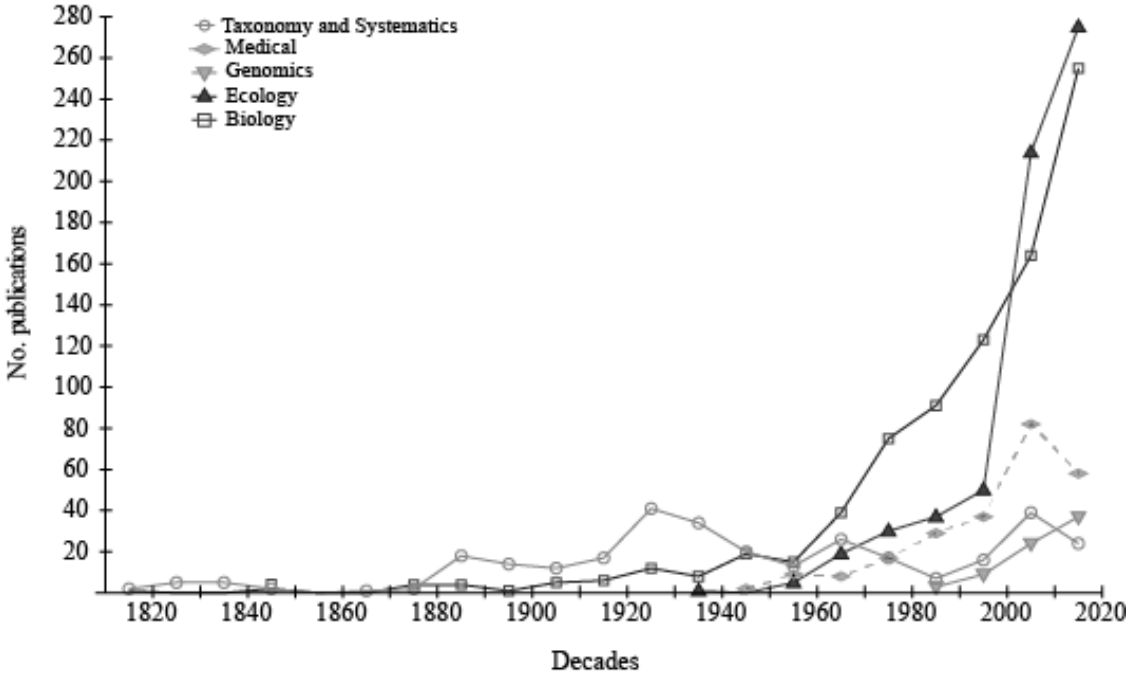

Figure 2. Overview of major research topics in Discomedusae through time. The total number of publication is 2092. Total number of publications per topic: taxonomy including systematics (320), Biology (826), Ecology (631), Medical (242), and Genomics (73). The maximum number of taxonomic and systematic publication is reached during the decades of 1920 and 1930; meanwhile, the maximum of biological and ecological publications is reached between 2010–2015. Genomic publications appear in the middle of the 1980 decade and increases in importance afterwards. Medical publications show an increment in number since 1970 decade. The information was generated using the search engines Zoological Records (Web of Science, Thomson Reuters), SCOPUS (Elsevier B.V.), and Biodiversity Heritage Library (Encyclopedia of Life) searching engines. We run four searches: (1) Taxonomy [Ecology (2), Biology (3), or Genomics (4)] + [Scyph* or Jellyfish* or Medus*], filtered: NOT topic: Hydro* + Cubo* + Ctenoph* + Fungi. Records for the medical research (toxicology and envenomation) were gathered from the Biology search. The resultant searches were concatenated into a single file and cleaned for duplicates. Publications focused exclusively on Coronatae jellyfish were excluded.

Chapter 2: Species richness of jellyfishes (Scyphozoa: Discomedusae) in the Tropical Eastern Pacific: missed taxa, molecules, and morphology match in a biodiversity hotspot

1. Abstract

Species richness in the seas has been underestimated due to the combined challenges presented by the taxonomic impediment, delimitation of species, preponderance of cryptic species, and uneven sampling effort. The mismatch between actual and estimated diversity varies by region and by taxon, leaving open questions such as: are hotspots for well-known taxa also hotspots for poorly-known taxa? We address these challenges and this question for shallow-water scyphozoan jellyfishes in the Tropical Eastern Pacific (TEP). We increased sampling effort at 34 coastal locations along the TEP, combined analyses of four molecular markers and up to 53 morphological characters. Phylogenetic analyses under the Bayesian and Maximum likelihood framework, barcoding, and statistical multivariate analyses for morphological data to estimate species richness. Where only five Discomedusae were reported previously, we found a total of 25 species. Of these, twenty-two species are new to science, two are non-indigenous, and one is a previous record; the other four prior records had been misidentified. Thus, by overcoming known challenges, we found that, as for well-known species, the TEP also is a hotspot for scyphozoans. The new discoveries demonstrate the need to evaluate the evolutionary relationships with neighboring regions to understand fully the origins of jellyfish diversity in the TEP and will lead to revision of the systematics and taxonomy of Scyphozoa.

2. Introduction

Global estimates of species richness are uncertain (Scheffers et al. 2012), with greatest imprecision about the number of species in the ocean (Costello et al. 2010; Mora et al. 2011; Appeltans et al. 2012). Many approaches have been applied to estimate the number of marine species, including prediction based on expert opinions (Gibbons et al. 1999; Gordon 2001; Bouchet 2006; Appeltans et al. 2012), extrapolation from well known taxa (Mora et al. 2011) and description rates and inventories (Costello et al. 2010), but recent estimates of marine eukaryotic diversity still vary from ~0.7–1.0 million species (Appeltans et al. 2012). Variation among estimates may be a consequence of the different methods used, but there also are factors that influence all methods of estimation, such as [a] the taxonomic impediment (Wheeler 2004; de Carvalho et al. 2007), [b] delimitation of species and definition of the species concept (De Queiroz 2005a; Frankham et al. 2012), [c] the presence of cryptic species (Appeltans et al. 2012), and [d] limited sampling effort (Costello et al. 2010). These problems are intertwined; understanding their relative impacts is therefore particularly timely for taxa currently attracting renewed attention and likely to undergo considerable revision as modern systematic approaches are adopted (Schlick-Steiner et al. 2010).

Among marine species, jellyfishes recently have increased relatively in profile (Condon et al. 2012) associated with efforts to understand species' dynamics, which has in turn raised questions about species diversity, distributions, evolutionary relationships, and ecology (Lucas and Dawson, 2014; Dawson et al. 2015). Despite these efforts and the continuous advancements in modern taxonomy and systematic approaches adopted in other taxa (Ellingson and Krug 2006; Caputi et al. 2007; Pfeiler et al. 2008; Leese et al. 2008; Lin et al. 2009; Schlick-Steiner et al. 2014), scyphozoan diversity is still underestimated (Appeltans et al. 2012) and its classification is in need of revision (Collins et al. 2006; Bayha et al. 2010). The cause is, at least in part, the so called "taxonomic impediment" (de Carvalho et al. 2005) which limited advances in the systematics and taxonomy of Discomedusae. By 1920, 35 taxonomists had described 80% of the valid species of Discomedusae (Gómez Daglio and Dawson, in prep.), after which the number of taxonomist and the number of

species' descriptions both declined until the 1990s. Although molecular and morphological tools, and the number of taxonomists, have increased slightly in the past two decades, only nine new species of Discomedusae (i.e. 5.7% of the valid species) have been described since the turn of the century (Matsumoto et al. 2003; Raskoff and Matsumoto 2004; Gershwin and Zeidler 2008a; 2008b; Bayha and Dawson 2010; Galil et al. 2010; Piraino et al. 2014; Nishikawa et al. 2014; Kolbasova et al. 2015).

A second reason for the shortfall is that, like most marine invertebrates, scyphozoan *species delimitation* has primarily used macro-morphological characters under the assumptions of the morphological species concept (Haeckel 1879; Vanhöffen, 1888; Mayer 1910; Kramp 1961). Larson (1990) emphasized the unstable taxonomic status of scyphozoans, which he attributed to vague descriptions, a shortage of diagnostic characters, and poor condition of type specimens (if they existed at all); he considered the problems acute in the order Semaeostomeae, particularly in the families Pelagiidae and Ulmaridae. In Scyphozoa, two general methods have been adopted to better resolve and stabilize the taxonomy of the group: (1) quantitative analysis of morphology, which also incorporated morphological characters of other life stages (larvae and polyps, Dong et al. 2008; Schiariti et al. 2008), and microscopic features (Östman 2000), resulting in the discrimination and/or description of new species in some genera (Gershwin and Collins 2002; Dawson 2003; Morandini and Marques 2010; Straehler-Pohl et al. 2011); (2) molecular analyses, which facilitated the delimitation and discovery of cryptic species confounded by morphological information alone (Greenberg et al. 1996; Dawson and Jacobs 2001). These advances have in turn led to consideration of alternative, more integrative, species concepts in scyphozoan taxonomy (e.g. Dawson 2005d) that are commensurate with challenges prevalent for the taxon.

Molecular analyses of even the familiar large jellyfishes then demonstrated that morphologically *cryptic species* complexes are commonplace. The moon jellyfish *Aurelia aurita* has at least 10 cryptic species (Dawson and Jacobs 2001; Dawson et al. 2005); the lion's mane jellyfish *Cyanea capillata* is a complex of at least four species (Dawson 2005b; Holst and Laakmann 2014); the name *Cassiopea andromeda* has at times been used to refer to at least five different species (Holland et al. 2004). Nevertheless, despite the advances made using quantitative morphological or molecular approaches, the systematics and taxonomy of the class Scyphozoa is still not resolved. Overall, scyphomedusae species diversity probably is approximately double the number of currently recognized species (i.e. ~400 species), with around 60 species being 'cryptic' (Dawson 2004; Hamner and Dawson 2009; Appeltans et al. 2012). Several authors have emphasized the need to unite morphological and molecular approaches to move toward an integrative taxonomy (Dayrat 2005; Dawson 2005c; Wiens 2007). A suggested advantage is that combining these different character types ameliorates the limitations of using only one or the other [e.g. *Mastigias* spp. (Dawson 2005d) and *Cyanea* spp. (Dawson 2005b)]. Indeed, quantitative and qualitative morphological data have been integrated with molecular data to distinguish species complexes in Scyphozoa (Bayha and Dawson 2010; Galil et al. 2010; Neethling et al. 2011; Piraino et al. 2014; Holst and Laakmann 2014), suggesting the problems associated with description—i.e. delimitation, definition, and crypsis—of scyphomedusae that have already been collected are largely surmountable.

A more basic challenge involves overcoming *limited sampling*, and adequately collecting species to describe. Appeltans et al. (2012) estimated that, after ~250 years of taxonomy, ~20–25% of scyphozoan species remain to be collected, possibly from relatively remote areas that are diversity hotspots for other taxa, such as the Indo-Pacific, Tropical Eastern Pacific, and the Caribbean Sea (Briggs 1961; 2005a; 2005b; Frey and Vermeij 2008; Bellwood and Meyer 2009; Esselstyn et al. 2013). Sampling effort in these areas has been limited to a few expeditions during the 19th and 20th

centuries (Segura-Puertas 1984; Segura-Puertas et al. 2003; Costello et al. 2010). For example, the Tropical Eastern Pacific (TEP) biogeographical region encompasses the west coast of America between 25° N (Bahía Magdalena) and 4° S (south of Golfo de Guayaquil), including the Gulf of California (GCA) (Robertson and Cramer 2009; Briggs and Bowen 2012). Its bathymetric and oceanographic patterns provide a wide range of suitable habitats for marine taxa (Roden 1958; Lavín et al. 2006), suggesting that biodiversity should be concomitantly high. Indeed, high species richness and a high rate of endemism have been identified for some marine taxa; for example, of 1,261 fish species (~7.5 % of the total marine fishes) 897 are endemic (~77.5% of the total of TEP species) in the Panamanian and Cortez provinces (Zapata and Robertson 2007; Robertson and Cramer 2009), and of 1,343 decapod species (~11% of all marine decapods) there are 420 (~31.2%) endemic in the Panamanian and Cortez provinces (Boschi 2000). In contrast, of 14 species of Scyphozoa reported in the TEP (~6.7% of the ~207 valid species globally) there is only one (~2 %) considered endemic in the TEP (Segura-Puertas 1984; Larson 1990; Cortés-Núñez 1997; Ocaña-Luna and Gómez-Aguirre 1999; Segura-Puertas et al. 2003). Of these 14 reported scyphozoans, only five, including the one proposed endemic, are Discomedusae (*Stellamedusa ventana*, *Pelagia noctiluca, Aurelia aurita*, *Stomolophus meleagris*, and *Catostylus ornatellus*; i.e. ~3.2% of ~155 Discomedusae described globally). The low species richness and low endemism of Discomedusae relative to other taxa in the TEP, suggests either that many species of Discomedusae remain to be discovered, particularly in the west coast of Mexico and Central America (Larson 1990), or that we require an alternative explanation for their low species diversity contrary to well-known taxa.

The uncertainty about the richness and distributions of scyphozoan species has potential to inhibit understanding of important historical and contemporary issues. For example, accurate phylogenies and species differentiation and delimitation are required to understand patterns and processes of speciation (Wiens and Donoghue 2004; Wiens 2011), ecological phenomena such as population dynamics of jellyfishes (Lee et al. 2012; Dawson et al. 2015; see also Brotz et al. 2012; Condon et al. 2012; Condon et al. 2013; Roux et al. 2013) and conservation decisions (Krupnick and Kress 2003; Terlizzi et al. 2003; Guzman et al. 2008). In this study, we ask three questions: How many species of Discomedusae inhabit the TEP? How are the species different genetically and morphologically? Are the species endemics new to science? We answer these questions by conducting the most intensive sampling effort for scyphozoans in the TEP to date and analyzing the resultant collections using quantitative morphological and molecular phylogenetic and barcoding techniques. We then integrate the morphological and molecular analyses to estimate species richness and taxonomic affinities of scyphozoan jellyfishes in the TEP.

3. Material and Methods

3.1 Sample collections

We collected samples from 34 locations along the Tropical Eastern Pacific (Gulf of California, west coast of México, and Central America), two locations in South Eastern Pacific and 14 sites along the Gulf of Mexico, Caribbean Sea and South Eastern Atlantic (Fig. 1; Table 1). Each site was georeferenced using a handheld GPSmap® 60CSx under the universal transverse Mercator coordinate system with a precision of ±3 m. We collected scyphomedusae by snorkeling, SCUBA diving, or with fishing nets or trawls. A piece of tentacle and/or oral arm was clipped and preserved in 95% ethanol before each jellyfish was preserved in 4% formalin.

For comparative purposes, to enable taxonomic identifications, we also included known specimens collected in the Caribbean, South America, and type material deposited in the National Museum of Natural History (Smithsonian, Washington, D.C.), Museum of Comparative Zoology (Harvard University, Massachusetts), University of Malaysia (Kuala Lumpur, Malaysia), and Instituto

Nacional de Investigación y Desarrollo Pesquero (Mar del Plata, Argentina) (Supplementary Table S1).

3.2 DNA extraction, amplification and sequencing

We extracted total genomic DNA from 367 tissue samples (Table 2) using a modified CTAB phenol-chloroform protocol (Dawson and Jacobs 2001). Two mitochondrial markers [cytochrome *c* oxidase subunit I (COI) and 16S rDNA] and two nuclear markers [18S rDNA (small subunit), 28S rDNA (large subunit)] were amplified using the primers listed in Supplementary Table S2. Each 25μL PCR contained: 0.5μL DNA template, 0.1 mM each dNTP (GeneAmp dNTP mix with dTTP, Applied Biosystems Inc., Bethesda, MD, USA), 2.5μL of 10X PCR buffer and 2.5μL $MgCl_2$, 0.63 μL each primer, and 0.05 units of Amplitaq (Applied Biosystems). The thermocycle conditions are given in Supplementary Table S3. Amplicons were sequenced directly using PCR primers when possible. If direct sequencing did not work, or reads revealed polymorphisms, amplicons were cloned using the TOPO TA Cloning Kit for sequencing (Invitrogen Inc.) or StrataClone PCR Cloning Kit (Stratagene) and sequenced using primers T7 and T3. Amplicons were sequenced by Cogenics Inc. (Houston, TX, USA), the University of Washington High-Throughput Genomics Unit (Seattle, WA, USA), Macrogen (Maryland, USA), or the DNA Sequencing Facility University of California, Berkeley (California, USA). All sequences were assembled, primers removed, and base calls manually corrected in SEQUENCHER v.5.2.4 (GeneCodes Corp., Ann Arbor). Sequences were compared by BLASTn searching GenBank (Benson et al. 2012) to affirm the amplification of the correct loci. All sequences were deposited in GenBank (**********).

We included the data set of Bayha et al. (2010) and samples from the Indo-Pacific (Supplementary Table S1) to situate the TEP samples within a global phylogenetic context. As necessary, we used the methods described above to complete sequencing of all four target loci for all specimens.

3.3 Phylogenetic analyses

16S, 18S and 28S were aligned in MAFFT v.7 (Katoh and Standley, 2013) under the iterative method of E-INS-I using the default parameter settings and tested using TRIMAL V.1.2 (Capella-Gutiérrez et al. 2009) under the automated parameters. For 16S and 28S we also used three additional approaches to assess reliability in alignments: (a) MUSCLE (Edgar 2004) with gap-open: gap-extension penalty combinations (-1000 : 0, -900 : -10, -800 : -5, -500 : -15); (b) T-COFFEE V.11 (Notredame et al. 2000) with the default parameters; and (c) MAFFT v.7 with the E-INS-I method with a combination of gap-opening penalties (1.0, 2.0, 3.0). Each resultant alignment was compared in GBLOCKS (Castresana 2000) with and without allowing a maximum of six contiguous non-conserved positions. Regions with ambiguous homology or poor alignment were omitted from further analyses. The best-fit substitution model for aligned sequences was chosen by the Akaike Information Criterion and Bayesian Information Criterion using jMODELTEST v.2.1.4 (Darriba et al. 2012).

We estimated (a) individual gene trees using Maximum likelihood (ML) and Bayesian inference (BY) and (b) species trees using the concatenated data set of 16S, 18S, and 28S. ML gene trees were constructed using the best fitting model of sequence evolution (16S—TPM2uf +I+G, 18S—GTR+I+G, 28S—TIM2+I+G) in GARLI v. 2.01 (Zwickl 2006) on the CIPRES PORTAL v. 3.1 (Miller et al. 2010); the best tree was selected from a minimum of six runs by comparing the log-likelihood scores and evaluating a symmetric difference (Robinson-Foulds) tree distance metric using PAUP v.4b10 (Swofford 2002). The robustness of the ML tree topologies was assessed by 1000 bootstrap

iterations. The bootstrap values (BS) were added into the best ML tree with SUMTREES (Sukumaran and Holder 2010) and plotted in FIGTREE v.1.4 (Rambaut 2013)
BY gene trees were generated using the BEAST v.1.8.1 software pipeline (Drummond et al. 2012). The Bayesian Markov Chain Monte Carlo (MCMC) method was run using priors from jMODELTEST, starting with random trees. Two runs, each with a hot and a cold MCMC chain were executed until the average deviation of split frequencies reached <0.01 (20^7 generations, sampling every 1,000th generation). Convergence and chain mixing were visualized using TRACER v.1.6 (Rambaut et al. 2014). To ensure recovery of the best-resolved tree (strict clock with a normal growing population), we reconstructed the BY trees under all the clock assumptions and population growth combinations. Trees from the stationary phase of the two runs were then pooled by LOGCOMBINER v.1.5.4 and the 50% maximum clade credibility trees were summarized. Assigning this tree as the target tree, the posterior probability (PP) of each node and the mean branch lengths were calculated with TREEANNOTATOR v.1.5.4 (Drummond et al. 2012).
Species trees were generated using the 16S (492 nt), 18S (1679 nt), and 28S (1099 nt) alignments concatenated using MESQUITE v.3.04 (Maddison and Maddison 2015). We partitioned the data set into different segments according to the best-fit substitution models (as listed above). Species trees were generated using the previously described ML and BY approaches.

3.4 Genetic barcoding
DNA barcoding is widely used for species recognition and discrimination (Hebert et al. 2003a; Ortman et al. 2010). Previous studies demonstrate that mitochondrial DNA in jellyfish (particularly a short fragment of COI), presents enough intraspecific variation to distinguish species of jellyfishes (Dawson and Jacobs 2001; Bayha and Dawson 2010). All the specimens were identified *a priori* using standard morphological criteria. Congeneric COI sequences were aligned using CLUSTALX v.2.0 (Larkin et al. 2007) and checked using JALVIEW (Waterhouse et al. 2009). Intraspecific and interspecific genetic distances were calculated among all individuals in PAUP v.4b10 (Swofford 2002)—grouping results across all Discomedusae and then within each family—using the K2P model of evolution (Hebert et al. 2003b; Hebert et al. 2010). The intra- and interspecific pairwise distance and its frequencies were plotted using IGOR PRO (Software Engineer, WaveMetrics, Inc.). For comparison with the barcoding method and for delimiting species, we also reconstructed the COI gene tree for each family using the ML framework described in the previous section.

3.5 Morphological data collection
We randomly selected five mature medusae per phylogenetic species (i.e. identified through the ML analyses plus genetic barcoding approach) and photographed each. We took two sets of pictures of ~53 features (*f*2–*f*70, *f*72–*f*101, *f*105–*f*107, *f*109–*f*119, *f*121–*f*156, Appendix 1). First, in an acrylic tank (with black background), each medusa was placed next to a scale bar, and pictures of the apical, ventral, and side view were taken; we also took detailed pictures of the oral arms, scapulae, tentacle insertion, manubrium, and mouth. Second, on a light table, each medusa was placed first oriented oral-aboral and then aboral-oral facing up and photographed under three different backgrounds and illuminations (black background with flash, black background without flash, full trans-illumination), we took close-ups of oral arms, muscles, gonads, stomach, oral pillars, manubrium, rhopalia, and lappets (Supplementary Table S4). The branching radial canals and stomach cavity were stained with a solution of food dye diluted with water. Example sets of photographs can be found on *The Scyphozoan *Wiki* (http://scyphozoan.ucmerced.edu/wiki/Main_Page, retrieved 12th January 2016).

Both sets of pictures were used to enumerate the meristic features and to measure other morphologic features (Appendix 1) using JMicrovision v.1.2.7 software (http://www.jmicrovision.com), except for features *f*71, *f*102–104, *f*108, *f*120, *f*158 that were measured with calipers and probes during the photographic session. For the genus *Drymonema*, we complemented our new measurements with the existing morphological data generated by Bayha and Dawson (2010), because this genus was represented by only one species in the TEP.

3.6 Morphological analyses

Measurements for each feature were analyzed for cross-correlations within each genus, using Spearman's Rank correlation, and all features were regressed on bell diameter, using ordinary least-squares regression, in STATISTICA v.12 (StatSoft Inc. 2013). To remove individual size as a factor, we standardized features which showed isometric growth as a ratio of bell diameter (Dawson 2003). The morphological matrix was tested for normality and homoscedasticity; invariant characters were excluded from subsequent analyses. Morphological similarity was tested using a principal component analysis (PCA) in STATISTICA v.12 and plotted using IGOR PRO (Software Engineer, WaveMetrics, Inc.).

4. Results

The species tree for Discomedusae (Fig. 2) supports the order Semaeostomeae as paraphyletic with respect to the order Rhizostomeae (100% BS and PP). Within the order Rhizostomeae, the suborder Dactyliophorae is paraphyletic with respect to the suborder Kolpophorae (branch support 100-99%, BS-PP respectively). Eight taxonomic groups were recovered as reciprocally monophyletic clades at the levels of family (Pelagiidae, Cyaneidae, Drymonematidae, and Ulmaridae) and superfamily (Scapulatae, Actinomyariae, Krikomyariae, and Kampylomyariae). Individual gene trees support these same clades (Supplementary Figure S1). Branch support for these deep nodes is 100% (BS and PP), except for the family Ulmaridae for which support is 75% BS and 80 % PP.

Shallow-water TEP species are present in six of the eight major family or superfamily level clades—three within the order Semaeostomeae (Drymonematidae, Pelagiidae, Ulmaridae) and three within the order Rhizostomeae (Scapulatae, Krykomyariae, Kampylomyariae; Table 2)—plus in the paraphyletic Inscapulatae. The lineage of pelagiids is the most diverse, with seven species recorded in the TEP. The genus *Chrysaora*, though, is paraphyletic with respect to *Pelagia* and *Sanderia*. Of the other Semaeostomeae families, the Ulmaridae is monophyletic, but its branch support is low (75-80%, BS-PP); Ulmaridae includes three species of *Aurelia* from the TEP. The family Drymonematidae is a strongly supported monophyletic group (100% BS and PP) represented by one species in the TEP.

Within Rhizostomeae, the superfamily Scapulatae is a monophyletic clade; within this clade are five TEP species identified as members of the family Stomolophidae (supported by 100% BS, PP). The superfamily Inscapulatae is paraphyletic with respect to Suborder Kolpophorae; within the superfamily Inscapulatae, the families Catostylidae, Lychnorhizidae, and Lobonematidae are polyphyletic, and comprise seven species from the TEP supported by 100-75% (BS, PP). The five families within the superfamily Kolpophorae are strongly supported (100% for BS and PP) reciprocally monophyletic clades. Two non-indigenous species are found in the TEP: *Cassiopea andromeda* and *Phyllorhiza punctata.*

The frequency histogram of pairwise genetic distances among all Discomedusae studied is tetramodal—with modes at ~0.01, ~0.05, ~0.11, and ~0.22 K2P—with two main discontinuities in the distribution: one 0.025–0.035 and the second at 0.055–0.095 K2P (Fig. 3). Discomedusae

intraspecific pairwise genetic distance ($\bar{x}$, SD) is 0.006 ± 0.005; the average interspecific distance ($\bar{x}$, SD) is 0.12 ± 0.04 (Fig. 3).

4.1 Species delimitation and differentiation

Order Semaeostomeae

Family Drymonematidae

Genus *Drymonema* Haeckel 1880 (Fig. 4a)

The COI ML tree supports three reciprocally monophyletic clades (100% BS) of which only *Drymonema* sp. 1 inhabits the TEP; the sister taxon, *D. larsoni*, inhabits the North Atlantic and Caribbean (Fig. 5a). The mean intraspecific genetic distance ($\bar{x}$ ± SD) for species of *Drymonema* is 0.002 ± 0.002; the mean interspecific genetic distance is 0.118 ± 0.019 (Fig. 5b). PCA analysis of 24 characters (eight categorical, 16 continuous) shows the discrimination of *Drymonema* sp. 1 from the Atlantic species, and the discrimination between Mediterranean and the Atlantic species, including *D. gorgo* (Fig. 5c). The most useful morphological characters for distinguishing the species, and the percentage of the variation they explain are: number of tentacles with 87% (*f*21), number of radial mesenteries by 3% (*f*35), and number of stomach pouches with 2% (*f38*).

Family Pelagiidae

The COI ML tree suggests three main groups (Fig. 6a), although this is at odds with the species tree (Fig. 2). Deeper branches in the COI ML tree have low support and the relative position of some taxa e.g. *Chrysaora* sp., *C. chinensis,* and the clade of the temperate north Pacific species (*C. achlyos*, *C. colorata*, *C. melanaster*, and *C. fuscescens)*, is unsettled. Seven species of pelagiids are present in the TEP.

Genus *Chrysaora* Péron and Lesueur 1809 (Fig. 4b–d)

The genus *Chrysaora* is monophyletic in the COI tree (Fig. 6a), but paraphyletic with respect to *Pelagia* in the species tree (Fig. 2). Four species of *Chrysaora* are distributed in the TEP (*Chrysaora* sp. 1, *Chrysaora* sp. 2, *Chrysaora* sp. 3, and *Chrysaora* sp. 4) and include the sister taxon of the Caribbean clade (*C. quinquecirrha, Chrysaora* sp. 5, and *Chrysaora* sp. 6; Fig. 6a). *Chrysaora* sp. 2 is not closely related to the other species in the TEP. Barcode analysis indicates the mean K2P intraspecific pairwise sequence distance ($\bar{x}$ ± SD) is 0.005 ± 0.004 ($\bar{x}$, SD) and the mean interspecific distance is 0.162 ± 0.05 (Fig. 6b). PCA analysis of 40 variable morphological characters (13 continuous, 27 categorical) allows the differentiation of six groups (Fig. 6c), from which five groups correspond to five of the phylogenetic species (Fig. 2; 6a); *C. quinquecirrha* and *Chrysaora* sp. 3 appear as a single group. The morphological variables that contribute the most to distinguishing the species and the percentage of the explained variance are: radial mesentery termination with 60% (*f*37), number of primary tentacles 20% (*f*21), presence of quadralinga 8% (*f*153), rhopaliar lappets shape 3% (*f*19), and velar lappets shape 2% (*f*11).

Genus *Sanderia* Goette 1886 (Fig. 4e)

A clade in the COI ML tree with robust basal branch support (100%, BS) (Fig. 6a) demonstrates that *Pelagia benovici* (Piraino et al. 2014) is more closely related to *Sanderia malayensis* than to *Pelagia*. Two other species of *Sanderia* are found in the TEP. Barcoding analyses show an average intraspecific K2P distance ($\bar{x}$ ± SD) of 0.0007 ± 0.0009 and interspecific distance of 0.199 ± 0.056 (Fig. 6b). The morphological discrimination (Fig. 7b) between *S. malayensis, Sanderia* sp. 1 and *Sanderia* sp. 2 is possible through the PCA analysis of 27 characters (20 continuous, seven categorical). The characters that contribute the most to differentiating the species and the

percentage of variation they explain are: velar lappets shape with 34.5% (*f*11), rhopaliar lappets shape 26% (*f*19), shape of the stomach/gonadal cavity 17% (*f*149); rhopalia position 4% (*f*116), structural shape of the gonads 4% (*f*151), and number of velar lappets 4% (*f*7).

Genus *Pelagia* (Forskål 1775)
Pelagia is represented in the TEP by one species: *Pelagia noctiluca* (Table 2). The ML tree shows two main clades (Fig. 6a), one for the TEP and Caribbean Sea species—*Pelagia* sp. 1—and the other for the western Pacific (see Supplementary Table S1). The mean K2P pairwise interspecific distance ($\bar{x}$ ± SD) is 0.041 ± 0.005, and the intraspecific pairwise distance is 0.008 ± 0.005 (Fig. 7c). PCA analysis of 19 morphological characters (two categorical and 17 continuous) discriminates between *P. noctiluca* and *P. panopyra* (Fig. 7d). The morphological characters that contributed the most to discriminate the species, and the percentage of the variation they explain are: bell thickness with 45% (*f*71), radial mesentery termination 33% (*f*37), longitudinal-sectional shape of exumbrella ornaments 13% (*f*140), and oral arm length 8% (*f*77).

Family Ulmaridae
Genus *Aurelia* Lamarck 1816
The ML tree (COI) shows two well-supported clades: (1) *A. aurita* and *Aurelia* sp. 14, and (2) two species from the TEP and three from the Atlantic basin (Fig. 8a). Three species are found in the TEP, of which two (*Aurelia* sp. 12, *Aurelia* sp. 13) are sister to species from the Atlantic basin (*Aurelia* sp. 9, *Aurelia* sp. 15, and *Aurelia* sp. 16). *Aurelia* sp. 14, however, is not closely related to other species in the TEP. The mean K2P intraspecific pairwise sequence distance ($\bar{x}$ ± SD) is 0.002 ± 0.002 while mean interspecific distance is 0.202 ± 0.032 (Fig. 8b). Morphological discrimination is possible through the PCA analysis of 33 morphological characters (32 continuous, one categorical) (Fig. 8c). The most useful morphological characters for distinguishing the species, and the percentage of the variation they explain are: number of terminations of adradial canals at the ring canal with 19% (*f*51), oral arm width 19% (*f*79), thickness of the subgenital porticus 16% (*f*152), number of perradial-perradial anastomoses 9% (*f*43), number of interradial canals origins at the gastrovascular cavity 9% (*f*41), and number of lobes 9% (*f*13).

Order Rhizostomeae
Family Lobonematidae
Genus 1
Eight specimens corresponded with the diagnosis of the family Lobonematidae *sensu* Mayer (1910). Phylogenetic analyses show two well-supported clades (100% BS), one corresponds to the type species of the family—*L. smithii*. The other clade includes four lineages from the TEP (Fig. 9a). The mean pairwise intraspecific distance ($\bar{x}$ ± SD) is 0.002 ± 0.002 and the average interspecific distance is 0.217 ± 0.059. (Fig. 9b). PCA analysis of 53 morphological variables (9 categorical, 44 continuous) shows the differentiation between *L. smithii* and Lobonematidae sp. 1 and sp. 3 (Fig. 9c). The most useful morphological characters for distinguishing the species, and the percentage of the variation they explain are: number of interradial-interradial anastomoses 42% (*f*44), number of perradial-perradial anastomoses 32% (*f*43), number of adradial-adradial anastomoses 16% (*f*45), and height of exumbrella protuberances 2% (*f*138).

Family Lychnorhizidae
Genus *Lychnorhiza* Haeckel, 1880 (Fig. 4f)

The COI ML tree shows three main clades (100% BS): (1) the TEP *Lychnorhiza* sp. 1, (2) *L. lucerna* and *Lychnorhiza* sp. 3, and (3) *Lychnorhiza* sp. 2 from the Caribbean (Fig. 10a). The mean K2P intraspecific pairwise distance ($\bar{x}$ ± SD) is 0.005 ± 0.004, and the average interspecific distance 0.129 ± 0.01 (Fig. 10b). PCA analysis of 32 morphological characters (25 continuous, seven categorical) distinguishes four groups (Fig 10c), two representing the phylogenetic species *L. lucerna* and *Lychnorhiza* sp. 2, the other two clusters correspond to *Lychnorhiza* sp. 1. The most useful morphological characters for distinguishing the species, and the percentage of the variation they explain are: rhopaliar lappet shape 2% (*f*19), presence of bifurcated velar lappets 3% (*f*6) and number of bifurcated velar lappets 3% (*f*8), distribution of intermediate filaments on the oral arm and oral disc with 3% (*f*109); distribution (*f*137), cross-sectional shape (*f*139), longitudinal-sectional shape of exumbrella ornaments (*f*140) with 3% each; length (*f*132), width (*f*133) and shape of subumbrella papillae (*f*134) by 2% each; subgenital ostia with ornaments 2% (*f*106), depth of the oral pillars 2% (*f*104), perradial of the stomach cavity 2% (*f*150), and velar lappets length 2% (*f*9).

Family Catostylidae
Genus 1 (Fig. 4g–h)
We identify these specimens as members of Catostylidae based on the diagnosis of the family *sensu* Kramp (1961). The COI ML tree supports the distinction of two species: Catostylidae sp. 1 and Catostylidae sp. 2 (Fig. 11a). The average intraspecific pairwise distance ($\bar{x}$ ± SD) is 0.002 ± 0.001, and the mean interspecific genetic distance is 0.131 ± 0.003 (Fig. 11b). PCA analysis of 38 characters (five categorical, 33 continuous) denotes the differentiation of the two species (Fig. 11c). The most useful morphological characters for distinguishing the species, and the percentage of the variation they explain are: number of adradial-adradial anastomoses 97% (*f*45), percentage of radius of medusa in which there is no branching radial canal 1% (*f*62); number (*f*97) and length (*f*100) of terminal clubs with 1% each; and number of interradial-adradial anastomoses 1% (*f*48).

Family Stomolophidae
Genus *Stomolophus* Agassiz 1869 (Fig. 4i–k)
The COI ML tree supports two reciprocal monophyletic groups—(1) TEP and (2) Caribbean (Fig. 12a)—which also receive some support in the species tree (BY alternative topology, Fig. 2). Five species are found in the TEP, which are nested by region: GCA—*Stomolophus* sp. 1 and sp. 2; Central America—*Stomolophus* sp. 3 and *Stomolophus* sp. 4; *Stomolophus* sp. 6 is a singleton that is closely related to the Central America clade. The average intraspecific pairwise distance ($\bar{x}$ ± SD) is 0.007 ± 0.005, and the mean interspecific genetic distance is 0.107 ± 0.028 (Fig. 12b). PCA analysis of 30 morphological variables (27 continuous, three categorical) discriminates six species (Fig. 12c). The most useful morphological characters for distinguishing the species, and the percentage of the variation they explain are: number of adradial canal origins at the gastrovascular cavity with 68% (*f*42), number of pigmented flecks in adradial canals 28% (*f*143), number of pigmented flecks in interradial canals 2% (*f*142), and subumbrella papillae length 2% (*f*132).

5. Discussion
Traditional estimates of the richness and distributions of scyphozoans have fallen far short of the true diversity. There is a concomitant shortfall in understanding of functional, evolutionary, and ecological diversity and commensurate misunderstanding of factors pertinent to contemporary issues (Gibbons and Richardson 2013; Lucas and Dawson 2014). To begin to address these shortfalls, we addressed four challenges to estimating biodiversity — the taxonomic impediment,

species delimitation, cryptic species, sampling effort — and posed three questions: How many species of Discomedusae inhabit the TEP? How are the species different genetically and morphologically? Are the species endemics new to science? We can now answer these questions, and also reflect on the completeness of our understanding of scyphozoan biodiversity in the TEP using integrative taxonomic approaches.

5.1 How many species of Discomedusae inhabit the TEP?

Though, historically, the Tropical Eastern Pacific has been reported as having low scyphozoan species diversity (Larson 1990; Segura-Puertas et al. 2003), this is emphatically not the case. Our extensive sampling effort in the region, coupled with molecular and statistical morphological approaches evince at least 25 species of shallow-water discomedusan jellyfishes in the TEP, a five-fold increase over previous records. These lineages represent nine of the 14 valid families of Discomedusae, and constitute ~7–8% of estimated total global scyphozoan species richness [~338–383 species, Appeltans et al. (2012)]. The diversity of scyphozoans in the TEP, as a proportion of global scyphozoan diversity, therefore matches closely with the proportions of global richness in other taxa that occur in the TEP (e.g. decapods—11%, fishes—7%). Our findings represent an increase of ~14% on the known 155 Discomedusae species, illustrating that the TEP is an area with a high species richness—a hotspot—of Discomedusae.

5.2 How are the species different genetically and morphologically?

A key finding of our analyses is that the large majority of new species are different both morphologically and genetically, yet the data types are complimentary rather than alternatives. Traditionally, scyphozoan taxonomy, with its roots established in the biological and morphological species concepts, employed macro-morphological characters to describe and delimit ~207 species, of which 155 species belong to the taxon Discomedusae (Gómez Daglio and Dawson in prep.). Adoption of molecular tools resulted in the recognition of 17 phylogenetic species (Gómez Daglio and Dawson in prep.). However, none of the phylogenetic species were formally described because of uncertainty about how phylogenetic species mapped to known and unknown species (Dawson 2003). Descriptions of new species of Discomedusae employing two or more lines of evidence and approaches number only a handful (Bayha and Dawson 2010; Galil et al. 2010; Piraino et al. 2014; Nishikawa et al. 2014; Kolbasova et al. 2015), and our results thus provide the clearest evidence yet, across diverse taxa, that genetic and morphological approaches yield highly congruent results in Discomedusae, understanding of jellyfish diversity can be improved through morphometric analyses and through molecular analyses, and that there is an added benefit in their integration.

5.3 Improvements through quantitative morphological analyses

Historically, morphological species delimitation and description of Discomedusae relied on the qualitative and quantitative description of few diagnostic meristic macro-morphological characters (e.g. numbers of tentacles, rhopalia, terminal clubs, oral arms, and velar lappets; general counts and descriptions of the canal system; see Mayer 1910; Stiasny 1921; Russell 1970). Such approaches led, for example, to the conclusion that a single species—*Stomolophus meleagris*—was distributed throughout the tropical and subtropical Americas (Kramp 1961; Segura-Puertas 1984; Ocaña-Luna and Gómez-Aguirre 1999). Our analyses demonstrate that the discrimination and delimitation of species is improved by the detailed assessment of quantitative (including morphometric) and qualitative morphological characters (Appendix 1). In the case of *Stomolophus*, such analyses reveal seven species, including four within the TEP (Fig. 12C).

The combination of different types of morphological characters and their quantitative analysis was previously addressed by Dawson (2003), who was able to differentiate and describe the intra- and interspecific morphological variation of a cryptic species complex (*Aurelia*) in different regions of the world. The quantitative analysis of morphological characters has since been applied to other taxa, for example in Catostylidae (Dawson 2005d), Cyaneidae (Dawson 2005b; Holst and Laakmann 2014), and Mastigiidae (Dawson 2005a). Likewise, this study morphologically discriminated 17 species in the TEP. These results demonstrate the utility of using morphological characters and quantitative morphometrics, although, morphological discrimination is not possible for all species, including three in our case. In two cases, we did not collect the full medusa. The third case, for example, discrimination was impossible within the *C. quinquecirrha* complex (includes *Chrysaora* sp. 3 and *Chrysaora* sp. 5, Fig. 6c) which concurs with the main conclusions made by Morandini and Marques (2010) that they could not differentiate between "Atlantic" and "Pacific" groups. This irresolution is due, in part, to the presence of homoplasies (e.g. the presence of the quadralinga in *C. achlyos*, *C. plocamia,* and *C. colorata*) and ontogenically variable features (e.g. the number or tentacles classified as primary, secondary or tertiary; Mayer 1910; Littleford and Truitt 1937; Morandini and Marques 2010).

5.4 Improvements through molecular analyses

Molecular data have proven invaluable in resolving a suite of difficult taxonomic challenges caused by morphological crypsis (Dawson and Jacobs 2001; Dawson 2005a) and phenotypic plasticity (Dawson 2005a) in jellyfishes. Today, molecular data are most commonly used in barcoding and phylogenetic approaches to infer taxonomic hypotheses and identify species (Bucklin et al. 2010; Ortman et al. 2010; Dellicour and Flot 2014).

The DNA barcoding approach is often used as a method to delimit, differentiate, and discover species. Its foundations, methodology, and taxonomic implications have been debated for more than 10 years (Wheeler 2005; Goldstein and DeSalle 2010; Bergsten et al. 2012; Collins and Cruickshank 2013), with critiques of the method focusing on key assumptions, such as the use of a single model of evolution (Kimura-2-parameter) to estimate the sequence divergences (Srivathsan and Meier 2011) and of a barcoding gap as a fixed parameter to delimit species (Meyer and Paulay 2005). Our estimation of the barcoding gap for Discomedusae falls within previous estimations for other invertebrates (Hebert et al. 2003b; Costa et al. 2009; Bucklin et al. 2010; Ortman et al. 2010). For example, COI genetic distances between *Drymonema* (Fig. 5b) and *Lychnorhiza* (Fig. 10b) show a distinctive barcoding gap that corresponds with the range of genetic distances (1.3–22.6%) estimated between other marine taxa—including anemones, crustaceans, echinoderms, fishes, and molluscs (Rocha et al. 2008; Lessios 2008; Miura et al. 2010; Miura et al. 2012)—that have sister taxa in the TEP and Caribbean Sea.

Nonetheless, there is high heterogeneity in the rate of molecular evolution (Figs. 2, 3), introducing some ambiguity into the choice of a 'barcode gap' for the Discomedusae. For example, *Pelagia* presents genetic distances which do not follow the trend shown in the con-familial genera *Sanderia* (Fig. 7a) and *Chrysaora* (Fig. 6b). Interspecific distances may be smaller in *Pelagia* (Fig. 7c) than in other pelagiids. This difference may be explained by differences in their life-cycles: *Pelagia* spp. are non-metagenic holoplanktonic species (Sandrini and Avian 1983) with a high dispersal potential while, on the other hand, *Sanderia* and *Chrysaora* are metagenic meroplanktonic scyphozoans (Arai 1997; Morandini et al. 2004; Widmer 2008; Schiariti et al. 2014; Ceh et al. 2015). Moreover, a single barcoding gap is not always evident, for example, the frequency distributions of genetic distances in *Chrysaora* (Fig. 6b) and *Stomolophus* (Fig. 12b) show two discontinuities. This supports the observation that DNA barcoding may not detect recently diverged

species (van Velzen et al. 2012), which here is the case for *Stomolophus* sp. 1 and *Stomolophus* sp. 2 with in the GCA, and *Chrysaora* sp. 5 and *Chrysaora* sp. 6. in the Caribbean Sea.

For these reasons, the phylogenetic approach to species delimitation remains a key component in the advancement of molecular taxonomy. The phylogenetic approach to species delimitation, which precedes barcoding (Cracraft 1983, 1992), is complemented by a well-developed species concept (Wheeler and Platnick 2000; De Queiroz 2005b; Mishler 2010), albeit of which details are debated (De Queiroz 2007; Dayrat et al. 2008; Velasco 2009; Platnick 2012), that is well-aligned with the long history of thought on species and speciation (Darwin 1859; Hennig 1966; Dobzhansky et al. 1977). In addition, the phylogenetic approach maintains some methodological advantages over barcoding, including (1) a growing suite of tools for including multiple loci that provide better delimitation and discrimination of species, (2) that phylogenies include estimates of uncertainty and are testable hypotheses, and (3) there are multiple methods available to test the hypotheses, such as maximum likelihood, Bayesian analyses, coalescence, and parsimony, for which the strengths and weaknesses are reasonably well-understood. Thus, for example, the family-level COI ML gene trees suggest a total of 25 species in the TEP (Figs. 5–6; 8–12). Testing these hypotheses by adding two loci in ML and BY analyses of the Discomedusae (Fig. 2) yields the same total number of TEP species and, for the most-part, a consistent species tree topology (excepting family Pelagiidae). These differences in relationships among the Pelagiidae appear attributable to the COI gene tree which reveals instability in the position of *C. chinensis* and the temperate clade of *Chrysaora* (*C. achlyos, C. colorata, C. fuscescens, C. melanaster*) suggesting saturation of COI and so long-branch attraction in this single gene tree analysis of Pelagiidae (Fig. 6a cf. Fig. 2). Thus, phylogenetic analyses can provide additional information over barcoding, though both can be sensitive to somewhat arbitrary decisions about the degree of difference that signifies a species (Sites and Marshall 2003; Mallet 2008; Velasco 2009; Mendelson and Shaw 2012).

5.5 The added benefit of integrative taxonomy

Integrative taxonomy arose as a philosophical and practical advance in the face of several challenges to traditional taxonomy, including perceptions that morphological taxonomy was arcane, archaic, and inadequate (Paterlini 2007), molecular analyses were broadly accessible and superior (Ellis et al. 2010; Hebert et al. 2010), and that limited funding during a biodiversity crisis necessitated a transition to a less specialized and more rapid approach (Ebach and Holdrege 2005). Integrative taxonomy addressed that decoupling of molecular methods from morphology, added value by inclusion of multiple lines of evidence, and aimed to provide new conceptual frameworks for delimiting, describing, classifying, and identifying biodiversity (Dawson 2004; Dayrat 2005; Schlick-Steiner et al. 2010). Despite the inevitable debates surrounding any new approach (Will et al. 2005; Valdecasas et al. 2008; Padial et al. 2010; Pires and Marinoni 2010; Yeates et al. 2010), integrative taxonomy has gained considerable attention including through exploration of new methods (Edwards and Knowles 2014) and their assumptions (Carstens et al. 2013). At the heart of these, variation in morphological characters in part reflects underlying additive and non-additive genetic variation (Felsenstein 2005; Lawing et al. 2008), so sampling morphological characters (quantitative or qualitative) therefore should enrich traditional descriptive taxonomy and also complement data on genetic variation.

Our analyses suggest that species delimitation, identification and discovery can be more complete and reliable when employing multiple lines of evidence. Whereas our morphological analyses clearly delineated 17 groups, i.e. morphospecies, and our molecular (barcoding and phylogenetic) analyses indicated 25 species, neither was always better than the other. In 17 cases, both

morphological and molecular analyses agreed. In three cases, post hoc comparison revealed clear morphological differences between clades that were only shallowly differentiated in molecular analyses (*Stomolophus* sp. 1; *Stomolophus* sp. 4; *P. noctiluca*). In three cases, molecular data differentiated morphotypes that could not clearly be discriminated using morphological data alone (*Chrysaora* sp. 3; Lobonematidae sp. 3; *Sanderia* sp. 2). The last 2 species are the non-native species (*Cassiopea andromeda* and *Phyllorhiza punctata*).

These results highlight the importance of developing an integrative taxonomy, perhaps especially in taxa that historically have proven difficult such as the medusae. Accurate species delimitation and identification are essential for accurate assessment of species richness and have important repercussions in other scientific disciplines. Species provide the foundational unit for framing hypotheses regarding factors that influence taxonomic diversification, the origin and radiation of functional diversity, and biogeographical patterns. Moreover, integrative taxonomy has important implications in areas of research which frequently underestimate biodiversity, assume genetic homogeneity over large geographic areas, and make equivocal conclusions about biological resources. For example, integrative taxonomy may reduce the misidentification of non-native versus native species (Graham and Bayha 2007), better inform about species responses to environmental change (Condon et al. 2012; see Dawson et al. 2015), suggest different management units for the exploitation of living marine resources (e.g. fisheries; Girón-Nava et al. 2015; see García de León et al. in prep.), and aid in the designation of protected areas which reliably reflect underlying assumptions such as that hotspots are a central conservation investment strategy (Marchese 2015).

5.6 Which of the species are endemics, new to science?

Of the 25 species in the TEP that our integrative analyses reveal, the question remains as to which are endemic and, therefore, whether the TEP represents a hotspot for Discomedusae, as it does for fishes, decapods, and perhaps other taxa. Of the 25 species from the TEP whose relationships with medusae from other locations are shown in Figure 2, we conclude that two are non-indigenous, one is a previous record (*P. noctiluca*) of a known indigenous species, and 22 are new to science.

Prior to this study, there were records of five Discomedusae in the TEP (Vanhöffen 1902; Bigelow 1940; Segura-Puertas 1984; Larson 1990; Cortés-Núñez 1997; Ocaña-Luna and Gómez-Aguirre 1999; Segura-Puertas et al. 2003; Raskoff and Matsumoto 2004; Rodríguez-Sáenz and Segura-Puertas 2009). One of these records was identified correctly for the TEP—*P. noctiluca*. Two records were of valid species: *Catostylus ornatellus* and the deep water *Stellamedusa ventana*. The remaining two species were misidentified (Table 2): *Aurelia* on the northwest coast of Mexico and Central America was misidentified as *A. aurita* (Segura-Puertas 1984; Gómez-Aguirre 1991; Cortés-Núñez 1997; Segura-Puertas et al. 2003) but is in fact *Aurelia* sp. 13; *Stomolophus* throughout the entire TEP was misidentified as *S. meleagris* (Bigelow 1914; Segura-Puertas 1984; Gómez-Aguirre 1991; Cortés-Núñez 1997; Segura-Puertas et al. 2003) but is in fact a complex of five undescribed species.

The two non-indigenous species are *Cassiopea andromeda* and *Phyllorhiza punctata*, both already well known as species introduced to other regions. *C. andromeda*, a cryptic species from the Red Sea, has been misidentified multiple times as a part of the regional fauna (Schembri et al. 2010) although Holland et al. (2004) identified this lineage as having multiple introductions to other oceanic regions including the Indo-Pacific, Hawaii, and the Caribbean. *P. punctata*, whose native range is Australia, has been mistakenly described as a new species (Moreira 1961; Schembri et al. 2010), but also is an invasive in many tropical oceanic regions, including the Gulf of Mexico, Hawaii, California, Brazil, and the Mediterranean (Graham et al. 2003; Graham and Bayha 2007).

We conclude that 22 lineages are endemic species that are new to science. However, six are represented by singleton specimens within the families Pelagiidae (*Sanderia* sp. 2), Lobonematidae (Lobonematidae sp. 2, Lobonematidae sp. 3, Lobonematidae sp. 4), Catostylidae (Catostylidae sp. 2), and Stomolophidae (*Stomolophus* sp. 6), and so we reserve judgment on the strength of this assessment. Although the evidence provided strongly suggests enough genetic and morphologic differentiation to be considered distinct species from their congeners, a single specimen cannot capture the morphological and genetic variation that is required to statistically delimit new species. In finding 22 endemic species, and 25 species total, we have increased the known endemic species richness of the TEP by five-fold. The shallow-water discomedusan jellyfishes have high endemicity at 88% in the TEP, similar to levels of endemism in other taxa such as fishes and mollusks (Briggs 1961; Vermeij and Petuch 1986; Laguna 1990; Palacios-Salgado et al. 2012). As such, we conclude that, as for these better known taxa, the TEP is also a biodiversity hotspot for jellyfishes. Moreover, considering that our sampling included only shallow water species, it is reasonable to conclude that the total diversity of Scyphozoa in the TEP—including Coronatae and mesopelagic Discomedusae—should be even higher.

5.7 Systematic implications

The updated phylogeny of the subclass Discomedusae (Fig. 2), with increased character and taxon sampling relative to earlier studies (Bayha et al. 2010), is better resolved for almost all nodes. Whereas Bayha et al. (2010) noted irresolution of relationships among Semaeostomeae families near the base of Discomedusae (particularly Cyaneidae and Pelagiidae), and among families at the base of Kolpophorae (particularly Cassiopeidae and Cepheidae), these all now appear well-supported (≥98% BS and PP). Likewise, we now have strong evidence—≥80%—that each of the families Catostylidae, Lobonematidae, and Lychnorhizidae are polyphyletic and the Superfamily Inscapulatae paraphyletic. We consider these advances to be attributable to both greater taxonomic representation and addition of characters.

In better resolving the overall tree of Discomedusae—including confirming which portions that need additional investigation—the new phylogeny renews emphasis on several broader challenges that have arisen in recent years. For example, while the molecular phylogenetic evidence—consistent with multiple morphological hypotheses—is now overwhelming that Semaeostomeae is paraphyletic due to a sister taxon relationship between Family Ulmaridae and Order Rhizostomeae (Collins et al. 2006; Bayha et al. 2010; Kayal et al. 2013), the relationship of Discomedusae (= Semaeostomeae + Rhizostomeae) to other medusozoans conflicts in whole mitochondrial genome analyses (Kayal et al. 2013) versus transcriptomes analyses (Zapata et al. 2015). Resolving this conflict may suggest intriguing patterns of evolution in mitochondrial DNA, particularly in coronates (Kayal et al. 2013), and will be key to correctly rooting the tree and polarizing family relationships and character evolution within Discomedusae.

In better resolving relationships, and the relative timing of species origins, we may also gain insight into whether diversification in multiple clades was driven by similar or different events. For example, Pelagiidae, particularly *Chrysaora*, and other diverse clades including *Aurelia*, *Lychnorhiza*, and *Stomolophus* each appear to have diversified in Caribbean and TEP seas. Likewise, we could ask whether similar morphologies arose at similar times in distinct lineages—such as the curtain-like oral arms of *Cyanea*, *Drymonema*, and *Phacellophora*—and whether this was a response to the diversification of other jellyfish lineages as potential prey. For example, did ontogenetic variation in Drymonematidae—which has been hypothesized to be consistent with a transition in primary food source—evolve in response to diversification of *Aurelia* (Bayha and Dawson 2010)? Such questions speak also to broader questions about evolutionary patterns of

diversity in the marine realm such as the frequency and causes of speciation (Palumbi 1994; Fitzpatrick et al. 2009; Norris and Hull 2011; Bowen et al. 2013) and of crypsis (Swift et al. 2016). To these issues, we add several taxonomic concerns that remain to be resolved. Family Pelagiidae, is in need of thorough taxonomic revision to remove the paraphyly of *Chrysaora*, and description of new genera for current members of the temperate group of *Chrysaora* (*C. colorata, C. achlyos, C. fuscescens*, and *C. melanaster*) and *Sanderia* from the TEP; the revision of the *Sanderia* clade will need to consider the recent description of *P. benovici* which is closely related to *S. malayensis* than *Pelagia noctiluca* (Fig. 2).

We also question the recent assignment of *Phacellophora camtschatica* to a new monogeneric family Phacellophoridae (Straehler-Pohl et al. 2011). While it has been common practice to erect new subfamilies for enigmatic deepwater medusae in the absence of molecular phylogenetic analyses (Matsumoto et al. 2003; Raskoff and Matsumoto 2004), creating a new family by fracturing a long-standing subfamily (Sthenoniinae; Kramp 1961; Larson 1986) that is well-supported by molecular phylogenetic analyses (Bayha *et al*., 2010) has the potential to undermine the organizational and informational role of taxonomy. The morphologically intriguing *Phacellophora* and the deep water ulmarid *Poralia* form a currently well-supported clade Sthenoniinae, which currently complements subfamilies Aureliinae and Deepstariinae in our phylogeny.

In all of these cases, further resolution requires more and better information than we currently have at hand. In many ways, resolving these outstanding issues will benefit from the lead taken in this study, i.e. by addressing historically limited sampling effort, by delimitating species in manners that are consistent with multiple species concepts, and by using techniques that can distinguish among otherwise cryptic species. In this vein, our choice of comparators for this analysis of the TEP already highlights considerable hidden diversity in the Caribbean (*Stomolophus* sp. 5, *Chrysaora* sp. 5, *Chrysaora* sp. 6, *Pelagia* sp. 1, *Lychnorhiza* sp. 2, and *Lychnorhiza* sp. 3) and in the Indo-Pacific region (*Acromitus* sp., *Pelagia* cf. *panopyra*, and *Phyllorhiza* cf. *pacifica*). Resolving uncertainty about Catostylidae, Lobonematidae, and Lychnorhizidae will require renewed sampling effort in their undersampled, biodiverse center of diversity: the Indo-Pacific. Resolving relationships among and taxonomy of the ulmarids will require increased sampling of the deep water discomedusae and their inclusion in more advanced morphological and molecular analyses. In all cases we suggest the most robust results and complete estimates of jellyfish species richness will be gained by supplementing expanded collections with integrated quantitative morphological and molecular genetic analyses.

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Table 1. Geographic position (Latitude and Longitude) for the sampling locations in the Tropical Eastern Pacific, Caribbean Sea, and South America, plus geographic information for reference samples from other oceanic regions. Map reference numbers refer to locations plotted in Figure 1. For locations marked with an asterisk, *, geographic coordinates were estimated using GOOGLE EARTH.

Country	Location	Latitude	Longitude	Location Code	Map reference no.	Sampling date
			Tropical Eastern Pacific			
México	Golfo de Santa Clara	31° 39' 40" N	114° 34' 34" W	MXSOGSC	1	Mar 2009
	Las Guásimas	28° 48' 34" N	111° 56' 27" W	MXSOGUY	2	Mar 2009
	Bahía Kino	27° 51' 34" N	110° 36' 37" W	MXSOBKN	3	Mar 2009
	Bahía San Luis Gonzaga	29° 47' 29" N	114° 22' 43" W	MXBNGOZ	4	Mar 2013
	Mulegé	26° 53' 56" N	111° 57' 39" W	MXBSMUL	5	Aug 2009
	Bahía Magdalena	26° 53' 56" N	111° 57' 39" W	MXBSMAG	6	Jul–Aug 2008
	Agonales	25° 08' 35" N	112° 08' 42" W	MXBSAGO	7	Aug 2009
	Canal Principal	25° 11' 20" N	112° 07' 45" W	MXBSCPC	8	Aug 2009
	Bahía de la Paz	24° 10' 24" N	110° 18' 57" W	MXBSBAP	9	Aug 2008; Mar–May 2009
	Canal de San Lorenzo	24° 28' 02" N	110° 12' 8" W	MXBSLOZ	10	Jul–Aug 2009
	Isla San José	24° 59' 00" N	110° 33' 00" W	MXBSISJ	11	Jul 2009
	Puerto Angel	16° 09' 46" N	95° 11' 19" W	MXOAPAN	12	Jan 2011; Mar 2011
	Salina Cruz	15° 39' 43" N	96° 29' 32" W	MXOASCZ	13	Mar 2011
El Salvador	Los Cóbanos	13° 29' 37" N	89° 51' 31" W	SVUSCOB	14	Oct 2010
	Bahía Jiquilisco	13° 12' 50" N	88° 26' 57" W	SVUSBJQ	15	Oct 2010; Feb 2011
	El Espino	13° 08' 07" N	88° 00' 12" W	SVUNESP	16	Feb 2011
	Bocana del Esterón	13° 09' 29" N	88° 04' 04" W	SVUNBES	17	Oct 2010; Feb 2011
	Las Tunas	13° 09' 30" N	87° 59' 24" W	SVUNTUN	18	Feb 2011
	El Coquito	13° 09' 33" N	88° 02' 37" W	SVUNCOQ	19	Feb 2011
	Golfo de Fonseca	13° 10' 22" N	87° 52' 53" W	SVUNGFO	20	Oct 2010; Feb 2011
Nicaragua	Golfo de Fonseca	13° 00' 30" N	87° 29' 21" W	NICIGFO	21	Nov 2010
	Puerto Sandino	12° 09' 59" N	86° 47' 40" W	NIMNSAN	22	Jul 2009; Nov 2010
	El Tránsito	12° 03' 11" N	86° 42' 15" W	NILETRA	23	Nov 2010; May 2011
	Masachapa	11° 40' 5" N	86° 34' 29" W	NIMNMAS	24	Nov 2010; May 2011
Costa Rica	Cuajiniquil	10° 57' 23" N	85° 43' 42"W	CRGUCUJ	25	Feb 2012
	Estero Culebras	10° 08' 55" N	85° 07' 53" W	CRGUCLB	26	Feb 2012

Table 1 Continued

Country	Location	Latitude	Longitude	Location Code	Map reference no.	Sampling date
			Tropical Eastern Pacific (cont.)			
Costa Rica	Isla Chira	10° 8' 48" N	85° 10' 01" W	CRPUICH	27	Feb 2012
	Golfo de Nicoya	10° 09' 06" N	85° 06' 33" W	CRPUNCY	28	Feb 2012
	Dominical	9° 14' 10" N	83° 52' 06" W	CRPUDOM	29	Feb 2012
Panamá	Gorgona	8° 33' 49" N	79° 49' 20" W	PAPAGOR	30	Dec 2011; Jan 2012
	Coronados	8° 32' 33" N	79° 52' 24" W	PAPACOR	31	Dec 2011
	Golfo de Panamá *	8° 59' 9" N	79° 29' 33" W	PAPAGPA	32	Jan 2012
	Panamá Viejo	8° 59' 47" N	79° 29' 16" W	PAPAPAV	33	Dec 2011
	Tocumen *	8° 00' 02" N	79° 29' 30" W	PAPATOC	34	Dec 2011
			South Eastern Pacific			
Chile	Marchant	33° 02' 05" S	71° 37' 05" W	CLVSMAR	35	Dec 2012
	Bahía Mejillones *	22° 49' 30" S	70° 30' 41" W	CLANMJL	36	Feb 2014
			Gulf of Mexico and Caribbean Sea			
Unites States of America	Oriental	35° 01' 32" N	76° 41' 59" W	USNCORI	37	Jul 2011
	Dauphin Island *	30°13' 59" N	88°13' 48" W	USALDIS	38	Mar–Jun 2005
México	Arrastradero-Redondo	18° 25' 03" N	93° 27' 10" W	MXTBARR	39	Aug 2009
	Mecoacán	18° 12' 44" N	93° 06' 04" W	MXTBMEC	40	Aug 2009
	Carmen-Machona	18° 10' 29" N	93° 30' 43" W	MXTBCAR	41	Aug 2009
Nicaragua	Uspan	14° 05' 54" N	83° 20' 03" W	NIANUSP	42	Oct–Nov 2010
	Guililanding	14° 04' 52" N	83° 23' 30" W	NIANGUI	43	Oct–Nov 2010
	Bilwi Tigni	14° 01' 33" N	83° 24' 08" W	NIANBWI	44	Oct–Nov 2010
	Estero Guililanding-Bilwi Tigni	14° 01' 22" N	83° 24' 12" W	NIANGBW	45	Oct–Nov 2010
Costa Rica	Punta Mona	9° 35' 35" N	82° 35' 54" W	CRLMPMO	46	Aug 2012; Sep 2012
	Gandoca	9° 37' 29" N	82° 37' 03" W	CRLMGAN	47	Aug 2012

Table 1 Continued

Country	Location	Latitude	Longitude	Location Code	Map reference no.	Sampling date
			Gulf of Mexico and Caribbean Sea (cont.)			
Panamá	Bocas del Toro	9° 13' 25" N	82° 13' 12" W	PABTBDE	48	Aug 2012; Sep 2012
Venezuela	Isla Margarita	11° 06' 36" N	63° 58' 16" W	VENEZIM	49	Aug 2009
			South Western Atlantic			
Argentina	Bahía Saborombón *	35° 56' 08" S	56° 59' 08" W	ARBABSB	50	Oct 2010
			Indo-Pacific			
Indonesia	Surabaya	7° 12' 58" S	112° 44' 50" E	IDJISUY		Nov–Dec 2010
Malaysia	Sg Janggut	3° 10' 23" N	101° 11' 26" E	MYSLJGG		Jun 2013
Thailand	Ko Panak	08° 11' 00" N	98° 29' 01" E	THKRKOP		May 2014
	Ko Panak Bat cave	08° 11' 00" N	98° 29' 00" E	THKRKOB		May 2014
	Racha Yai	07° 35' 00" N	98° 21' 01" E	THKRRAY		May 2014
			North Western Pacific			
Japan	Unknown	Not available	Not available	JPXXXXX		Mar 2014
			East Atlantic			
Nigeria	Gulf of Guinea	4° 05' 18" N	3° 47' 33" E	NGXXGGI		Summer 2012
Great Britain	North Sea	51° 45' 02" N'	1° 45' 11' E	GBXXNTS		Aug 2013

Table 2. Classification of specimens and other details of samples included in this study. Taxonomic names were assigned following the classification proposed by Kramp (1961) and Mianzan and Cornelius (1999) with one emendation: inclusion of the family Drymonematidae (Bayha and Dawson 2010). Records for the Tropical Eastern Pacific (TEP) are labeled "New" if a species has not previously been mentioned in the literature; for previously recorded species the references are cited. Details of the location codes are given in Figure 1 and Table 1. Specimen codes include the Museum of Comparative Zoology, Harvard University (MCZ); National Museum of Natural History, Smithsonian (NMNH); California Academy of Sciences, San Francisco, CA (CAS); Instituto Nacional de Investigación y Desarrollo Pesquero, Mar del Plata, Argentina (INIDEP). * = species misidentified by the authors. § = data from Bayha and Dawson (2010). † = data from Piraino et al. (2014). ‡ = data from Dawson et al. (2015).

Family	Species	Location	Sample sizes (*n*)					Oceanic Region	Records TEP
			COI	16S	28S	18S	Specimens		
Order Semaeostomeae									
Pelagiidae	*Chrysaora achlyos*	MXBSMAG	8	3	3	3	1	North Eastern Pacific	
	Chrysaora colorata	USCAMBQ	3	1	1	1		North Eastern Pacific	
	Chrysaora chinensis	MYSLJGG; THKRKOP	5	3	3	3		Strait of Malacca	
	Chrysaora fulgida	NGXXGGI	1	1	1	1		Gulf of Guinea	
	Chrysaora fuscescens	USCAMBQ, USCAXXX	2					North Eastern Pacific	
	Chrysaora hysoscella	NGXXGGI		1	1	1		Gulf of Guinea	
	Chrysaora lactea	BRRJRIO	1					South Western Atlantic	
	Chrysaora melanaster	USALBER	5‡					Bering Sea	
	Chrysaora pacifica	JPXXXX	1	1	1	1		Japan	
	Chrysaora plocamia	CLANMJL	3	3	3	3		Southern Eastern Pacific	
	Chrysaora quinquecirrha	USNCORI, MXTBARR, MXTBMEC	6	3	3	3	3	North Western Atlantic, Gulf of Mexico	
	Chrysaora sp. 1	MXSOGSC	10	3	3	3	5	TEP	New
	Chrysaora sp. 2	MXSOBKN	15	3	3	3	5	TEP	New
	Chrysaora sp. 3	SVUNBES, SVUNGFO, NIMNSAN, NIMNMAS,	23	3	3	3	7	TEP	New
	Chrysaora sp. 4	PAPAGOR	6	3	3	3	5	TEP	New
	Chrysaora sp. 5	NIANBWI, NIANUSP	5	3	3	3	5	Caribbean	
	Chrysaora sp. 6	CRLMPMO, PABTBDE	3	3	3	3	5	Caribbean	
	Chrysaora sp.	NGXXGGI	1	1	1	1		Gulf of Guinea	

Table 2. Continued

Family	Species	Location	Sample sizes (*n*)				Specimens	Oceanic Region	Records TEP
			COI	16S	28S	18S			
Pelagiidae	*Sanderia malayensis*	USCTNOQ	2	2	2	2	MCZ-1927, NMNH-29772	Indo-Pacific	
	Sanderia sp. 1	SVUNBES, SVUNESP, NIMNMAS	8	3	3	3	5	TEP	New
	Sanderia sp. 2	CRGUCLB	1	1	1	1	1	TEP	New
	Pelagia benovici		6†					Adriatic Sea	
	Pelagia noctiluca	MXBSLOZ, MXOAPAN, CRPUDOM	8	3	3	3	5 MCZ1404, MCZ3436, MCZ (***)	TEP, Sargasso Sea, Bermuda, North Western Atlantic	Bigelow (1940); Segura-Puertas (1984); Gómez-Aguirre (1991); Cortés (1997); Segura-Puertas *et al*. (2003)
	Pelagia panopyra cf	IDPAGFC, IDPAGFI, IDPAPYK	9	3	3	3	5 MCZ1944	South Western Pacific, China Sea	
	Pelagia sp. 1	VENEZIM	3	3	3	3		Caribbean	
Drymonematidae	*Drymonema dalmatinum*	TRIZFOC	5 §				7§	Aegean Sea	
	Drymonema gorgo	ARBABBL					1§ MCZ8079, MCZ8080	Caribbean, South Western Atlantic	
	Drymonema larsoni	USFLAMS, USALDIS	9 §				10 §	North Western Atlantic	
	Drymonema sp. 1	MXOASCZ, NIMNMAS, NIMNSAN, NILETRA	6	3	3	3	5	TEP	New
Ulmaridae	*Aurelia aurita*	CLVSMAR	1	1	1	1	MCZ3307, MCZ6015	North Western Atlantic	
	Aurelia sp. 9	MXTBCAR	5	3	3	3	3	Gulf of Mexico	
	Aurelia sp. 12	MXBSBAP	8	3	3	3	4	TEP	New
	Aurelia sp. 13	SVUNESP, CRGUCUJ, NILETRA	9	3	3	3	4	TEP	Segura-Puertas (1984); Gómez-Aguirre (1991); Cortés (1997); Segura-Puertas *et al*. (2003) *
	Aurelia sp. 14	PAPAGPA	3	3	3	3		TEP	New
	Aurelia sp. 15	PATBBDE	3	2	2	2	3	Caribbean	

Table 2. Continued

Family	Species	Location	Sample sizes (*n*)					Oceanic Region	Records TEP
			COI	16S	28S	18S	Specimens		
Ulmaridae	*Aurelia* sp. 16	ARBABSB	3	3	3	3		South Western Atlantic	
	Aurelia sp.						CAS108710	Caribbean	
			Orden Rhizostomeae						
Cassiopeidae	*Cassiopea andromeda*	MXBSISJ	6	3	3	3		TEP	New
	Cassiopea frondosa	PATBBDE	2	2	2	2		Caribbean	
Mastigiidae	*Phyllorhiza punctata*	MXBSAGO, MXBSCPC	3	3	3	3		TEP	New
	Phyllorhiza pacifica	THKRKOP		1	1	1		Strait of Malacca	
Lobonematidae	*Lobonema smithii*	MYSLJGG, THKRKOP	5	3	3	3	1	Indo-Pacific	
	Gen. 1 sp. 1	MXBSBAP, MXBSAGO, MXBSCPC	5	3	3	3	5	TEP	New
	Gen. 1 sp. 2	SVUNCOQ	1	1	1	1		TEP	New
	Gen. 1 sp. 3	PAPATOC	1	1	1	1	1	TEP	New
	Gen. 1 sp. 4	SVUNGFO	1	1	1	1		TEP	New
Catostylidae	*Acromitus flagellatus*	IDJISUY		1	1	1		Java	
	Catostylus townsendi	MYSBJGG, IDJISUY		1	1	1		Strait of Malacca, Java	
	Catostylus sp. 1	SVUNBES, SVUNGFO, SVUSBJQ, NIMNSAN, NICIGFO	15	3	3	3	13	TEP	Vanhöffen (1888); Larson (1990)
	Catostylus sp. 2	CRPUDOM	1	1	1	1	1	TEP	New

Table 2. Continued

Family	Species	Location	Sample sizes (*n*)					Oceanic Region	Records TEP
			COI	16S	28S	18S	Specimens		
Lychnorhizidae	*Lychnorhiza lucerna*	NIANGUI, NIANGBW, NIANTUP, ARBABSB	5	3	3	3	5 INIDEP	Caribbean	
	Lychnorhiza sp. 1	MXOASCZ, SVUNTUN, NIMNMAS, CRPUDOM, PAPAGOR, PAPATOC	16	3	3	3	11	TEP	New
	Lychnorhiza sp. 2	CRLMGAN	3	3	3	3	5	Caribbean	
	Lychnorhiza sp. 3	VENEZIM	2	2	2	2		Caribbean	
Stomolophidae	*Stomolophus meleagris*	USNCXXX, USALDIS, MXTBARR, MXTBCAR, MXTBMEC	28	3	3	3	5 MCZ3310 MCZ383	Gulf of Mexico, North Western Atlantic	
	Stomolophus sp. 1	MXSOGSC	24	3	3	3	5	TEP	New
	Stomolophus sp. 2	MXBSBAP, MXBSLOZ, MXSOGUY, MXBNGOZ, MXBSCPC, MXBSMUL, MXSOBKN	53	3	3	3	8	TEP	New
	Stomolophus sp. 3	SVUNBES, SVUSCOB, CRGUCLB, CRPUNCY, CRPUDOM, PAPACOR	33	3	3	3	6	TEP	New
	Stomolophus sp. 4	PAPATOC, PAPAPAV	12	3	3	3	5	TEP	Vanhöffen (1888)
	Stomolophus sp. 5	NIANGBW, NIANGUI, NIANTUP	15	3	3	3	5	Caribbean	
	Stomolophus sp. 6	MXSOBKN	1	1	1	1		TEP	New

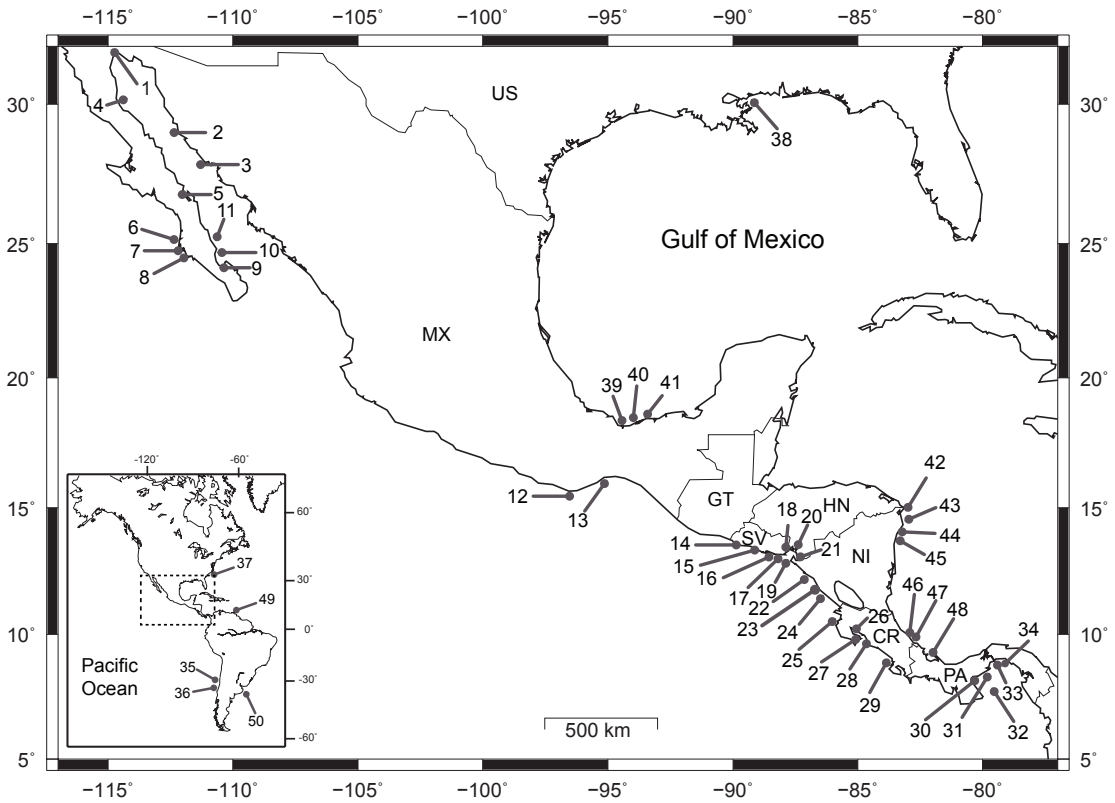

Figure 1. Map of sample collection sites for our study of scyphozoan diversity in the Tropical Eastern Pacific (TEP) and Caribbean. We sampled at 34 locations in the TEP, four in the Gulf of Mexico, and eight locations in the Caribbean. Sites in South America (2) and the northeast United States of America (2) are shown in the inset map. The reference numbers for each location also appear in Table 1 with additional information for each sample site. Country codes are as follows: Costa Rica (CR); El Salvador (SV); Guatemala (GT); Honduras (HN); México (MX); Nicaragua (NI); Panamá (PA); United States of America (US).

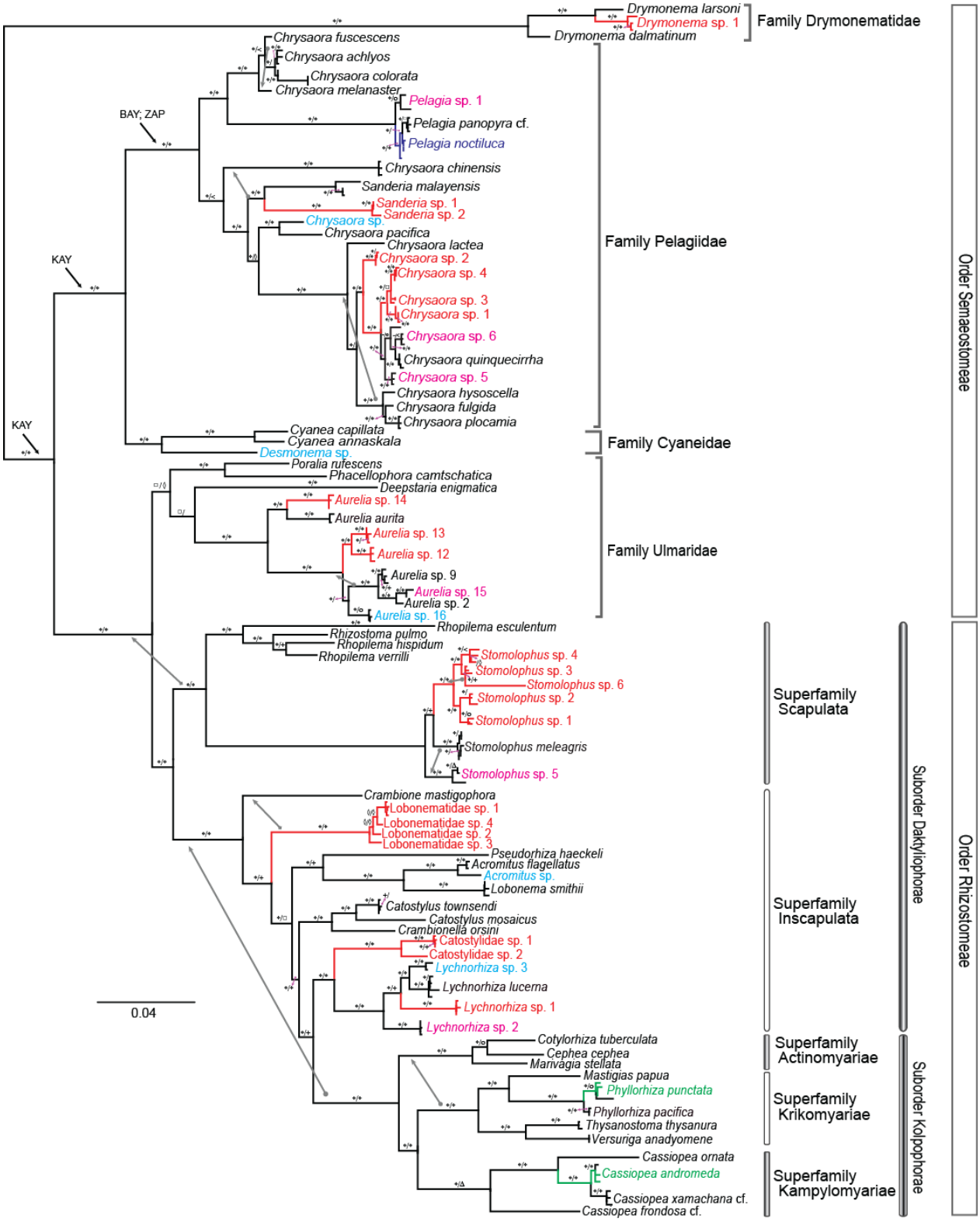
Drymonema larsoni
Drymonema sp. 1
Drymonema dalmatinum
Family Drymonematidae
Chrysaora fuscescens
Chrysaora achlyos
Chrysaora colorata
Chrysaora melanaster
Pelagia sp. 1
Pelagia panopyra cf.
Pelagia noctiluca
BAY; ZAP
Chrysaora chinensis
Sanderia malayensis
Sanderia sp. 1
Sanderia sp. 2
Chrysaora sp.
Chrysaora pacifica
Chrysaora lactea
Chrysaora sp. 2
Chrysaora sp. 4
Chrysaora sp. 3
Chrysaora sp. 1
Chrysaora sp. 6
Chrysaora quinquecirrha
Chrysaora sp. 5
Chrysaora hysoscella
Chrysaora fulgida
Chrysaora plocamia
Family Pelagiidae
KAY
KAY
Cyanea capillata
Cyanea annaskala
Desmonema sp.
Family Cyaneidae
Poralia rufescens
Phacellophora camtschatica
Deepstaria enigmatica
Aurelia sp. 14
Aurelia aurita
Aurelia sp. 13
Aurelia sp. 12
Aurelia sp. 9
Aurelia sp. 15
Aurelia sp. 2
Aurelia sp. 16
Family Ulmaridae
Order Semaeostomeae
Rhopilema esculentum
Rhizostoma pulmo
Rhopilema hispidum
Rhopilema verrilli
Stomolophus sp. 4
Stomolophus sp. 3
Stomolophus sp. 6
Stomolophus sp. 2
Stomolophus sp. 1
Stomolophus meleagris
Stomolophus sp. 5
Superfamily Scapulata
Crambione mastigophora
Lobonematidae sp. 1
Lobonematidae sp. 4
Lobonematidae sp. 2
Lobonematidae sp. 3
Pseudorhiza haeckeli
Acromitus flagellatus
Acromitus sp.
Lobonema smithii
Catostylus townsendi
Catostylus mosaicus
Crambionella orsini
Catostylidae sp. 1
Catostylidae sp. 2
Lychnorhiza sp. 3
Lychnorhiza lucerna
Lychnorhiza sp. 1
Lychnorhiza sp. 2
Superfamily Inscapulata
Suborder Daktyliophorae
Order Rhizostomeae
0.04
Cotylorhiza tuberculata
Cephea cephea
Marivagia stellata
Superfamily Actinomyariae
Mastigias papua
Phyllorhiza punctata
Phyllorhiza pacifica
Thysanostoma thysanura
Versuriga anadyomene
Superfamily Krikomyariae
Cassiopea ornata
Cassiopea andromeda
Cassiopea xamachana cf.
Cassiopea frondosa cf.
Superfamily Kampylomyariae
Suborder Kolpophorae

Figure 2. Unrooted maximum likelihood species tree for Discomedusae, based on analyses of 16S, 28S, and 18S genes, highlighting the 25 records for the TEP. Geographic information on the collecting sites is provided in Table 1. Black arrows show three different hypotheses for rooting the tree according to Bayha *et al*. (2010) [BAY], Kayal *et al*. (2013) [KAY], and Zapata *et al.* (2015) [ZAP]. Gray arrows represent alternative topologies present in the Bayesian analyses. Branches: black, specimens from Bayha *et al*. (2010) and additional specimens from other oceanic regions (Supplementary Table S1); red, 22 new endemics from the TEP; blue, one previously recorded and correctly identified species in the TEP; green, two non-indigenous species in the TEP. Leaves: magenta, five new taxa from the Caribbean Sea; cyan, four new taxa from other oceanic regions (e.g. Indo-West Pacific). Bootstrap and posterior probabilities are shown on branches: * 100–99%, + 98–95%, Δ 94–90%, O 89–85%; ◊ 84–80%; □ 79–75%; < 74–70%; not shown if < 70%.

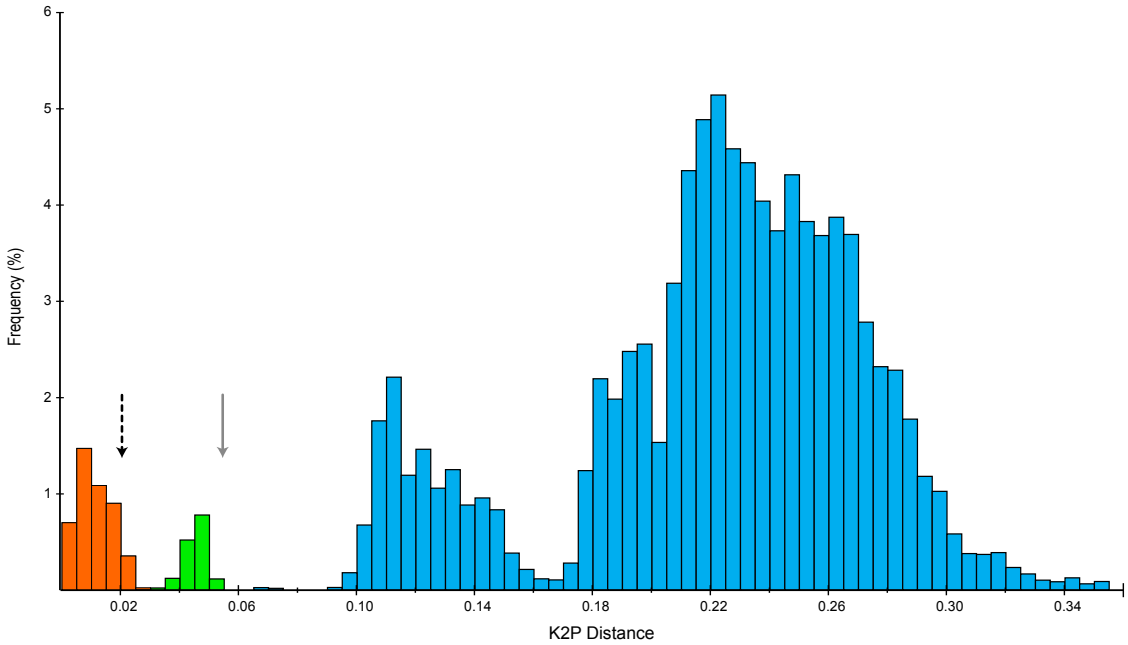

Figure 3. Representation of the barcoding gap for Discomedusae. Frequency histogram of COI pairwise sequence distances (using the K2P model of evolution) between 433 individuals (see Table 2 for the complete list of specimens). Orange bars show the frequency distribution of inferred intraspecific distances. Blue bars show the frequency distribution of inferred interspecific distances. Green bars highlight intermediate distances that fall between previously proposed barcode gaps, as indicated by arrows. Gray arrow: approximate maximum medusozoan barcoding gap of 0.057 estimated by Ortman *et al.* (2010). Black dashed arrow, approximate minimum barcode gap based on the finding that 98% of congeneric species pairs showed ≥2% divergence (Bucklin *et al.* 2010). Barcode gaps for other taxa have been estimated at ~0.03–0.035 (Hebert et al. 2003a, Hebert et al. 2003b) and ≤0.043 (Costa et al. 2009).

Figure 4. *In situ* photographs of 11 new Discomedusae collected in the TEP and Caribbean. a) *Drymonema* sp. 1 from Puerto Sandino, Nicaragua, Pacific. b) *Chrysaora* sp. 5 from Uspan, Nicaragua, Caribbean. c) *Chrysaora* sp. 2 from Bahía Kino, Gulf of California, México. d) *Chrysaora* sp. 3 from Puerto Sandino, Nicaragua, Pacific. e) *Sanderia* sp. 1 from la Bocana del Esterón, El Salvador. f) *Lychnorhiza* sp. 1 from Golfo de Fonseca, Nicaragua, Pacific. g) Catostylidae sp. 1 from Puerto Sandino, Nicaragua, Pacific. h) Catostylidae sp. 2 from El Dominical, Costa Rica, Pacific. i) *Stomolophus* sp. 2 from Mulegé, Golfo de California, México. j) *Stomolophus* sp. 3 from El Dominical, Costa Rica, Pacific. k) *Stomolophus* sp. 5 from Bilwi Tigni, Nicaragua, Caribbean.

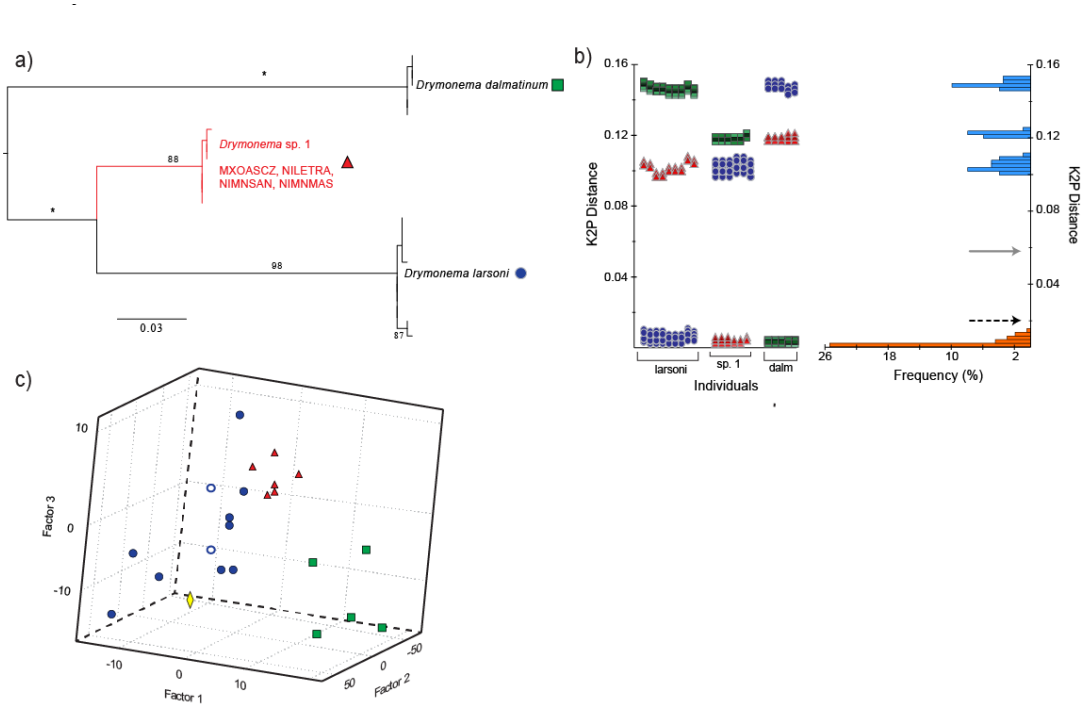

Figure 5. *Drymonema* spp. genetic and morphological differentiation. a) Maximum likelihood gene tree reconstructed using ~600 nt of COI from 20 individuals and the GTR+I model of sequence evolution with midpoint rooting. Geographic information of the collecting sites is provided in Table 1. Red branches represent new endemics from the TEP. Bootstrap values are shown on branches: * 100–99%; not shown if < 70%. b) DNA barcoding plot: left-most plot represents the K2P distance matrix, separated by species on the x-axis and the genetic distance on y-axis. Right-most plot represents the frequency distribution of the intra- and inter-specific distances (as a percentage of all comparisons). Orange bars show the distribution of intraspecific distances; blue bars show the distribution of interspecific distances. Gray arrow: approximate maximum medusozoan barcoding gap by Ortman *et al.* (2010). Black dashed arrow, approximate minimum barcode gap of Bucklin *et al.* 2010. Abbreviations: *Drymonema* sp. 1 (sp.1); *D. dalmatinun* (dalm); *D. larsoni* (larsoni). c) PCA of standardized morphological data, for which three factors explained 98.58% of the variance. Filled markers correspond with the species shown in the tree; open markers are two non-identified museum specimens from Bermuda (Table 2). *D. gorgo* (diamond) was represented by only one specimen (Table 2).

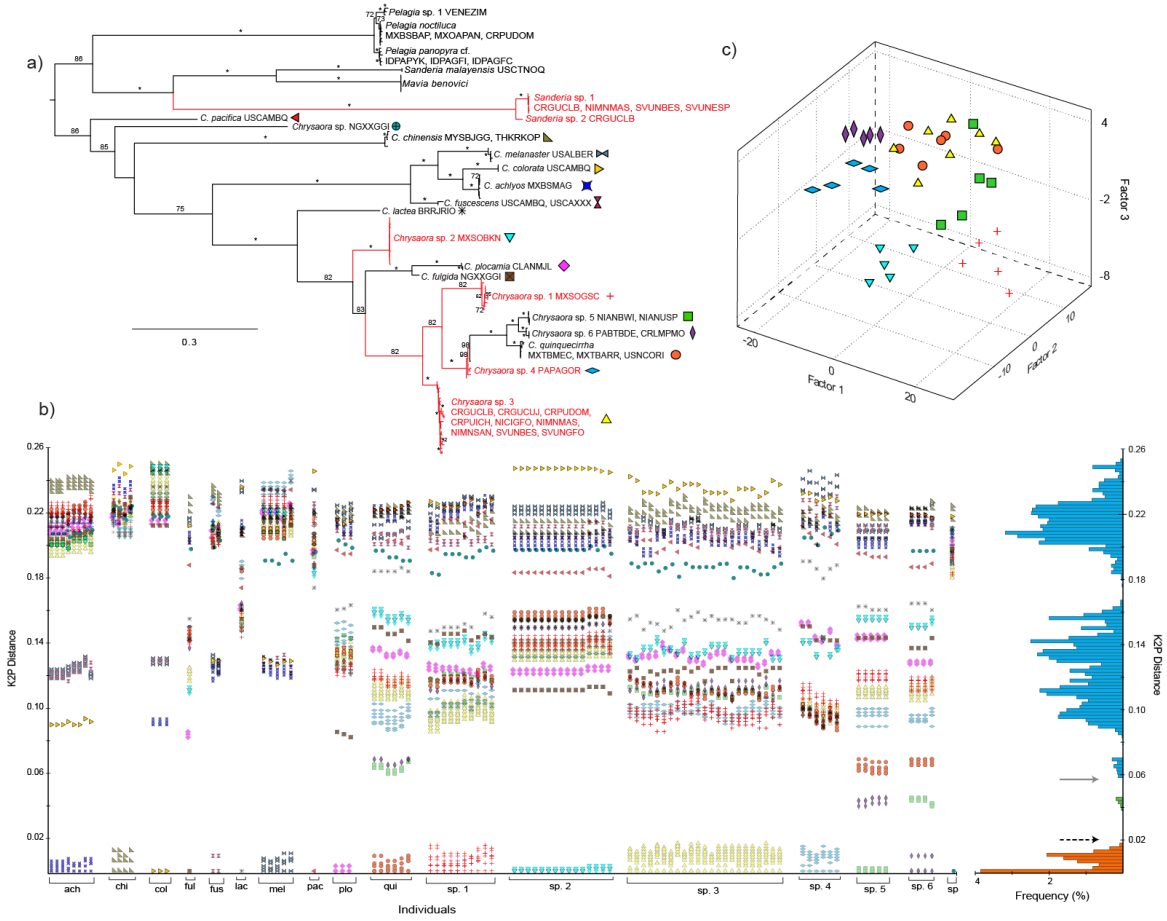

Figure 6. Pelagiidae genetic and morphological differentiation. a) Midpoint rooted maximum likelihood COI gene-tree of 132 individuals, using the TVM+I+G model of evolution; bootstrap values are shown on branches: * 100–99%; not shown if < 70%. Geographic information for the collecting sites is provided in Table 1. Red branches emphasize new endemics from the TEP. b) Plot of the barcode gap of 17 *Chrysaora* species (98 individuals) reconstructed using the K2P pairwise distance; plots as described in Fig. 5. Abbreviations: *C. achlyos* (ach); *C. chinensis* (chi); *C. colorata* (col); *C. fulgida* (ful); *C. fuscescens* (fus); *C. lactea* (lac); *C. melanaster* (mel); *C. pacifica* (pac); *C. plocamia* (plo); *C. quinquecirrha* (qui); *Chrysaora* sp. 1 (sp. 1); *Chrysaora* sp. 2 (sp. 2); *Chrysaora* sp. 3 (sp. 3); *Chrysaora* sp. 4 (sp. 4); *Chrysaora* sp. 5 (sp. 5); *Chrysaora* sp. 6 (sp. 6); *Chrysaora* sp. (sp). c) PCA of standardized morphological data for eight species of *Chrysaora* distributed in the TEP and Caribbean, for which three factors explained 92.8% of the variance. Symbols correspond to the clades labeled in the phylogenetic tree.

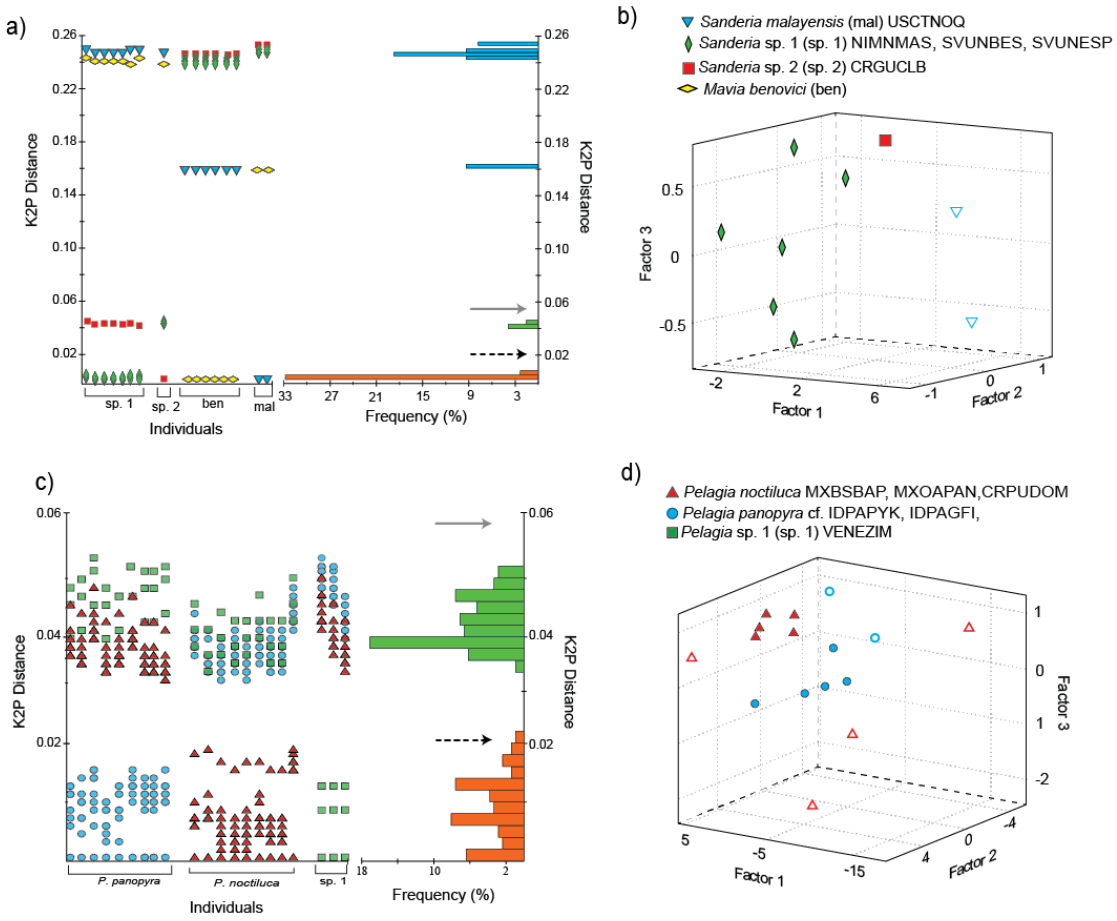

Figure 7. Morphological and genetic discrimination of *Sanderia* spp. and *Pelagia* spp. a) Plot of the barcode gap of 16 *Sanderia* specimens reconstructed using the K2P pairwise distance; plots as described in Fig. 5. b) PCA of standardized morphological data for *S. malayensis* and *Sanderia* spp. *Pelagia benovici* is not included because specimens were not available. Differentiation of samples was possible with three factors that explain 98.61% of the variance. Filled markers represent specimens in Fig. 6a; open markers are specimens from museums and therefore not included in Fig. 6a. c) Plot of the barcoding gap for 21 *Pelagia* specimens using K2P genetic distances; plots as described in Fig. 5. d) PCA of standardized morphological data for *Pelagia* species. *Pelagia* sp. 1 is not included because we did not have a complete specimen; open markers are museum specimens (MCZ and NMNH Table 2); filled markers correspond to samples used in Fig. 6a.

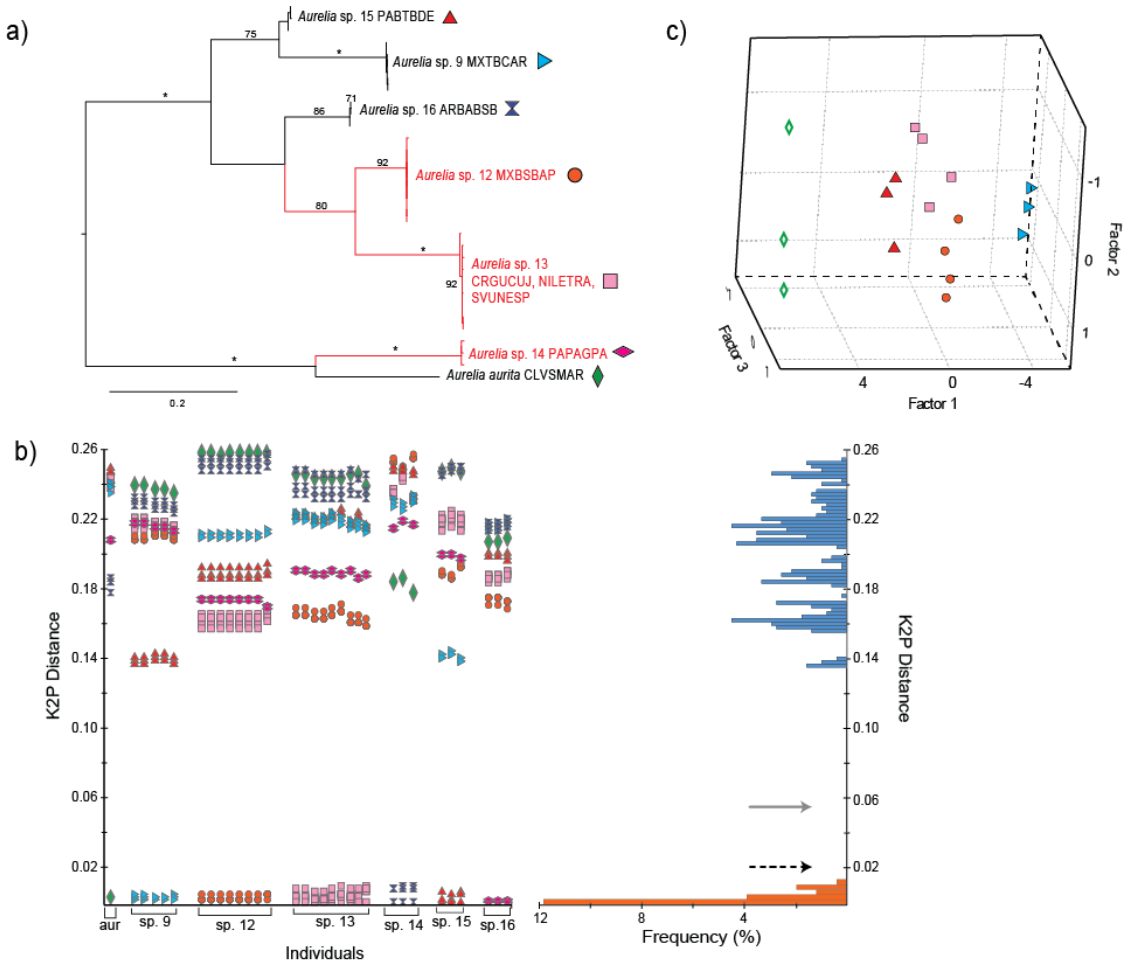

Figure 8. Genetic and morphological discrimination of *Aurelia* spp. a) Maximum likelihood midpoint rooted COI gene tree (~650 nt) of 32 individuals, using the TPM1uf+I model of sequence evolution. Geographic information for the collecting sites is provided in Table 1. Red branches highlight new endemics from the TEP. Bootstrap values are shown on branches, * 100–99%; not shown if < 70%. b) Plot of the barcode gap of 7 *Aurelia* species (32 individuals) reconstructed using the K2P pairwise distances; plots as described in Fig. 5. Abbreviations: *Aurelia aurita* (aur); *Aurelia* sp. 9 (sp. 9); *Aurelia* sp. 12 (sp. 12); *Aurelia* sp. 13 (sp. 13); *Aurelia* sp. 14 (sp. 14); *Aurelia* sp. 15 (sp. 15); *Aurelia* sp. 16 (sp. 16). c) PCA of standardized morphological data for five species distributed in the TEP, Gulf of Mexico, South America, and the Caribbean; three factors explain 98.24 % of the variance. Symbols represent the species listed in the ML tree. Filled symbols correspond to samples used in the ML tree; open markers are specimens from museums (Table 2).

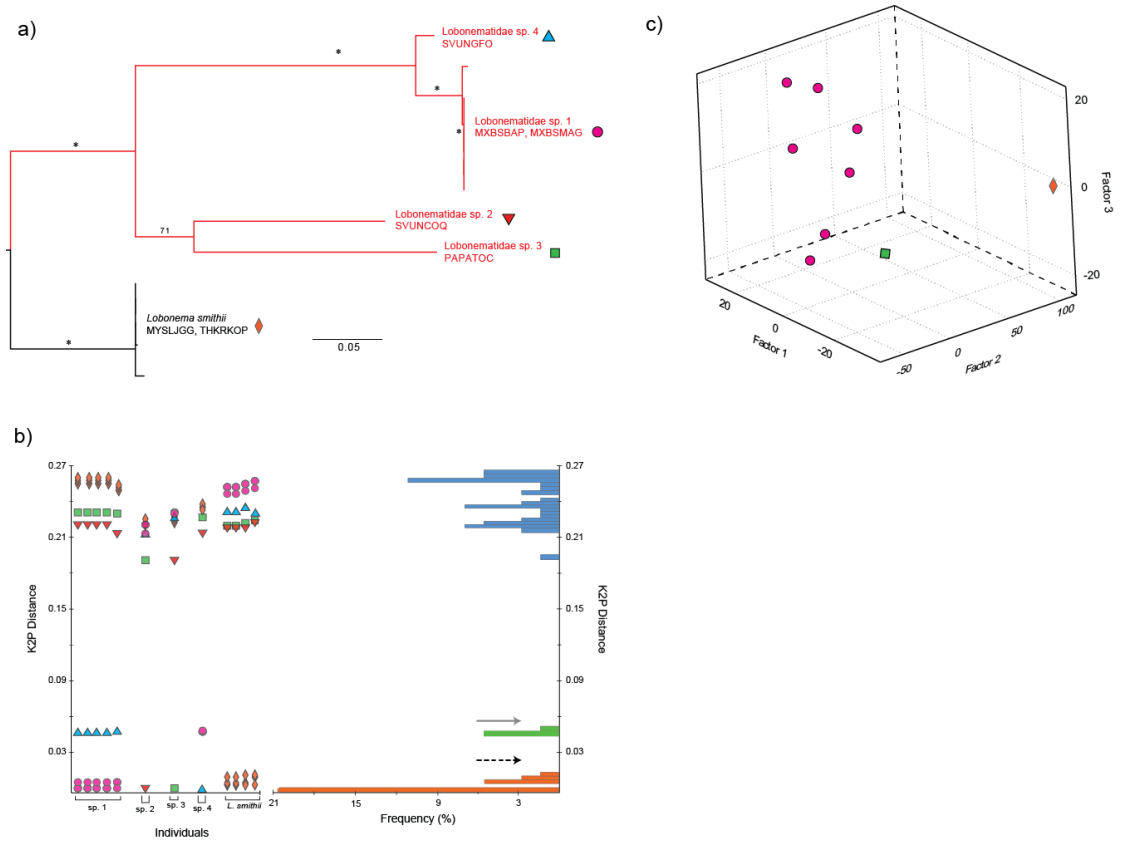

Figure 9. Lobonematidae spp. genetic and morphological discrimination. a) Maximum likelihood midpoint rooted gene tree reconstructed using ~650 nt of COI from 12 individuals, and the TIM2+I model of sequence evolution. Geographic information for the collecting sites is provided in Table 1. Red branches emphasize new endemics from the TEP. Bootstrap values are shown on branches, * 100–99%; not shown if < 70%. b) DNA Barcoding plots using the K2P pairwise distances; plots as described in Fig. 5. Abbreviations: Lobonematidae sp. 1 (sp. 1); Lobonematidae sp. 2 (sp. 2); Lobonematidae sp. 3 (sp. 3); Lobonematidae sp. 4 (sp. 4). c) PCA of standardized morphological data. Differentiation of three species was possible with three factors, which explain 93.48% of the variance. Symbols represent the species listed in the gene tree.

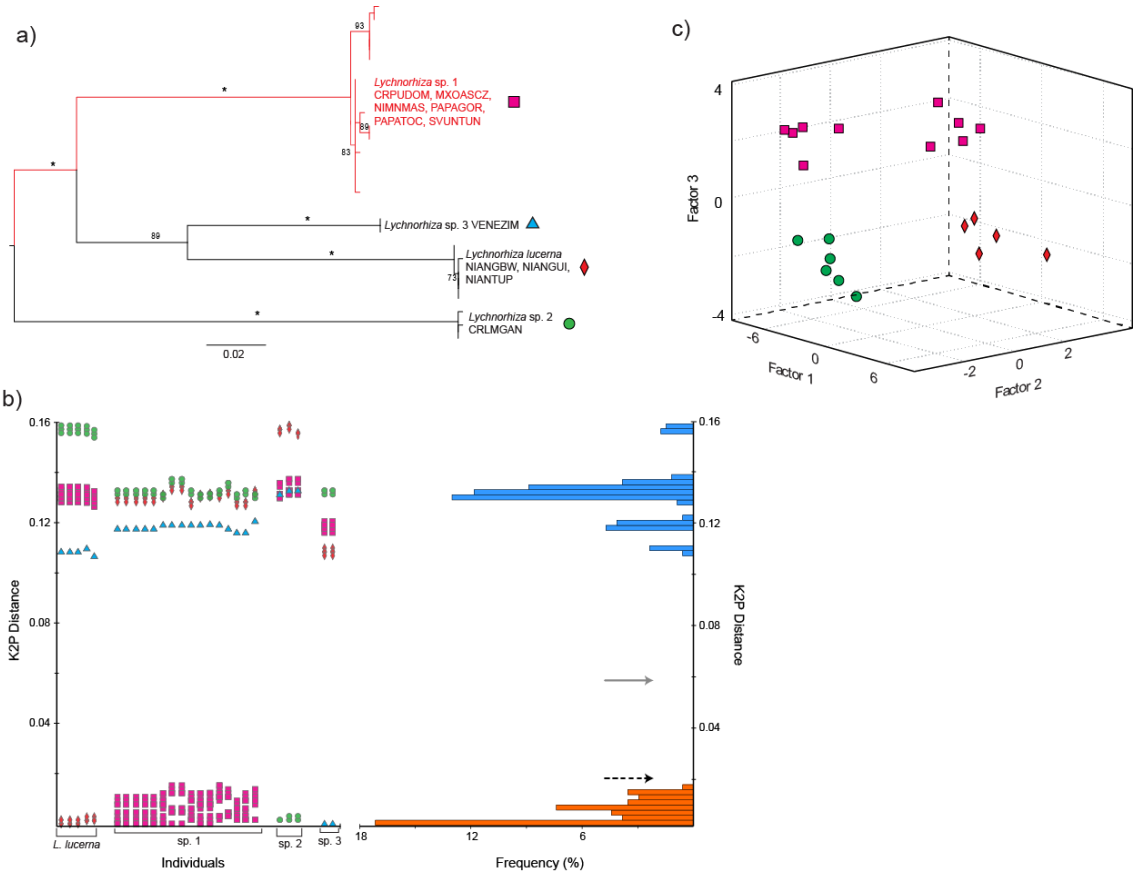

Figure 10. Morphological and genetic differentiation of Lychnorhizidae species. a) Maximum likelihood midpoint rooted gene tree reconstructed using 650 nt of COI from 26 individuals, and the TIM2+I model of sequence evolution. Geographic information for the collecting sites is provided in Table 1. Red branches, emphasize new endemics from the TEP. Bootstrap values are shown on branches: * 100–99%; not shown if < 70%. b) DNA Barcoding plot using the K2P pairwise distances; plots as described in Fig. 5. Abbreviations: *Lychnorhiza* sp. 1 (sp. 1); *Lychnorhiza* sp. 2 (sp. 2); *Lychnorhiza* sp. 3 (sp. 3). c) PCA of standardized morphological data for *Lychnorhiza* species. Morphological discrimination was possible with three factors, which explain 71.58%. Symbols represent the species listed in the gene tree.

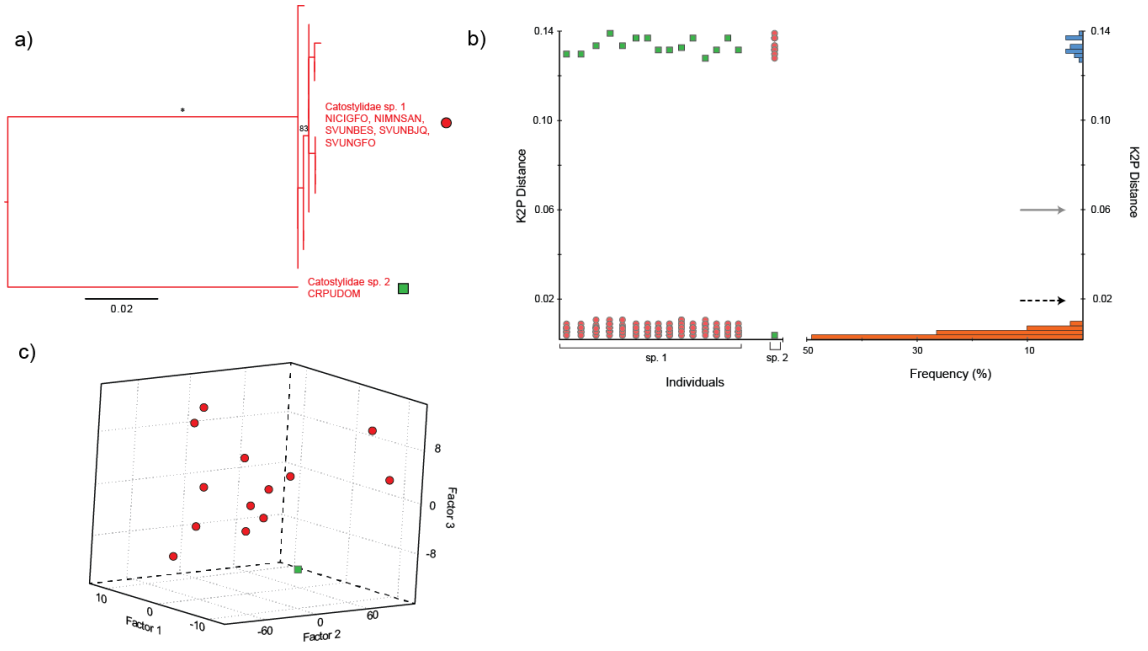

Figure 11. Catostylidae spp. genetic and morphological differentiation. a) Maximum likelihood midpoint rooted tree reconstructed using 650 nt of COI from 16 individuals, and the GTR+I+G model of sequence evolution. Red branches highlight new endemics from the TEP. Geographic information on collection sites is provided in Table 1. Bootstrap values are shown on branches, * 100–99%; not shown if < 70%. b) DNA Barcoding plot using the K2P pairwise distances; plots as described in Fig. 5. Abbreviations: Catostylidae sp. 1 (sp. 1); Catostylidae sp. 2 (sp. 2). c) PCA of standardized morphological data. Discrimination was possible with three factors, which explain 98.46% of the total variance. Symbols correspond to those used in the gene tree.

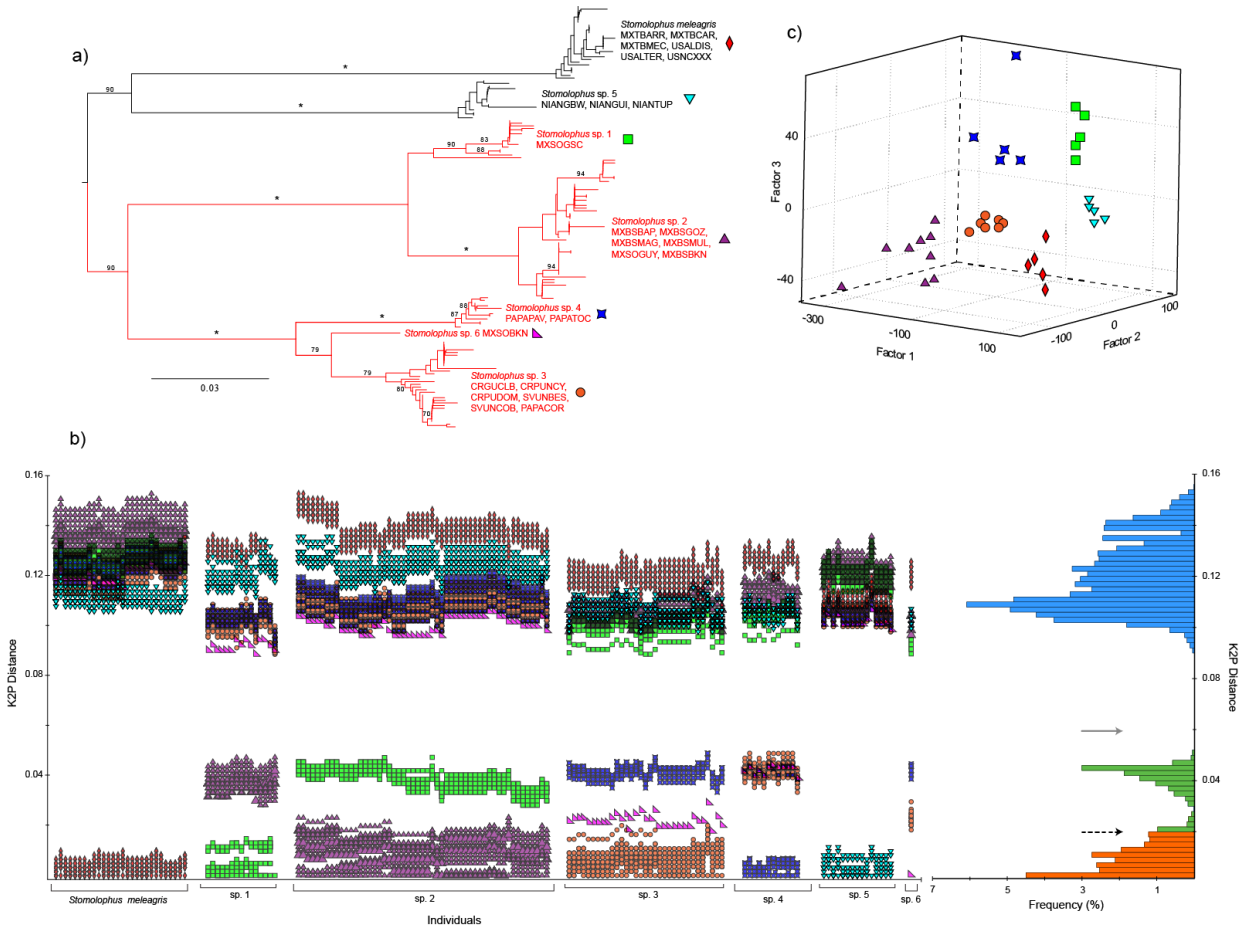

Figure 12. *Stomolophus* spp. genetic and morphological differentiation. a) Maximum likelihood midpoint rooted gene tree reconstructed using ~650 nt of COI from 157 individuals, and the HKY+I model of sequence evolution. Geographic information for the collection sites is provided in Table 1. Red branches emphasize new endemics from the TEP. Bootstrap values are shown on branches, * 100–99%; not shown if < 70%. b) Plots of the barcode gap estimated using the K2P model of sequence evolution; plots as described in Fig. 5. c) PCA of standardized morphological data. Morphological discrimination was possible with three factors, which explain 98.58% of the variance. Symbols correspond to the species plotted in the ML tree.

8. Supplementary Material

Appendix 1. Morphometric and meristic morphological features and their states. The morphological matrix is a modification and compilation of those characters that have been previously proven to be helpful to assess the morphological differences in several families and genera of scyphozoans (Gershwin and Collins 2002; Dawson 2003; Marques and Collins 2004; Dawson 2005b, c, d; Morandini and Marques 2010), and new features that primary literature suggest may be informative (Mayer 1910; Stiasny 1921, 1922; Rao 1932).

No.	Description	Features states
1	Symmetry of medusa	radial = 0, biradial = 1, tri-radial = 2 radial tetramerous = 3, pentamerous = 4
2	Gastric filaments	absent = 0, present = 1
3	Coronal muscle	well developed = 0, marginal and tiny = 1
4	Velum-like structure	absent = 0, velum = 1, velarium = 2
5	Umbrellar margin	smooth and continuous (no clefts, no lappets) = 0, clefts (or peronia) and lappets = 1, clefts and lobes = 2
6	Velar lappets bifurcated	no = 0, yes = 1
7	Number of velar lappets	count per octant
8	Number of bifurcated lappets	count per octant
9	Velar lappet length	millimeters
10	Velar lappet width	millimeters
11	Velar lappet shape	symmetric square = 0, symmetric semi-circular = 1, symmetric semi-oval=2, symmetric tapering = 3, asymmetric square = 4, asymmetric semi-circular = 5, asymmetric semi-oval = 6, asymmetric tapering = 7
12	Velar lappets in heterogenous size classes	no = 0, yes = 1
13	Number of lobes	count per quadrant
14	Primary lobe cleft depth	milimiters
15	Secondary lobe cleft depth	milimiters
16	Number of rhopalial lappets	count per rhopalium
17	Rhopalial lappet length (cleft depth)	millimeters
18	Rhopalial lappet width	millimeters
19	Rhopalial lappet shape	symmetric square = 0, symmetric semi-circular = 1, symmetric semi-oval=2, symmetric tapering = 3, asymmetric square = 4, asymmetric semi-circular = 5, asymmetric semi-oval = 6, asymmetric tapering = 7
20	Rhopalia in marginal clefts	no = 0, yes = 1
21	Number of umbrella tentacles	count per quadrant
22	Number of secondary tentacles	count per quadrant
23	Number of tertieary tentacles	count per quadrant
24	Tentacular insertion	at umbrella margin = 0, proximally on exumbrella = 1, distally on exumbrella = 2, proximally on subumbrella = 3, distally on subumbrella = 4
25	Structure of medusoid tentacles	hollow = 0, solid = 1
26	Tentacular morphology	straight = 0, with angular inflection = 1, capitate = 2
27	Number of tentacle whorls or rows	count

Appendix 1. Continued

No.	Description	Features states
28	Tentacle position	perradial only = 0, interradial only = 1, adradial only = 2, perradial + interradial = 3, perradial + adradial = 4, interradial + adradial = 5, perradial + interradial + adradial = 6
29	Tentacle arrangement	single/continuous = 0, clumped = 1
30	Tentacular bulbs	absent = 0, present = 1
31	Tentacles with terminal knob	absent = 0, present (i.e. capitate) = 1
32	Gastric mesenteries	absent = 0, present = 1
33	Number of gastric ostia	count
34	Gastric ostia position	perradial only = 0, interradial only = 1, adradial only = 2, perradial + interradial = 3, perradial + adradial = 4, interradial + adradial = 5, perradial + interradial + adradial = 6
35	Number of radial mesenteries	count
36	Radial mesentery shape	straight = 0, bent distally = 1, paired forming Y proximally = 2
37	Radial mesentery termination	percent of distance from tentacle (0%) to rhopalium (100%)
38	Number of radiating stomach pouches	count
39	Radial canals	absent = 0, present = 1
40	Number of perradial canal origins at the gastrovascular cavity	count per quadrant
41	Number of interradial canal origins at the gastrovascular cavity	count per quadrant
42	Number of adradial canal origins at the gastrovascular cavity	count per quadrant
43	Number of perradial-perradial anastomoses in radial canals that are circumscribed by the ring canal	count per quadrant
44	Number of interradial-interradial anastomoses in radial canals that are circumscribed by the ring canal	count per quadrant
45	Number of adradial-adradial anastomoses in radial canals that are circumscribed by the ring canal	count per quadrant
46	Number of perradial-interradial anastomoses in radial canals that are circumscribed by the ring canal	count per quadrant
47	Number of perradial-adradial anastomoses in radial canals that are circumscribed by the ring canal	count per quadrant
48	Number of interradial-adradial anastomoses in radial canals that are circumscribed by the ring canal	count per quadrant
49	Number of terminations of perradial canals at the ring canal	count per quadrant
50	Number of terminations of interradial canals at the ring canal	count per quadrant
51	Number of terminations of adradial canals at the ring canal	count per quadrant
52	Number of perradial canals originating distally at the circular canal	count per quadrant
53	Number of interradial canals originating distally at the circular canal	count per quadrant
54	Number of adradial canals originating distally at the circular canal	count per quadrant
55	Number of sinuses originating at the gastrovascular cavity	count per quadrant
56	Number of sinuses originating at the perradial canal	count per quadrant
57	Number of sinuses originating at the interradial canals	count per quadrant
58	Number of sinuses originating at the adradial canals	count per quadrant
59	Number of sinuses originating proximally at the circular canal	count per quadrant

Appendix 1. Continued

No.	Description	Features states
60	Number of anastomoses circumscribed by the circular canal that lead to two sinuses	count per quadrant
61	Number of sinuses originating distally at the circular canal	count per quadrant
62	Percentage of radius of medusa in which there is no branching radial canal	per quadrant
63	Ring canal	absent = 0, weakly developed chain of enlarged branches circumscribes bell = 1, a primary artery easily distinguishable from other canals circumscribes bell = 2
64	Furrow in bell	absent = 0, coronal groove = 1, laingiomedusan type = 2
65	Number of gonads	count
66	Gonads are paired	no = 0, yes = 1
67	Gonad position axis	perradial only = 0, interradial only = 1, adradial only = 2, perradial + interradial = 3, perradial + adradial = 4, interradial + adradial = 5, perradial + interradial + adradial = 6
68	Lateral distance from center to most proximal portion of gonad	millimeters
69	Lateral distance from center to most distal portion of gonad	millimeters
70	Gonad associated with particular structure	manubrium = 0, radial canals = 1, gastric septa or quadralinga = 2, radial septa = 3, pouch = 4, out folded pockets = 5, stomach arms = 6
71	Bell thickness	millimeters (center; 1/3; edge)
72	Mouth lips	absent = 0, simple lips = 1, gelatinous or curtain-like arms = 2, oral arms with suctorial mouths =3
73	Manubrium	absent = 0, basal in arms = 1, basal and extended beyond arms = 2, pillars and disk = 3
74	Manubrium depth	millimeters
75	Manubrium width at base	millimeters
76	Manubrium width at mouth	millimeters
77	Length of the simple, unwinged portion of the oral arm	millimeters
78	Length of the winged portion of the oral arm	millimeters
79	Oral arm width	millimeters
80	Cross-sectional form of oral arm	sheet-like = 0, two-winged = 1 three-winged = 2
81	Secondary structure of oral arm	absent = 0, spiral =1
82	Number of fenestrations in oral arm	count
83	Scapulae	absent = 0, present = 1
84	Point of scapula attachment to oral mass	at disk = 0, both disk and oral arm = 1, on smooth portion of oral arm = 2
85	Length of attachment to oral mass	millimeters
86	Length of scapula (smooth part)	millimeters
87	Length of scapula (mouthed part)	millimeters
88	Distribution of mouths on scapula	top = 0, bottom = 1, entire surface = 2
89	Shape of scapula	straight = 0, scimitar-shaped, curved up = 1, finger-like, curved up = 2
90	Scapulae occurrence per oral arm	one per arm = 0, two per arm = 1
91	Scapulae branched	no= 0 ; yes = 1

Appendix 1. Continued

No.	Description	Features states
92	Number of filaments per scapulae	count
93	Distribution of filaments on scapulae	absent = 0, scapula exterior only = 1, scapula interior only = 2
94	Shape of scapular filaments	rod-like = 0, tapering = 1, string-like = 2, string-like with terminal bulb (capitate) = 3, spatula = 4
95	Length of scapular filaments	millimeters
96	Width of scapular filaments	millimeters
97	Number of terminal clubs	count
98	Cross-sectional shape of terminal clubs	circular = 0, planar = 1, convex planar (ovoid) = 2, concave planar = 3, triangular = 4, convex triangular = 5, concave triangular = 6
99	Longitudinal-sectional shape of terminal clubs	rod-like = 0, tapering = 1, string-like = 2, string-like with terminal bulb = 3, spatula = 4
100	Length of terminal clubs	millimeters
101	Width of terminal clubs	millimeters
102	Length of the oral pillars	millimeters
103	Width of the oral pillars	millimeters
104	Depth of the oral pillars	millimeters
105	Width of the subgenital ostia	millimeters
106	Subgenital ostia with ornamentations	no= 0 ; yes = 1
107	Perradial diameter of the oral disc	millimeters
108	Depths of the oral disc	millimeters
109	Distribution of intermediate filaments on the oral arm and oral disc	absent = 0, oral arm exterior only = 1, oral arm interior only = 2, oral disk only = 3, oral arm = 4, oral arm and disk = 5
110	Number of intermediate filaments on the oral arm	count
111	Number of intermediate filaments on the oral disc	count
112	Shape of intermediate filaments	rod-like = 0, tapering = 1, string-like = 2, string-like with terminal bulb (capitate) = 3, spatula = 4
113	Length of intermediate filaments	millimeters
114	Width of intermediate filaments	millimeters
115	Number of rhopalia	count per quadrant
116	Rhopalia position	perradial only = 0, interradial only = 1, adradial only = 2, perradial + interradial = 3, perradial + adradial = 4, interradial + adradial = 5, perradial + interradial + adradial = 6
117	Rhopalia location	at umbrella margin = 0, distally on exumbrella = 1, median on subumbrella = 2, distally on subumbrella = 3
118	Rhopalium pit length	millimeters
119	Rhopalium pit width	millimeters
120	Rhopalium pit depth	millimeters
121	Number of coronal muscle folds	count
122	Coronal muscle covers radial septa or canals on proximal-distal axis	not at all = 0, partially = 1, exactly = 2, exceeds = 3
123	Coronal muscle is continuous circularly over radial septae or canals	no = 0, yes = 1, mixed depending on position = 2

Appendix 1. Continued

No.	Description	Features states
124	Coronal muscle pits	count per octant (averaged per centimeter band)
125	Radial muscles	absent = 0, weakly developed = 1, strongly developed = 2
126	Radial muscle distribution	subumbrellar proximal = 0, subumbrellar distal = 1, subumbrella proximal-to-distal = 2
127	Number of radial muscle folds	count per octant
128	Gastrovascular pits in radial muscle folds	count per cm of muscle
129	Number of subumbrellar sacs/saccules	count
130	Number of rows of subumbrellar sacs/saccules	count
131	Subumbrellar papilla width	millimeters
132	Subumbrellar papilla length	millimeters
133	Subumbrellar papilla height	millimeters
134	Subumbrellar papilla shape	dome = 0, pyramidal = 1, conic = 2, cylindrical = 3, hernia/scrotum-like = 4, wishbone = 5, horse shoe = 6, leaf = 7
135	Type of exumbrella ornamentation	none (smooth) = 0, protuberance = 1, crenulation = 2
136	Number of exumbrella ornaments	count per octant
137	Distribution of exumbrella ornaments	crown of bell = 0, toward bell margin = 1, crown and margin = 2
138	Height of protuberances (depth of crenulations)	millimeters
139	Cross-sectional shape of exumbrella ornaments	circular = 0, rectangular = 1, convex planar (ovoid) = 2, concave planar = 3, triangular = 4, convex triangular = 5, concave triangular = 6
140	Longitudinal-sectional shape of exumbrella ornaments	globose nobs = 0, tapering filaments = 1, mesa-like = 2, mound = 3, conic = 4
141	Number of pigmented flecks in perradial canal	count per quadrant
142	Number of pigmented flecks in interradial canal	count per quadrant
143	Number of pigmented flecks in adradial canal	count per quadrant
144	Shape of pigment on exumbrella	none = 0, dot = 1, circle = 2, uneven patch = 3, radiating lines = 4, star = 5,
145	Number of pigmented spots, patches, shapes on exumbrellar surface	count per octant
146	Distribution of color spots/patches/shapes on exumbrella	crown of bell = 0, toward bell margin = 1, crown and margin = 2
147	Bell diameter	millimeters
148	Ring canal diameter	millimeters
149	Shape of the stomach/gonadal cavity	circular = 0, cruciform = 1, pouched = 2, outfolded pockets = 3, horseshoe = 4
150	Perradial diameter of the stomach cavity	millimeters
151	Structural form of gonad	digitate = 0, ribbon = 1, floret = 2, flame = 3, kidney = 4
152	Thickness of the subgenital porticus	millimeters
153	Quadralinga present	no = 0, yes = 1
154	Quadralinga length	millimeters
155	Quadralinga diameter	millimeters
156	Quadralinga shape	scooped = 0, tri-lobed = 1
157	Subumbrella radial furrows	absent = 0, present = 1
158	Number of subumbrellar radial furrows	count per octant

Table S1. List of all samples included in the study. Details of the locations codes are given in Table 1. Museum of Comparative Zoology, Harvard University (MCZ); National Museum of Natural History, Smithsonian (NMNH); California Academy of Sciences, San Francisco, CA (CAS); Instituto Nacional de Investigación y Desarrollo Pesquero, Mar del Plata, Argentina (INIDEP), University of California, Merced (M0D).

Species	Location Code	Catalogue No./Accession No.	Specimens
		Family Pelagiidae	
Chrysaora achlyos	MXBSMAG	M0D006019M, M0D006020N, M0D006030X, M0D006031Y, M0D006032Z, M0D006033A, M0D006034B, M0D006035C	M0D006019M
Chrysaora colorata	USCAMBQ	M0D022665S, M0D022666T, M0D022667U	-
Chrysaora chinensis	MYBJJGG; THKRKOP	M0D022639S, M0D022643W, M0D022644X, M0D022683I; M0D022671Y	-
Chrysaora fulgida	NGXXGGI	M0D022655I	-
Chrysaora fuscescens	USCAMBQ; USCAXXX	M0D014611Y; M0D020074B	-
Chrysaora hysoscella	GBXXNTS	M0D037294V	-
Chrysaora lactea	BRRJRIO	M0D014610X	-
Chrysaora melanaster	USALBER	KJ026151.1, KJ026152.1, KJ026153.1, KJ026154.1, KJ026155.1	-
Chrysaora pacifica	JPXXXX	M0D022681I	-
Chrysaora plocamia	CLANMJL	M0D022677E, M0D022678F, M0D022679G	-
Chrysaora quinquecirrha	USNCORI; MXTBARR, MXTBMEC	M0D020068V–M0D020070X; M0D014126H; M0D014731O, M0D014732P	M0D020069W, M0D020070X; M0D014126H; M0D014731O, M0D014732P
Chrysaora sp. 1	MXSOGSC	M0D014009U–M0D014012X, M0D014015A–M0D014018D, M0D014021G, M0D014026L	M0D014007S, M0D014008T, M0D014011W, M0D014020F, M0D014021G
Chrysaora sp. 2	MXSOBKN	M0D014070D, M0D014077K, M0D014078L, M0D014081O, M0D014082P, M0D014087U, M0D014088V, M0D014091Y, M0D014093A, M0D014099G, M0D0140100H, M0D0140101I, M0D0140102J, M0D014158N, M0D014159O	M0D014070D, M0D014072F, M0D014075I, M0D014077K, M0D014078L

Table S1. Continued

Species	Location Code	Catalogue No./Accession No.	Specimens
Chrysaora sp. 3	SVUNBES; SVUNGFO; NICIGFO; NIMNMAS; NIMNSAN; CRGUCUJ; CRPUICH; CRGUCLB; CRPUDOM	M0D015866F; M0D015910X, M0D015931S, M0D015932T; M0D016154H–M 0D016156J; M0D016185M, M0D016186N; M0D018435A, M0D018452R; M0D020169S–M0D020171U, M0D020192P; M0D020196T, M0D020197U; M0D020199W, M0D020200X, M0D021266X; M0D021294Z, M0D021296B, M0D021297C	M0D015866F; M0D015910X; M0D016154H; M0D020171U; M0D020197U; M0D020200X; M0D021294Z
Chrysaora sp. 4	PAPAGOR	M0D020100B, M0D020101C, M0D020107I, M0D020111M, M0D020112N, M0D020114P	M0D020100B, M0D020101C, M0D020107I, M0D020111M, M0D020112N
Chrysaora sp. 5	NIANBWI; NIANUSP	M0D016023G, M0D016025I; M0D016141U–M0D016143W	M0D016023G, M0D016025I; M0D016141U–M0D016143W
Chrysaora sp. 6	PABTBDE; CRLMPMO	M0D021394V–M0D021396X; M0D021364R	M0D021394V–M0D021398Z
Chrysaora sp.	NGXXGGI	M0D022654H	-
Sanderia malayensis	USCTNOQ	M0D022660N, M0D022661O, M0D022664R	MCZ1927; NMNH29772
Sanderia sp. 1	SVUNBES; SVUNESP; NIMNMAS	M0D015848N–M0D015850P; M0D018393K; M0D018437C, M0D018451Q, M0D018456V	M0D015848N–M0D015850P; M0D018437C; M0D018456V
Sanderia sp. 2	CRGUCLB	M0D020198V	M0D020198V
Pelagia benovici		KJ573410.1–KJ573414.1	-
Pelagia noctiluca	MXBSLOZ; MXOAPAN; CRPUDOM	M0D006021O–M0D006023Q; M0D020012R, M0D020013S, M0D020021A; M0D021332L–M0D021334N	M0D020013S; M0D021333M, M0D021335O, M0D021336P; M0D014612Z; MCZ1404; MCZ3436; MCZ (###)
Pelagia panopyra cf	IDPAGFC; IDPAGFI; IDPAPYK	M0D001464H; M0D001483A; M0D007198V, M0D007202Z–M0D007204B	M0D007198V–M0D007203A; MCZ1944
Pelagia sp. 1	VENEZIM	M0D014907I–M0D014909K	-
		Family Drymonematidae	
Drymonema dalmatinum	TRIZFOC	HQ234621.1, HQ234617.1, HQ234616.1, HQ234615.1 HQ234614.1	7§
Drymonema gorgo	ARBABBL	-	1§ MCZ8079, MCZ8080

Table S1. Continued

Species	Location Code	Catalogue No./Accession No.	Specimens
Drymonema larsoni	USFLAMS; USALDIS	HQ234618.1–HQ234620.1; HQ234622.1, HQ234610.1, HQ234611.1, HQ234612.1, HQ234613.1, HQ234650.1	10 §
Drymonema sp. 1	MXOASCZ; NIMNMAS; NIMNSAN; NILETRA	M0D020005K, M0D020010P, M0D020011Q; M0D018444J; M0D016153G; M0D020002H	M0D020005K, M0D020010P; M0D018444J; M0D016153G; M0D020002H
		Family Ulmaridae	
Aurelia aurita	CLVSMAR	M0D020052F	MCZ3307, MCZ6015
Aurelia sp. 9	MXTBCAR	M0D014701K–M0D014705O	M0D014701K–M0D014703M
Aurelia sp. 12	MXBSBAP	M0D006054V–M0D006059A, M0D006068J, M0D014842V	M0D006054V, M0D006058Z, M0D006059A, M0D006068J
Aurelia sp. 13	SVUNESP; NILETRA; CRGUCUJ	M0D018376T–M0D018379W; M0D018460Z, M0D020000F; M0D020159I–M0D020161K	M0D020163M–M 0D020168R
Aurelia sp. 14	PAPAGPA	M0D014904F–M0D014906H	-
Aurelia sp. 15	PATBBDE	M0D021365S–M0D021367U	M0D021370X, M0D021374B, M0D021377E
Aurelia sp. 16	ARBABSB	M0D014936L–M0D014938N	-
Aurelia sp.	Caribbean	-	CAS108710
		Family Cassiopeidae	
Cassiopea andromeda	MXBSISJ	M0D006024R–M0D006026T	-
Cassiopea frondosa	PATBBDE	M0D021380H–M0D021382J	-
		Family Mastigiidae	
Phyllorhiza punctata	MXBSAGO; MXBSCPC	M0D014780L, M0D014781M; M0D014783O	-
Phyllorhiza pacifica	THKRKOP; THKRKOB	M0D022673A; M0D022675C	-

Table S1. Continued

Species	Location Code	Catalogue No./Accession No.	Specimens
		Family Lobonematidae	
Lobonema smithii	MYSLJGG; THKRKOP	M0D021410L, M0D021411M, M0D22651E, M0D022652F; M0D022670X	M0D022652F
Gen. 1 sp. 1	MXBSBAP; MXBSAGO; MXBSCPC	M0D0006067I, M0D014775G, M0D014776H; M0D014768Z, M0D014770B	M0D0006067I; M0D014775G, M0D014777I; M0D014768Z, M0D014770B
Gen. 1 sp. 2	SVUNCOQ	M0D018374R	-
Gen. 1 sp. 3	PAPATOC	M0D020075C	M0D020075C
Gen. 1 sp. 4	SVUNGFO	M0D018375S	-
		Family Catostylidae	
Acromitus flagellatus	IDJISUY	M0D21416R, M0D021418T	-
Catostylus townsendi	IDJISUY; MYSLJGG	M0D021427C; M0D022653G, M0D022684L	-
Catostylidae sp. 1	SVUNBES; SVUNGFO; SVUSBJQ; NIMNSAN; NICIGFO	M0D015851Q–M0D015854T; M0D015886Z, M0D015890D, M0D015948J, M0D016248X; M0D015997G, M0D015999I, M0D0160050; M0D016158L–M0D016160N; M0D016161O, M0D016162P	M0D015851Q–M0D015854T, M0D015856J; M0D015997G, M0D015999I; M0D016159M, M0D016160N; M0D016175C, M0D016182J
Catostylidae sp. 2	CRPUDOM	M0D021319Y	M0D021319Y
		Family Lychnorhizidae	
Lychnorhiza lucerna	NIANGUI, NIANGBW, NIANTUP, ARBABSB	M0D016016Z; M0D016088T; M0D016128H–M0D016130J	M0D016016Z; M0D016088T; M0D016128H – M0D016130J; INIDEP-CC0106-EG38, INIDEP-CC0500-GG27-MR2
Lychnorhiza sp. 1	MXOASCZ; SVUNTUN; NIMNMAS; CRPUDOM; PAPAGOR; PAPATOC	M0D020006L–M0D020008N; M0D016187O; M0D018419K–M0D018422N; M0D021295A, M0D021299E, M0D021303I; M0D020086N, M0D020087O, M0D020089Q; M0D020093U	M0D020006L; M0D016187O; M0D018420L–M0D018422N; M0D021295A; M0D020078F, M0D020086N–M0D020088P, M0D020092T
Lychnorhiza sp. 2	CRLMGAN	M0D021350D–M0D021352F	M0D021350D–M0D021354H
Lychnorhiza sp. 3	VENEZIM	M0D014910L, M0D014911M	-

Table S1. Continued

Species	Location Code	Catalogue No./Accession No.	Specimens
		Family Cepheidae	
Marivagia stellata	THKRRAY	M0D022674B	-
		Family Rhizotostomidae	
Rhopilema hispidum	MYSLJGG	M0D022638R	-
		Family Stomolophidae	
Stomolophus meleagris	USNCXXX; USALDIS; MXTBARR; MXTBCAR; MXTBMEC	M0D020054H; M0D014966P–M0D014979C; M0D014113U, M0D014640B–M M0D014645G, M0D014147C, M0D014148D, M0D014668D–M0D014700J, M0D014728L, M0D014729M; M0D014730N	MCZ3310, MCZ383; M0D014147C, M0D014641C, M0D014669E; M0D014700J; M0D014730N
Stomolophus sp. 1	MXSOGSC	M0D006069K–M0D006075Q, M0D006086B–M0D006088D, M0D014000L–M0D014003O, M0D014005Q	M0D006072N, M0D006075Q, M0D006088D, M0D014003O, M0D014005Q
Stomolophus sp. 2	MXBSBAP; MXBSLOZ; MXSOGUY; MXBSCPC; MXBSMUL; MXSOBKN; MXBNGOZ	M0D006060B–M0D006065G; M0D006066H; M0D014029O, M0D014030P, M0D014032R, M0D014040Z, M0D014041A, M0D014046F–M0D014049I, M0D014056P, M0D014063W, M0D014066Z–M0D014068B; M0D014795A–M0D014797C, M0D014800F, M0D014806L–M0D0148090, M0D014815U–M0D014819Y, M0D014822B, M0D014823C; M0D014849C–M0D014851E, M0D014853G–M0D014855I, M0D014864R–M0D014866T, M0D014873A–M0D014879G; M0D014090X; M0D021412N	M0D006060B, M0D006061C; M0D014047G, M0D014063W; M0D014796B, M0D014797C; M0D014865S, M0D014866T
Stomolophus sp. 3	SVUNBES; SVUSCOB; CRGUCLB; CRPUNCY; CRPUDOM; PAPACOR	M0D015960V–M0D015974J; M0D016012V; M0D020193Q–M0D020195S; M0D021275G, M0D021276H, M0D021278J–M0D021286R; M0D021293Y; M0D020122X, M0D020123Y	M0D015960V, M0D015961W, M0D015964Z; M0D020195S; M0D021277I, M0D021279K
Stomolophus sp. 4	PAPATOC, PAPAPAV	M0D020076D, M0D020077E, M0D020079G, M0D020080H; M0D020142R–M0D020147W, M0D020152B, M0D020153C	M0D020076D, M0D020079G, M0D020080H; M0D020144T, M0D020145U
Stomolophus sp. 5	NIANGBW, NIANGUI, NIANTUP	M0D016089U–M0D016092X; M0D016014X, M0D016015Y, M0D016094Z, M0D016099E, M0D016102H, M0D016106L, M0D016112R, M0D016114T; M0D016125E–M0D016127G	M0D016102H, M0D016113S, M0D016117W, M0D016118X; M0D016126F
Stomolophus sp. 6	MXSOBKN	M0D014112T	-

Table S2. List of primers. Primer combinations are denoted by the superscript number ([n]); primers used for sequencing only (*).

Loci	Primer	Sequence (5'-3')	Source
COI	LCOjf [1]	GGTCAACAAATCATAAAGATATTGGAAC	Dawson, 2005
	HCO2198[1, 2, 3, 8, 9, 11]	TAAACTTCAGGGTGACCAAAAAATCA	Folmer et al. 1994
	St COI F10 31 [2]	GATATTCGGAGCT	This study
	Cass COI 120375 F [3]	ATYAGGAGCAGGATTCAGTATG	This study
	Acro LCOI 8 [4, 6]	CGGTGCTTTTTCAGCAATGAT	K. Bayha unpublish data
	Acro LCOI 8deg [5, 7]	CGGTGCYTTTYTCHGCWATGAT	K. Bayha unpublish data
	Acro HCO 611 [4, 5]	AGCAGGGTCGAAGAAAGATGTATT	K. Bayha unpublish data
	Acro HCO 611deg [6, 7]	AGCAGGRTCGAARAADGABGTATT	K. Bayha unpublish data
	Chry sp5 F COI [8]	GAT TGG CACAGCTTTTAGTAT G	This study
	Chry sp3 F COI [9]	GATTGGCACAGCTTTTAGTATG	This study
	Chry Atlan F2 [10, 11]	GCATTCTCCGCAATGATAGG	This study
	Chry Atlan R1 [10]	TTCTGGGTGACCAAAGAACC	This study
16S	16sL [1]	GACTGTTTACCAAAAACATA	Ender and Schierwater, 2003
	Aa H16S 15141H [1]	AGATTTTAATGGTCGAACAGAC	Bayha and Dawson, 2010
	Hydro16Sar [2]	TCGACTGTTTACCAAAAACATAGC	Cunningham and Buss, 1993
	Hydro16Sbr [2]	ACGGAATGAACTCAAATCATGTAAG	Cunningham and Buss, 1993
28S	Aa L28S 21 [1, 3]	GAACRGCTCAAGCTTRAAATCT	Bayha et al. 2010
	Aa H28S 1078 [1]	GAAACTTCGGAGGGAACCAGCTAC	Bayha et al. 2010
	Aa L28S 48 [2]	GCTTGCAACAGCGAATTGTA	Bayha et al. 2010
	Aa H28S 1039 [2, 3, 4]	GTCTTTCGCCCCTATACCCA	Bayha et al. 2010
	Cassiopea 28S F[4]	GRCGGCGAATTGTAGTCTCGA	This study
18S	18Sa [1, 4]	AACCTGGTTGATCCTGCCAGT	Medlin et al.1988
	18Sb [1]	GATCCTTCTGCAGGTTCACCTAC	Medlin et al.1988
	L *	CCAACTACGAGCTTTTTAACTG	Apakupakul et al. 1999
	C *	CGGTAATTCCAGCTCCAATAG	Apakupakul et al. 1999
	Aa L18S 1159 *	CGGAAGGGCACCACCAGGAG	Bayha et al. 2010
	Aa H18S 1318 *	CAGACAAATCACTCCACCAAC	Bayha et al. 2010
	Aa L18S 12 [1, 2]	TCCTGCCAGTAGTCATATGCTTG	Bayha et al. 2010
	Aa H 18S 1798 [2]	CCTACGGAAACCTTGTTACGA	Bayha et al. 2010
	Cassiopea 18S L [3]	GCACTTGTACTGTGAAACTGCG	This study
	Cassiopea 18S H [1, 3]	CTTCCTCTAAATGATCG	This study

Table S3. Thermocycle conditions used to amplify COI, 16S, 28S, and 18S.

Loci	Holds	Denaturation	Annealing	Elongation	Number of cycles	Final elongation	Reference
COI	94°C for 480 s 51–57°C for 120 s 72°C for 120 s 94°C for 240 s 51–56°C for 120 s 72°C for 120 s	94°C for 45 s	50–55°C for 45–60 s	72°C for 60 s	33-35	72°C for 600 s	Modified from Dawson and Jacobs (2001)
	94°C for 240 s	94°C for 45 s	47–52°C for 50–70 s	72°C for 60 s	33-35	72°C for 600 s	
	94°C for 480 s 48–50°C for 120 s 72°C for 120 s 94°C for 240 s 49–54°C for 120 s 72°C for 120 s	94°C for 45 s	50–52°C for 45–60 s	72°C for 60 s	33-35	72°C for 600 s	
16S	94°C for 480 s	94°C for 45 s	50–52°C for 45 s	72°C for 60 s	33-35	72°C for 300 s	
28S	94°C for 240 s	94°C for 45 s	47–55°C for 60–90 s	72°C for 70–90 s	38	72°C for 600 s	
	94°C for 120 s	94°C for 45 s	48°C for 60 s	72°C for 90 s	38	72°C for 600 s	Modified from Bayha et al. (2010)
	94°C for 480 s 49–54°C for 120 s 72°C for 120 s 94°C for 240 s 50–54°C for 120 s 72°C for 120 s	94°C for 45 s	50–54°C for 60 s	72°C for 70–90 s	38	72°C for 600 s	
18S	94°C for 120 s	94°C for 45 s	48°C for 60 s	72°C for 90 s	38	72°C for 600 s	Modified from Bayha et al. (2010)
	94°C for 240 s	94°C for 45–50 s	47–54°C for 70 s	72°C for 70–90 s	38	72°C for 600 s	
	94°C for 240 s	94°C for 15–20 s	45–47°C for 15-20 s	70°C for 90 s	35	72°C for 420 s	Modified from Apakupakul et al. (1999)

Table S4. Picture list for the photographic session, including the quantitative and meristic features take during the photographic session. The use of the color swatch (CMYK) is only for specimens collected recently. Specimens from museums or that have been preserved in formalin for long periods of time then the color swatch is not necessary.

No.	Description
Tank	
1	Label (catalogue number)
2	Lateral view whole animal, camera in the front
3	Lateral view whole animal, camera in the front (include a CMYK)
4	Lateral view whole animal Side
5	Whole animal, camera from top (include a CMYK)
6	Close-up front/right quadrant, camera in the front or side
7	Close-up bell margin, camera in the front or side
8	Close-up of tentacle, camera in the front or side
9	Close-up of oral arms, camera in the front or side
10	Close-up of oral arm filaments
11	Close-up of terminal clubs, camera in the front or side
12	Close-up of mouthlets, camera in the front
13	Close-up of scapulae, camera in the front or side
14	Close-up of scapular filaments
15	Close-up of scapular filaments (include a CMYK)
Position	Lift up bell, drape oral arms to expose ends of manubrium
16	Close-up of manubrium/mouth
17	Oral side up from top
Acrylic Table	
Position	Subumbrella up (face up oral-aboral), tentacles/oral arms out
	Measure diameter of the bell diameter _____
	Measure bell thickness 1)____ 2)____ 3)____
	Measure of the oral disk thickness 1)____ 2)____ 3)____
	Measure diameter of oral disk _____
18	Whole medusa, (include a CMYK) with flash
19	Whole medusa, bottom illuminated with black background
20	Whole medusa, full transillumination
Position	Move tentacles and/or oral arms from quadrant
21	Close-up of quadrant, bottom illuminated with black background
22	Close-up of quadrant, no illumination with black background
23	Picture of center, bottom illuminated with black background
24	Picture of center, full transillumination
25	Close-up of velar lappets, bottom illuminated with black background
26	Close-up of velar lappets, full transillumination
27	Close-up of rhopaliar lappets, bottom illuminated with black background
28	Close-up of rhopaliar lappets, full transillumination
29	Close-up of rhopalium, bottom illuminated with black background
30	Close-up of rhopalium, full transillumination

Table S4. Continued

No.	Description
	Rhopalium pit depth _____millimeters
31	Close-up of coronal muscles, bottom illuminated with black background
32	Close-up of coronal muscles, full transillumination
33	Close-up of radial muscles, bottom illuminated with black background
34	Close-up of radial muscles, full transillumination
35	Close-up of gonad, bottom illuminated with black background
36	Close-up of gonad, full transillumination
37	Close-up of canals two quadrants, bottom illuminated with black background
38	Close-up of canals two quadrants, full transillumination
Position	Move oral arms to top in a group - reveal oral pillars
39	Close-up of oral pillar, bottom illuminated with black background
40	Close-up of oral pillar, full transillumination
	Measure depth of oral pillar _____
	Measure height of oral pillar_____
	Measure the wide of oral pilar_____
41	Close-up of subumbrellar papillae, bottom illuminated with black background
42	Close-up of subumbrellar papillae, full transillumination
Position	Splay the half of oral arms and terminal clubs out
43	Close-up of oral arms/terminal clubs, bottom illuminated with black background
44	Close-up of oral arms/terminal clubs, full transillumination
Position	Splay single oral arm out (winged portion), expose fenestrations
45	Close-up of single oral arm, bottom illuminated with black background
46	Close-up of single oral arm, full transillumination
Position	Splay out scapulae as with oral arm (above)
47	Close up of scapula, bottom illuminated with black background
48	Close up of scapula, full transillumination
	Quadralinga present or absent
	Number of subumbrellar radial furrows_____(per quadrant)
Position	Flip animal so exumbrella faces up (face up aboral-oral)
49	Close-up of exumbrella quadrant, bottom illuminated with black background
50	Close-up of exumbrella quadrant, full transillumination
51	Whole animal exumbrella, bottom illuminated with black background
52	Whole animal exumbrella, full transillumination
Position	If dying canals possible - flip animal and dye canals
53	Close-up of dyed canals 2 quadrants, full transillumination

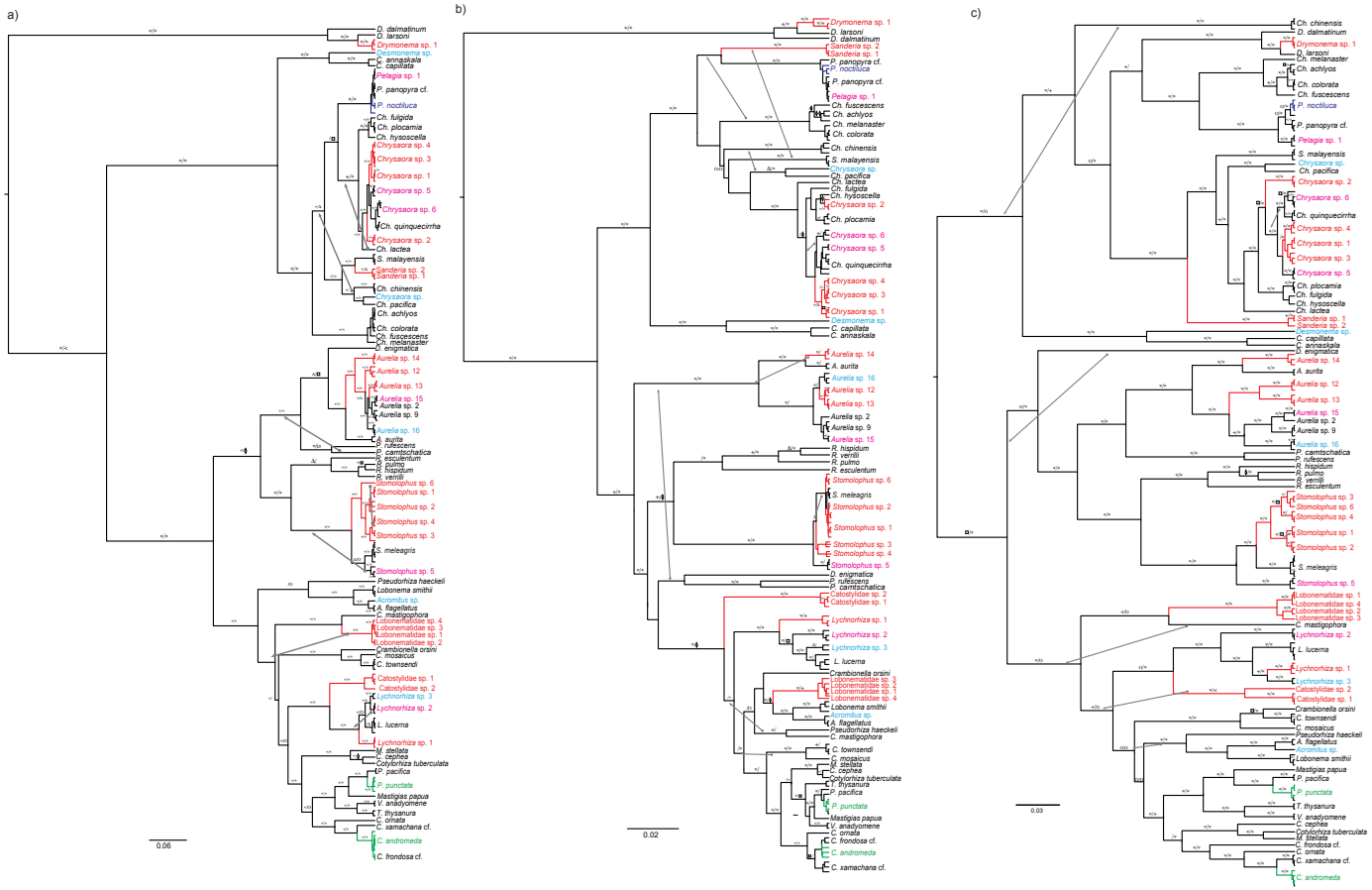

Figure S1. Gene-trees for 16S, 28S and 18S of 171 Discomedusae individuals, highlighting the 25 records for the TEP. a) Midpoint rooted Bayesian 16S tree, using the TPM2uf+I+G model of evolution. b) Midpoint rooted Bayesian 28S tree, using the TIM2+I+G model of evolution. c) Midpoint rooted Bayesian 18S tree, using the GTR+I+G model of evolution. Geographic information on the collecting sites is provided in Table 1. Gray arrows represent alternative topologies present in the Maximum Likelihood analyses. Branches: black, specimens from Bayha *et al*. (2010) and additional specimens from other oceanic regions (Supplementary Table S1); red, 22 new endemics from the TEP; blue, one previously recorded and correctly identified species in the TEP; green, two non-indigenous species. Leaves: magenta, five new taxa from the Caribbean Sea; cyan, four new taxa from other oceanic regions (e.g. Indo-West Pacific). Posterior probabilities and bootstrap are shown on branches: * 100–99%, + 98–95%, Δ 94–90%, O 89–85%; ◊ 84–80%; □ 79–75%; < 74–70%; not shown if < 70%.

Chapter 3: On the Origin of Cryptic Species: Taxonomic Radiation without Morphological Diversification in Jellyfishes (Discomedusae, Scyphozoa)

1. Abstract

The processes and patterns associated with evolutionary radiations have been assessed under different perspectives, with the aim to understanding the biodiversity patterns. The lack of clarity in key concepts (such as adaptive radiation, its principles, and methods to assess it) have caused the misinterpretation of the evolutionary processes and mechanisms. Here we explore if evolutionary radiation was the driver of the high species richness in planktonic shallow-water marine invertebrates in a recognized hot spot area (Tropical Eastern Pacific). Also, we question whether the radiation was associated with a diversification and if it involves a morphological innovation. We built a time-calibrated phylogeny for Discomedusae and estimated the net diversification rates. To identify a key innovation, we mapped 40 morphological characters onto a Pelagiidae phylogeny. The divergence times for the extant taxa occurred within the past ~25 – 8 Mya. Three primary diversification rate shifts are present within the families (Stomolophidae, Pelagiidae, and Ulmaridae). The rate shifts coincide with the closure of the Panamanian Isthmus. Ancestral trait reconstruction did not show any synapomorphic characters for the genus *Chrysaora* (in the Caribbean-TEP clade). We speculate the heterogeneous pelagic environment between the Caribbean and the Tropical Eastern Pacific caused the radiation. However, the further evaluation of the ecology and life history of the species is needed to affirm a geologic radiation.

2. Introduction

Adaptive radiation is a core and familiar concept in evolutionary biology (Simões et al. 2016). Iconic adaptive radiations of cichlid fishes in, Africa's rift lakes (Brawand et al. 2014), Darwin's finches on the Galapagos Islands (Grant and Grant 2003), and *Anolis* lizards of the Caribbean Islands (Losos and Glor 2003; Rabosky and Glor 2010), provide the foundations for adaptive radiations as one of the most plausible explanations for modern patterns of biodiversity (Olson and Arroyo-Santos 2009). Yet the definition, the frequency of invocation, and the methods used to identify adaptive radiation have fueled controversy (Olson and Arroyo-Santos 2009; Glor 2010; Soulebeau et al. 2015).

Other types of evolutionary radiations have received much less attention. Moreover, non-adaptive, geographic, and climatic radiations are often confused with and misreported as adaptive radiations (Soulebeau et al. 2015; Simões et al. 2016). In part, this confusion results from hazy definitions of evolutionary radiations and the lack of testable hypotheses to distinguish among them. Therefore, non-adaptive, geographic, and climatic radiations may play important but under-appreciated roles in explaining patterns of biodiversity globally (Rundell and Price 2009; Simões et al. 2016).

There also is a bias in the subjects of studies of adaptive radiations (Simões et al. 2016). The majority of studies of evolutionary radiations are in mainland, archipelagos, and oceanic island systems; mostly, studies are in tropical regions; and mostly the taxa investigated are terrestrial (Blackledge et al. 2004; Givnish et al. 2009; Lerner et al. 2011;

Soulebeau et al. 2015). The relative dearth of studies in other geographic regions, environments, and taxa may exist for multiple reasons: evolutionary radiations elsewhere truly are few, evolutionary radiations elsewhere are common but overlooked, or evolutionary radiations elsewhere are of a different type. Evidence suggests that evolutionary radiations occur in marine ecosystems too, and potentially have played important roles in shaping the biodiversity patterns we observe today. For example, in some marine systems (e.g. coastal) along some geographic areas (e.g. Tropical Eastern Pacific and Indo-Pacific) species richness is spectacularly high for marine invertebrate and bony fishes (Morato et al. 2010; Bowen et al. 2013; Marchese 2015; Huang et al. 2015), in addition they present high rates of diversification (Kelly and Eernisse 2008; Tittensor et al. 2010; Hallas et al. 2016).

Here we explore the role of evolutionary radiation in explaining the high species diversity in a group of planktonic shallow-water marine invertebrates. Particularly, we explore the recently discovered high diversity of scyphozoan jellyfishes in the Tropical Eastern Pacific (Gómez Daglio and Dawson, in review) and ask, if there was a radiation, whether radiation was associated with diversification, and whether radiation involved morphological innovation. We ask these questions in part because of long-standing interest in how patterns of evolution on land compare with those in the seas (e.g.Vermeij and Grosberg 2010; Carrete Vega and Wiens 2012; Dawson 2012; Grosberg et al. 2012) and in the prevalence and sources of cryptic species in marine systems (e.g. Hamner 1995; Knowlton 2000; Pfenninger and Schwenk 2007; Swift et al. 2016). We build a time-calibrated phylogeny and estimate the diversification rates of Discomedusae—classical metagenetic invertebrates that live in all marine environments (Arai 1997; Morandini et al. 2016; but see Ceh et al. 2015)—that paleontological records indicate appear around the pre-Cambrian (Chen et al. 2002; Waggoner and Collins 2004; Park et al. 2012) and that now show a striking morphological diversity (Arai 1997; Marques and Collins 2004; Morandini and Marques 2010). However, the species richness of Discomedusae, when their diversity arose, and whether their diversity is functional and contributed to persistence through more than 550 million years rather than diversification across all different types of marine environments are topics of recent conjecture (Bayha and Dawson 2010).

3. Material and methods

3.1 Taxonomic collection

The taxonomic sampling included 171 individuals, representing all 13 valid families in Discomedusae (a total of 82 species) published by Gómez Daglio and Dawson (in review). Due to uncertainty about the sister taxon of Discomedusae (Bayha et al. 2010; Kayal et al. 2013; Zapata et al. 2015), we included species from the Order Coronatae (*Atolla wyvillei, Periphylla peryphilla* and *Linuche unguiculata*; (Bayha et al. 2010), Class Hydrozoa (*Zanclea prolifera, Bougainvillia fulva* and *Limnocnida tanganyicae*; (Cartwright et al. 2008), and Class Cubozoa (*Tripedalia cystophora, Carybdea mora* and *Chironex fleckeri*; (Bentlage et al. 2009). All sequences were retrieved from GenBank (Supplementary Material Table S1).

3.2 Phylogenetic analyses

Sequences of a mitochondrial marker (16S rDNA) and two nuclear markers (18S rDNA [small subunit], 28S rDNA [large subunit]) were aligned in MAFFT V. 7 (Katoh and Standley 2013) under the iterative method of FFT-INS-I using the default parameter settings and tested using GBLOCKS (Castresana 2000) allowing a maximum of six contiguous non-conserved positions. Regions with ambiguous homology or poor alignment were omitted from further analyses. The best-fit substitution model for aligned sequences was chosen by the Akaike Information Criterion and Bayesian Information Criterion using jMODELTEST v.2.1.4 (Darriba et al. 2012).

We estimated the species tree using the concatenated alignments of 16S (306 nt), 18S (1665 nt), and 28S (731 nt). The maximum likelihood (ML) tree was constructed using the best fitting model of sequence evolution (16S— GTR+I+G, 18S—TIM2+I+G, 28S—TIM2+I+G) in GARLI v. 2.01 (Zwickl 2006) on the CIPRES PORTAL v. 3.1 (Miller et al. 2010); the best tree was selected from a minimum of four runs by comparing the log-likelihood scores and evaluating asymmetric difference (Robinson-Foulds) tree distance metric using PAUP v.4b10 (Swofford 2002). The robustness of the ML tree topologies was assessed by 1000 bootstrap iterations. The bootstrap values (BS) were added into the best ML tree with SUMTREES (Sukumaran and Holder 2010) and plotted in FIGTREE v.1.4 (Rambaut 2013).

The Bayesian (BY) tree was generated using BEAST v.2.3.2 software pipeline (Bouckaert et al. 2014). Two runs were executed for 20^7 generations with Markov chains sampled every 1000^{th} generation. Convergence and chain mixing were visualized using TRACER v.1.6 (Rambaut et al. 2014). Trees from the stationary phase of the two runs were then pooled by LOGCOMBINER v.2.3.2 and the 50% maximum clade credibility tree was summarized. Assigning this tree as the target tree, the posterior probability (PP) of each node and the mean branch lengths were calculated with TREEANNOTATOR v.2.1.3 (Bouckaert et al. 2014).

3.3 Molecular clock analysis

Calibration of the molecular clock was performed in BEAST v.2.1.3 (Bouckaert et al. 2014). Tree topology was constrained based on the results of the BY and ML analyses. The 16S tree and clock were unlinked from the 28S and 18S, according to the resultant model of evolution used in the phylogenetic analyses. We employed the relaxed log normal clock with a birth-death incomplete sampling prior. The calibration nodes are listed in Table I and described in Figure 1. The MCMC chains were run twice for 200 million generations, storing every 5000^{th} tree. Post BY analyses followed the pipeline described in the phylogenetic section (2.2).

3.4 Diversification rates

We used BAMM v.2.5.0 (Rabosky 2014) to estimate the speciation, extinction, and net diversification rates across the Discomedusae phylogeny. The analysis was conducted using the BY time-calibrated phylogeny, excluded the outgroups, employed two chains running simultaneously for a total of 50 million generations, and sampled tree space every 2000^{th} generation. We discarded 10% as burn-in and checked for MCMC convergence using the BAMMTOOLS package (Rabosky 2014) in the R statistical environment (R Core

Team 2014). The data were processed, visualized, and edited using the R package BAMMTOOLS (Rabosky et al. 2014).

3.5 Ancestral reconstruction within Pelagiidae

Character reconstruction was mapped onto the best ML phylogeny generated including all the members of the family Pelagiidae. We chose 40 morphological traits (Supplementary Material S2), that are taxonomically and evolutionarily informative (Gershwin and Collins 2002; Morandini and Marques 2010; Gómez Daglio and Dawson submitted). We performed our analyses using two methods: Parsimony (PY), where the character states were treated as unordered, and Maximum Likelihood (MLT) reconstruction, with equal probability for any particular character change (Ekman et al. 2008). All analyses were performed in Mesquite v.3.04 (Maddison and Maddison 2015).

4. Results

4.1 Phylogenetic systematics

The ML and BY analyses recovered phylogenetic trees displaying concordant topologies (Fig. 1), except: (a) the family Drymonematidae clade is sister to family Cyaneidae in the ML analysis but basal in the BY analysis, and (b) genus *Chrysaora* is paraphyletic with respect to *Pelagia* in the ML tree but monophyletic in the BY tree. The time-calibrated phylogeny shows the subclass Coronatae as sister taxon to Discomedusae. The divergence time of Order Rhizostomeae from the paraphyletic order Semaeostomeae occurred 212 Mya (95% highest density interval, HPD). The divergence time of the semaeostomes' families occurred during the Jurassic (157 Mya, 95% HPD); whereas the divergence times of rhizostome families occurred later, during the Cretaceous – Paleogene (115 – 25 Mya, 95% HPD).

4.2 Diversification rates

The phylorate plot of Discomedusae shows a disparity in diversification rates between the orders Semaeostomeae and Rhizostomeae (Fig. 2). Within Order Semaeostomeae, two of the four families (Pelagiidae and Ulmaridae) present the highest diversification rates (0.93 and 0.85, respectively); on the other hand, only one of five superfamilies in Rhizostomeae (Scapulatae), and 2 of 10 families, have high diversification rate (0.90 – 0.93). The remaining rhizostome superfamilies and semaeostomes families show a great variability in the rates within each taxon.

We find three main rate shifts across the phylorate plot (Fig. 2) with the highest marginal probability of 0.75 under the best configuration (f=0.69); under different configurations (f=0.45; f=0.32) the same number of shifts and marginal probabilities were found. The first shift corresponds to the diversification of the genus *Chrysaora* (in Family Pelagiidae) around 20 Ma (range between 25 – 15 Mya) during early Neogene. The second shift appears on the tropical clade of *Aurelia* (in Family Ulmaridae) almost at the same time of the *Chrysaora* diversification (22 – 13 Ma). The third main shift in rate occurs at the node basal for the family Stomolophidae (in Superfamily Scapulatae) around 15 – 9 Mya. The diversification rates decrease on those lineages that diverged before or early Cretaceous (70 – 125 Mya), such as the monospecific taxon of *Phacellophora* and the

families Cyaneidae (divergence time 157 – 137 Mya) and Drymonematidae (212 – 254 Mya).

4.3 Ancestral reconstruction within Pelagiidae

Genus *Chrysaora* shows a higher diversification rate for a short period of time (~6 Mya) within Pelagiidae (Fig. 2). Overall, the reconstruction of the 40 characters' states agrees whether reconstructed using the PY or MLT framework. Character evolution mapped in the Pelagiidae phylogeny does not show any synapomorphic character for the genus *Chrysaora* (Fig. 3a, b). For example, characters such as gastric filaments (*f*1), number of bifurcated lappets (*f*4), presence of quadralinga (*f*40), and secondary structure in the oral arms (*f*31) are present in different species from tropical (e.g. *C. quinquecirrha*) and temperate (e.g. *C. achlyos*) clades of *Chrysaora* species.

Other characters such as the number of radial mesenteries (*f*21, Fig. 3c), rhopalia (*f*13) and tertiary tentacles (*f*17) are unique to the *Sanderia* clade. The length and width of the rhopaliar (*f*11, *f*12) and velar lappets (*f*5, *f*6; Fig. 3b) distinguish *Pelagia* from the temperate water *Chrysaora* species (C. *achlyos, C. colorata, C. melanaster,* and *C. fuscescens*). *Chrysaora* sp. 1 is the only species with autapomorphic characters: presence of subgenital ornamentations (*f*32), tentacles present in clusters (*f*20), and tentacles inserted distally in the subumbrella (*f*18).

5. Discussion

The role of evolutionary radiations in increasing and shaping planktonic shallow-water marine diversity has, like the magnitude of marine biodiversity itself, been obfuscated by inadequate collections, insufficient human resources, the challenges of delimiting species, and the presence of cryptic species (Costello et al. 2010; Appeltans et al. 2012). This has been as true of scyphozoan jellyfishes, as of other invertebrate taxa, and so discovery of a hotspot of scyphozoan diversity in the Tropical Eastern Pacific (TEP) and largely consistent inferences of species boundaries from genetic and morphological data (Gómez Daglio and Dawson, in review) provided an opportunity to explore whether the TEP hotspot was due to a radiation, if yes, what type of radiation (adaptive, non-adaptive, geographic, or climatic), and was there morphological innovation. Our results suggest that, at least in this case, modern diversity is a complex of ancient radiation of major taxa which are functionally different (and may now be represented by single or many species) and recent radiation of new species which are functionally similar.

5.1 Discomedusae systematics

Our time-calibrated phylogeny displays a very similar topology to previously published trees (Bayha et al. 2010) suggesting the higher-level systematics of Discomedusae is stable and sufficient to support robust analyses of patterns and rates of radiation. The principle areas of uncertainty are [1] the superfamily Inscapulata, which is not well resolved phylogenetically, nor taxonomically (families Lobonematidae, Lychnorhizidae and Catostylidae are polyphyletic; Fig. 1) and is likely undersampled, and [2] the position of the family Drymonematidae, basal in the BY reconstruction but sister to Cyaneidae in the ML analysis,, as was previously published (Bayha and Dawson 2010). We consider the heterogeneity of rates and long-branch attraction a common problem in phylogenetic

inference that is driving this inconsistency (Mueller 2006; Baele et al. 2013; Bielejec et al. 2014; Su and Townsend 2015). However, these systematics issues do not prohibit the analyses with which we are concerned here, although undersampling bias the estimation of the diversification shifts (e.g. Gubry-Rangin et al. 2015; Looney et al. 2016; Liu et al. 2016). Our phylogeny does support Coronatae as sister taxon to Discomedusae (branch support 100% bootstrap and posterior probability), concordant with morphological phylogenies proposed by (Marques and Collins 2004; Van Iten et al. 2006), as well as phylogenomic analysis (Zapata et al. 2015). Our results do not support Hydrozoa as sister taxon, as was proposed by (Kayal et al. 2013) using mitochondrial genomic data. This is an important result as it helps clarify ancestral states and polarize patterns of evolutionary change.

5.2 Discomedusae radiations

The divergence between Coronatae and Discomedusae is estimated around 512 Mya (95% HDP), only shortly after Scyphomedusae split from the Medusozoan crown group during the Pre-Cambrian (571 – 670 Mya, (Park et al. 2012). The diversification rates (of modern taxa) remain slow up to the Mesozoic (Fig. 2). Patterns of diversification in Discomedusae likely were influenced by global patterns during the Mesozoic. The diversification rates of modern scyphozoan taxa increased, which is corroborated by the multiple fossil records of macrozooplankton found during the Jurassic period (Barthel et al. 1990). This suggests a massive plankton radiation occurred during this time, caused by the split of continents which increase the upwelling systems (Rigby and Milsom 2000). The mass extinction at the end of the Cretaceous/Paleogene (C/P, ~ 65 Mya) had influenced the diversification rates, particularly for those epipelagic lineages and form obligate symbiotic relationship with microalgae (e.g. zooxanthellae), such as the species of the suborder Kolpophorae—*Mastigias* and *Cassiopea* (Fig. 2). In the pelagic environment the top predators, such as non-photosymbiotic jellyfish, ray-fishes, marine mammals and elasmobranches, were killed by starvation (Sibert and Norris 2015). After the mass extinction, the diversification rates rose in the Families Pelagiidae and Ulmaridae (Order Semaeostomeae) and Super families Inscapulata and Scapulata (Order Rhizostomeae); three main rate shifts are denoted during the Neogene (23.03 – 0 Mya, Fig. 2). The increment in diversity levels is found in other zooplanktonic and pelagic taxa, whether the diversity was partially recovered after the C/P event, but did not return to its former levels (Rigby and Milsom 2000; Sibert et al. 2016).

The rate shift occurred in parallel in three different clades—Stomolophidae (superfamily Scapulata), *Chrysaora* (family Pelagiidae), and *Aurelia* (tropical clade, family Ulmaridae)—during the Neogene (20 – 15 Mya; Fig. 2). The most plausible driver of this radiation is the geologic event of the closure of the Panamanian Isthmus (Montes et al. 2015). After the closure of the isthmus, the Caribbean and Eastern Pacific evolved into very different environments (Collins et al. 1996; Lavín et al. 2006; Leigh et al. 2013), which created empty niches in each basin into which species were able to evolve (Leigh et al. 2013). An increase in origination rates has been hypothesized for other benthic and planktonic taxa (e.g. mollusks, crustaceans, ray-fishes, echinoderms) along the Caribbean and TEP (Lessios 2008), and high diversification rates for these groups has been confirmed in several instances (Hurt et al. 2009; Miura et al. 2010; Miura et al. 2012).

5.3 Morphological innovations

Evolutionary radiations can be detected with a time-calibrated phylogeny and an estimation of the diversification rates (Glor 2010; Blankers et al. 2013). The distinction between the different types of evolutionary radiations is still a puzzle (Soulebeau et al. 2015), and the definition of the popular concept "adaptive radiation" debatable (Olson and Arroyo-Santos 2009). Probably the simplest way to identify an adaptive radiation is by the presence of a "key innovation". Our results indicate that a radiation occurred 20 – 15 Mya. However, our morphological analyses for the family Pelagiidae did not reveal any key morphological innovation (Fig. 3). According to Assis and de Carvalho (2010) a key innovation must represent a derived character (i.e. should represent a synapomorphic character for clades with high rates of diversification with respect to sister taxa) and it should be functionally advantageous.

The character mapping on the Pelagiidae phylogeny did not result in the finding of any synapomorphic character for the TEP-Caribbean clade of *Chrysaora,* however, synapomorphies were found for the *Pelagia* and *Sanderia* genera. Taxonomically, the genus *Chrysaora* represents a challenge, in some instances species cannot be distinguished morphologically (Morandini and Marques 2010). The lack of morphological innovation coupled with the genetic diversification suggests a geographic radiation within *Chrysaora*. We speculate this radiation was caused by a homogeneous pelagic environment in which the species are not selected to develop characters that are functionally novel and advantageous and allopatric speciation initiated cladogenesis.

This study highlights the importance of understanding the morphology, functionality, and genetic diversity of a species when describing and classifying a potential radiation. Scyphomedusae present a particularly interesting case given their success as a taxon for more than ~600 Mya, diversity, and role as a top predator in the pelagic food web. We find no evidence of the radiation in Scyphomedusae being adaptive in nature, but highlight that other types of radiations may be important contributors to biodiversity and potentially underrepresented in the literature.

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Table 1. Calibration points used for the molecular clock analyses. Geologic events parameters follow the recommendations delineated by Ho et al. (2015). Million years ago (Mya). Node numbers can be visualized in Figure 1.

Calibration points	Node	Mean (Mya)	St dev (Mya)	References
Geologic events				
Closure of Panamanian Isthmus	1	14	2	Montes et al. 2015
Eastern Pacific Barrier	2	65	5	Briggs 1961; Grigg and Hey 1992
Origin of the Gulf of California	3	5.5	1	Helenes and Carreño 1999; Ledesma-Vazquez 2002
Fossils				
Semaeostomeae, Coronatae, and Cubozoa	4	504	3	Cartwright et al. 2007
Medusozoa	5	570	63	Chen et al. 2002

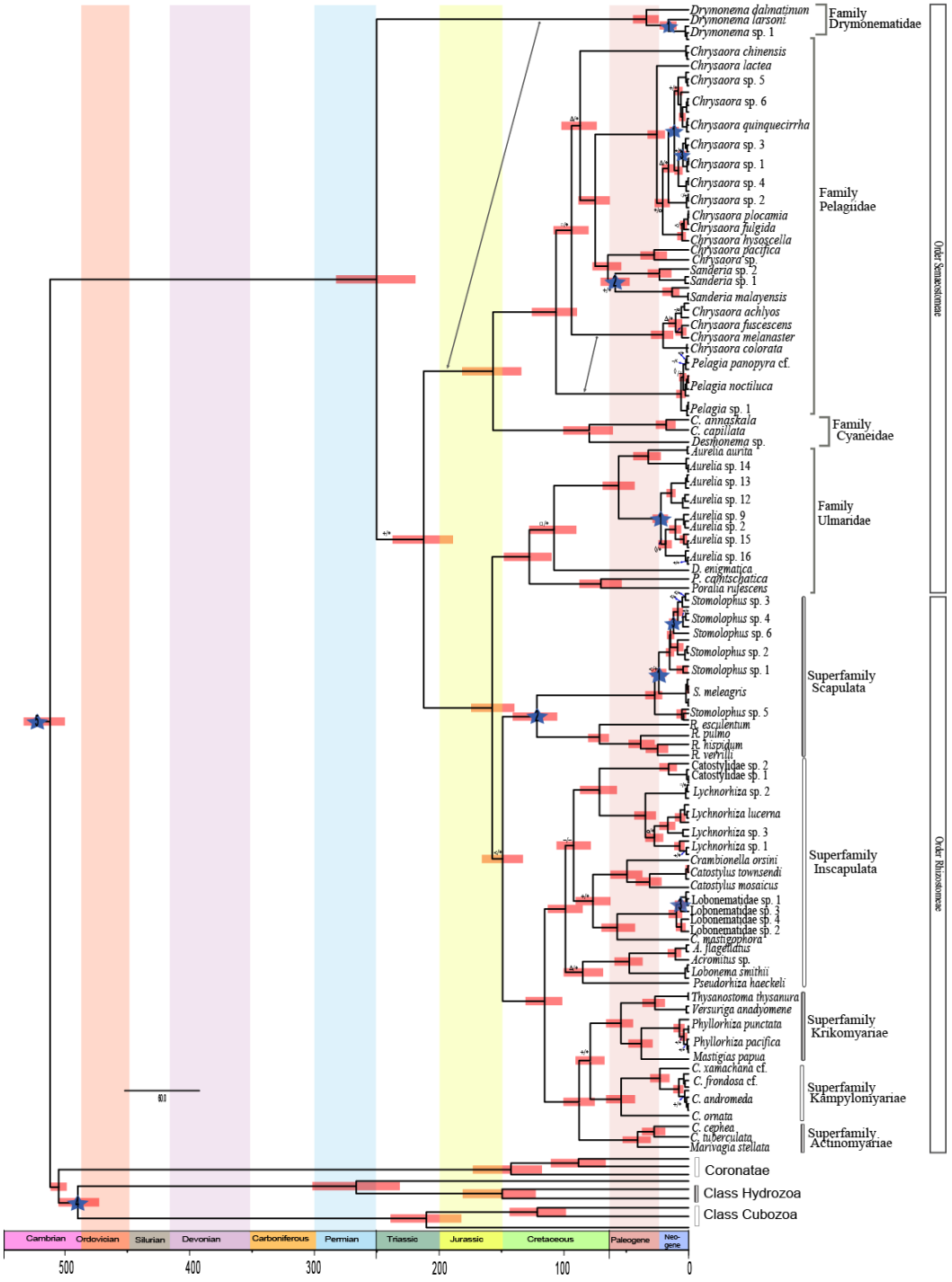

Figure 1. Time-calibrated phylogeny for 82 species of Discomedusae, based on analyses of 16S, 28S, and 18S genes. Outgroups are 3 species from each of three taxa: Coronatae, Hydrozoa, and Cubozoa. Gray arrows show alternative topology returned using ML analysis. Red/orange bars indicate 95% posterior probability densities (HPD) of each node. Numbers in blue stars indicate fossil calibration points from Table 1. Bootstrap and posterior probabilities are shown by symbols on branches: * 98–95%, + 94–90%, Δ 89–85%; O 84–80%; ◊ 79–75%; □ < 74%; not shown if 100–99%.

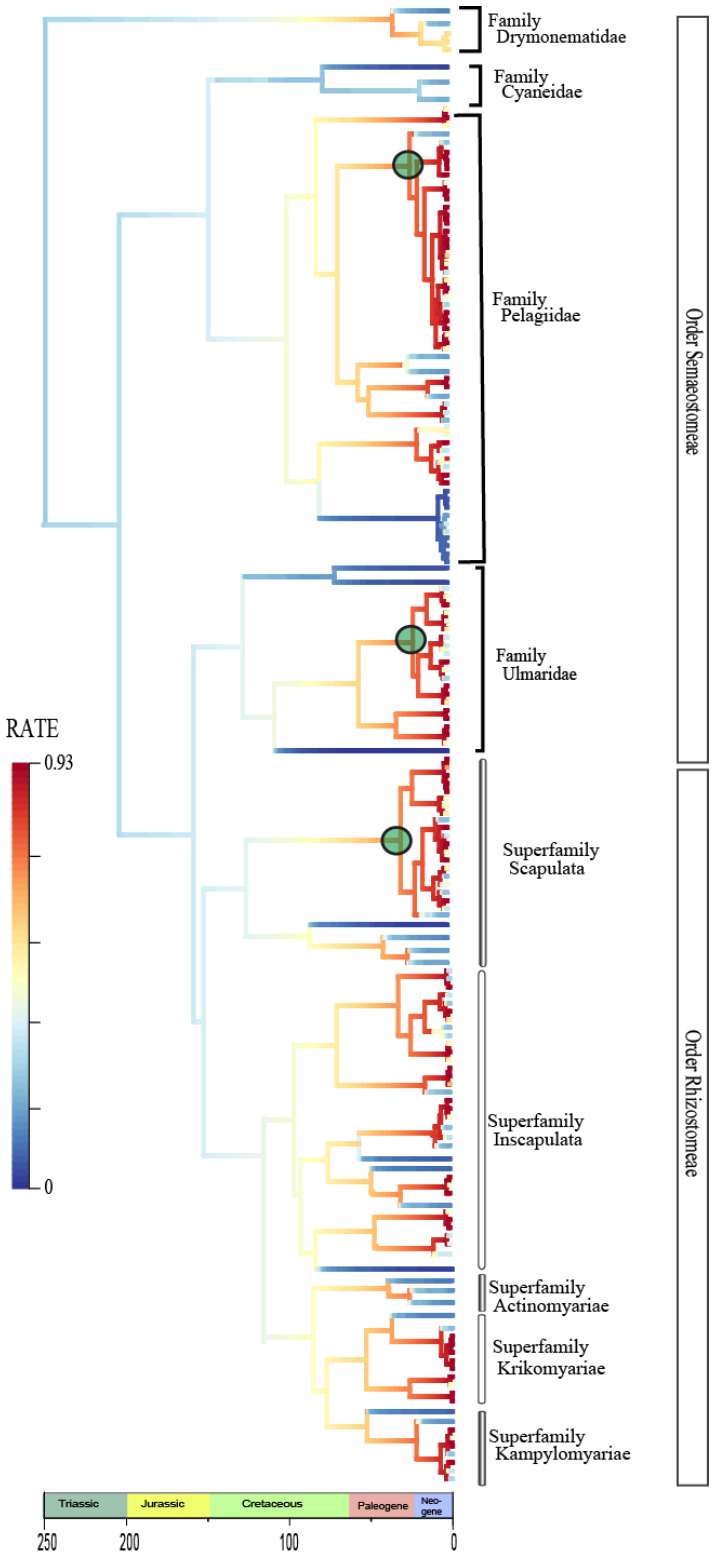

Figure 2. BAMM phylorate plot showing the average net diversification rate. Warmer colours denote faster diversification rates (lineages per Ma). Green circles show the location of rate shifts with a marginal shift probability of 0.75 under the best configuration (f=0.69).

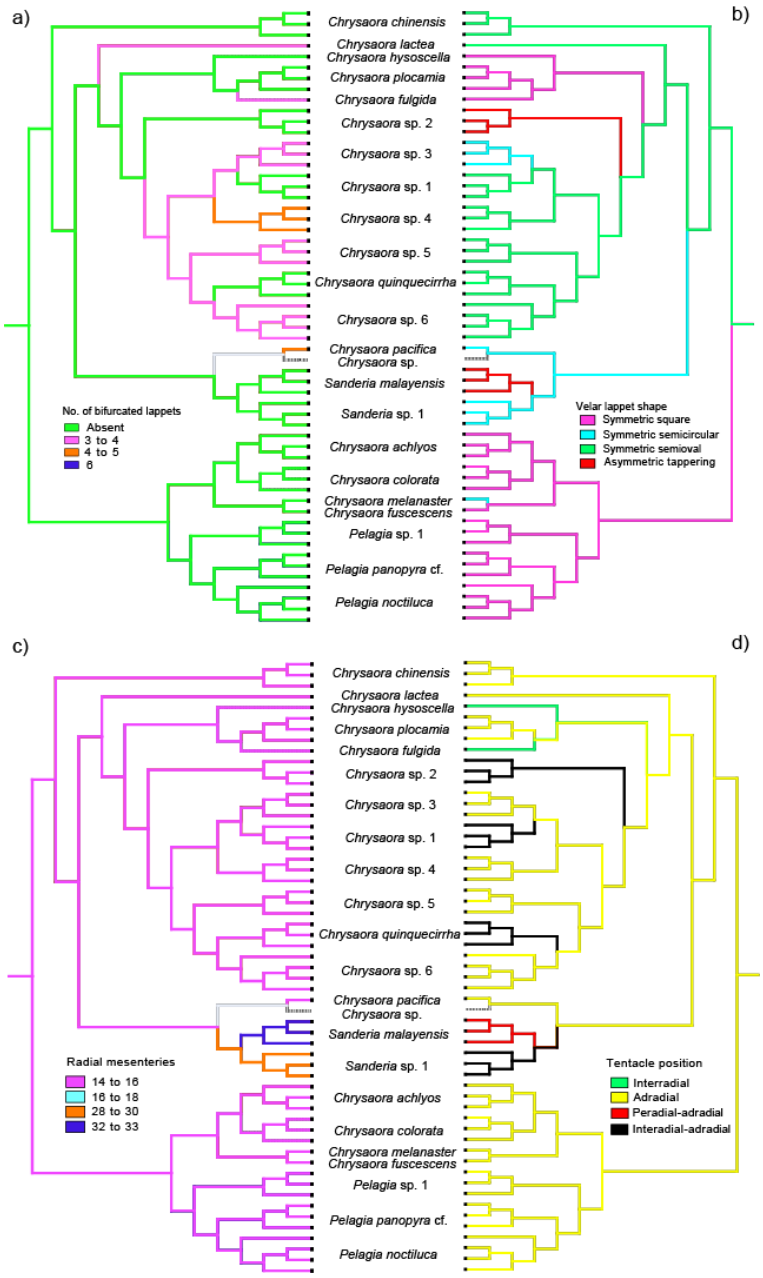

Figure 3. Ancestral reconstruction of morphological characters plotted on the ML phylogeny of Family Pelagiidae. The plots are the summary of PY and MLT analyses generated using MESQUITE. (a) Representation of the number of bifurcated lappets (Table S1, character 4). (b) Representation of velar lappet shape (Table S2, character 7). (c) Representation of the number of radial mesenteries (Table S2, character 21). Representation of the tentacle position (Table S2, character 19).

8. Supplementary Material

Table 1. GenBank accession number

Taxa	Locus		
	28S	18S	16S
Order Coronatae			
Atolla wyvillei	HM194841.1	HM194776.1	****
Periphylla peryphilla	HM194775.1	HM194842.1	****
Linuche unguiculata	HM194830.1	HM194777.1	****
Class Hydrozoa			
Zanclea prolifera	EU272598.1	EU272639.1	EU305488
Bougainvillia fulva	EU305507.1	EU305490.1	EU305470.1
Limnocnida tanganyicae	AY920795.1	AY920755.1	EU293972.1
Class Cubozoa			
Tripedalia cystophora	GQ849065	GQ849088	GQ849123
Chironex fleckeri	GQ849074	GQ849051	GQ849102
Carybdea mora	GQ849092	GQ849069	GQ849106

Table S2. Morphometric and meristic morphological features and their states. The morphological matrix is a modification and compilation of those characters that have been previously proven to be helpful to assess the morphological differences in Family Pelagiidaefamilies and genera of scyphozoans (Gershwin and Collins 2002; Morandini and Marques 2010; Gómez Daglio and Dawson, in prep.).

No.	Description	Features states
1	Gastric filaments	absent = 0, present = 1
2	Velar lappets bifurcated	no = 0, yes = 1
3	Number of velar lappets	count per octant
4	Number of bifurcated lappets	count per octant
5	Velar lappet length	millimeters
6	Velar lappet width	millimeters
7	Velar lappet shape	symmetric square = 0, symmetric semi-circular = 1, symmetric semi-oval=2, symmetric tapering asymmetric square = 4, asymmetric semi-circular = 5, asymmetric semi-oval = 6, asymmetric tapering = 7
8	Velar lappets in heterogenous size classes	no = 0, yes = 1
9	Number of rhopalial lappets	count per rhopalium
10	Rhopalial lappet length (cleft depth)	millimeters
11	Rhopalial lappet width	millimeters
12	Rhopalial lappet shape	symmetric square = 0, symmetric semi-circular = 1, symmetric semi-oval=2, symmetric tapering asymmetric square = 4, asymmetric semi-circular = 5, asymmetric semi-oval = 6, asymmetric tapering = 7
13	Number of rhopalia	count per quadrant
14	Number of umbrella tentacles	count per quadrant
15	Number of primary tentacles	count per quadrant
16	Number of secondary tentacles	count per quadrant
17	Number of tertiary tentacles	count per quadrant
18	Tentacular insertion	at umbrella margin = 0, proximally on exumbrella = 1, distally on exumbrella = 2, proximally on subumbrella = 3, distally on subumbrella = 4
19	Tentacle position	perradial only = 0, interradial only = 1, adradial only = 2, perradial + interradial = 3, perradial + adradial = 4, interradial + adradial = 5, perradial + interradial + adradial = 6
20	Tentacle arrangement	single/continuous = 0, clumped = 1
21	Number of radial mesenteries	count
22	Radial mesentery shape	straight = 0, bent distally = 1, paired forming Y proximally = 2
23	Radial mesentery termination	percent of distance from tentacle (0%) to rhopalium (100%)
24	Shape of the stomach/gonadal cavity	circular = 0, cruciform = 1, pouched = 2, outfolded pockets = 3, horseshoe = 4

Table S2. Continued

No.	Description	Features states
25	Lateral distance from center to most proximal portion of gonad	millimeters
26	Lateral distance from center to most distal portion of gonad	millimeters
27	Gonad position axis	perradial only = 0, interradial only = 1, adradial only = 2, perradial + interradial = 3, perradial + adradial = 4, interradial + adradial = 5, perradial + interradial + adradial = 6
28	Gonad associated with particular structure	manubrium = 0, radial canals = 1, gastric septa or quadralinga = 2, radial septa = 3, pouch = 4, out folded pockets = 5, stomach arms = 6
29	Length of the simple portion of the oral arm	millimeters
30	Oral arm width	millimeters
31	Secondary structure of oral arm	absent = 0, spiral =1
32	Subgenital ostia with ornamentations	no= 0 ; yes = 1
33	Width of the subgenital ostia	millimeters
34	Rhopalia position	perradial only = 0, interradial only = 1, adradial only = 2, perradial + interradial = 3, perradial + adradial = 4, interradial + adradial = 5, perradial + interradial + adradial = 6 at umbrella margin = 0, distally on exumbrella = 1, median on subumbrella = 2, distally on subumbrella = 3
35	Rhopalium pit length	millimeters
36	Rhopalium pit width	millimeters
37	Shape of the stomach/gonadal cavity	circular = 0, cruciform = 1, pouched = 2, outfolded pockets = 3, horseshoe = 4
38	Perradial diameter of the stomach cavity	millimeters
39	Structural form of gonad	digitate = 0, ribbon = 1, floret = 2, flame = 3, kidney = 4
40	Quadralinga present	no = 0, yes = 1

Chapter 4: Comparative phylogeography of jellyfishes (Scyphozoa, Discomedusae) in the Tropical Eastern Pacific

1. Abstract

In an oceanographic sense, the Tropical Eastern Pacific (TEP) and its provinces (Cortez and Panama) are defined based on the distribution patterns of bony fishes, missing the inclusion of another type of data and taxa (e.g. marine invertebrates, phylogenetics, and population genetics). The integration of multiple sources of data can provide a better understanding and suggest alternative hypotheses to explain the biodiversity patterns in the marine realm. Here we compare the phylogeographic patterns of nine species of jellyfish (*Stomolophus* spp. and *Chrysaora* spp.) with biogeographic barriers and genetic discontinuities (or breaks) in the TEP. We analyzed sequence data from 25 localities in the TEP. To infer population differentiation, we estimated of the pairwise genetic distance, F_{ST} and molecular variance (AMOVA) among and between species and populations. Our findings support the TEP biogeographic regionalization based on physicochemical factors to delimit species distributions. However, the intraspecific genetic structure shows a discordance between the phylogeographic boundaries. That suggest that the life history and ecology of the species are important to define the population dynamics in the TEP.

2. Introduction

Marine biogeographic regions typically are defined according to the distributions of species described most often within single-taxon studies (e.g. bony fishes) and coupled with characterization of water masses and currents (Briggs and Bowen 2012). If the water masses and currents are in part responsible for shaping the species' distributions, then the boundaries for multiple taxa should coincide (e.g. Avise et al. 1987; Avise 1992; Reygondeau et al. 2011; Brante et al. 2012) and enable delineation of Provinces. However, the lack of obvious physical boundaries to define the biogeographic regions have made it difficult to recognize the mechanisms and processes that shape patterns of biodiversity in the ocean (Palumbi 1994; Hurtado et al. 2010; Hallas et al. 2016). The implementations of new methodologies (e.g. assessment of multiple genetic markers) and integration of other scientific disciplines (e.g. ecology, population genetics, and phylogenetics) are rejuvenating biogeographic hypotheses and regionalization of marine systems (Hickerson et al. 2003; Richards et al. 2007; Knowles 2009; Hickerson et al. 2010; Cutter 2013).

Phylogeographic studies describe the genealogical relationships, typically within a single species, and can provide insights about the limits and boundaries of gene flow, migration, and speciation processes. They also inform about the past and present evolutionary dynamics of species (Stepien et al. 2001; Craig et al. 2006; Lessios et al. 2012). Yet, multi-taxon comparisons are essential for establishing the generality of processes in historical biogeography (Riddle et al. 2008; Cutter 2013).

Particular value may exist in multi-taxon comparisons when the taxa have different modes of reproduction and life cycles. For example, fishes and jellyfishes possessing two life stages that alternate between benthic and pelagic habitats—but opposing life-phases disperse: the larvae of fishes but the adults of jellyfishes (Arai 1997; Hastings 2000; Zapata and Robertson 2007). Other intriguing relationships may exist between such taxa — for example adult jellyfishes are voracious predators of larval fishes (Purcell 1991), yet jellyfishes also are commensals providing some fishes protection under their umbrellas in

the otherwise refuge-free pelagic environment (Hamner 1995; Ohtsuka et al. 2009) — further strengthening the proposition of biogeographic regions as natural units that represent underlying commonalities in the distributions of species.

The Tropical Eastern Pacific (TEP) is a model system for studying the distribution and population assemblages that are affected by habitat discontinuities (Craig et al. 2006). The TEP is distinguishable from other biogeographic regions by (a) steep thermal gradients—separating the TEP from the temperate regions in the north [Bahía Magdalena, Gulf of California (GCA)] and south (Golfo de Guayaquil, Ecuador); (b) the East Pacific barrier—5400 km of deep water between the central Pacific and the TEP); and (c) the Isthmus of Panama—separating the Caribbean from the TEP (Hastings 2000; Fiedler and Lavín 2006). Within the TEP, two major discontinuities are known to play a major role in the benthic fish community assemblages: 1) the Sinaloan gap—370 km of sandy and muddy shoreline and (2) The Central American Gap—extending from the Golfo de Tehuantepec to the Golfo de Fonseca ~1000 km of sandy, muddy-mangrove shore line (Walker 1960; Hastings 2000; Mora and Robertson 2005). TEP biogeographic regionalization is supported by the distribution, diversification, and biogeographical affinities of bony fishes (Craig et al. 2006; Zapata and Robertson 2007; Rocha et al. 2008; Robertson and Cramer 2009; Briggs and Bowen 2012). However, fewer studies have used marine invertebrates in single taxon studies (Laguna 1990; Tam et al. 1996; Arnaud et al. 2000; Hurtado et al. 2007; Dawson et al. 2011; Meyers et al. 2013; Hurtado et al. 2013). The most recent assessment of the biogeographic regions divide the TEP into two provinces Panamanian and Cortez (Briggs and Bowen 2012), without contemplating the distribution, species richness, and divergence of marine invertebrates.

Here we compare biogeographic barriers, habitat discontinuities, and genetic differentiation in marine organisms, and introduce new phylogeographic data on jellyfishes. By integrating multi-taxon datasets across a variety of temporal and geographical scales, we aim to answer two questions: (1) are the Central American and Sinaloan gaps the only "phylogeographic breaks" in the TEP, or is there a range of "filters" of varying strengths? (2) How do the phylogeographic "breaks/filters" influence the community assemblages, leading to the high endemism in the TEP and GCA?.

3. Material and Methods

3.1 Study group

Discomedusan jellyfish (Scyphozoa) are metagenetic invertebrates that live in all marine environments (Arai 1997). According to Gómez Daglio and Dawson (in review), *Chrysaora* spp. (Semaeostomeae, Pelagiidae) and *Stomolophus* spp. (Rhizostomeae, Stomolophidae), are the genera that best represent the scyphofauna in the TEP. *Chrysaora* species are fragile organisms and voracious plankton predators (Purcell 1991; Purcell and Decker 2005). They are common in all pelagic and coastal environments, including estuarine systems with records in fresh water (Kramp 1961; Morandini and Marques 2010). *Stomolophus* species have a well-defined spherical umbrella shape (tough texture) and are filter feeding organisms (Larson 1991). Their distribution is in shallow-water coastal environments (Kramp 1961; Larson 1990). *Chrysaora* and *Stomolophus* species, like many other scyphozoans, can increase their biomass and abundance for a short period—natural phenomena known as "blooms" (Arai 1997; Hamner and Dawson 2009). Their differences

in habitat selection and feeding modes, in addition to the similarities in life cycles (Calder 1972; 1982) provide an interesting scenario to compare their distribution and community assemblages in the TEP.

3.2 Taxon sampling

We used the collections made in the TEP by Gómez Daglio and Dawson (in review). We selected 97 individuals of the four registered species of *Chrysaora* spp. and 159 of the five registered *Stomolophus* spp. from 25 locations along the TEP (Table 1; Fig. 1).

3.3 Loci selection, amplification, and sequencing

Previous studies demonstrate that the mitochondrial markers cytochrome *c* oxidase subunit I (COI) and 16S rDNA are variable enough for phylogenetic and population genetic analyses in Scyphozoans (Dawson and Jacobs 2001; Bayha and Dawson 2010; Ortman et al. 2010; Bucklin et al. 2010). We retrieved the sequences of the COI data set published by Gómez Daglio and Dawson (in review, GenBank accession numbers are in Supplementary Material 1) and complemented it with amplification of 16S for the same individuals (Table 1). PCR was carried out using the primer pairs 16sL: 5' GACTGTTTACCAAAAACATA 3' (Ender and Schierwater 2003) and Aa H16S 15141H: 5' AGATTTTAATGGTCGAACAGAC 3' (Bayha and Dawson 2010), on a reaction of 25μL: 0.5μL DNA template, 0.1 mM each dNTP (GeneAmp dNTP mix with dTTP, Applied Biosystems Inc., Bethesda, MD, USA), 2.5μL of 10X PCR buffer and 2.5μL $MgCl_2$, 0.63 μL each primer, and 0.05 units of Amplitaq (Applied Biosystems). The thermocycle condition consisted of one hold 94°C for 8min, 33 cycles of 94°C for 45 s, 52°C for 45 s, and 72°C for 60 s; followed by final extension step of 72°C for 300 s. Amplicons were sequenced by the University of Washington High-Throughput Genomics Unit (Seattle, WA, USA), Macrogen (Maryland, USA), or the DNA Sequencing Facility University of California, Berkeley (California, USA). All sequences were assembled, primers removed, and base calls manually corrected in SEQUENCHER v.4 (GeneCodes Corp., Ann Arbor). All sequences were deposited in GenBank (Accession numbers ********).

3.4 Data analyses

For each taxon, we concatenated sequences from COI and 16S using MESQUITE v.3.04 (Maddison and Maddison 2015). The sequences were aligned using MAFFT v.7 (Katoh and Standley 2013) under the iterative method of E-INS-I using the default. Pairwise sequence difference (PSD) and the mean ± SD (standard deviation) between species and locations were calculated in PAUP v.4b10 (Swofford 2002).

Haplotype diversity (h), nucleotide diversity (π), and population pairwise F_{ST} and significance were verified through 10,000 permutations computed using ARLEQUIN v.3.5.1.2 (Excoffier and Lischer 2010). Partitioning of genetic variability among and within species and locations was tested by means of hierarchical analysis of molecular variance AMOVA—1000 permutations using ARLEQUIN v.3.5.1.2 (Excoffier and Lischer 2010). To visualize the relationship between the haplotypes we built a minimum spanning haplotype network using the TCS v.1.20 software (Clement et al. 2005).

4. Results

4.1 Chrysaora *phylogeography and population structure*

The haplotype diversity analyses show the presence of 45 haplotypes; haplotype diversity was high for all the locations ($h > 0.733$) except for the population of Bahía Kino (BKN, $h = 0.133$) which presents two haplotypes and a low nucleotide diversity (Table 2). Nucleotide diversity for *Chrysaora* sp. 2, *Chrysaora* sp. 3, and *Chrysaora* sp. 4 ranged from 0.005 to 0.009 (Table 2).

F_{ST} values and pairwise genetic distance values are high among the four species (Table 3). The greater pairwise genetic distance is between *Chrysaora* sp. 2 and the other three species (mean ± SD, 71.274 ± 0.895). Within the locations of *Chrysaora* sp. 3 the genetic distance was small, ranges between 0.02 to 5.10, and the F_{ST} values are low (<0.63). The haplotype network revealed four main clusters, one per each species of *Chrysaora*. *Chrysaora* sp. 3 cluster presents highest haplotype diversity, however, there is no pattern by location (Fig. 2). The AMOVA analysis revealed that 94.3% of the genetic variation could be explained by the variation between species, whereas the remaining (5.76%) came from variation among populations (Table 5).

4.2 Stomolophus phylogeography and population structure

The haplotype diversity analyses showed the presence of 64 haplotypes; haplotype diversity was high for all the locations—$h > 0.789$ (excluding the sample from Isla Tiburón n=1). Nucleotide diversity was low, ranging from 0.007 to 0.011 (Table 2).

F_{ST} values and pairwise genetic distance values are high among the five species (Table 4). Within the Gulf of California, *Stomolophus* sp. 2 had small genetic distances between the locations, ranging between 1.30 to 7.91, and the F_{ST} values varied between the locations, the comparison between BAP and BKN showed a high F_{ST} value (0.96), meanwhile the comparisons between the rest of the locations are low values. The comparisons between the populations of species 3 revealed high F_{ST} values and genetic distances (Table 4) between the locations from El Salvador (BES, COB, COQ, ESP) and Costa Rica (CLB, NCY, DOM, including one locality from Panama—COR).

The haplotype network revealed four main clusters, two in Central America (*Stomolophus* sp. 3, *Stomolophus* sp. 6 and *Stomolophus* sp. 4), and two in the GCA (*Stomolophus* sp. 1 and *Stomolophus* sp. 2). Within the *Stomolophus* sp. 3 cluster, three main groups are distinguished: (1) haplotypes from El Salvador, (2) haplotypes from Costa Rica and Panama, and (3) haplotype from the GCA (IST). The AMOVA analysis revealed that 84.56% of the genetic variation could be explained by the variation within species, whereas the remaining (15.44%) came from variation among populations (Table 5).

5. Discussion

Phylogeographic patterns in the ocean do not always resemble the biogeographic boundaries hypothesis, because intraspecific genetic structure cannot be predicted only from oceanographic patterns (e.g. currents, eddies, water masses, (Burton 1998; Dawson 2001; Dawson et al. 2002). The biogeographic boundaries described for the TEP concur with the jellyfishes' species distribution; for example, the Cortez province (GCA) is represented by the presence of five endemic species, and the Panamanian province includes the Central American species (four species) whose distribution is limited within the known

boundaries for this province (Briggs and Bowen 2012). On the other hand, the intraspecific structure does not concur the proposed biogeographic hypothesis (e.g. mouth of the Gulf of California as barrier for gene flow). Other factors, such as the life history and species' ecology might explain the patterns found in this study.

5.1 Chrysaora *phylogeography and population structure*

Chrysaora is one of the most common jellyfish genera around the world; its distribution ranges from cold-temperate regions to the tropics (Morandini and Marques 2010). Four species inhabit the TEP, three of them are closely related (*Chrysaora* sp. 1; *Chrysaora* sp. 3, and *Chrysaora* sp. 4) and are geminate species with the Caribbean lineage (Gómez Daglio and Dawson, in review). Meanwhile, *Chrysaora* sp. 2 from BKN is not closely related with the TEP clade (Gómez Daglio and Dawson, in review). *Chrysaora* sp. 2 has the greatest genetic distance (mean ± SD, 71.406 ± 0.982) with respect the other species, and the highest F_{ST} values (Table 3). These results support the high endemism recorded for other taxa in the Cortez province (Boschi 2000; Saarman et al. 2010; Palacios-Salgado et al. 2012).

Within the Central America group, the intraspecific genetic structure of *Chrysaora* sp. 3 does not show a pattern. Overall, the *Chrysaora* sp. 3 cluster presents the highest haplotype and nucleotide diversity values (Table 2). The moderate F_{ST} values between almost all Costa Rica localities (DOM, CIR, ICH, CLB) indicate the individuals conform a single population, however the location of CUJ, which is the northern and pelagic, hence shares more haplotypes with the Nicaraguan locations (SAN, POT, MAS) and the genetic distances are smaller with respect the former Costa Rica's locations (Fig. 2, Table 3). The locations from Nicaragua and Golfo de Fonseca, present low F_{ST} values and genetic distance indicate a high gene flow between all the locations. The location of BES appears slightly different from the Golfo de Fonseca and Nicaragua locations (Fig. 2, Table 3). However, the values are biased by the small sample size (n=1). Hence it is difficult to conclude whether the Golfo de Tehuantepec is a phylogeoghraphic break or not (Hastings 2000; Hurtado et al. 2007).

5.2 Stomolophus *phylogeography and population structure*

The cannonball jellyfish (*Stomolophus* spp.) inhabit the tropical and temperate coastal waters of the north and south American continents (Kramp 1961). Previous phylogenetic studies revealed two main clades for the family Stomolophidae: one in the Caribbean and the second in the TEP (Gómez Daglio and Dawson, in review). In the TEP there are five species: three in the GCA—*Stomolophus* sp. 1, *Stomolophus* sp. 2, and *Stomolophus* sp. 6, and two in Central America—*Stomolophus* sp. 3 and *Stomolophus* sp. 4 (Gómez Daglio and Dawson, in review). The estimation of the genetic distances and F_{ST} values (Table 4) supports phylogenetic findings, where the highest F_{ST} (mean ± SD, 0.896 ± 0.007) and greatest genetic distances (mean ± SD, 52.591 ± 2.556) are between the GCA species (*Stomolophus* sp. 1 and *Stomolophus* sp. 2) and the Central American species (*Stomolophus* sp. 3 and *Stomolophus* sp. 4) + *Stomolophus* sp. 6 from Isla Tiburón (GCA).

Within the GCA group, *Stomolophus* sp. 1 inhabits exclusively the northern part of the GCA, which contradicts the results of Girón-Nava et al. (2015), who state that the population from the GSC has a high connectivity favored by the oceanographic conditions

with the GUY population. *Stomolophus* sp. 2 inhabits the west and east coast of the GCA including the Pacific side of the peninsula (MAG). The F_{ST} values and genetic distances demonstrate the presence of three populations (BAP, MUL, GUY; Table 4, Fig. 3). BKN is part of the GUY population (F_{ST} 0.23; genetic distance 4.16), and MAG does not show a strong differentiation with respect the other populations.

These results contradict other phylogeographic patterns described for GCA: (1) several examples of bony fishes show disjunct population distributions—species are present in the central and north regions of the GCA and the Pacific coast of the Baja California Peninsula (Stepien et al. 2001; Bernardi and Lape 2005; Bernardi 2014), which suggests that the divergence between those populations might have occurred during the mid-peninsular seaway opening (Upton and Murphy 1997; Bernardi et al. 2003); (2) the populations on the Pacific coast of the Baja California Peninsula present a high gene flow and strong connectivity with the Northern Baja California Peninsula populations (Hurtado et al. 2007; 2010). Hence, the population of MAG might keep a certain degree of gene flow with the GCA populations, potentially through a transient population in the Cape region. We suggest that a finer resolution scale study (e.g. microsatellites, SNPs or ddRAD data) is needed to understand the complexity of the population in the GCA, as has been accomplished for other taxa (Glynn and Ault 2000; Selkoe and Toonen 2006; Liu et al. 2015; DaCosta and Sorenson 2016).

The Central America group displays four clusters, which are well supported by high F_{ST} values between species (*Stomolophus* sp. 3, *Stomolophus* sp. 4, and *Stomolophus* sp. 6) and regions (Table 4, Fig. 3). Among El Salvador locations (COB, BES, ESP, COQ) the pairwise genetic distances are small with low differentiation (F_{ST}). On the other hand, the population from El Salvador is different from those distributed below the Golfo de Fonseca: Costa Rica locations + COR (Panama). This suggests the Golfo de Tehuantepec as a phylogeographic break, that restricts gene flow between the populations from El Salvador and Nicaragua. *Stomolophus* sp. 4 represents a single population within the Gulf of Panama (low F_{ST} and small genetic distances, Table 4), which is genetically distinct from the Panamanian population (COR).

5.3 Biogeographic patterns in the TEP

The comparison between the phylogeographic patterns of *Chrysaora* and *Stomolophus* provides new insights and reaffirms, in part, the regionalization of the TEP. For example, the Cortez province (GCA) is characterized by the high differentiation of species, particularly in bony fishes and benthic invertebrates (Hastings 2000; Hurtado et al. 2007; 2010; Palacios-Salgado et al. 2012; Meyers et al. 2013; Hurtado et al. 2013). Our results show, a great differentiation between both planktonic jellyfishes in the GCA compared with the rest of the TEP (Fig. 2, 3). This differentiation is attributed to the thermal barrier present in the mouth of the GCA (Roden 1958; Castro et al. 2000) and the presence of the Sinaloan gap in mainland coast (Walker 1960; Hastings 2000) by restricting gene flow between mainland and the peninsula.

The GCA is divided into two regions: north and south. The north region presents a high number of species and populations that are restricted to the area (such as *Stomolophus* sp. 1 and *Chrysaora* sp. 1). Other taxa, for example, benthic fishes and rocky intertidal invertebrates, show the same distribution pattern (Riginos and Nachman 2001; Riginos

2005; Hurtado et al. 2007; 2010). The area of the Great Islands (Isla Angel de La Guarda and Isla Tiburón) presents contrasting oceanographic conditions that separate the Gulf into two regions and shape the assemblages of benthic and planktonic communities (Walker 1960; Gutiérrez et al. 2004). An unexpected result was the similarity between species from the area of the Great Islands—IST (*Stomolophus* sp. 6) and BKN (*Chrysaora* sp. 2)—with the Central American groups; this pattern has not been recorded for bony fishes or marine invertebrates.

We identify a plausible scenario, where the populations diverge from the Pacific lineage colonizing the proto-Gulf (~11.6 Ma, Helenes and Carreño 1999); during the glacial-interglacial periods, the sea surface temperature drops to 6° to 10° and the sea-level low stand (Mortyn et al. 2010; Dolby et al. 2015). Thus, the proto-Gulf have been a refugee for the warm-temperate species and trap the species in the northern portion of the proto-Gulf (~1.8 – 0.7 Ma); meanwhile, the populations in the TEP might contract. After the inter-glacial period the species: (a) recolonized the TEP occupying the empty niches in the TEP, or (b) they were trapped in the area of the Great Islands, due to the strong oceanographic dynamic established at this time. To test those scenarios, we need samples from the populations that inhabit the Mexican Pacific mainland area, including the areas of the Golfo de Tehuantepec and Guatemala.

In Central America, the regionalization and population structure differs between both species. *Stomolophus* species present a strong population structure where Golfo de Tehuantepec is a discontinuity for the populations from El Salvador and Costa Rica + COR (Panama) (Table 4, Fig. 3). On the other hand, *Chrysaora* sp. 3 do not show a population structure (Table 3, Fig. 2), the estimations suggest a high gene flow between the different localities, do not support the presence of phylogeographic barriers or break points. The difference in the population dynamics between these jellyfishes result from their ecological needs. *Stomolophus* are coastal species, hence the oceanography of the Gulf of Tehuantepec (Fiedler and Lavín 2006; Willett et al. 2006) might be a moderate break point to restrict the gene flow between the El Salvador and Costa Rica populations. *Chrysaora* species can inhabit coastal or pelagic waters (Morandini and Marques 2010). The medusae allow *Chrysaora* to disperse more efficiently than the coastal *Stomolophus*. However, we need more information about the natural history of the species (e.g. diet, swimming behavior, reproduction) to put phylogeographic differences between species into an ecological context.

The Golfo de Panama appears to be a moderate break point. Analyses of both jellyfishes show well established populations (high haplotype and nucleotide diversity, Table 2), differentiated by a great genetic distance and high F_{ST} values (Table 3, 4) from the closest northern population (COR—*Stomolophus*, and DOM—*Chrysaora*). This is the first phylogeographic and demographic study in the Central America area, previous studies are records of presence/absence (e.g. Hastings 2000; Zapata and Robertson 2007; Robertson and Cramer 2009) of bony fishes, and the taxon and geographic sampling is very limited (e.g. Hurtado et al. 2007; Frey and Vermeij 2008; Meyers et al. 2013). Thus, studies of additional taxa are needed to test this hypothesis.

The most recent biogeographic regionalization of the TEP follows Briggs and Bowen (2012), who contradict Hastings' regionalization (2000) that includes a third province Mexican—from the Golfo de Tehuantepec up to the mouth of the GCA.

According to Briggs and Bowen (2012), the Mexican province does not present a sufficiently high number of endemics to be considered as a different province. Our analyses in Central America and the GCA suggest there might be a high probability that more species of *Chrysaora* and *Stomolophus* can inhabit the area of southwest Mexico, and the Central American gap might be a strong phylogeographic break not only for benthic organisms (Zapata and Robertson 2007; Meyers et al. 2013). This scenario will remain unclear until we increase the sampling locations in the Mexican province.

The integration of multiple taxa on comparative analyses enriches the plausible hypotheses that might explain the ecology and evolution of the species. Here, the results from metagenetic benthic-planktonic species (with different life histories) supports some new and some well-known pattern of regionalization for the TEP. We evince the need for more information about the natural history of the species that inhabit the TEP, and the use of new technology (e.g. next generation sequencing) to evaluate the hypotheses proposed for this area.

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Table 1. Sampling locations along the Tropical Eastern Pacific, including geographic position (Latitude and Longitude). Map reference numbers are shown in Figure 1. Numbers of sequences amplified for COI and 16S per locations from *Chrysaora* spp. and *Stomolophus* spp. Parenthetical numbers are species assignations following Gómez Daglio and Dawson (in review). Not present (N/P)—species has no anecdotal or sighting record after the locality was surveyed at least three times at different times during the year. Not observed (N/O)— the location was surveyed once, hence the location may be a potential collecting area. * Geographic coordinates were estimated using GOOGLE EARTH.

Location	Latitude	Longitude	Location Code	Map reference no.	*Chrysaora*	*Stomolophus*
		México				
Golfo de Santa Clara	31° 39' 40" N	114° 34' 34" W	GSC	1	10 (sp. 1)	15 (sp. 1)
Las Guásimas	28° 48' 34" N	111° 56' 27" W	GUY	2	N/O	15 (sp. 2)
Isla Tiburón	28° 53' 40" N	112° 14' 11" W	IST	3	N/O	1 (sp. 6)
Bahía Kino	27° 51' 34" N	110° 36' 37" W	BKN	4	15 (sp. 2)	1 (sp. 2)
Mulegé	26° 53' 56" N	111° 57' 39" W	MUL	5	N/O	15 (sp. 2)
Bahía de la Paz	24° 10' 24" N	110° 18' 57" W	BAP	6	N/O	7 (sp. 2)
Bahía Magdalena	26° 53' 56" N	111° 57' 39" W	MAG	7	N/O	15 (sp. 2)
		El Salvador				
Los Cóbanos	13° 29' 37" N	89° 51' 31" W	COB	8	N/O	1 (sp. 3)
El Espino	13° 08' 07" N	88° 00' 12" W	ESP	9	N/O	7 (sp. 3)
Bocana del Esterón	13° 09' 29" N	88° 04' 04" W	BES	10	1 (sp. 3)	15 (sp. 3)
El Tamarindo	13° 10' 14" N	87° 52' 44" W	TAM	11	7 (sp. 3)	N/P
El Coquito	13° 09' 33" N	88° 02' 37" W	COQ	12	N/O	14 (sp. 3)
Golfo de Fonseca	13° 10' 22" N	87° 52' 53" W	GFO	13	5 (sp. 3)	N/P
		Nicaragua				
Potosi	13° 00' 30" N	87° 29' 21" W	POT	14	2 (sp. 3)	N/P
Puerto Sandino	12° 09' 59" N	86° 47' 40" W	SAN	15	6 (sp. 3)	N/P
Masachapa	11° 40' 5" N	86° 34' 29" W	MAS	16	8 (sp. 3)	N/P
		Costa Rica				
Cuajiniquil	10° 57' 23" N	85° 43' 42"W	CUJ	17	9 (sp. 3)	N/P
Estero Culebras	10° 08' 55" N	85° 07' 53" W	CLB	18	2 (sp. 3)	3 (sp. 3)
Isla Chira	10° 8' 48" N	85° 10' 01" W	ICH	19	9 (sp. 3)	N/O
El Cirialito	10° 09' 05" N	85° 06' 33" W	CIR	20	4 (sp. 3)	12 (sp. 3)
Dominical	9° 14' 10" N	83° 52' 06" W	DOM	21	7 (sp. 3)	1 (sp. 3)
		Panamá				
Gorgona	8° 33' 49" N	79° 49' 20" W	GOR	22	12 (sp. 4)	1 (sp. 3)
Coronados	8° 32' 33" N	79° 52' 24" W	COR	23	N/O	3 (sp. 3)
Panamá Viejo	8° 59' 47" N	79° 29' 16" W	PAV	24	N/O	12(sp. 4)
Tocumen *	8° 00' 02" N	79° 29' 30" W	TOC	25	N/O	7 (sp. 4)

Table 2. Estimates of haplotype diversity (h) and nucleotide diversity (π) in the concatenate data set of COI and 16S for *Chrysaora* and *Stomolophus* species. *Stomolophus* sp. 6 from Isla Tiburón (IST) is not included in the table because the sample size is 1.

	Chrysaora			*Stomolophus*		
	No. of haplotypes	h (± SD)	π (± SD)	No. of haplotypes	h (± SD)	π (± SD)
Species 1	8	0.733 ± 0.077	0.008 ± 0.077	10	0.895 ± 0.07	0.007 ± 0.004
Species 2	2	0.133 ± 0.112	0.002 ± 0.003	20	0.920 ± 0.02	0.009 ± 0.005
Species 3	30	0.952 ± 0.013	0.009 ± 0.003	18	0.789 ± 0.054	0.011 ± 0.005
Species 4	5	0.803 ± 0.095	0.005 ± 0.003	15	0.904 ± 0.056	0.011 ± 0.006

Table 3. Species and population differentiations of *Chrysaora* spp. from the TEP. Above the diagonal pairwise F_{ST} values. Below the diagonal corrected average pairwise difference. Bold numbers show p values = 0.050. Locations information is provided in Table 1.

	Sp. 1	Sp. 2	Sp. 3											Sp. 4
	GSC	BKN	BES	TAM	GFO	SAN	MAS	POT	CUJ	CLB	ICH	CIR	DOM	GOR
GSC	*	0.97	0.90	0.92	0.91	0.92	0.92	0.91	0.92	0.91	0.94	0.91	0.93	0.93
BKN	49.04	*	1.00	0.99	0.99	0.99	0.98	1.00	0.98	1.00	0.99	0.99	0.99	0.98
BES	48.70	70.91	*	**0.95**	**0.06**	**0.08**	**0.09**	**0.32**	**0.07**	**0.06**	**0.02**	**0.03**	**0.06**	**0.24**
TAM	49.67	70.70	2.41	*	0.44	0.02	0.38	0.38	0.45	0.01	0.14	0.07	0.03	0.74
GFO	49.22	70.27	2.50	0.19	*	**0.95**	**0.22**	**0.35**	**0.64**	0.33	0.04	0.07	0.06	0.89
SAN	49.20	70.14	2.60	0.09	0.10	*	**0.04**	**0.35**	**0.57**	**0.36**	0.37	0.04	0.30	0.69
MAS	49.00	72.00	3.39	0.19	0.29	0.08	*	**0.94**	**0.63**	**0.36**	0.43	0.44	0.03	0.65
POT	48.30	69.67	2.00	0.21	0.30	0.10	0.39	*	**0.94**	**0.34**	0.39	**0.53**	0.46	0.87
CUJ	48.94	72.00	3.11	0.09	0.12	0.12	0.02	0.33	*	0.94	0.40	0.46	0.49	0.85
CLB	47.97	71.72	6.00	2.66	3.10	2.27	2.27	3.00	2.11	*	0.94	0.50	0.46	0.79
ICH	48.90	71.67	5.17	1.90	2.22	1.53	1.32	2.17	1.44	0.17	*	0.45	0.46	0.78
CIR	54.30	71.81	5.67	2.33	2.67	1.93	1.93	2.67	1.83	0.33	0.06	*	0.45	0.78
DOM	54.20	72.00	5.10	1.85	2.20	1.48	1.36	2.10	1.44	0.38	0.02	0.05	*	0.94
GOR	75.07	72.40	53.00	51.41	51.03	50.93	50.64	52.0	50.11	52.00	51.72	51.08	51.24	*

Table 4. Species and population differentiations of *Stomolophus* spp. from the TEP. Above the diagonal pairwise F_{ST} values. Below the diagonal corrected average pairwise difference. Bold numbers show p values = 0.050. Locations information is provided in Table 1.

	Sp. 1	Sp. 2					Sp. 6	Sp. 3								Sp. 4	
	GSC	BAP	GUY	BKN	MAG	MUL	IST	BES	COB	COQ	ESP	CLB	NCY	DOM	COR	TOC	PAV
GSC	*	0.87	0.82	0.82	0.79	0.83	**0.91**	0.95	0.92	0.80	0.94	0.93	0.94	**0.92**	0.93	0.94	0.94
BAP	22.13	*	0.58	0.96	0.65	0.79	**0.99**	0.99	1.00	0.79	0.99	0.99	0.98	**1.00**	0.99	0.98	0.98
GUY	19.77	4.15	*	0.23	0.30	0.57	**0.92**	0.96	0.93	0.81	0.95	0.94	0.95	**0.93**	0.93	0.95	0.95
BKN	23.15	6.71	1.30	*	0.37	0.67	**1.00**	0.98	1.00	0.60	0.98	0.97	0.98	**1.00**	0.97	0.97	0.97
MAG	16.52	5.48	1.72	4.34	*	0.40	**0.93**	0.96	0.93	0.82	0.95	0.94	0.95	**0.93**	0.94	0.95	0.95
MUL	19.04	7.91	4.49	7.20	2.23	*	**0.95**	0.97	0.95	0.83	0.96	0.96	0.96	**0.95**	0.96	0.96	0.96
IST	48.35	56.00	52.23	53.00	53.08	55.33	*	**0.89**	**1.00**	**0.13**	**0.90**	**0.83**	**0.88**	**1.00**	**0.83**	**0.93**	**0.93**
BES	55.28	61.23	57.45	58.50	58.30	60.56	9.63	*	0.56	0.23	0.07	0.68	0.67	**0.63**	0.68	0.94	0.94
COB	54.09	60.00	56.23	57.00	57.08	59.33	10.00	2.03	*	0.55	0.60	0.67	0.76	**1.00**	0.67	0.93	0.93
COQ	45.20	51.16	47.17	48.48	48.04	50.17	9.36	1.94	3.61	*	0.08	0.03	0.25	**0.57**	0.04	0.56	0.64
ESP	55.25	61.17	57.39	58.50	58.24	60.50	9.67	0.06	2.00	1.82	*	0.62	0.62	**0.60**	0.62	0.94	0.94
CLB	53.42	59.33	55.56	58.33	56.37	58.67	11.00	2.50	5.00	3.19	2.00	*	0.07	**1.00**	0.00	0.93	0.93
NCY	53.76	59.67	55.90	58.67	56.75	59.01	11.04	2.53	5.04	3.30	2.04	0.04	*	**0.09**	0.07	0.94	0.94
DOM	54.09	60.00	56.23	59.00	57.08	59.33	11.00	2.50	5.00	3.36	2.00	0.00	0.04	*	**1.00**	**0.93**	**0.93**
COR	53.42	58.67	55.42	58.33	55.79	58.04	11.00	2.50	5.00	3.36	2.00	0.00	0.04	0.00	*	0.85	0.93
TOC	55.35	63.29	58.63	60.57	60.65	62.90	22.00	21.50	22.00	18.25	21.62	22.71	22.57	22.71	0.93	*	0.02
PAV	55.27	62.49	57.89	59.78	59.86	62.11	21.70	21.03	21.53	17.82	21.17	22.36	22.22	22.36	22.25	22.71	*

Table 5. Analyses of molecular variance (AMOVA) for the concatenated data set of COI and 16S for *Chrysaora* spp. and *Stomolophus* spp.

Source of variation	d.f.	Sum of squares	Variance components	% of variation	Fixation index
Chrysaora spp.					
Among species	4	1651.517	29.504 Va	94.3	F_{ST} = 0.95846
Among populations between species	10	40.326	0.505 Vb	1.61	
Within populations	84	109.259	1.300 Vc	4.15	
Stomolophus spp.					
Among species	4	2057.266	20.902 Va	84.56	F_{ST} = 0.92189
Among populations between species	12	185.979	1.887 Vb	7.63	
Within populations	118	227.836	1.930 Vc	7.81	

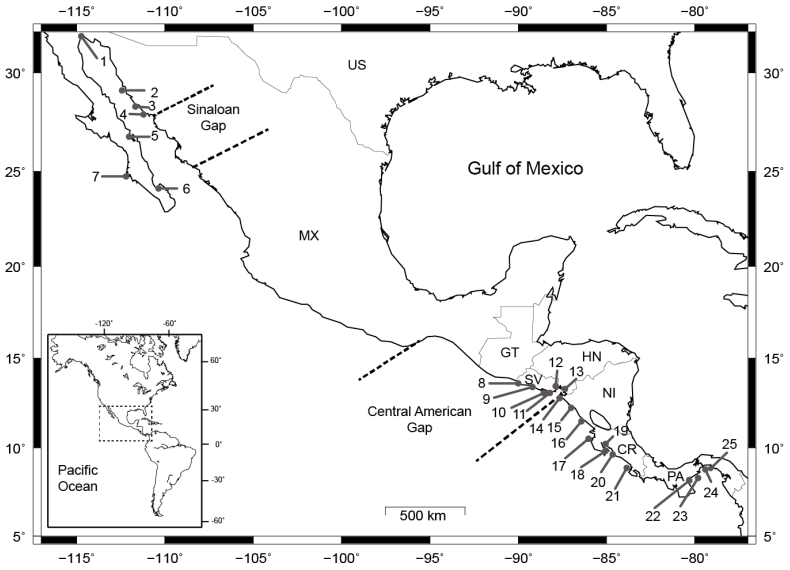

Figure 1. Sampling locations along the Tropical Eastern Pacific (TEP). Geographic information and corresponding location numbers are provided in Table 1. Break lines show the limits of the Sinaloan and Central American gaps to Hastings (2000). Abbreviations: Costa Rica (CR); El Salvador (SV); Guatemala (GT); Honduras (HN); Mexico (MX); Nicaragua (NI); Panamá (PA).

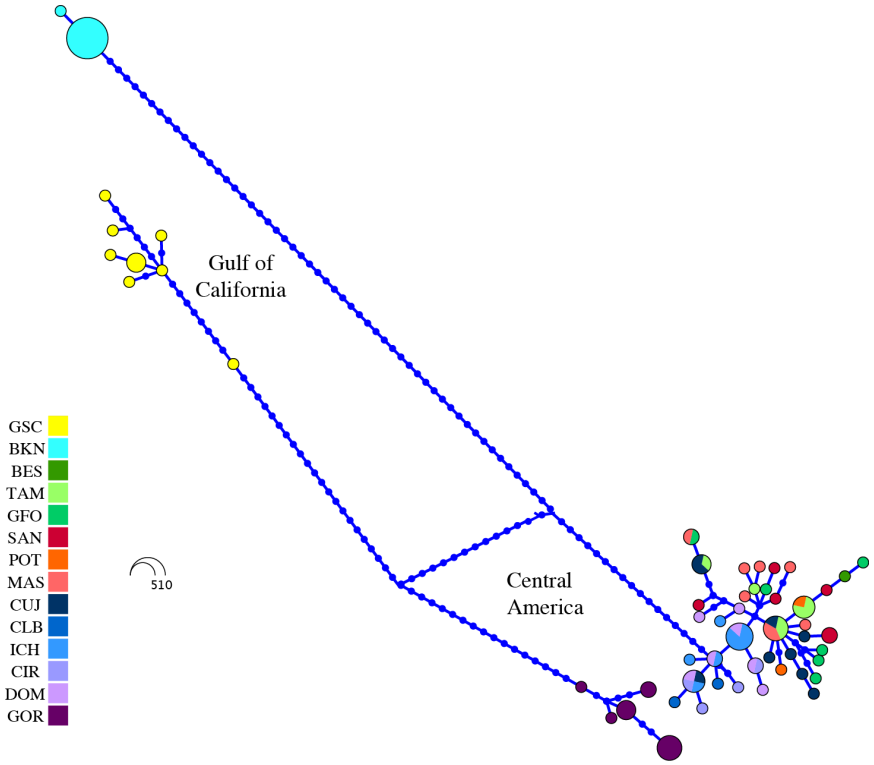

Figure 2. *Chrysaora* spp. minimum spanning haplotype network of the concatenate set COI and 16S. Blue dots represent unsampled haplotypes. The area of circles and circle sections are directly proportional to the number of individuals sharing the same haplotype sequence. Colors follow the legend of the last three letters of the locations (Table 1).

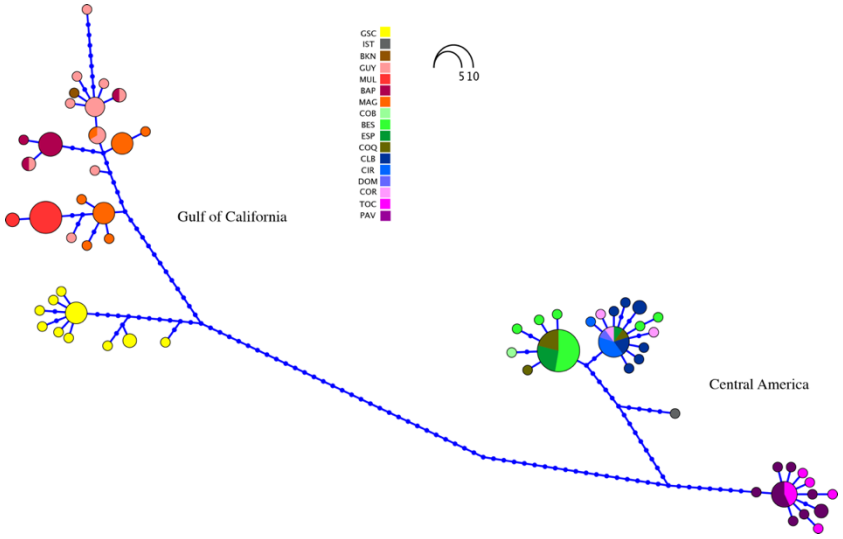

Figure 3. *Stomolophus* spp. minimum spanning haplotype network of the concatenate set COI and 16S. Blue dots represent unsampled haplotypes. The area of circles and circle sections are directly proportional to the number of individuals sharing the same haplotype sequence. Colors follow the legend of the last three letters of the locations (Table 1).

Chapter 5: Synthesis and Future Directions

Marine species richness is estimated at ~226,000; another ~0.5-0.7 million species may be undescribed (Appeltans et al. 2012). Imprecision in these estimates is ascribed to the so-called *taxonomic impediment*, a penurious understanding of taxonomy as a scientific discipline, and under-sampling in high diversity areas (e.g. tropics—Tropical Eastern Pacific; Will et al. 2005; de Carvalho et al. 2007; Wheeler 2009). It is becoming increasingly important to have accurate species descriptions and identifications in economic, environmental, and evolutionary contexts, especially of those species that play an important role in marine ecosystems (e.g. jellyfish; Omori and Nakano 2001; Lynam et al. 2005; Purcell et al. 2007; Hamner and Dawson 2009; Gibbons and Richardson 2013).

Scyphozoan jellyfish may have substantial effects on food-webs and marine communities through predation on zooplankton (e.g. eggs and larvae of fishes) and economically important invertebrates (Purcell 1991; 2003; Riascos et al. 2014). Additionally, in some cases, scyphomedusae are known to 'bloom' (rapid increase in biomass) in response to climate change, anthropogenic introduction, and reduction of larval fish populations due to overfishing (Graham et al. 2001; Purcell et al. 2007; Lucas and Dawson 2014). However, accurate species identification, including morphological and molecular studies have allowed clarification of biological aspects of invasive species, such as *Phyllorhiza*, *Aurelia* (Bolton and Graham 2004; Graham and Bayha 2007), and *Cassiopea* (Holland et al. 2004). The accurate identification of the jellyfish diversity help to determine places of origin of invasive species and recognition of pandemic ecological effects of invasive species (Hamner and Dawson 2009), this information will be important and relevant for management strategies.

The contribution includes the discovery of 25 new species and increasing the species richness for Discomedusae by 16%. Moreover, I demonstrated that the integration of multiple lines of evidence (e.g. molecular and morphological) resulted in a reliable method to identify and delimit the species, in comparison with the common approach to employ a single line of evidence (Wheeler 2005; Lohse 2009; Straehler-Pohl et al. 2011). The approaches applied in this work, provide the opportunity to standardize the taxonomic and systematic methods, that will lead to the revision of the systematics and taxonomy of Scyphozoa. The results from this assessment of the species richness provide the foundations for ecological and biogeographical hypotheses, which are necessary to contextualize the evolutionary patterns of marine taxa.

Once the correct species assignation and an accurate species richness estimation are met, they provide the necessary context to question the origin and processes that shape the biodiversity patterns. The timing of the processes and origins is estimated with a time-calibrated phylogeny of Discomedusae, which provides enough information to describe better the evolutionary relationships, divergence times, and question whether the radiation was associated with a diversification and if it involves a morphological innovation. The diversification of several families within the Discomedusae taxa occurred in parallel during the late Cretaceous and early Paleogene. Evolutionary radiation leads the diversification of taxa in the TEP (e.g. *Chrysaora, Stomolophus,* and *Aurelia*), by the closure of the Panamanian Isthmus. However, this radiation, might not include a morphological key innovation. This pattern follows hypotheses proposed for other Discomedusae taxa which

present a high number of cryptic species (Dawson 2003; 2005; Swift et al. 2016).

Knowing that an evolutionary radiation triggers the high diversity of some jellyfish taxa (*Chrysaora* and *Stomolophus*) in the Tropical Eastern Pacific (TEP), we were able to assess the biodiversity patterns on a microevolutionary scale. The biogeographic and phylogeographic patterns for the Tropical Eastern Pacific are described according to the distribution of bony fishes (Hastings 2000; Briggs and Bowen 2012), and often the biogeographic regionalization do not couple the phylogeographic patterns (Avise et al. 1987; Dawson 2001; Brante et al. 2012). Here we provide enough evidence to consider alternative hypotheses to describe the biodiversity patterns in the TEP, whether the oceanographic and geological factors cannot explain those patterns. I emphasize the importance of the life history and species ecology to understand population structure better.

This dissertation proves the necessity to increase taxonomic and systematic knowledge, other scientific disciplines (e.g. ecology and biogeography) are largely benefited and set the foundations to propose alterative hypothesis. In the future, it is necessary to increase the knowledge of the life history of the species (e.g. physiology, reproduction, feeding, diet), which can explain much of the biodiversity patterns at the regional scale. In addition, it is necessary to the taxonomic sampling in other marine hot spots, such as the Indo-Pacific, which will provide enough context to re-evaluate the evolutionary relationships of jellyfish on a global scale.

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