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Research Article

Genetic and morphological evidence of the presence of *Phyllorhiza punctata* in the southwestern Gulf of California (NE Pacific Ocean)

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Abstract

The jellyfish *Phyllorhiza punctata* is native to Australia and has been widely regarded as a successful invader. In the Mexican Pacific, this species has been recorded in the Gulf of California, specifically in La Paz Bay and the coast of the Baja California peninsula, but the origin of its introduction to the Mexican Pacific is unknown. There is a poor baseline of knowledge and monitoring of this species; therefore, the present study provides genetic and morphological evidence of the reappearance of this exotic species in La Paz Bay, Mexico. The taxonomic identification of the specimen was carried out based on the morphological descriptions, and its identity was corroborated with the BLAST search tool and phylogenetic analyses. The collected specimen showed genetic and morphological evidence indicating that it belongs to *P. punctata*, whose distribution has expanded to tropical and subtropical coastal waters of 16 countries. Therefore, this study allows the establishment of a baseline for future studies of this invasive jellyfish.

Key words: Medusozoa, Scyphozoa, invasive jellyfish, non-native species, La Paz Bay

Introduction

The Australian spotted jellyfish *Phyllorhiza punctata* von Lendenfeld, 1884 (Cnidaria, Scyphozoa, Rhizostomeae) is a species of the family Mastigiidae Stiasny, 1920, considered to be native to Australia (Graham et al. 2003; Haddad and Nogueira Jr. 2006), and widely recorded as alien species in tropical and subtropical waters worldwide (Moreira 1961; Bolton and Graham 2004; Deidun et al. 2017). This species has a metagenetic life cycle (Rippingale and Kelly 1995) with rapid growth rates of marked seasonality and is mainly characterized by the presence of two main body forms known as polyp (or scyphistoma) and medusa (Arai 1997; Haddad and Nogueira Jr. 2006).

The polyp stage is small (~ 4 mm, maximum height) (Schiariti et al. 2008), has benthic habits, and arises from the attachment of a planula (previous larval stage) to a substrate (Jufri 2001). Polyps exhibit asexual reproduction through an exclusive ciliated bud (characteristic of the order Rhizostomeae Cuvier, 1800), which for *P. punctata* is the planuloid bud type (Jufri 2001; Schiariti et al. 2014), or by strobilation (Jufri 2001). The asexual reproduction rate increases with the availability of food and low temperatures (18 °C for budding and > 20 °C for strobilation) (Jufri 2001). The mature polyp has 16 tentacles, and, under a long photoperiod condition, it produces a single ephyra through monodisc strobilation, thus giving rise to a juvenile medusa (Rippingale and Kelly 1995).

The medusa stage is conspicuous (2–470 mm, bell diameter) (Haddad and Nogueira Jr. 2006), has planktonic habits, and it can present zooxanthellae in the umbrella, which give medusae a yellowish-brown color and provide the necessary nutrients for their development (García 1990; De Souza et al. 2007). However, there are populations without zooxanthellae, and their diet changes from symbiotic to zooplanktivorous organisms (Galil et al. 2009; Graham et al. 2003). Mature medusae reproduce sexually under high-temperature conditions, with planulae produced after internal fertilization, a common feature of the order Rhizostomeae (Peach and Pitt 2005).

In subtropical waters, the medusa stage is absent during winter–spring (Haddad and Nogueira Jr. 2006), while in tropical waters different life stages are present indistinctly during any season (García 1990). Currently, *P. punctata* is distributed in the Mediterranean Sea, Atlantic, Indian, and Pacific Oceans, and it is hypothesized that its range expansion began with the merchant marine fleet of the 19th century (Galil et al. 1990; Silveira and Cornelius 2000; Bolton and Graham 2004; Zenetos et al. 2017).

There are two records of *P. punctata* from the Mexican Pacific. The first in the Gulf of California, and specifically in La Paz Bay, occurring in 2008 (Swift et al. 2016). The second record corresponds to the coast of the Baja California peninsula in 2017 (Gómez Daglio and Dawson 2017). The origin of its introduction to the Pacific coast of Mexico, including La Paz Bay is unknown, but the primary vector in other localities (e.g. United States and Gulf of Mexico) has been attributed to the transport of polyps in ballast water (Carlton and Geller 1993; Goldberg 1995; Okolodkov et al. 2007), ship hulls, or medusae drift through ocean currents (Larson and Arneson 1990). More than a decade after its first record, there is a poor baseline of knowledge and monitoring of *P. punctata* in the Mexican Pacific coast; therefore, the present study focuses on generating genetic and morphological evidence of the reappearance of this exotic species in La Paz Bay, Mexico, thereby allowing the establishment of a baseline for future studies of this invasive jellyfish.

Materials and methods

In July 2019, we found a medusa of *P. punctata* at the Punta Prieta terminal (24°13′15″N; 110°18′48″W), which is used for the unloading of oil and the port service to tow ship tanks (DOF 2019). This area is situated in La Paz Bay, a southwestern region of the Gulf of California, Mexico. The bay is delimited by the Espiritu Santo Archipelago and has a water exchange with the Gulf of California through two vias: the northern mouth and the southern San Lorenzo Channel (Obeso-Nieblas et al. 2004). The bay has a national and international maritime communication network made up of ports, terminals, and marinas (APIBCS 2020; SCT 2020).

Sea surface temperature, salinity, and dissolved oxygen concentration (DO) were recorded simultaneously *in situ* with a Horiba U-52 Multiparameter probe. The specimen collection was carried out with the help of a scoop net. The medusa was transported to the Marine Physiology Laboratory of the *Centro de Investigaciones Biológicas del Noroeste S. C.* (CIBNOR), where it was placed in an aquarium with filtered seawater and kept alive for 48 h. Subsequently, two fragments were excised, one from the terminal appendage (club) and another from the umbrellar margin, and preserved in 96% ethanol for extraction of genetic material. Then, the whole medusa was preserved in 4% formaldehyde solution in seawater.

The taxonomic identification of the specimen was carried out based on the morphological descriptions of Mayer (1910), Light (1921), Kramp (1961), Silveira and Cornelius (2000), and Jarms and Morandini (2019). Also, squash-type preparations were made to identify and measure (length × width) the nematocysts. A total of 10 measurements were performed by type of nematocyst from the exumbrella, umbrellar margin, oral arm, and terminal appendages. The nematocyst preparations were observed in a ZEISS optical microscope, and images were taken with a ZEISS AxioCam ICc camera and Zen software (edition 2.3 lite). Nematocyst types were identified according to Calder (1977), Östman (2000), Peach and Pitt (2005), and Yap and Ong (2012). The specimen was deposited in the Necton and Reef Ecology Laboratory at CIBNOR under the code PAZ_20190705. Furthermore, in order to know the species' distribution area, an exhaustive literature search in Google Scholar of global records was carried out, identifying locations within records where the species is native or exotic.

DNA extraction

Genomic DNA was extracted using a salt-extraction protocol (Sambrook et al. 1989) with RNase treatment. Briefly, lysis solution (200 µL) containing 10 mM Tris-HCl, pH 8.0; 100 mM EDTA, pH 8.0; 20 µg mL^{-1} RNase (Qiagen), 90 µL 10% SDS, and 20 uL proteinase K (15 µg/µL) were added to each sample. Samples were homogenized with the pistil and incubated at

55.0 °C for 2 h, mixing briefly every 15 min. Then, samples were centrifuged at 14,000 rpm for 5 min, and DNA was recovered from the supernatant fraction. Afterward, 350 μL chloroform/isoamyl alcohol was added, mixed by inversion, and centrifuged at 14,000 rpm for 3 min. Finally, DNA was precipitated with 700 μL cold absolute ethanol and centrifuged at 14,000 rpm for 10 min. The pellet was dried and eluted in 10 μL nuclease-free water. DNA was quantified at 260 nm by spectrophotometry.

PCR amplification

Two fragments of mitochondrial genes, 16S ribosomal DNA and COI, were amplified for terminal appendage and umbrellar margin tissues (n = 4) using the primer pairs C&B1-C&B2 for 16S (Cunningham and Buss 1993) and LCOI490-JJ2-HCOI2198-JJ2 for COI (Astrin et al. 2016), respectively. Amplification of PCR was performed in a Bio-Rad T-100 Thermal Cycler in a total volume of 12.5 μL including 6.25 μL GoTaq® Green Master Mix (Promega), 10.0 μM of each primer, and 10.0 ng of total genomic DNA. PCR conditions were as follows: 3 min at 94.0 °C followed by 5 cycles of denaturation at 94.0 °C for 30 s, annealing at 45.0 °C for 50 s, and extension at 72.0 °C for 1 min, and 30 cycles of denaturation at 94.0 °C for 30 s, annealing at 55.0 °C for 45 s, and extension at 72.0 °C for 1 min, with a final extension step at 72.0 °C for 10 min. PCR products were analyzed on 1% (w/v) Agarose/Synergel™ gels and visualized under UV light after staining with UView™ loading dye (Bio-Rad). PCR products were sequenced for both strands.

In silico analyses

Contiguous sequences were assembled and trimmed using Geneious 6.1.8 (Biomatters Ltd.). Once assembled, each sequence was analyzed using BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch; Altschul et al. 1990) to check for sequencing errors and/or contamination. Sequences were deposited in GenBank (Table 1).

The dataset for the phylogenetic analyses included the newly generated sequences and other sequences obtained from GenBank of *Phyllorhiza* spp. (ingroup) and Rhizostomeae (outgroup; *Lychnorhiza lucerna* Haeckel, 1880 was used as the root, because phylogenetically it is close to *Phyllorhiza* and following the proposal of Gómez Daglio and Dawson (2017)). The phylogenetic placement of the specimen we collected was inferred by concatenating 16S and COI sequences (all COI sequences were translated to amino acid sequences to check for stop codons using Geneious 6.1.8; genetic code: Invertebrate Mitochondrial). Similarity alignments were performed using Mafft v7.271 (Katoh and Standley 2013). The 16S sequences were aligned using the E-INS-i method and the COI sequences using the L-INS-i method. Sequences were concatenated using SequenceMatrix v1.8

Table 1. Terminal taxa ID, species and GenBank accession numbers for the specimens used in the combined dataset (16S+COI). GenBank accession numbers of sequences obtained in this study are bolded. * Chimeric terminal taxon.

Terminal taxa ID	Species	GenBank accession number	
		16S	COI
*Phyllorhiza pacifica*_1_Malaysia	*Phyllorhiza pacifica*	JN202945	JN203010
*Phyllorhiza pacifica*_2_Malaysia*	*Phyllorhiza pacifica*	JN184783	JN202999
*Phyllorhiza pacifica*_3_Malaysia*	*Phyllorhiza pacifica*	JN202946	JN203007
*Phyllorhiza punctata*_1_PBCP_Mexico	*Phyllorhiza punctata*	KY610625	KY611062
*Phyllorhiza punctata*_1_SYD_Australia*	*Phyllorhiza punctata*	KY610624	EU363342
*Phyllorhiza punctata*_1_WA_Australia	*Phyllorhiza punctata*	KU901024	KU900938
*Phyllorhiza punctata*_2_PBCP_Mexico	*Phyllorhiza punctata*	KY610626	KY611060
*Phyllorhiza punctata*_2_WA_Australia	*Phyllorhiza punctata*	KU901025	KU900939
*Phyllorhiza punctata*_3_PBCP_Mexico	*Phyllorhiza punctata*	KY610627	KY611061
*Phyllorhiza punctata*_GCA_Mexico	*Phyllorhiza punctata*	**MT902932**	**MT904380**
*Phyllorhiza punctata*_R_GCA_Mexico	*Phyllorhiza punctata*	**MT902935**	**MT899235**
*Phyllorhiza punctata*_USA/PuertoRico*	*Phyllorhiza punctata*	JX393272	MF742376
Cassiopea andromeda	*Cassiopea andromeda*	KY610609	KY610551
Cassiopea xamachana	*Cassiopea xamachana*	KY610615	KY610557
Lychnorhiza lucerna	*Lychnorhiza lucerna*	KY610592	KY611035
Mastigias papua	*Mastigias papua*	KU901021	KU901397
Mastigias sp.	*Mastigias* sp.	KU900968	KU900912
Versuriga anadyomene	*Versuriga anadyomene*	KX904852	KX904853

(Vaidya et al. 2010). Most taxa have sequences for all markers, but some are chimeras (Table 1). Nucleotide sequences were submitted to phylogenetic analysis under Maximum Likelihood (ML) in IQ-TREE multicore v1.6.10 (Nguyen et al. 2015) with the optimal partition scheme and substitution models selected by ModelFinder (Kalyaanamoorthy et al. 2017) (Supplementary material Table S1). Ultrafast bootstrap and the Shimodaira-Hasegawa approximate likelihood-ratio test (SH-aLRT) were measured from 1000 pseudoreplicates. Individual markers phylogenies (16S, COI) were also inferred using IQ-TREE (Tables S1, S2) and plotted alongside locality maps using the R package "phytools" (Revell 2012) (Table S3).

We also performed the concatenated phylogenetic analysis within the Bayesian Inference (BI) framework in MrBayes v3.2.6 (Ronquist et al. 2012) with the optimal partition scheme and substitution models selected by PartitionFinder2 (Lanfear et al. 2016) (Table S1). The analysis was run for five million generations, and two independent runs were generated, each consisting of four chains (one cold and three heated). Trees were sampled every 1,000 generations. Convergence of the runs was assessed by examining the standard deviation of split frequencies, which fell below 0.01, and checking the effective sample size values of each parameter in Tracer v1.7.1 (Rambaut et al. 2018). After checking for convergence and stationarity, the first 10% of the sampled trees were excluded as burn-in and a consensus tree was built; nodal numbers represent the posterior probabilities. PartitionFinder and MrBayes analyses were run on CIPRES Scientific Gateway (Miller et al. 2010).

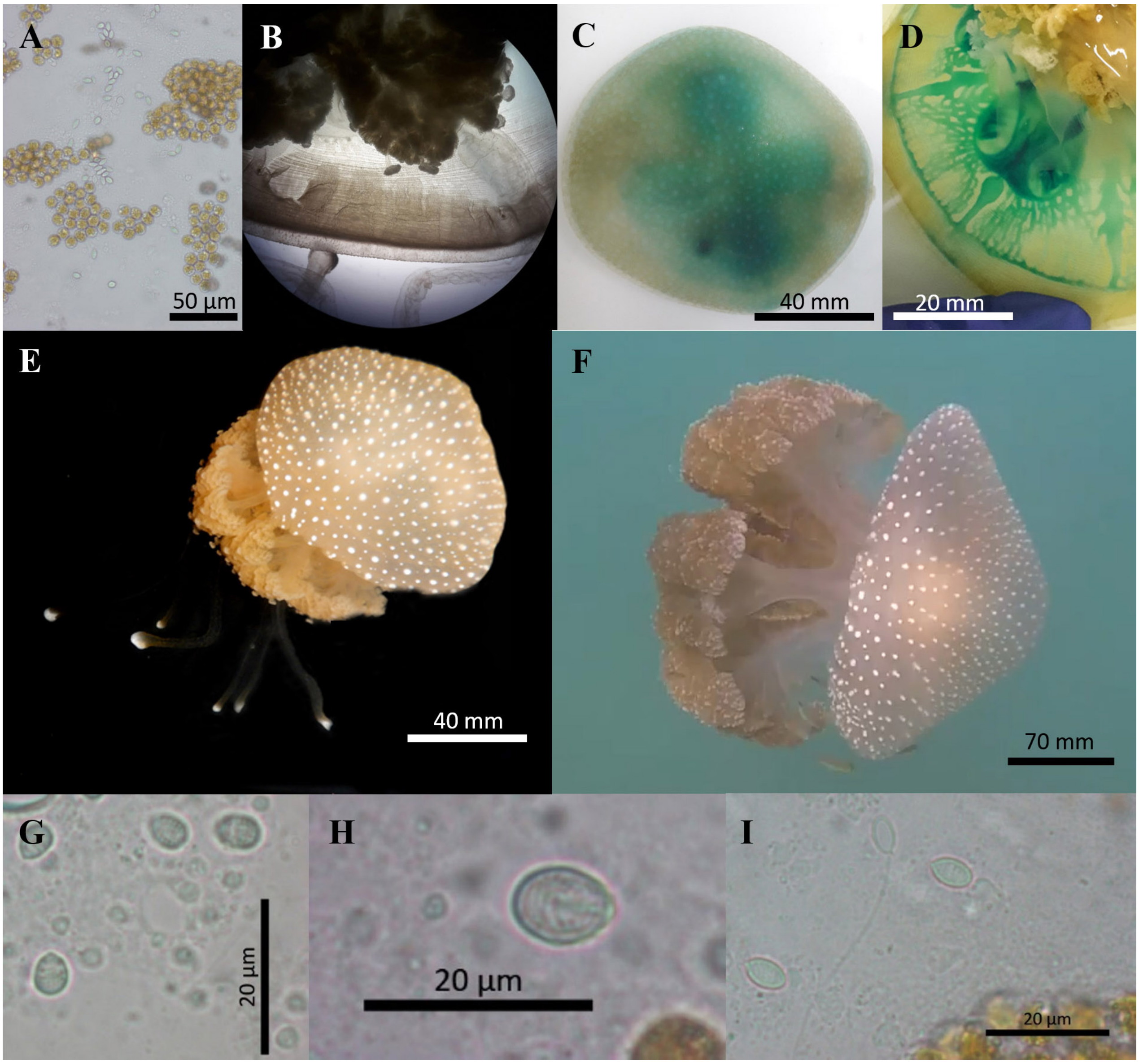

Figure 1. *Phyllorhiza punctata* von Lendenfeld, 1884. (A–D) Morphology, (A) zooxanthellae from oral arms; (B) claviform intermediate filaments from oral arms; (C) central cruciform stomach, seen from exumbrella; (D) oral view, showing gastrovascular canal system with connecting interradial canals, ring canal, and stomach; (E) specimen collected in the present study; (F) specimen observed in La Paz Bay, Mexico; (G–I) Nematocysts, (G) Oval-shaped holotrichous isorrhizae; (H) Rhopaloids; (I) Holotrichous isorhiza. Photographs by (A–D, G–I) Estrada-González ME and Rosales Catalán L, (E) Mendoza-Becerril MA, (F) Jay Gittens.

Results

Morphological description

The collected specimen was brownish in color due to the presence of zooxanthellae (Figure 1A). Umbrella hemispherical, diameter 98.0 mm. Granulated exumbrellar surface with warts and numerous white spots. Eighty marginal and broad rounded lappets, ten per octant, connected by a fine membrane, and arranged as follows: four simple, four double, and two rhopaliar. Eight rhopalia with two rhopaliar lappets, each. Octagonal oral disc with six long whip-like transparent appendages, length 80.0 mm; six three-winged mouth arms, J-shaped, length 55.0 mm, with abundant claviform intermediate filaments (Figure 1B) and each arm with a terminal

Table 2. Size of nematocyst types of *Phyllorhiza punctata.* L, length; W, width, measures in µm.

Region / Nematocyst type	Holotrichous isorhiza, oval shaped		Rhopaloid		Holotrichous isorhiza		Microbasic mastigophore	
	L	W	L	W	L	W	L	W
Exumbrella	2.4–3.1	2.1–2.8	4.3–5.9	3.8–5.3	4.7–6.7	2.9–4.2	–	–
Bell margin	2.3–2.7	2.0–2.5	4.4–5.2	3.9–4.3	4.4–5.8	2.9–3.4	–	–
Oral arm	2.5–3.4	2.2–3.0	4.6–5.6	4.1–4.9	5.5–7.5	2.8–4.7	7.6–10.6	2.3–3.3
Terminal appendages	2.4–2.7	2.1–2.6	4.9–5.6	4.0–4.8	4.9–7.4	2.4–4.0	8.7–10.8	2.5–3.1

white club-shaped appendage (Figure 1E–F). Four oval subgenital ostia, length 34.0 mm. Gastrovascular system consisting of a central cruciform stomach (Figure 1C), oral disc, diameter 44.0 mm. Eight rhopaliar canals (four perradial and four interradial), ring canal present with 9–11 canal roots per octant, connecting with interradial canals, ring canal, and stomach but not to perradial canals (Figure 1D). Circular musculature interrupted over radial canals.

Nematocysts

Three nematocyst types were identified (Table 2). Three nematocyst types were observed in all the structures analyzed: oval-shaped holotrichous isorhiza (Figure 1G), rhopaloids (Figure 1H), and holotrichous isorhiza (Figure 1I). Oval-shaped holotrichous isorhiza nematocyst was the smallest (length 2.3 × diam. 2.0 µm), while microbasic mastigophore nematocyst was the largest (length 10.7 × diam. 3.1 µm).

Distribution

Phyllorhiza punctata shows an expansion of its range outside its native distribution area (SE Australia) to tropical and subtropical coastal waters (40°N to 34°S) of 16 countries belonging to the Mediterranean Sea, Atlantic, Indian, and Pacific Oceans (Table 3). Therefore, of all specific localities recorded, 39 present an exotic registry (Table 3). The *P. punctata* specimen we collected was recorded at 28.7 °C, 34.6 PPT, and a DO of 4.6 mg/L. Previous records worldwide establish that estuaries and coastal lagoons with the incidence of marine traffic are the habitats in which this medusa was mostly seen, perhaps driven by its tolerance to wide ranges of temperature (11.0–39.7 °C), salinity (10.0–109.3 PPT), and DO (4.6–9.2 mg/L) (Table 3).

Genetics

Analyses of the newly generated sequences using the BLAST search tool indicate that these sequences match with other specimens of *P. punctata* recorded in GenBank (Table 1), and the ML and BI analyses also support this hypothesis.

Based on the ML tree, our specimen nested in the *P. punctata* clade (Figure 2), which includes other specimens from Mexico (the Pacific coast of the Baja California peninsula), the Eastern Atlantic (USA/Puerto Rico)

Table 3. Worldwide records of *Phyllorhiza punctata* von Lendenfeld, 1884 and temperature, salinity, and dissolved oxygen of sites where the species was recorded. ♦Native locality.

Country	Location	Ocean or Sea	Temperature (°C)	Salinity (PPT)	Dissolved oxygen (mg/L)	References
Australia	Fremantle Harbor	Indian				Swift et al. 2016
	Sydney♦	Pacific				von Lendenfeld 1884; Stiasny 1924; Stiasny 1926; Daryanabard and Dawson 2008; Bayha et al. 2010; Gómez-Daglio and Dawson 2017
	Port Jackson♦	Pacific				Galil et al. 1990
	Peel-Harvey Estuary	Indian	–	30.0–35.0		Rippingale and Kelly 1995
Brazil	Cananéia	Atlantic				Tronolone et al. 2002; Morandini et al. 2005
	Fortaleza	Atlantic				Morandini et al. 2006
	Guaratuba	Atlantic				De Souza et al. 2007
	(1) Guarujá, (2) Ubatuba	Atlantic				Morandini et al. 2005
	Itanhaém	Atlantic				Moreira 1961
	Santos	Atlantic				Morandini et al. 2005; Carneiro et al. 2011
	Southern Brazil	Atlantic				Haddad and Nogueira 2006
Greece	Igoumenitsa Harbor	Mediterranean				Abed-Navandi and Kikinger 2007
	Lefkada	Mediterranean	13.0–30.0	34.0–37.0		
Israel	Ashdod	Mediterranean	17.0–31.5	39.5		Galil et al. 2009
	Beit Yanai	Mediterranean				Galil et al. 1990
Italy	Cala Sauraccia	Mediterranean				Boero et al. 2009
Lebanon	Sarafand	Mediterranean				Dailianis et al. 2016
Libya	Misratah	Mediterranean	16.0–17.0			Rizgalla and Crocetta 2020
Malaysia	Klang Strait	Indo-Pacific				Rizman-Idid et al. 2016
	Matang	Indo-Pacific				
	Pengkalan Parit Jawa	Indo-Pacific				
	Palau	Indo-Pacific				
	Teluk	Indo-Pacific				
Malta	Island of Malta	Mediterranean	15.3	102.3		Deidun et al. 2017
Mexico	(1) Agonales, (2) Canal Principal	Pacific				Gómez Daglio and Dawson 2017
	La Paz Bay	Pacific	28.7*	34.6*	4.6*	Swift et al. 2016; This study*
	Laguna Mandinga	Atlantic	33.0	34.0		Ocaña-Luna et al. 2010
Philippines	Danajon Bank	Pacific				Heeger et al. 1992
Puerto Rico	Cabo Rojo	Atlantic	24.0–32.0	10.0–35.0		García 1990
	No data	Atlantic	24.0–31.0	19.0–35.0		García and Durbin 1993
Syria	Latakia Port	Mediterranean	25.6–39.7	38.6–38.0		Durgham 2011
Turkey	Koycegiz-Dalyan	Mediterranean	21.0–28.0	21.0–30.0	8.7–9.2	Gülşahin and Tarkan 2012
	Iskenderun Bay	Mediterranean	25.7	38.6		Cevik et al. 2011
Tunisia	South of Bizerte	Mediterranean				Gueroun et al. 2015
	Gulf of Gabès	Mediterranean				Stamouli et al. 2017
USA	Galveston Bay	Pacific	24.0	22.0		Barord et al. 2007
	Mississippi	Pacific				Graham et al. 2003
	San Diego Bay	Pacific				Larson and Arneson 1990
	(1) St. Catherines, (2) Wassaw	Pacific	17.0–29.0	34.0		Verity et al. 2011
	Hawaii	Pacific				Doty 1961; Clarke and Aeby 1998

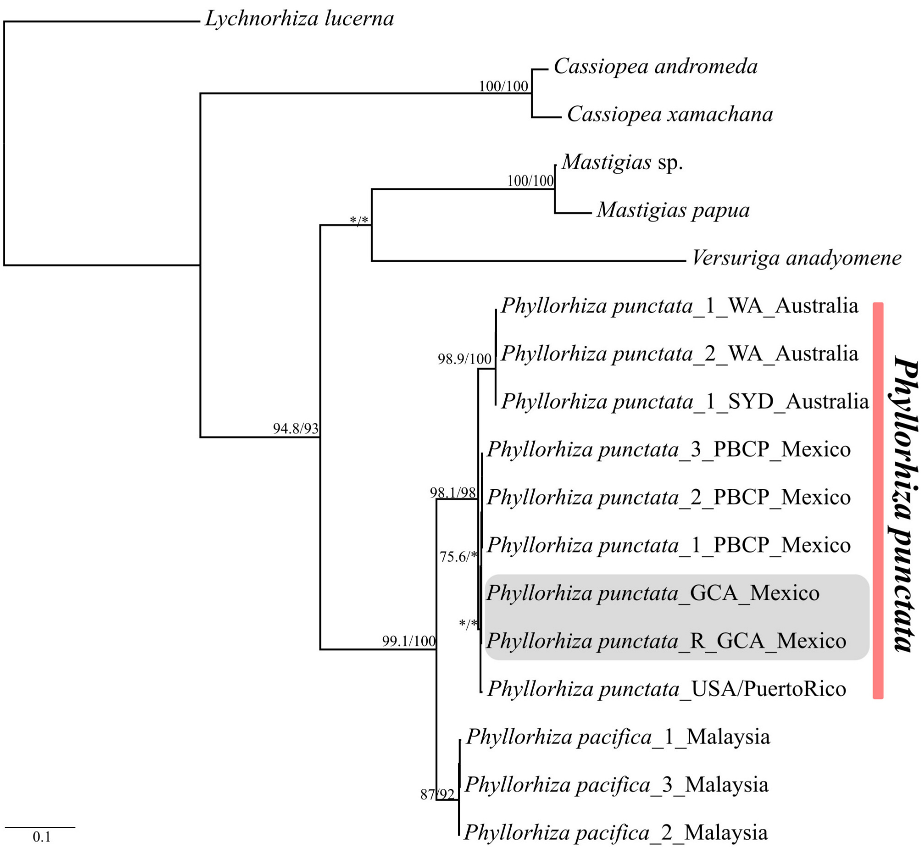

Figure 2. IQ-TREE optimal tree (log likelihood = −4633.546) derived from the combined dataset (16S + COI). Nodal numbers represent, as in figure order, the ultrafast bootstrap and the Shimodaira-Hasegawa approximate likelihood-ratio test (* = less than 70). The newly sequenced specimen is highlighted in gray ("*Phyllorhiza punctata*_R_GCA_Mexico" and "*Phyllorhiza punctata*_GCA_Mexico" represent two samples of the same specimen). GCA: Gulf of California, PBCP: Pacific coast of the Baja California peninsula, SYD: Sydney, USA: United States of America, WA: Western Australia. Tables 1 and SM1 contains further details on the data used to reconstruct this phylogeny.

and Australia (Figure 2). In the *P. punctata* clade it is visible that all the specimens registered in Mexico form unique clade. This Mexican clade was recovered as the sister group of the terminal taxa from the Eastern Atlantic Region, albeit with low ultrafast bootstrap and SH-aLRT values (~ 60; Figure 2). Mexican + Eastern Atlantic *Phyllorhiza* clade conforms to the sister group of the clade that contains specimens from Australia (Figure 2). *Phyllorhiza punctata* was recovered as the sister group of *Phyllorhiza pacifica* (*sensu* Gómez Daglio: MN395673, KY610622–23). The same topology was found in the BI analysis (Figure S4).

With a larger number of terminal taxa, the COI phylogeny corroborates the patterns of distribution found in the concatenated phylogeny and additionally shows the presence of *P. pacifica* in Indonesia (Figures 3A, S5). In addition to the concatenated and COI phylogenies, the 16S phylogeny shows the presence of *P. punctata* in Israel and Thailand, and the presence of *P. pacifica* in Thailand, and shows *Phyllorhiza* sp. (*sensu* Dong et al. 2019; from southern Yellow Sea, China) as the sister species of *P. punctata* (Figures 3B, S5).

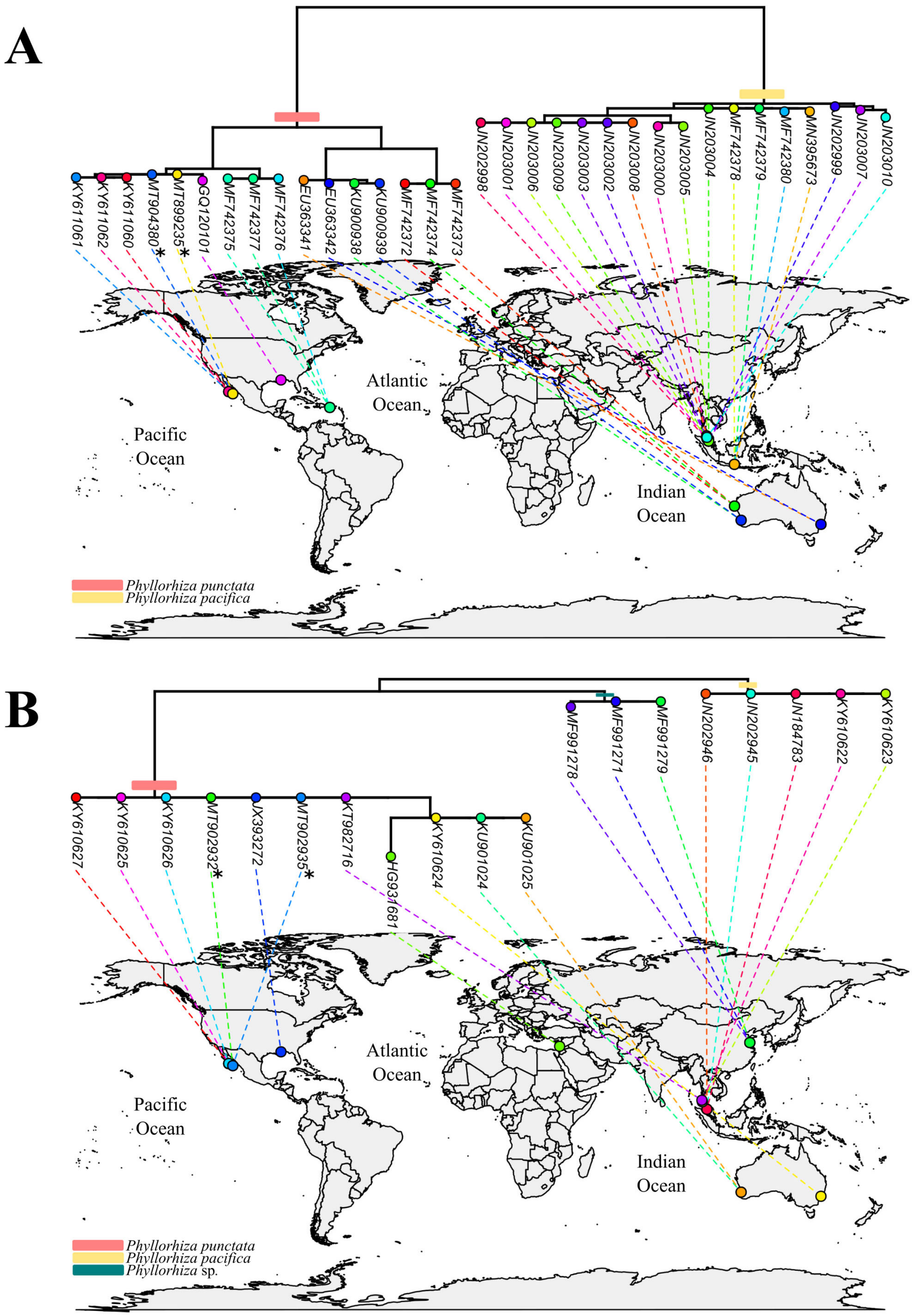

Figure 3. Phylogenetic trees and locality maps for 51 *Phyllorhiza* specimens. Dashed lines connect each specimen to its collection locality. Nodal horizontal colored lines (pink, yellow, green) denote the species identified. These trees are shown without the outgroup, supplementary figure SM5 contains the whole phylogenies (ingroup + outgroup and the ultrafast bootstrap and the Shimodaira-Hasegawa approximate likelihood-ratio test values) and the supplementary tables SM1–2 contain further details on data used to reconstruct COI and 16S phylogenies. A – COI phylogeny and locality map for 33 *Phyllorhiza* specimens. B – 16S phylogeny and locality map for 18 *Phyllorhiza* specimens. *, sequences from this study.

Discussion

In the present study, macroscopic and microscopic (Figure 1) morphological characteristics of *P. punctata* were integrated, expanding the information available for this species. In the microscopic characters, the three types of nematocysts reported here coincide with those reported for the species, according to Peach and Pitt (2005). Furthermore, genetic analyses based on a combined dataset corroborated the taxonomic identity of *P. punctata* in the southwestern Gulf of California.

Like other scyphozoans, *P. punctata* is known to exhibit variable morphology within its populations (Bolton and Graham 2004), which, together with ambiguous descriptions and the absence of discriminating characters for the medusa, hampers its taxonomic identification (Miller et al. 2010; Yap and Ong 2012; Gómez Daglio and Dawson 2017). To avoid taxonomic misidentification, are employed morphological characters, incorporating stages of its life history (Gómez Daglio and Dawson 2017), microscopic characters (Östman 2000), and molecular analysis (Gómez Daglio and Dawson 2017). In this context, to help identify new records, it is advisable to maintain a solid taxonomic and molecular base of this species recognized as invasive.

According to molecular analyses and based on available records, it is possible that the species entered into the Gulf of California from a nearby region, such as Magdalena Bay, Baja California Sur, Mexico (Gómez Daglio and Dawson 2017) or Mission Bay, California, USA (Larson and Arneson 1990), having possibly first been introduced from Australia into some of these regions, however more sequences are necessary to confirm this.

Records of its distribution indicate a wide tolerance to temperature (11.0–39.7 °C, Durgham 2011; Gueroun et al. 2015) and salinity (21.0–102.3 PPT, Gülşahin and Tarkan 2012; Deidun et al. 2017), so it is considered an eurythermic and euryhaline species. Its seasonal presence in La Paz Bay can be associated with the metagenetic life cycle. An increase in density due to asexual reproduction has been observed in scyphozoan polyps at a temperature of 20.0 °C (Schiariti et al. 2014). Moreover, the medusa stage's presence occurs between April and August (García 1990; Graham et al. 2003; Haddad and Nogueira Jr. 2006), which coincides with the stage and temporality reported in this study.

The environmental conditions of temperature (28.7 °C) and salinity (34.6 PPT) in the Gulf of California could favor the establishment of *P. punctata*, which are very similar to those reported by Ocaña-Luna et al. (2010) in the Gulf of Mexico. However, in La Paz Bay, no large aggregations have been observed, as reported in invaded sites: Georgia, USA, with greater abundance in spring and summer (Verity et al. 2011); Lake Borgne, Louisiana, USA, with a large aggregation of medusae between May and September (Graham et al. 2003). The reason why there is not yet a bloom of *Phyllorhiza* in La Paz Bay is still unknown. However, it seems that the presence of the species has been somewhat constant since it was registered for the first time in 2008 (Swift et al. 2016) and now in 2019 (our specimen and another reported by the biologist Jay Gittens – *personal communication*, November 21, 2019 – Figure 1F).

The consequences of blooms have a direct impact on the fisheries industry (Daryanabard and Dawson 2008; Graham et al. 2003). An example of this is the blooms registered in Georgia, USA (Verity et al. 2011), Veracruz, Mexico (Ocaña-Luna et al. 2010), and Brazil (Silveira and Cornelius 2000; Haddad and Nogueira Jr. 2006), where they have directly affected zooplankton, as well as the physicochemical properties of water by increasing viscosity (Carneiro et al. 2011). Also, the presence of non-indigenous species poses a risk to the trophic structure due to competition for food and its impact on fisheries (Verity et al. 2011) and native species (Kleitou et al. 2021). Therefore, detecting exotic species in the Gulf of California is essential to preventing the establishment of non-native species (Tovar-Hernández et al. 2014).

Given the specie's erratic presence (Boero et al. 2009), the scientific monitoring of medusae in La Paz Bay with citizen science strategies should be continued. For example, another specimen of *P. punctata* was observed during tourist activities (Figure 1F) (*personal communication*, November 21, 2019). Moreover, greater efforts for the study, monitoring, and collection of specimens should be made. Likewise, there should be development of phylogeographic works, which provide greater certainty about the dispersal routes of this invasive species, as has been evidenced for other gelatinous organisms (cf. Ghabooli et al. 2013).

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Authors' contribution

LRC – investigation and data collection, data analysis and interpretation, writing, review, editing manuscript; MCEG – investigation and data collection, data analysis and interpretation, writing, review, editing manuscript; CRP – data analysis and interpretation, writing and review manuscript; MARS – data collection; EGM – data analysis and interpretation, writing and review manuscript; ACM – data interpretation, review, editing manuscript; MAMB – research conceptualization, investigation and data collection, data analysis and interpretation, writing, review, editing manuscript.

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Supplementary material

The following supplementary material is available for this article:

Table S1. Number of terminals, alignments length, partition schemes, and best-fit models for the phylogenetic analyses.
Table S2. Species, GenBank accession numbers and terminal taxa ID for the specimens used in the single marker phylogenies (16S, COI).
Table S3. GenBank accession numbers and latitude and longitude information.
Figure S1. BI tree determined from concatenated sequences of 16S and COI genes.
Figure S2. IQ-TREE gene trees for 16S (log likelihood = −2115.109) and COI (log likelihood = −3035.668).
Appendix 1. References for Table S2.

This material is available as part of online article from:
http://www.reabic.net/aquaticinvasions/2021/Supplements/AI_2021_Rosales-Catalan_etal_SupplementaryMaterial.pdf