diversity

MDPI

Article

Crambe insularis sp. nov. (Crambeidae: Poecilosclerida) a New Crambeid from the Eastern Tropical North Pacific: Morphological, Molecular and Ontogenetic Approach

Eric Bautista-Guerrero [1,*], José Luis Carballo [2,3] and Alma Paola Rodríguez-Troncoso [1]

[1] Laboratorio de Ecología Marina, Centro de Investigaciones Costeras, Centro Universitario de la Costa, Universidad de Guadalajara, Av. Universidad No. 203, Puerto Vallarta 48280, Jalisco, Mexico
[2] Instituto de Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México (Unidad Académica Mazatlán), Av. Joel Montes Camaren S/N, Mazatlán 82000, Sinaloa, Mexico; jlcarballo@ola.icmyl.unam.mx
[3] Laboratorio de Biología Marina, Departamento de Zoología, Universidad de Sevilla, Avenida Reina Mercedes, 641012 Sevilla, Spain
* Correspondence: eric.bautista0177@academicos.udg.mx

Abstract: Specimens of Poecilosclerida taxa, collected from an insular coral community on the Pacific coast of Mexico, were identified as members of the family Crambeidae Lévi, 1963. They were associated with larvae and rhagon phases by using morphological characters, the nucleotide relationship and genetic divergence of three independent loci, two mitochondrial (COI and 16S rDNA) and one ribosomal (28S rDNA C3–C5). *Crambe insularis* sp. nov. differs from the general skeletal architecture in the genus *Crambe* Vosmaer, 1880, by its reduced spiculation defined by the presence of ectosomal and choanosomal monactinal megascleres, and the absence of microscleres. Bayesian and Maximum–Likelihood analyses of three loci supported the clustering of larvae, rhagon and adult sponge, all closely related to Mediterranean *Crambe crambe* (type species of the genus *Crambe*), and with South American *Crambe* species (*C. chilensis*, *C. maldonadoi* and *C. amarilla*) as sister species. The larva of *C. insularis* sp. nov. corresponded to the typical parenchymella larvae poecilosclerid species but with the presence of subtylostyles and styles. Ontogenetic process about the larval and rhagon of this new crambeid are provided. The morphological characters and molecular affinities of *Crambe insularis* sp. nov. are similar to *Monanchora* genus, and the implications are further discussed. This is the first taxonomic and molecular study with an integrative approach that includes other diagnostic features such as larval and rhagon development for the description of new species in Porifera.

Keywords: Porifera; molecular marker; marine biodiversity; 16S rRNA; COI; crambeid

Citation: Bautista-Guerrero, E.; Carballo, J.L.; Rodríguez-Troncoso, A.P. *Crambe insularis* sp. nov. (Crambeidae: Poecilosclerida) a New Crambeid from the Eastern Tropical North Pacific: Morphological, Molecular and Ontogenetic Approach. *Diversity* **2023**, *15*, 608. https://doi.org/10.3390/d15050608

Academic Editor: Stuart Kininmonth

Received: 17 March 2023
Revised: 18 April 2023
Accepted: 18 April 2023
Published: 29 April 2023

1. Introduction

The family Crambeidae Lévi, 1963 is characterized by the presence of a complex skeleton with a full complement of spicules: ectosomal and choanosomal monactinal megascleres also as complementary microscleres, and if present, the inclusion of a wide variety of acanthostyles, unguiferate–anchorate or monodentate chelae, sigmas, spined microrhabds, microxeas and astro- or sphaeroclone desmas [1,2]. Currently, four genera are considered valid: *Discorhabdella* Dendy, 1924, *Litochela* Burton, 1929, *Crambe* Vosmaer, 1880 and *Monanchora* Carter, 1883 [3]. Specifically, the genera *Crambe* and *Monanchora* share a similar external morphology, skeletal architecture, two categories of megascleres [4], and a wide repertoire of secondary metabolites, i.e., crambescidins [5,6]; but, only *Crambe* present hypersilicified microscleres (e.g., asterose desmas) [7–9] which is a diagnostic characteristic that allocate species in either of these two genera [1]. However, the taxonomical and diagnostic features may vary widely and the microscleres (desmas, isochelae, and microxeas) may or may not be present in response to silica concentration in the water column, such as previously observed for *Crambe crambe* and *Crambe acuta*, which shows an intraspecific skeletal variability [10–13].

Currently, the genus *Crambe* is integrated by eleven species distributed along the Indian [9,11,14], Atlantic [15–18] and Pacific Ocean [8,19]; but only nine are recognized as valid [8,9,11,19,20]. The Pacific region is widely regarded as the primary centre of diversity for *Crambe* [19], with the Temperate South America province being particularly significant. This is due to the recent identification of three *Crambe* species in this area that possess a full complement of spicules, including astro/sphaeroclones desmas and additional microscleres: *C. chilensis* Esteves, Lôbo-Hajdu & Hajdu, 2007, *C. amarilla* Esteves, Lôbo-Hajdu & Hajdu, 2007, and *C. maldonadoi* Esteves, Lôbo-Hajdu & Hajdu, 2007. Furthermore, the Tropical Eastern Pacific is home to an endemic species of *Crambe*, *Crambe panamensis* Maldonado, Carmona, van Soest & Pomponi, 2001 [8]. Despite the fact that these species have a limited distribution and that suitable locations such as oceanic islands and archipelagos along Eastern Tropical North Pacific have yet to be fully explored, no species of the *Crambe* have been recorded in the rocky and coral reefs of the Mexican Pacific despite extensive efforts to study sponge diversity in this area [21–25]. This study represents the first record of a new species of a *Crambe* Vosmaer, 1880 with no development of microscleres and distributed in a remote oceanic island from the Mexican Pacific coast. In addition to the taxonomical characterization of adult phase, the larva metamorphosis, and details of the rhagon morphology are also included. Additionally, the systematic position of this new species was assessed within the Poecilosclerida Order using molecular data of three independent loci (COI, 16S and 28S) in an integrative approach.

2. Materials and Methods

2.1. Sampling

Adults and larvae collection: specimens described herein were manually collected by SCUBA diving in a coral community of Isabel Island, Mexico; 21°52′30″ N, 105°54′54″ W (Figure 1), between, July–December 2010 and July–December 2011. These specimens were photographed in situ using an underwater camera (GoPro) before collection and later fixed in ethanol 96% at room temperature and transported to the laboratory for morphological and molecular characterization. Larvae in full planktonic conditions were collected with three horizontal hauls performed at noon using two 22 cm diameter plankton nets with an 80 µm mesh size. The nets were towed manually and driven nearby the coral community in a depth range of 1–7 m. The total water volume filtered during each haul was ~4.5 m^3, and then transferred to sealed plastic containers (3 L), and filtered with a 100 µm mesh net, and kept in Petri dishes with filtered seawater for observation under a dissecting microscope (Stemi 508–Zeiss®, Oberkochen, Germany) to check for the presence of sponge larvae. All collected larvae were individually documented, considering the external morphology, size and swimming movements. Some larvae maintained in observation, were settled within 12 h and post-settlement stages (rhagon) were monitored by 72 h and photo–documented using a Canon Elph PowerShot 180 camera (Canon Inc., Tokyo, Japan).

2.2. Morphological Description

Spicule and skeleton preparation for light and electron microscopy (SEM) of adults were prepared following the techniques described by Boury–Esnault and Rützler [26]. Additionally, spicules of adults and larvae were obtained by dissolving a small piece of each specimen and complete larvae in 1% nitric acid, after which the residue was three–times rinsed with distiller water, and once with 96% ethanol. The spicules were air–dried on microscopic slides and fixed with mounting medium (Entellan™). Twenty–five spicules of each specimen were randomly chosen and measured using a Canon EOS Rebel T8i camera mounted on a Zeiss Stemi 508 stereomicroscope. The minimum, maximum, and mean values for each spicule category were calculated. Holotypes and paratypes were deposited in the "Colección de Esponjas del Pacífico Mexicano" (LEB–ICML–UNAM).

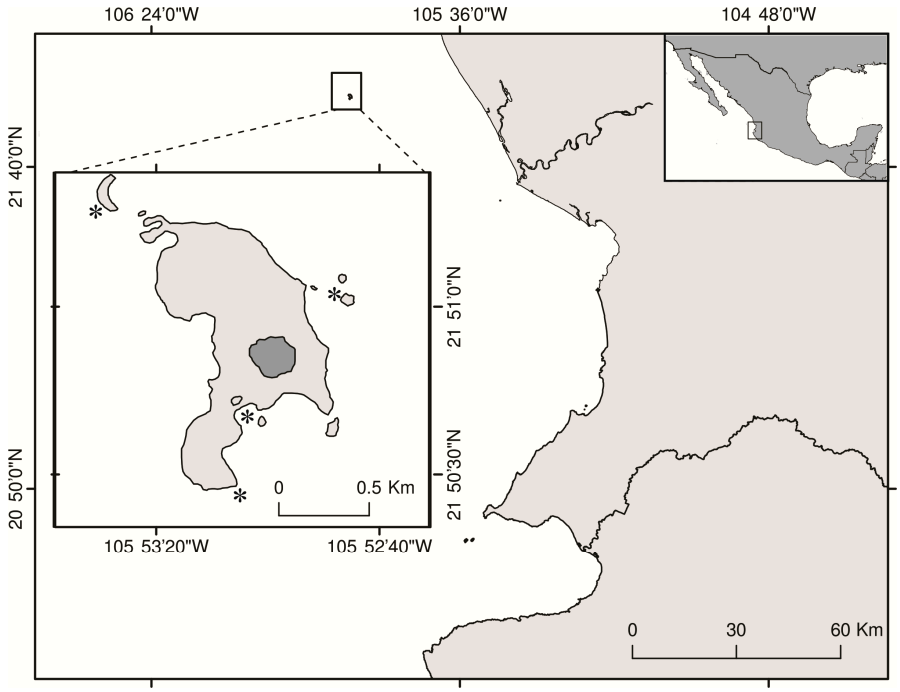

Figure 1. *Crambe insularis* sp. nov. collection sites in the Eastern Tropical Pacific. The black asterisk indicates the collecting site in the coral communities of Isabel Island, Mexico.

2.3. Molecular Identification

Whole genomic DNA was extracted from adult tissue and larvae, and rhagon specimens that were ethanol-preserved, using PROMEGA Wizard®Genomic DNA Purification Kit. A partial sequence of the cytochrome c oxidase subunit 1 gene (COI, ~650 bp) was amplified using degenerate primers: LCOI490 5-′GGTCAACAAATCATAAAGAYATYGG-3′ and HCOI21908 5′-TAAACTTCAGGGTGACCAAARAAYCA-3′ [27]. The thermocycling protocol PCR conditions were as follows: one cycle at 94 °C for 5 min; 35 cycles at 94 °C for 1 min; 48 °C for 1 min, 72 °C for 1 min, and a final one cycle at 72 °C for 5 min, rendering a product of ~650 bp. One set of primers was synthetized to amplify the gen 28S rRNA region D3–D5 (~990 bp), using the primers NL3R 5′-TTTACCGGACATTCAACCCT-3′ NL4F 5′-GACCCGAAAGATGGTGAACTA′ [28]. Thermocycler conditions were as follows: one cycle at 94 °C × 5 min initial denaturation; 35 cycles at 94 °C × 1 min; 50 °C × 1 min, 72 °C × 1 min, and a final extension of one cycle at 72 °C × 5 min. Finally, a fragment of ~630 bp of the mitochondrial 16S rRNA gene was amplified with primers diplo-rnl-f1 TCGACTGTTTACCAAAAACATAGC and diplo-rnlr1 AATTCAACATCGAGGTSG-GAAAC [29]. The thermocycler conditions were as follows: one cycle at 94 °C × 5 min initial denaturation; 35 cycles at 94 °C × 1 min; 50 °C × 1 min, 72 °C × 1 min, and a final extension of one cycle at 72 °C × 5 min. Each amplification reaction master mix contained 6.1 µL nuclease-free H_2O, 1.8 µL $MgCl_2$, 0.70 µL dNTPs, 2.5 µL 10× PCR buffer, 0.15 µL of primer forward and reverse, 0.10 µL Taq polymerase (Promega), and 1 µL of a 1:100 dilution of the DNA template. PCR products were visualized on 2%-TAE (Tris-acetate-EDTA) agarose gel electrophoresis, and all positive amplicons were purified using the Wizard SV Gel and PCR Clean–Up System (Promega). PCR clean products of 100 ng μL^{-1} and 25 µL were sequenced in both directions (forward and reverse) by external service at Macrogen Inc., Seoul, Korea. Forward and reverse sequences were manually edited using the Geneious Prime 2022.2., to obtain consensus sequences for each sample. Sequences were submitted to GenBank® from NCBI (National Center for Biotechnology Information), with the following accession numbers: OQ788445 (Larva), COI: OQ788444 (Rhagon), OQ788446 (Adult); 28S: OQ789402 (Larva), OQ789400 (Rhagon), OQ789401 (Adult) 16S: OQ789394 (Larva), OQ789395 (Rhagon), OQ789393 (Adult).

2.4. Sequence Alignment and Phylogenetic Analysis

Sequences from GenBank® of species belonging to the Order Poecilosclerida were downloaded and aligned with the sequences of the new species using MEGA v. X [30]. GTR + G (COI), Kimura 2 parameter + G + I (28S) and Tamura 3 parameter + G + I (16S), were the best fitting evolutionary substitution model for each selected region of the three datasets according to the JModelTest 2.0 Software [31]. Maximum Likelihood (ML) analyses were executed using the MEGA v. X with 1000 bootstrap replicates [30]. Bayesian Inference was estimated using Mr. Bayes 3.2.1 [32], with two Markov Chain Monte Carlo (MCMC) simulations of over 400,000 generations carried out with sampling every 1000 generations. The appropriate burnin value was determined by examining the standard deviation of split frequencies dropped below 0.01. A 50% majority rule consensus tree was constructed from all generations sampled after the burnin and after discarding 25% of the original samples. Sequences of Polymastida, Suberitida, Axinellida and Trachycladida Orders were used as outgroups to root the tree. The trees from Mr. Bayes (BI) and those from MEGA (ML) are shown in posterior probability (PP) and bootstrap proportions (BP) in the node of each tree, respectively. Finally, genetic distances with Kimura's two parameter model (K2P) among congeneric species of *Crambe* and mean intergeneric genetic distances among genera (*Crambe* vs *Monanchora*) were obtained using MEGA v. X [30].

3. Results

3.1. Taxonomic Descriptions

SYSTEMATICS
Class DEMOSPONGIAE
Subclass HETEROSCLEROMORPHA
Order Poecilosclerida
Family Crambeidae Lévi 1963
Genus *Crambe* Vosmaer 1880
Diagnosis

Thin, encrusting, or thicker tubercular sponges; surface translucent, slightly hispid, occasionally conulose, detachable, with clear veinal channel pattern in life. Sponges are characterized by a complex skeleton with a full complement of spicules: ectosomal styles–subtylostyles (occasionally strongyles), which may fan out into a tangential layer; choanosomal stylote with plumose organization, interlocking with desmas (often absent) forming a semi–rigid basal skeleton. Complementary microscleres are pseudastrose desmoid spicules (often absent) anchorate isochelae, spined microxeas [1].

Type species *Crambe crambe* (Schmidt, 1862).
Diagnosis

Thinly encrusting *Crambe,* red–orange. Surface microscopically hispid, with evident radiating and scattered whitish subdermal channels. Skeleton plumose, with ectosomal (subtylo) styles thick up to 200 µm long and choanosomal styles/tylotes fusiform up to 245 µm long. Microscleres are completely absent [1].

Crambe insularis sp. nov.
Type material

Holotype: LEB–ICML–UNAM (3187), 24/04/2007, Las Monas (Isla Isabel, Nayarit) 5 m (21°50′59″ N, 105°52′46″ W). Paratypes: LEB–ICML–UNAM (3188), Las Monas (Isla Isabel, Nayarit) 5 m (21°50′59″ N, 105°52′46″ W). Paratypes: LEB–ICML–UNAM (3189), Las Monas (Isla Isabel, Nayarit) 5 m (21°50′59″ N, 105°52′46″ W).

Description

External morphology: encrusting to cushion-shaped sponge 5–15 cm long and 8–23 mm thick on rocks (Figure 2A) and shell oysters (Figure 2B). Color in vivo bright red or orange; pale in preservation. Surface hispid, consistency fleshy, and easy to tear. Subdermal channels are red–translucent and converge radially towards the oscula 0.6–1.2 mm diameter that are slightly raised, an evident from the rest of the body, scattered over the ectosome.

(Figure 2C). Ostia 60–90 µm in diameter, visible only under optical magnification, and distributed over the sponge surface (Figure 2D).

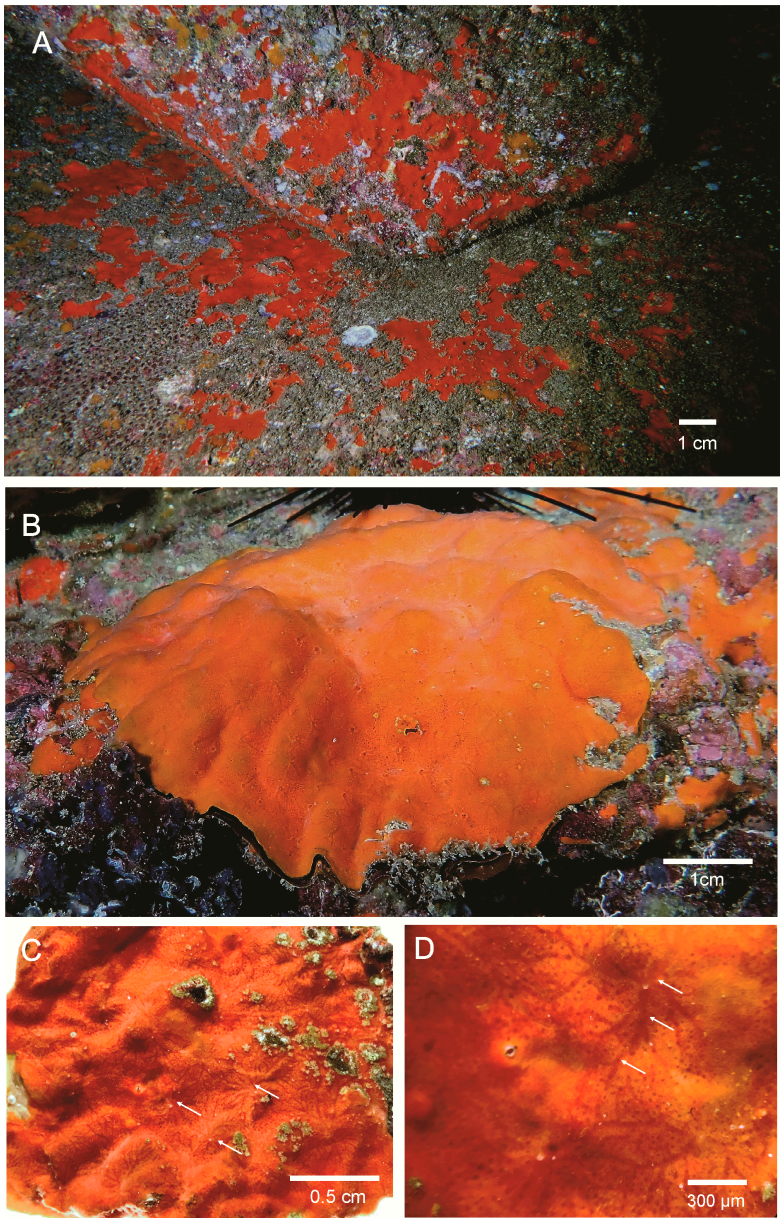

Figure 2. External morphology of *Crambe insularis* sp. nov. (**A**) Thinly encrusting specimens in situ were growing on rocks. (**B**) Encrusting specimen growing on shell oyster. (**C**) Details of oscules distributed with radial pattern (white arrows). (**D**) Surface magnification showing the scattered ostia and subdermal channels (white arrows).

Skeleton: the ectosomal skeleton was epithelium–like with a 40–60 µm thick layer composed of brushes of subtylostyles and single spicules dispersed with no apparent organization (Figure 3A). The choanosomal skeleton has a plumose organization arranged as multispicular tracts interconnected by uni-, bi or paucispicular tracts (30 µm) (Figure 3B) resulting in irregular meshes (40–60 µm).

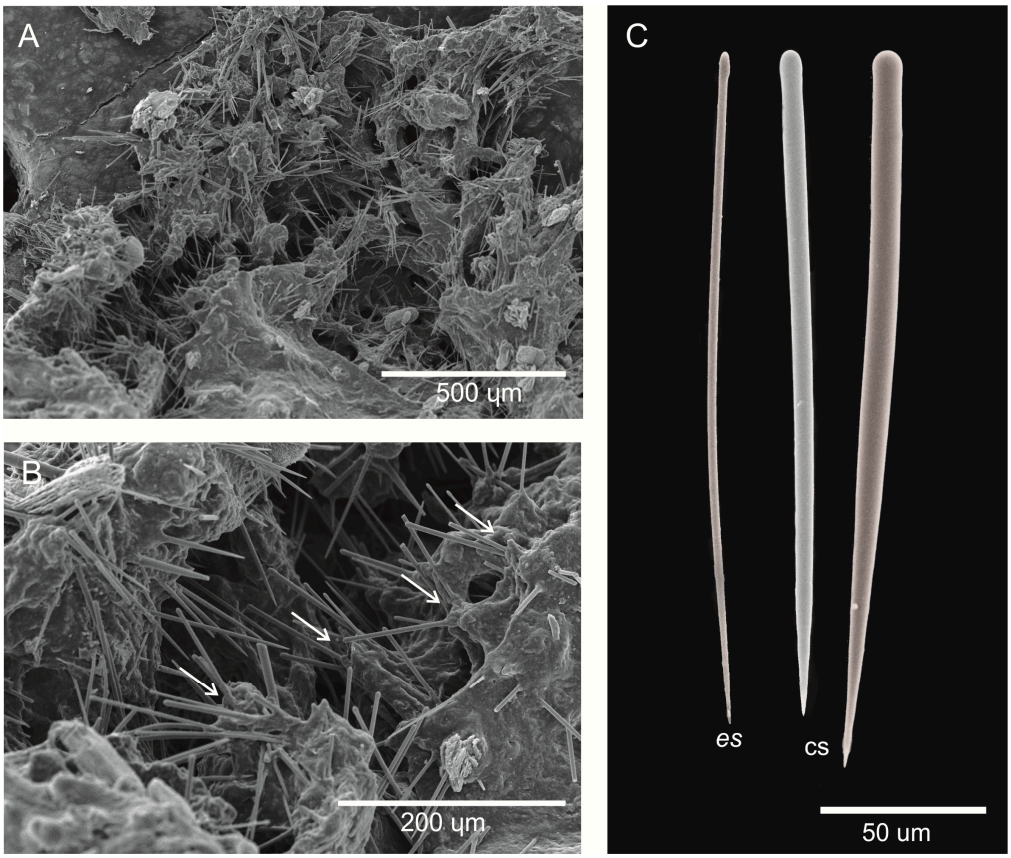

Figure 3. SEM image of the skeleton of *Crambe insularis* sp. nov. (**A**) Hispid surface details of the holotype (**B**) choanosomal plumoreticulate skeleton (the arrow points to the multispicular spicule tracts); (**C**) spicules (cs) choanosomal subtylostyle; (es) ectosomal subtylostyle.

Spicules. Megascleres (Figure 3C): ectosomal (subtylo) styles were thick, slightly curved near the base (with or without telescopic apices), with a sharp point: 155–264 × 2.5–7.5 µm. Choanosomal styles or modified to tylotes fusiform, straight, or curved and sharp pointed: 178–325 × 5.0–10.5 µm (Table 1). Microscleres were completely absent.

Ecology

Crambe insularis sp. nov. is very common in coral communities from Isabel Island and its distribution apparently is restricted to this island territory. Specimens can be found in caves or shady places encrusting rocky zones and overgrowing oyster shell. Its bathymetric distribution range 4 m to 13 m deep.

Distribution. Recorded known only from type locality, Isabel Island, eastern tropical North Pacific.

Etymology

Named *insularis* refers to the island territory where the new specie was collected.

Remarks: *Crambe insularis* sp. nov. is characterized by the occurrence of only ectosomal subtylostyles, choanosomal styles/tylotes, but the complete absence of microscleres.

In the south–east Pacific, there are four species of *Crambe* bearing a full complement of spicules composed by megascleres: ectosomal and choanosomal (sub)(tylo) styles and a wide variety of microscleres; anchorate isochelae, sphaeroclones as asterose desmas and spined microxeas [8,19]. Morphologically, *C. insularis* sp. nov. differs from *C. chilensis*, *C. maldonadoi* and *C. amarilla*, in its smaller choanosomal styles, and lack of anchorate isochelae, asterose/sphaeroclones desmas and spined microxeas, which are absent in the new species here described (Table 2). *Crambe panamensis* Maldonado, Carmona, van Soest & Pomponi, 2001 is the closest species to *C. insularis* sp. nov. in possessing similar size–categories spicules of the choanosomal tylostyles (275–483 µm × 12–16 µm). However,

beside the wide variety of growth stages of astroclone desmas and anchorated isochelae, which are totally absent in *Crambe insularis* sp. nov., the Panamanian species has smaller ectosomal subtylostyles with a well–marked tyle and point with feeble spines [8]. The new species has morphological and skeletal features similar to some members of the genus *Monanchora*, but it lacks the sigmoid chelea and anchorate isochelae, which are the most important diagnostic characters in *Monanchora* species from the Eastern and Central Pacific [33–36].

Table 1. Morphometric measurements of spicules of *Crambe insularis* sp. nov. Values: minimum—(mean)—maximum in micrometers (μm) (length × width); *n* = 25.

Material Examined	Choanosomal Styles/Tylotes	Ectosomal Subtylostyles
Holotype LEB–ICML–(3187)	200–(241.4)–275 × 5.0–(6.8)–10	155–(190.1)–225 × 2.5–(2.9)–5
Paratype LEB–ICML–(3188)	178–(245.5)–325 × 5.0–(9.4)–10.5	175–(221.6)–264 × 3.5–(6.5)–6
Paratype LEB–ICML–(3189)	198–(231.5)–287 × 5.0–(6.9)–9.8	169–(210.6)–256 × 3.6–(5.7)–7.5

Table 2. Morphometric characters of the valid species in the genus *Crambe.* Measurements of spicules size range (length × width or diameter in desma) are given in micrometers (μm).

Species	Locality	Ectosomal (Sub) (Tylo) Styles		Choanosomal (Acantho) (Tylo) Styles		Desmas	Anchorate Isochelea	Additional Microscleres	Skeletal Arrangement	References
***Crambe insularis* sp. nov.**	Pacific coast of Mexico	211–411	5.5–6.8	275–483	12–16	Absent	Absent	Absent	Plumose	[*]
C. crambe Schmidt, 1862	Mediterranean Sea	316–281	7	423–539	11–17	Poorly developed Astroclone or Sphaeroclone	Unguiferate with reduced shortened three-toothed I: 28–43 II: 8–11	Absent	Plumose	[1,13]
C. erecta Pulitzer-Finali, 1993	Adriatic Sea	230–340	6–9	350–500	14–38	Astroclone 120–180	Anchorate I: 33 II:16–19	Absent	Plumose	[1,8,12, 37]
C. paramensis Maldonado, Carmona, van Soest & Pomponi, 2001	Pacific coast of Panama	117–208	2.5–3.5	180–300	6.5–12	Astroclone 26–65	Spatulate Three/five-toothed 20–22	Absent	Hymedesmioid	[1,8]
C. acuata Lévi, 1958	Red Sea and Aldabra southern Madagascar	180–220	2–7	120–175	8–10	Astroclone 15–75	Three/five-toothed I: 20–22 II: 8–10	Spined microxeas 16–90, rare raphides	–	[11,15]
C. acuata Lévi, 1958	South Atlantic, East coasts of Africa	267.7	10.5	315	10.75	Astro/sphaeroclone 105	Anchorate 35	Spined microxeas, rare raphides	–	[15,16]
C. chilensis Esteves et al.,2007	South–east Pacific, coast of Chile	186–387	4–14	245–833	10–42	Astroclone (4–7 arms) 50–232	Spatuliferous–five–toothed 21–37	Spined microxeas. 30–67	Hymedesmioid	[19]
C. amarilla Esteves et al.,2007	South–east Pacific, coast of Chile	189–402	4–10	315–877	9–24	Astroclone (6 arms) 43–178	Unguiferate/Spatuliferous five–toothed 21–33	Rare or even absent spined microxeas 45–58, Raphides	Hymedesmioid	[19]
C. maldonadoi Esteves et al.,2007	South–east Pacific, coast of Chile	400–785	4–15	255–769	12–34	Sphaeroclone (5–6 arms) 94–211	Spatuliferous–three/five–toothed 22–31	Spined microxeas 22–43	Hymedesmioid	[19]
C. tailliezi Vacelet & Boury–Esnault, 1982	Western Mediterranean	Microspiny point 205–330	2.5–4	350–460	6–8	Astro/sphaeroclone 25–120	Unguiferate three toothed 23–26	Spined microxeas 7.8–15.6 Desmoi	–	[38]
C. tuberosa Maldonado & Benito, 1991	Mediterranean Alboran Island	87–104	4.6–5.8	Tuberose tyle I: 500–700 II: 150–300	I: 20–30 II: 5–10	Sphaeroclone (4–7 arms) 94–134	Five–toothed isoanchorae 27–30	Special asters	Hymedesmioid	[18]

Present paper [*]; Not reported (–).

3.2. Molecular Phylogenetic Analyses and Pairwise Genetics Distance Inferences

The genetic distance (K2P) calculated from ribosomal (28S) and mitochondrial (COI and 16S) genes within congeneric species of *Crambe* and among intergeneric species of *Monanchora* are resumed in Table 3. The lowest interspecies genetic distances values computed of 28S sequences of *Crambe* were observed between *Crambe insularis* sp. nov. and *Crambe crambe* (0.014 ± 0.004) from the Western Mediterranean. On the other hand,

C. insularis sp. nov. showed major distances (0.017 ± 0.004) from all South American *Crambe* species (*C. chilensis*, *C. maldonadoi* and *C. amarilla*), which were genetically identical among them (0.000 ± 0.000). The same pattern was recorded with interspecies genetic distances values computed of 16S sequences, *C. insularis* sp. nov. is genetically closer to *Crambe crambe* (0.006 ± 0.003), but the new species showed a greater genetic distance with respect to all South American *Crambe* species (*C. chilensis*, *C. maldonadoi* and *C. amarilla*) which were almost genetically identical among them (0.002 ± 0.002) with this molecular marker.

Interspecies KP2 genetic distances (Table 3) using COI sequences proved that *C. insularis* sp. nov. is genetically closer to Mediterranean *Crambe crambe* (0.005 ± 0.003) and distant to species of the genus *Monanchora* (0.025–0.043 ± 0.006–0.0.08). Comparing between two genera, the pairwise genetic distances average among species of *Crambe* and *Monanchora* were statistically different for the 28S gen ($K2P_{Crambe}$ = 0.0158 ± 0.001, $KP2_{Monanchora}$ = 0.0197 ± 0.002; $t = 3.419$, $p = 0.005$) and COI ($K2P_{Crambe}$ = 0.006 ± 0.005, $KP2_{Monanchora}$ = 0.0304 ± 0.004; $t = 9.669$, $p < 0.001$), but no statistical differences between *Crambe* and *Monanchora* using 16S gene was found ($K2P_{Crambe}$ = 0.080 ± 0.085, $K2P_{Monanchora}$ = 0.026 ± 0.001; $t = 1.502$ $p = 0.021$).

Table 3. Pairwise genetic distance (K2P) matrix among selected *Crambe* species using mitochondrial (COI and 16S) and nuclear (28S rRNA) genes. Values of genetic distances (K2P) are in bold (lower left diagonal) and standard error estimated(s) (upper right diagonal).

		28s						
		1	2	3	4	5	6	7
1	*Crambe insularis* sp. nov.	–	0.004	0.005	0.004	0.004	0.005	0.005
2	*Crambe amarilla*	**0.017**	–	0.000	0.000	0.004	0.005	0.004
3	*Crambe maldonadoi*	**0.017**	**0.000**	–	0.000	0.005	0.005	0.004
4	*Crambe chilensis*	**0.017**	**0.000**	**0.000**	–	0.004	0.005	0.004
5	*Crambe crambe*	**0.014**	**0.012**	**0.012**	**0.012**	–	0.004	0.005
6	*Monanchora arbuscula*	**0.022**	**0.025**	**0.025**	**0.025**	**0.017**	–	0.004
7	*Monanchora clathrata*	**0.024**	**0.020**	**0.020**	**0.020**	**0.018**	**0.006**	–
		16S						
		1	2	3	4	5	6	7
1	*Crambe insularis* sp. nov.	–	0.021	0.021	0.021	0.003	0.007	0.006
2	*Crambe amarilla*	**0.156**	–	0.000	0.002	0.022	0.023	0.022
3	*Crambe maldonadoi*	**0.156**	**0.000**	–	0.002	0.022	0.023	0.022
4	*Crambe chilensis*	**0.159**	**0.002**	**0.002**	–	0.022	0.023	0.023
5	*Crambe crambe*	**0.006**	**0.160**	**0.160**	**0.163**	–	0.007	0.005
6	*Monanchora arbuscula*	**0.027**	**0.172**	**0.172**	**0.175**	**0.026**	–	0.007
7	*Monanchora coccinea*	**0.015**	**0.166**	**0.166**	**0.169**	**0.013**	**0.020**	–
		COI						
		1	2	3	4	5	6	7	8
1	*Crambe insularis* sp. nov.	–	0.003	0.005	0.008	0.007	0.007	0.006	0.007
2	*Crambe crambe*	**0.005**	–	0.004	0.008	0.007	0.006	0.005	0.007
3	*Crambe* sp.	**0.016**	**0.011**	–	0.008	0.006	0.006	0.005	0.006
4	*Monanchora* sp.	**0.043**	**0.037**	**0.036**	–	0.004	0.004	0.008	0.005
5	*Monanchora uningulata*	**0.032**	**0.027**	**0.026**	**0.013**	–	0.000	0.007	0.004
6	*Monanchora clathrata*	**0.032**	**0.027**	**0.025**	**0.012**	**0.000**	–	0.007	0.004
7	*Monanchora arbuscula*	**0.025**	**0.020**	**0.018**	**0.047**	**0.036**	**0.036**	–	0.007
8	*Monanchora quuadrangulata*	**0.034**	**0.029**	**0.024**	**0.021**	**0.011**	**0.011**	**0.035**	–

The systematics position of *Crambe insularis* sp. nov. within the Poecilosclerida Order was determined using three independent loci: one nuclear (28S rRNA D3–D5) and two mitochondrial (COI mDNA, 16S rRNA). All phylogenetic reconstructions with both BI and ML methods are congruent with moderate to high resolution for a family-level relationship, retrieved sequences of *Crambe* + *Monanchora* (Family Crambeidae) in a well-supported monophyletic clade (Figures 4–6).

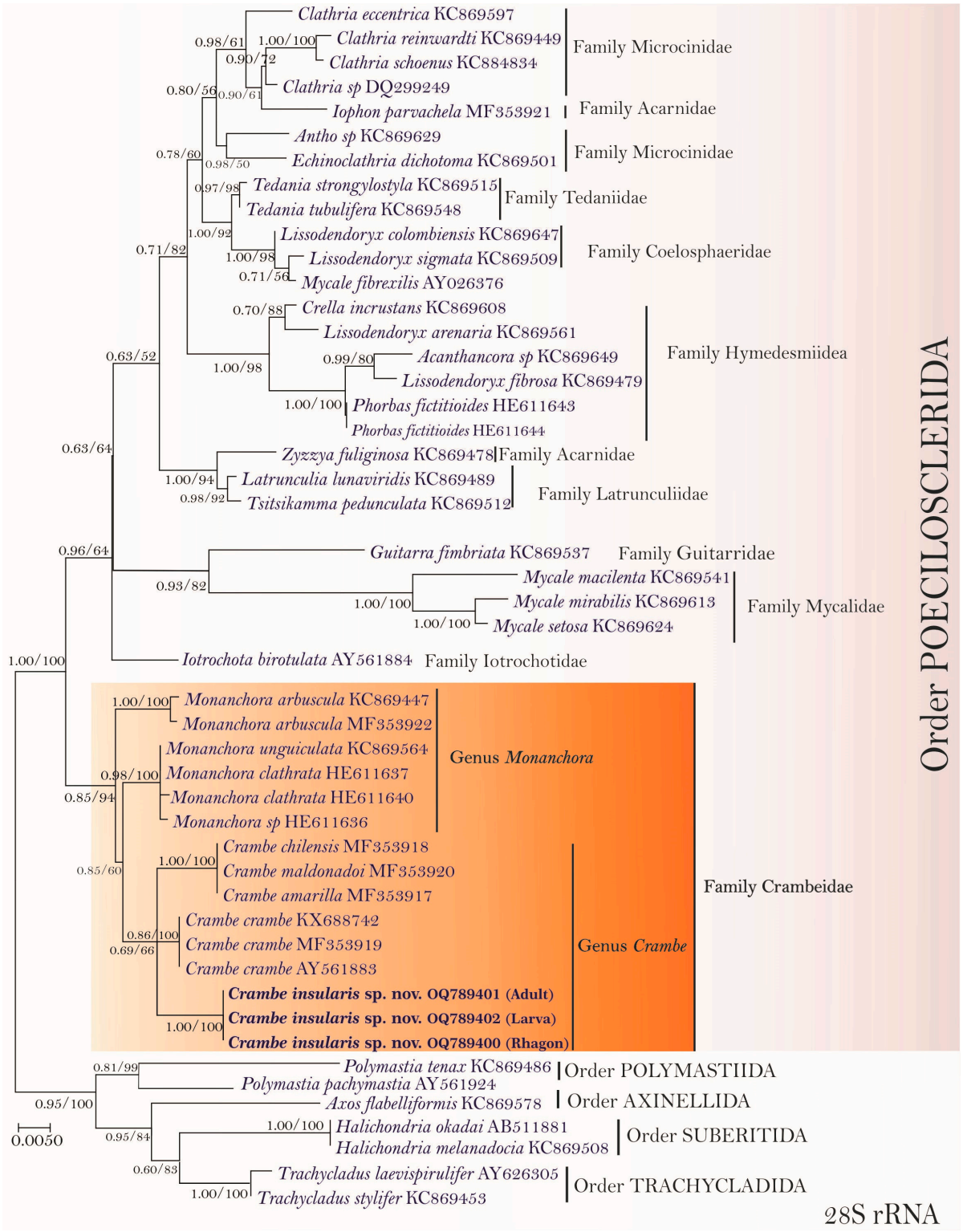

Figure 4. Molecular phylogenetic analyses showing the systematic positions of *Crambe insularis* sp. nov. resulted from the analysis of partial sequences from the large ribosomal subunit 28S D3–D5. The topology was inferred from the results of the Bayesian inference and Maximum likelihood method. The values of each node correspond to posterior probability and bootstrap values of major clades that were reconstructed with BI and ML strict consensus tree, respectively (BI/ML). The dark grey boxes correspond to the families belonging to Order Poecilosclerida, the orange box to species of the family Crambeidae, and light grey box to sequences of species used as outgroup to root the tree. GenBank accession numbers are given after each taxa name. New species sequences are in bold.

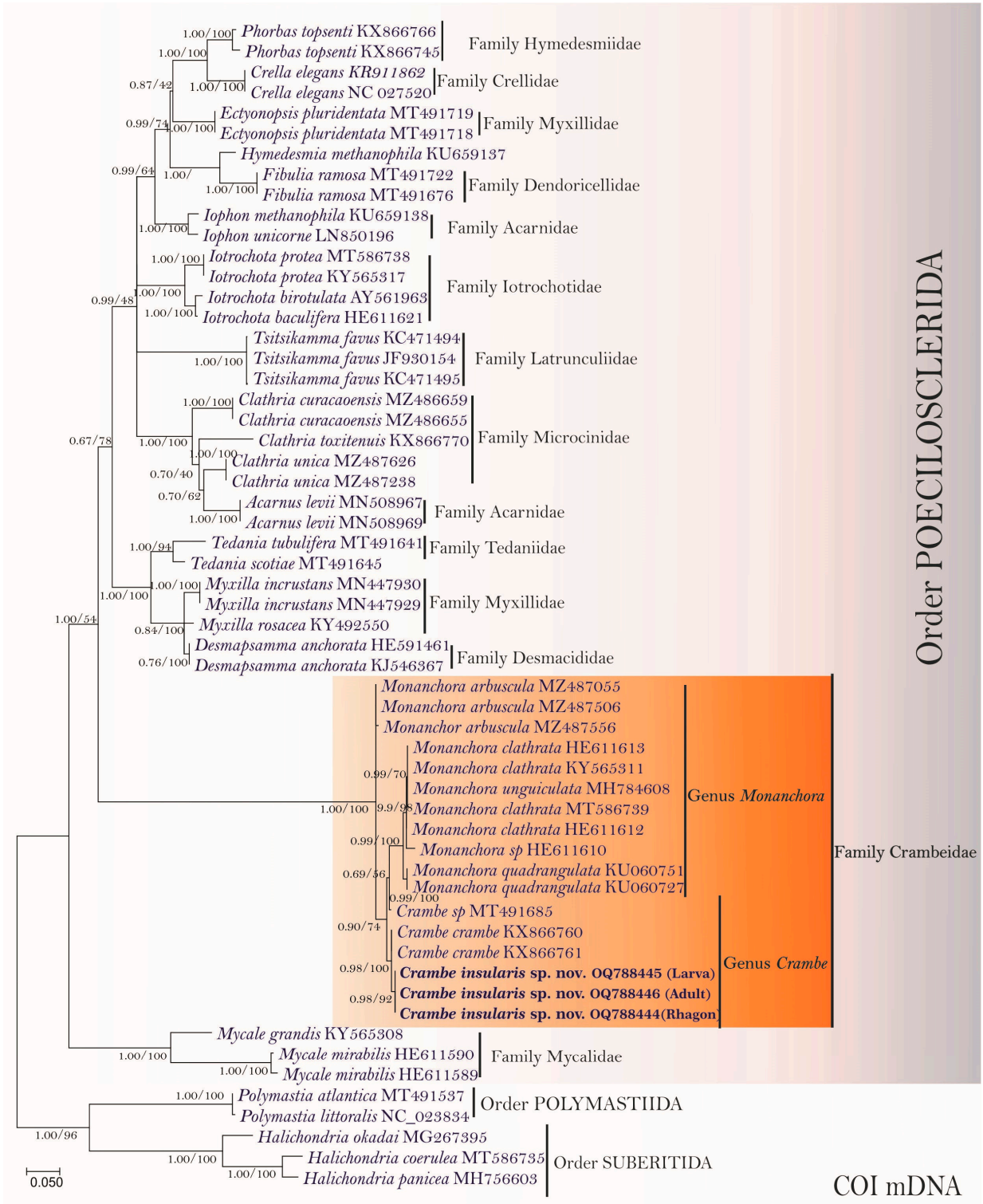

Figure 5. Molecular phylogenetic analyses showing the systematics position of *Crambe insularis* sp. nov. resulted from the analysis of partial sequences of mitochondrial COI gene. The topology was inferred from the Bayesian inference and Maximum likelihood method results. The values of each node correspond to posterior probability and bootstrap values of major clades that were reconstructed with BI and ML strict consensus tree, respectively (BI/ML). The dark grey boxes correspond to the families belonging to Order Poecilosclerida, orange box to species of the family Crambeidae, and light grey box to sequences of species used as outgroup to root the tree. GenBank accession numbers are given after each taxa name. New species sequences are in bold.

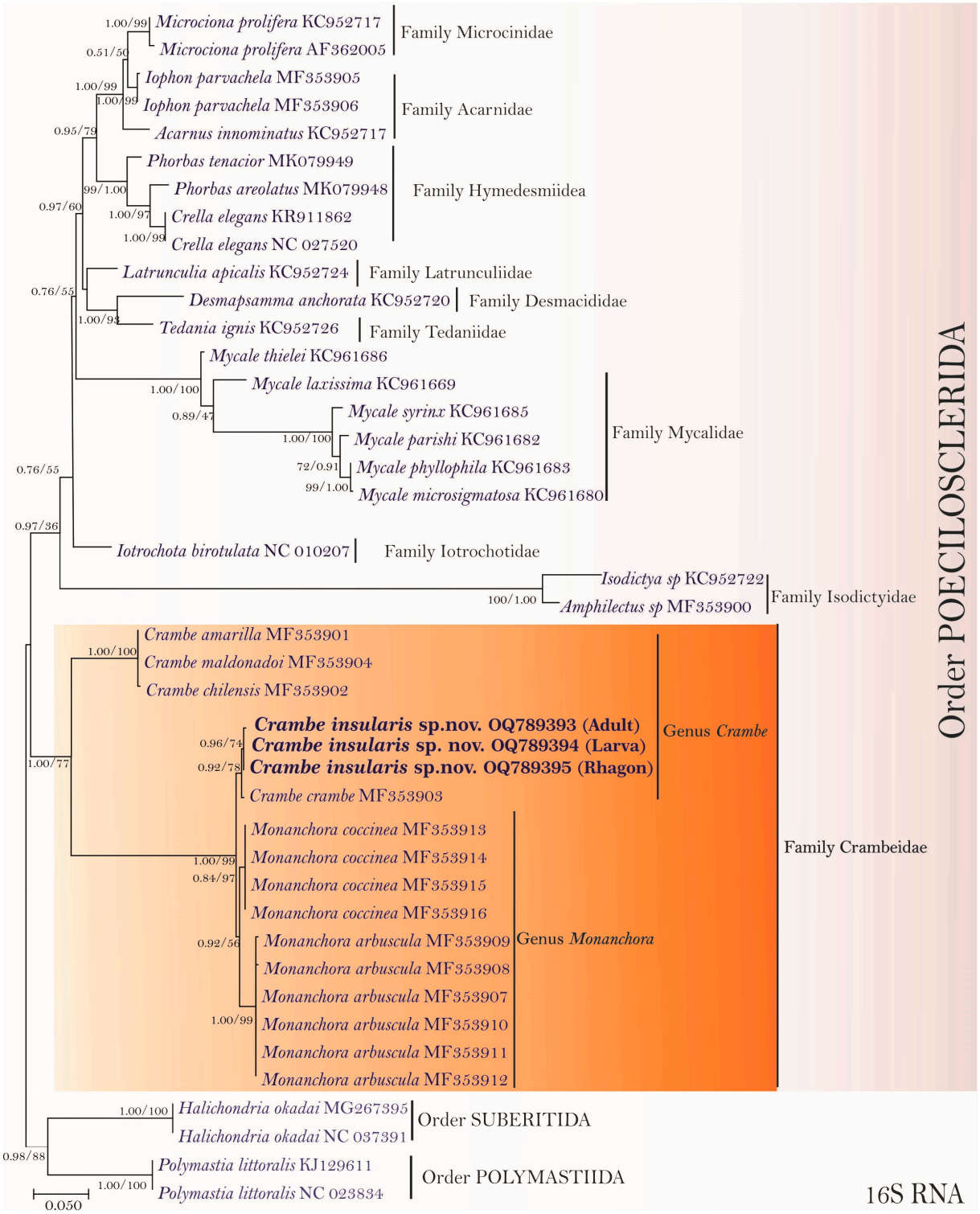

Figure 6. Molecular phylogenetic tree showing the position of *Crambe insularis* sp. nov. resulted from the analysis of partial sequences of mitochondrial 16S RNA gene. The topology was inferred from the Bayesian inference and Maximum likelihood method results. The values of each node correspond to posterior probability and bootstrap values of major clades that were reconstructed with BI and ML strict consensus trees, respectively (BI/ML). The dark grey boxes correspond to the families belonging to Order Poecilosclerida, orange box to species of the family Crambeidae, and light grey box to sequences of species used as outgroup to root the tree. GenBank accession numbers are given after each taxa name. New species sequences are in bold.

The molecular phylogeny of the 28S rRNA (D3–D5) was constructed using 48 sequences (909 selected sites; 759 sites were conservative, 150 variables and of which 120 were parsimony informative). In addition, the phylogenetic-based BI and ML analyses on this locus (Figure 4) place *Crambe insularis* sp. nov. in a well-supported monophyletic clade with species belonging to the genera *Crambe* and *Monanchora* (family Crambeidae). The monophyletic clade is subdivided in three well-supported subclades: the first contains all the sequences of South American *Crambe* species (*C. chilensis*, *C. maldonadoi* and *C. amarilla*) the Mediterranean *C. crambe* and *Crambe insularis* sp. nov.; the second with the sequences of *Monanchora* species (*Monanchora clathrata, M. unguiculata* and *Monanchora* sp.) and the third only sequences of *M. arbuscula*. The molecular analysis with the 28S data set retrieved *Crambe* as monophyletic and *Monanchora* as polyphyletic.

Phylogenetic reconstructions resulting from BI and ML analyses of COI mDNA were constructed with 58 sequences (647 selected sites; 372 sites were conservative, 257 variables and 255 were parsimony informative). The topology based on this gene (Figure 5), place *Crambe insularis* sp. nov. in a well-supported monophyletic clade with other members of Crambeidae: the first subclade contains three species of *Monanchora*: *M. clathrata*, *Monanchora* sp., *M. unguiculata* and two specimens of *M. quadrangulata*; the second with sequences of *Crambe insularis* sp. nov. that fell in a polytomy with two specimens of type species of *Crambe crambe,* a third subclade with sequences of *M. arbuscula,* and a fourth subclade a single specimen of *Crambe* sp. The phylogenetic analyses of CO1 data, retrieved *Crambe* + *Monanchora* as polyphyletic and *Crambe insularis* sp. nov. and *Crambe crambe* as sister species.

Finally, the molecular phylogeny using the 16S rRNA was constructed with 42 sequences (531 selected sites; 278 sites were conservative, 253 variables, and 230 were parsimony informative). Similarly, the phylogenetic hypothesis of BI and ML analyses of 16S retrieve to *Crambe* and *Monanchora* (Family Crambeidae) in a well-supported monophyletic clade that is subdivided in two principal subclades: the first contains sequences of *Crambe insularis* sp. nov. that fell in a polytomy with the Mediterranean type species *Crambe crambe* and a sister group with sequences of *Monanchora* species: *M. coccinea* and *M. arbuscula*. The second contains only sequences of South American *Crambe* species*: C. chilensis*, *C. maldonadoi* and *C. amarilla*. The molecular analysis with 16S rRNA data set retrieved *Monanchora* as monophyletic and *Crambe* as polyphyletic (Figure 6).

3.3. External Morphology of the Larva

Parenchymella larvae elongated or oval and slightly flattened in anterior to posterior direction; color burnt orange or red. The size ranges 240–763 μm length to 160–365 μm width. This larva shows a clear polarity, and the body is evenly covered with small cilia of equal length (~25 μm), except for the anterior pole, which is bulbous, bare, and densely granulate (Figure 7A). Active swimming of the larva could be detected in counter-clockwise spirals or corkscrew patterns, and it may sink, stay still, or move rapidly or slowly on the bottom of the dish (Supplementary material). During the observation, some morphological changes occur in the larva body, such as temporal protraction anterior or posterior pole retraction without apparent cause. In digested–acid larvae, several megascleres were found in the posterior pole of the larvae; styles/subtylostyles were thick, fusiform, straight, and sharply pointed: 28.73–145.19 × 3.56–6.28 μm, and tylostyles straight or slightly curved: 59.42–156.64 × 1.02–2.44 μm.

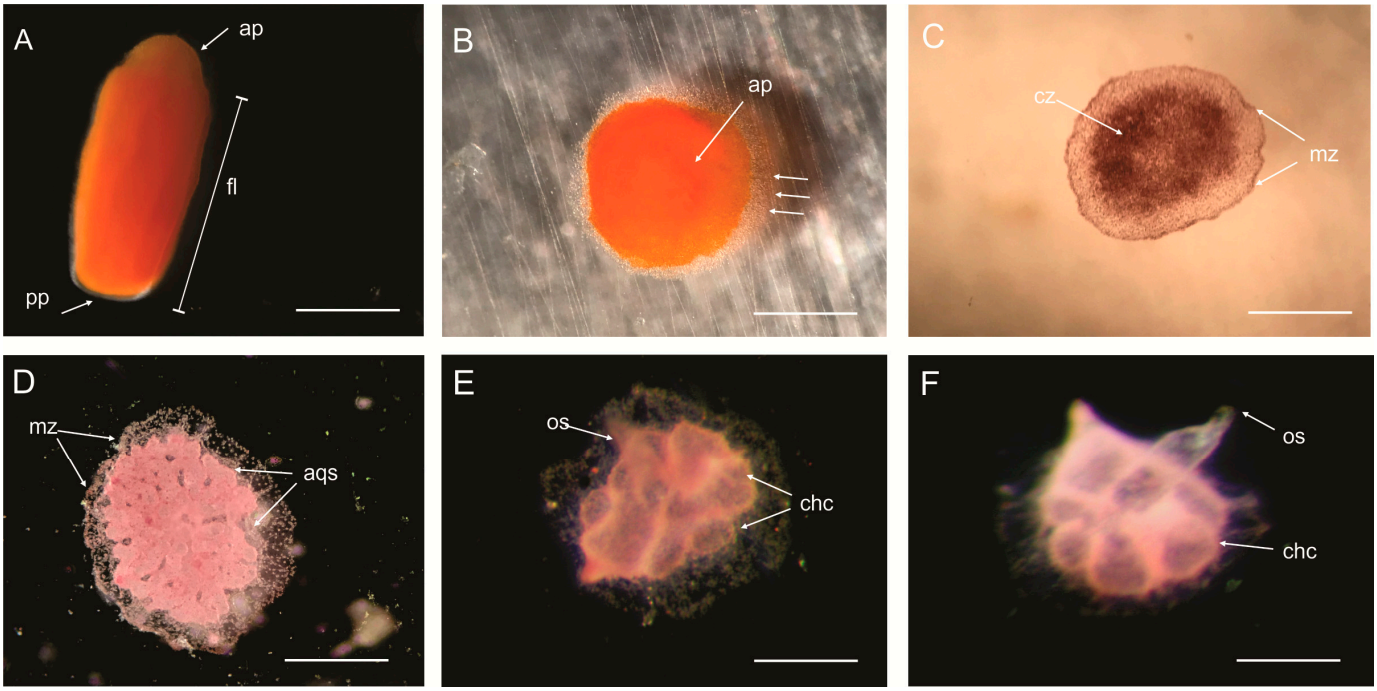

Figure 7. Larval morphology and post–settlement stages of *Crambe insularis* sp. nov. (**A**) Parenchymella free-swimming larva showing the (ap) anterior pole, (pp) posterior pole, and (fl) flagellated layer. (**B**) Larva recently settled; note the incipient (ap) anterior pole and the zone of cellular proliferation around the marginal zone (white arrows). (**C**) Larva completely flattened 18 h post–settlement showing the evident regionalization with high cellular density in their (cz) central zone surrounded by a thin translucent layer in the (mz) marginal zone with low cell density. (**D**) Pre-rhagon showing the development of incurrent and excurrent channels of (aqs) aquiferous system and the marginal growth zone. (**E**) Early rhagon 2 days after larval settlement with (chc) choanocytes chamber in development. (**F**) Rhagon or young sponge in vivo 3-days post-settlement with (os) osculum and (chc) choanocytes chamber completely developed and functioning aquiferous system. Scale bar: 300 µm.

3.4. Rhagon Phase

Some larvae in overnight observation (16 h after collection) ceased their horizontal counter-clockwise spiral movement and remained vertical and stationary, rotating slowly around their axis anti-clockwise over the base Petri dishes. In subsequent hours (16–18 h), all larvae were finally attached via the anterior pole and settled into the bottom and the corner of the Petri dish. Initially (18 h after settlement), the settled larvae (pre-rhagon) were almost flattened, forming a homogeneous cell patch of burnt orange color of 0.5–0.8 µm in diameter (Figure 7B). One day after settlement, pre-rhagon was wholly flattened and, an evident cellular regionalization could be observed in tow zones: a thick inner conglomerate with a high cellular density that is surrounded by a thin translucent marginal zone with rounded edges and low cell density (Figure 7C). After two days, cell proliferation and evident development of the aquiferous system elements (formation of choanocytes chambers) were observed in their central part (Figure 7D). The complete metamorphosis was recorded three days after larval settlement, with the marked development of a central osculum and a functional aquiferous system (Figure 7E). The mature rhagon had a syconoid morphology, with radial organization and apical–basal polarity. It was 0.8–1 mm wide with a single large central finger–shaped osculum and 6–8 lobulated neighboring choanocytes chambers of 125–190 µm length (Figure 7F). Its surface is pink-translucent and probably formed by pinacocytes. A faint water circulation was observed inside the lobulated choanocytes chamber and across the central osculum.

4. Discussion

The description of a new species of *Crambe* Vosmaer, 1880 is highly relevant at different levels. First, the increase in the richness of *Crambe* species, with a total of five species distributed along the Pacific Ocean, and a total of ten with worldwide distribution; more important for the Eastern Tropical Pacific is the Northern record and the only one for the region of Mexican Pacific. *Crambe insularis* sp. nov. possess an external morphology and skeletal architecture similar to the rest *Crambe* spp., i.e., live–color, hispid surface with distinctly swollen veins in life and a plumose skeleton structure composed by ectosomal and choanosomal monactines that suggests its appropriate placement in Crambeidae Leví, 1963 [1,4,8,11,19].

Morphologically, *Crambe insularis* sp. nov. does not possess a full complement of spicules that characterize all *Crambe* species [1], and instead, it only bears two shapes of megascleres (styles/tylotes and subtylostyles) and no microscleres (i.e., desmas, isochelae and microxeas). Nevertheless, the external features and the plumose skeletal arrangement of *C. insularis* sp. nov. is similar to other members of the genera *Crambe* and *Monanchora* [1,9]. The new species of *Crambe* described herein show some similarities with the type species *Crambe crambe* Schmidt, 1862 such as thin subtylostyles dispersed with no special organization in the ectosome and choanosomal styles/anisostrongyles, with a plumose arrangement formed by multispicular tracts, with the exception that some specimens of *C. crambe* have interlocking microscleres that are scarce or even absent in some natural populations [1,12,13].

The new species share virtually the same external morphology and some skeletal elements with similar size-range with its congeneric Panamanian species *Crambe panamensis*. However, *C. panamensis* has tylostyles with a well–marked tyle in combination with very scarce anchorated isochelae in the choanosome, besides a well-developed basal substratum conformed by abundant interlocked astroclone desmas [8,12], which are absent in *C. insularis* sp. nov. Contrastingly, the Panamanian species has smaller ectosomal subtylostyles, as well as the absence of choanosomal styles, which are characteristics of the new species. The distribution pattern locates both species in the Tropical Eastern Pacific (TEP) region, however, *C. panamensis* distribution is limited to the Panama Bight Ecoregion, in the central TEP while *C. insularis* sp. nov. has been exclusively found in a single island locality Mexican Tropical Pacific, which is the North–limit of the TEP [39].

Additionally, the skeletal reduction observed in all current specimens studied, clearly distinguishes *Crambe insularis* sp. nov. from the rest of the East Pacific *Crambe* species (*C. chilensis*, *C. amarilla*, *C. maldonadoi* and *C. panamensis*), as also all Mediterranean species (*C. crambe*, *C. tuberosa* and *C. tailliezi*) which bearing (depending on the species) well-developed astro/sphaeroclones desmas, anchorate isochelae and the occasional occurrence of sigmoid elements and spined microxeas in a variable abundance [1,8,18,19,37,38].

From a morphological perspective, the taxonomy of poecilosclerid sponges is based on categories-type and size of microscleres [1,4]. However, the presence/absence of these spicular complements have been reported as a hyper–variable character in the whole family Crambeidae [1,11,12,15,17]. This phenotypic skeletal inhibition is a response to the local environmental conditions, where a silica depletion in the water column may promote skeletal inhibition, suppressing the production of hypersilicified desmas and affecting both the size and shape of other supplementary spicules [13]. The fact that the skeleton composition may change in response to local conditions, instead of a species-specific character, has been the main promoter of misidentification in the family Crambeidae [36]. In this regard, the lack of a full complement of spicules as characterized for *C. insularis* sp. nov., led to the fact that the traditional taxonomy based on the comparison of skeleton organization and spicular elements resulted in an incorrect and therefore unreliable diagnosis, evidencing the relevance of the use of integrative taxonomy.

The inclusion of *C. insularis* sp. nov. in Poecilosclerida was supported principally by pairwise genetic distances and molecular analysis of three independent loci: two mitochondrial (COI mDNA, 16S rRNA) and one nuclear (28S rRNA D3–D5). All phylogenetic analysis supports that *Crambe insularis* sp. nov. is closely related to the type species *Crambe crambe* and other sister species belonging to *Crambe* and *Monachora.* The genetic analysis supports our morphological identification and its inclusion within Crambeidae, despite not sharing the full complement of spicules characteristics of the genus *Crambe* [1,7,12]. Additionally, the molecular data allowed us to establish the proximity of *Crambe* and *Monachora* as both genera are retrieved in robust clades with high bootstrap support and posterior probability as a sister group inside Crambeidae. This family was recovered as a basal and strong monophyletic clade with other families (i.e., Isodictydiae, Mycalidae, Hymedesmiiae, Iotrochotidae Tedaniidae), which belongs to the Order Poecilosclerida. Previously, the monophyly of Crambeidae was recovered using independent nuclear (28S and 18S), mitochondrial (COI and 16S), and housekeeping genes [36,40–42]. Nevertheless, there are no records of sequences of nuclear and mitochondrial genes of *Litochela* and *Discorhabdella* species, which are essential groups to solve internal relationships among *Crambe* and *Monanchora* in Crambeidae.

Regarding the relationship between members of *Crambe*, the topologies indicated no consensus between mitochondrial (16S and COI) and nuclear (28S) phylogenies. The BI and ML analysis with the nuclear gen (28S) recovered to *C. insularis* sp. nov. + Mediterranean and South American *Crambe* species in a well-supported monophyletic clade whereas, the mitochondrial genes (16S and COI) recovered to *Crambe* species as paraphyletic (Figures 5 and 6). The inconsistency between topologies as result of a phylogenetic reconstruction has been previously reported [36] and may be a consequence of the use of different matrices with diverse taxonomic composition (OUTs) which could be showing an incomplete family view or different sister group relationships between Crambeidae.

However, these differences should not be taken lightly, since interestingly, the phylogenetic reconstruction with three deferens loci (16S, COI and 28S) (Figures 4–6), showed that *C. insularis* sp. nov. is closely related to Mediterranean species *Crambe crambe,* which seems to be more reasonable, as not only both species are genetically closer (Table 3) but share morphological characteristics rather than to congeneric species from the South American Pacific coast. Contrastingly, previous molecular phylogenies using 28S [43], COI [42] and 16S [36] genes have been retrieved to *Crambe crambe* in a clade with others Atlantic species of *Monachora* (*M. arbuscula*, *M. coccinea*), but still there is no a clear pattern of the monophyly of crambeid genera neither the internal relationships between *Crambe* and *Monanchora* until molecular data of *Litochela* and *Discorhabdella* species become available.

Finally, the present analyses clarified the relative position of a new species of *Crambe* that lacks microscleres, within the family Crambeidae (Poecilosclerida) using independent molecular data sets. *Crambe insularis* sp. nov. represents the fifth species of genus recognized in the Pacific Ocean and the first endemic *Crambe* in the Mexican Pacific coast. This may indicate that the diversity of crambeids is, to date, underestimated. The distribution limits of *Crambe* spp. have already been recorded to the South and Central Pacific Ocean [8,19], and with the present report, their distribution expanded southwards to the South-eastern tropical Pacific. More importantly, it emphasizes the ability of the *Crambe* genus to modify its skeletal structures genetically as a response to local environmental conditions [13], considering the different scenarios of climate change, this is an adaptive advantage, and it could be expected that other species of the same genus could modify their type of skeleton. Indeed, this is the first taxonomic and molecular study with an integrative approach that includes other diagnostic features for the description of new species in the phylum Porifera, e.g., larval and rhagon, with this being an approximation that must be considered for all organisms that have a phenotypic plasticity such as those found in *Crambe*.

Parenchymella Larva

The general morphology of the larva of *Crambe insularis* sp. nov. corresponded to the typical parenchymella larva with anterior-posterior polarity similar to other closely related poecilosclerid species described previously [44], e.g., *Crambe crambe* [45] or *Hamiguera hamigera* [46]. Anatomically, external morphological differences among the larvae of *C. insularis* sp. nov. and their sister species *C. crambe* were observed, although both parenchymella larvae presented an evident anatomic polarity, all larvae of *C. insularis* sp. nov. described in this study have the posterior pole evenly ciliated, and the anterior pole is bare. These, however, do not occur in the four subtypes larvae described of the Poecilosclerida [44]. In *Crambe crambe* for example, large larvae dissected from brooding individuals had an anterior pole uniformly ciliated with a bare posterior zone; in contrast, the larvae in early developmental stages are completely flagellated [45]. Indeed, the larvae of *C. insularis* sp. nov. are smaller than those described by Uriz et al. [45], who reported larval size of 1200 µm in length and 600 µm wide, which are larger than those reported for other poecilosclerid members [46,47].

Additionally, two different spicule types (styles and tyles) were found in the posterior region of the larvae, 2–3 orders of magnitude smaller than those present in the skeleton of the adult stage. The larvae of *C. insularis* sp. nov. analysed in this study were collected in planktonic conditions in an advanced stage of maturation. Contrastingly, in immature larvae dissected from brooding individuals of *C. crambe,* spicules were not found; however, in the ultrastructure analysis, some sclerocytes with an axial filament were detected in the posterior pole of the larvae [45]. The last observation suggested that the lack of spicular elements in the early larval phase can be attributed to larval immaturity. The presence of spicules in the planktonic larvae of *C. insularis* sp. nov., suggests that the production and quantity of elements may be critical during the dispersal phase or in the terminal stages of larvae to facilitate the settlement process. Maldonado et al. [48] suggested that the contents and increased spicular elements in late-stage larvae may increase the larval density and favour its vertical sinking towards the bottom.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d15050608/s1.

Author Contributions: Conceptualization, E.B.-G. and J.L.C.; methodology E.B.-G., J.L.C. and A.P.R.-T.; resources, J.L.C.; original draft preparation, E.B.-G. and J.L.C. writing—review and editing, E.B.-G., J.L.C. and A.P.R.-T.; funding acquisition J.L.C.; performed field E.B.-G. All authors have read and agreed to the published version of the manuscript.

Funding: The present study was funded by the project Ecología larvaria y dispersión de algunas esponjas destructoras de corales CONACYT–SEP (254806). E.B.G. received support from the Programa de Apoyo a la Mejora en las Condiciones de Producción de los Miembros del SNI y SNCA–PROSNI.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: We thank the authorities of the Islas Isabel National Park through the Comisión Nacional de Areas Naturales Protegidas (CONANP) for the permission to conduct this research and their logistical support in field samplings. Finally, the authors thank to Lourdes Palma Tirado (INB–UNAM) for the processing of the SEM images and Benjamin Yáñez-Chávez (ICMyL–UNAM) and Jeimy Denisse Santiago-Valentín for help with the specimen's collection and their assistance in field.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Van Soest, R.W.M. Family Crambeidae Lévi, 1963. In *Systema Porifera: A Guide to the Classification of Sponges*; Hooper, J.N.A., Van Soest, R.W.M., Eds.; Kluwer Academic/Plenum Publishers: New York, NY, USA, 2002; pp. 547–555. ISBN 0-306-47260-0.
2. Esteves, E.L.; Lerner, C.; Lôbo-Hajdu, G.; Hajdu, E. *Monanchora brasiliensis* sp. nov. (Poecilosclerida: Demospongiae), new crambeid from the Brazilian coast, south-western Atlantic, with monodentate anchorate chelae. *J. Mar. Biol. Assoc. UK* **2012**, *92*, 869–876. [CrossRef]
3. Van Soest, R.W.M.; Boury-Esnault, N.; Hooper, J.N.A.; Rützler, K.; de Voogd, N.J.; Alvarez, B.; Hajdu, E.; Pisera, A.B.; Manconi, R.; Schönberg, C.; et al. World Porifera Database. 2019. Available online: http://www.marinespecies.org/porifera (accessed on 29 April 2019).
4. Esteves, E.L.; de Paula, T.S.; Lerner, C.; Lôbo-Hajdu, G.; Hajdu, E. Morphological and molecular systematics of the '*Monanchora arbuscula* complex' (Poecilosclerida: Crambeidae), with the description of five new species and a biogeographic discussion of the genus in the Tropical Western Atlantic. *Invertebr. Syst.* **2018**, *32*, 457–503. [CrossRef]
5. Arevabini, C.; Crivelenti, Y.D.; de Abreu, M.H.; Bitencourt, T.A.; Santos, M.F.C.; Berlinck, R.G.S.; Hajdu, E.; Beleboni, R.E.; Fachin, A.L.; Marins, M. Antifungal activity of metabolites from the marine sponges *Amphimedon* sp. and *Monanchora arbuscula* against *Aspergillus flavus* strains isolated from peanuts (*Arachis hypogaea*). *Nat. Prod. Commun.* **2014**, *9*, 33–36. [CrossRef] [PubMed]
6. Santos, M.F.; Harper, P.M.; Williams, D.E.; Mesquita, J.T.; Pinto, E.G.; da Costa-Silva, T.A.; Hajdu, E.; Ferreira, A.G.; Santos, R.A.; Murphy, P.J.; et al. Anti-parasitic guanidine and pyrimidine alkaloids from the marine sponge *Monanchora arbuscula*. *J. Nat. Prod.* **2015**, *78*, 1101–1112. [CrossRef]
7. Uriz, M.J.; Maldonado, M. A reconsideration of the relationship between polyaxonid and monaxonid spicules in Demospongiae: New data from the genera *Crambe* and *Discorhabdella* (Porifera). *Biol. J. Linn. Soc.* **1995**, *55*, 1–15. [CrossRef]
8. Maldonado, M.; Carmen Carmona, M.; Van Soest, R.W.; Pomponi, S.A. First record of the sponge genera *Crambe* and *Discorhabdella* for the eastern Pacific, with description of three new species. *J. Nat. Hist.* **2001**, *35*, 1261–1276. [CrossRef]
9. Leví, C. Résultats scientifiques des campagnes de la 'Calypso'. V. Campagne en Mer Rouge (1951–1952). 11. Spongiaires de Mer Rouge. *Ann. Inst. Océanographique Monaco.* **1958**, *34*, 3–46.
10. Boury-Esnault, N. Spongiaires de la zone rocheuse de Banyuls-sur-Mer. II. Systématique. *Vie Milieu* **1971**, *22*, 287–349.
11. Vacelet, J.; Vasseur, P.; Lévi, C. Spongiaires de la Pente Externe des Récifs coralliens de Tulear (sud-ouest de Madagascar). *Mémoires Muséum Natl. D'Histoire Nat. Série A Zool.* **1976**, *99*, 1–116.
12. Maldonado, M.; Uriz, M.J. Skeletal morphology of two controversial poecilosclerid genera (Porifera, Demospongiae): *Discorhabdella* and *Crambe*. *Helgoländer Meeresunters* **1996**, *50*, 369–390. [CrossRef]
13. Maldonado, M.; Carmona, M.C.; Uriz, M.J.; Cruzado, A. Decline in Mesozoic reef-building sponges explained by silicon limitation. *Nature* **1999**, *401*, 785–788. [CrossRef]
14. Lévi, C. Résultats scientifiques des campagnes de la 'Calypso'. XIV. Campagne 1954 dans l'Océan Indien. 2. Les spongiaires de l'Ile Aldabra. *Ann. Inst. Océanographique Monaco* **1961**, *39*, 3–32.
15. Lévi, C. Les Spongiaires à desmes astéroïdes. *Bull. Inst. Océanographique Monaco* **1960**, *57*, 1–8.
16. Lévi, C. Spongiaires d'Afrique du Sud (1). Poecilosclerides. *Trans. R. Soc. South Afr.* **1963**, *37*, 1–71. [CrossRef]
17. Uriz, M.J. Deep-water sponges from the continental shelf and slope of Namibia (South-West Africa): Classes Hexactinellida and Demospongiae. *Monogr. Zool. Mar.* **1988**, *3*, 9–157.
18. Maldonado, M.; Benito, J. *Crambe tuberosa* n. sp. (Demospongiae, Poecilosclerida): A new Mediterranean poecilosclerid with lithistid affinities. *Cah. Biol. Mar.* **1991**, *32*, 323–332.
19. Esteves, E.L.; Lôbo-Hajdu, G.; Hajdu, E. Three new species of *Crambe* (Crambeidae: Poecilosclerida: Demospongiae) from the south-eastern Pacific, with a review of morphological characters for the genus. *J. Mar. Biol. Assoc. UK* **2007**, *87*, 1367–1378. [CrossRef]
20. de Voogd, N.J.; Alvarez, B.; Boury–Esnault, N.; Carballo, J.L.; Cárdenas, P.; Díaz, M.-C.; Dohrmann, M.; Downey, R.; Hajdu, E.; Hooper, J.N.A.; et al. World Porifera Database. Crambeidae Lévi. 1963. Available online: https://www.marinespecies.org/porifera/porifera.php?p=taxdetails&id=1316 (accessed on 11 January 2023).
21. Gómez, P.; Bakus, G. *Aplysina gerardogreeni* and *Aplysina aztecus* (Porifera: Demospongiae) new species from the Mexican Pacific. *An. Inst. Cienc. Mar Limnol. Univ. Autónoma México* **1992**, *19*, 175–180.
22. Gómez, P. First record and new species of *Gastrophanella* (Porifera: Demospongiae: Lithistida) from the central East Pacific. *Proc. Biol. Soc. Wash.* **1998**, *111*, 774–780. Available online: https://biostor.org/reference/69064 (accessed on 11 January 2023).
23. Cruz-Barraza, J.A.; Carballo, J.L. Taxonomy of sponges (Porifera) associated with corals from the Mexican Pacific Ocean. *Zool. Stud.* **2008**, *47*, 741–758.
24. Aguilar-Camacho, J.M.; Carballo, J.L.; Cruz-Barraza, J.A. Acarnidae (Porifera: Demospongiae: Poecilosclerida) from the Mexican Pacific Ocean with the description of six new species. *Sci. Mar.* **2013**, *77*, 677–696. [CrossRef]
25. Carballo, J.L.; Gómez, P.; Cruz-Barraza, J.A. Biodiversidad de Porifera en México. *Rev. Mex. Biodivers.* **2014**, *85*, 143–153. [CrossRef]
26. Boury-Esnault, N.; Rützler, K. Thesaurus of Sponge Morphology. *Smithson. Contrib. Zool.* **1997**, *596*, 1–55. [CrossRef]
27. Folmer, O.; Black, M.; Hoeh, W.; Lutz, R.; Vrijenhoek, R. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol. Mar. Biol. Biotechnol.* **1994**, *3*, 294–299.

28. Nichols, S.A. An evaluation of support for order-level monophyly and interrelationships within the class Demospongiae using partial data from the large subunit rDNA and cytochrome oxidase subunit I. *Mol. Phylogenet. Evol.* **2005**, *34*, 81–96. [CrossRef]
29. Lavrov, D.V.; Wang, X.; Kelly, M. Reconstructing ordinal relationships in the Demospongiae using mitochondrial genomic data. *Mol. Phylogenet. Evol.* **2008**, *49*, 111–124. [CrossRef]
30. Kumar, S.; Stecher, G.; Li, M.; Knyaz, C.; Tamura, K. MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Mol. Phylogenet. Evol.* **2018**, *35*, 1547–1549. [CrossRef]
31. Posada, D. jModelTest: Phylogenetic model averaging. *Mol. Biol. Evol.* **2008**, *25*, 1253–1256. [CrossRef]
32. Ronquist, F.; Teslenko, M.; van der, P.M.; Ayres, D.L.; Darling, A.; Höhna, S.; Larget, B.; Liu, L.; Suchard, M.A.; Huelsenbeck, J.P. MrBayes 3.2: Efficient Bayesian Phylogenetic Inference and Model Choice Across a Large Model Space. *Syst. Biol.* **2012**, *61*, 539–542. [CrossRef]
33. Stone, R.P.; Lehnert, H.; Reiswig, H. A guide to the deep-water sponges of the Aleutian Island Archipelago. In *NOAA Professional Paper NMFS12*; NOAA: Silver Spring, MA, USA, 2011; Volume 12, 187p. Available online: http://hdl.handle.net/1834/30422 (accessed on 3 September 2021).
34. Carter, H.J. New Genus of Sponges. *Ann. Mag. Nat. Hist.* **1883**, *11*, 369–370. [CrossRef]
35. Hechtel, G.J. New species and records of shallow water Demospongiae from Barbados, West Indies. *Postilla* **1969**, *132*, 1–38. [CrossRef]
36. Van Soest, R.W.M. *Monanchora stocki* n. sp. (Porifera, Poecilosclerida) from the Mid-Atlantic islands. *Bijdr. Dierkd.* **1990**, *60*, 249–255.
37. Pulitzer-Finali, G. A collection of marine sponges from East Africa. *Ann. Mus. Civ. Stor. Nat. Giacomo Doria* **1993**, *89*, 247–350. Available online: https://biostor.org/reference/100342 (accessed on 1 January 2022).
38. Vacelet, J.; Boury-Esnault, N. Une nouvelle éponge du genre *Crambe* (Demospongiae, Poecilosclerida) de Méditerraneée, *C. tailliezi* n. sp. *Trav. Sci. Parc. Nation Port-Cros Fr.* **1982**, *8*, 107–113.
39. Spalding, M.D.; Fox, G.R.; Allen, N.; Davidson, Z.A.; Ferdaña, M.; Finlayson, B.S.; Halpern, M.A.J.; Al, L.; Sara, A.L.; Kirsten, D.M.; et al. Marine Ecoregions of the World: A Bioregionalization of Coastal and Shelf Areas. *BioScience* **2007**, *57*, 573–583. [CrossRef]
40. Redmond, N.E.; Morrow, C.C.; Thacker, R.W.; Diaz, M.C.; Boury-Esnault, N.; Cárdenas, P.; Hajdu, E.; Lôbo-Hajdu, G.; Picton, B.E.; Pomponi, S.A.; et al. Phylogeny and systematics of Demospongiae in light of new small-subunit ribosomal DNA (18S) sequences. *Integr. Comp. Biol.* **2013**, *53*, 388–415. [CrossRef]
41. Hill, M.S.; Hill, A.L.; Lopez, J.; Perterson, K.J.; Pomponi, S.; Diaz, M.C.; Thacker, R.W.; Adamska, M.; Boury-Esnault, N.; Cárdenas, P.; et al. Reconstruction of family level phylogenetic relationships within Demospongiae (Porifera) using nuclear encoded housekeeping genes. *PLoS ONE* **2013**, *8*, e50437. [CrossRef]
42. Vargas, S.; Erpenbeck, D.; Göcke, C.; Hall, K.A.; Hooper, J.N.A.; Janussen, D.; Wörheide, G. Molecular phylogeny of *Abyssocladia* (Cladorhizidae: Poecilosclerida) and *Phelloderma* (Phellodermidae: Poecilosclerida) suggests a diversification of chelae microscleres in cladorhizid sponges. *Zool. Scr.* **2013**, *42*, 106–116. [CrossRef]
43. Thacker, R.W.; Hill, A.L.; Hill, M.S.; Redmond, N.E.; Collins, A.G.; Morrow, C.C.; Spicer, L.; Carmack, C.A.; Zappe, M.E.; Pohlmann, D.; et al. Nearly complete 28S rRNA gene sequences confirm new hypotheses of sponge evolution. *Integr. Comp. Biol.* **2013**, *53*, 373–387. [CrossRef]
44. Ereskovsky, A.V. Typization of Sponge Development and Its Significance for Phylogeny. In *The Comparative Embryology of Sponges*; Springer: Dordrecht, The Netherlands, 2010; pp. 209–230. [CrossRef]
45. Uriz, M.J.; Turon, X.; Becerro, M.A. Morphology and ultrastructure of the swimming larvae of *Crambe crambe* (Demospongiae, Poecilosclerida). *Invertebr. Biol.* **2001**, *120*, 295–307. [CrossRef]
46. Boury-Esnault, N. Ultrastructure de la larve parenchymella d'*Hamigera hamigera* (Schmidt) (Démosponge, Poecilosclerida). Origine des cellules grises. *Cah. Biol. Mar.* **1976**, *17*, 9–20.
47. Bergquist, P.R.; Sinclair, C.R.; Green, C.R.; Silyn-Roberts, H. Comparative morphology and behavior of larvae of Demospongiae. In *Biologie des Spongiaires*; Colloq. Int. CNRS n. 291; Lévi, C., Boury-Esnault, N., Eds.; CNRS: Paris, France, 1979; pp. 103–111.
48. Maldonado, M.; George, S.B.; Young, C.M.; Vaquerizo, I. Depth regulation in parenchymella larvae of a demosponge: Relative roles of skeletogenesis, biochemical changes, and behavior. *Mar. Ecol. Progr. Ser.* **1997**, *148*, 115–124. [CrossRef]