Copula lucentia sp. nov., a new box jellyfish (Cnidaria: Cubozoa: Carybdeida) from Western Mediterranean Sea

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Abstract

A new species of box jellyfish, *Copula lucentia* sp. nov., is described from El Campello, Alicante, Spain and compared to the holotype of *Copula sivickisi* that was successfully recovered after being lost for almost 60 years. So far, the only cubozoan species recorded in the Western Mediterranean was *Carybdea marsupialis.* The genus *Copula* just included the type species (*Copula sivickisi*) reported from different temperate, tropical and subtropical localities in the Pacific, Atlantic and Indian Oceans. Morphologically, this new species possesses the typical characteristics of the genus *Copula* (four adhesive pads on the apex and a vertical keyhole-shaped rhopalial niche ostium) but it can be differentiated from the *C. sivickisi* holotype by the velarial canal pattern. In *C. lucentia* the velarial canal roots taper towards the velarial rim and each root bears 1–2 narrow triangular canals with sharp tips, resembling a bird-beak. In contrast, the velarial canal roots of *C. sivickisi* bear 2 short, broad canals that increase breadth towards the velerial rim and split up into 3 to 6 lobed, finger-like canals with rounded tips, giving the canal pattern a paw-like appearance. Molecular analyses of mitochondrial cytochrome c oxidase subunit I (COI) and nuclear 16S ribosomal DNA genes show that there are even more *Copula* species than expected, but which will not be focused on here. This study highlights not only that there are species yet to be discovered in the Mediterranean Sea, one of the most studied seas worldwide, but that a revision of the genus *Copula* is necessary.

INTRODUCTION

Cubozoa, also known as Cubomedusae or box jellyfish, is the smallest cnidarian class with approximately 50 currently accepted species, half of them described in the past two decades (Collins & Jarms, 2022). Distinctive by their cube-shaped bell, they possess unique attributes such as complex lens eyes (Coates 2003, Nilsson et al. 2005, O'Connor et al. 2009), ability to swim actively and horizontally against currents that defines them as nekton and not as plankton which drifts passively (Haeckel 1890, Gordon & Seymour 2009, Garm et al. 2007, Colin et al. 2013, Schlaefer et al. 2020, Bordehore et al. 2022), complex mating behavior (Lewis & Long 2005, Lewis et al. 2008), associative learning (Bielecki et al. 2023), and powerful venoms (Kintner et al. 2005, Yanagihara et al. 2012, Jouiaei et al. 2015) that can even result in fatalities (Fenner & Hadok 2002, Tibballs 2006, Carrete & Seymour, 2013, Sucharitakul et al. 2017). Because of these fascinating traits, box jellyfishes garner a great deal of attention from both the scientific community and the general public.

Among this class, the genus *Copula* was recently introduced by Bentlage and others after a comprehensive phylogenetic analysis (Bentlage et al. 2010) and included hitherto a single valid species, *Copula sivickisi* (Stiasny, 1926). So far, it has been reported from Thailand (Stiasny 1922), Philippines (Stiasny 1926), Vietnam (Kramp, 1962), New Zealand (Hoverd, 1985), Japan mainland and Okinawa (Uchida 1929, 1970, Lewis & Long 2005, Garm et al. 2012, Morandini et al. 2014, Straehler-Pohl et al. 2014, Toshino et al. 2014), Hawaii (Matsumoto 2002, Crow 2006), Guam (Gershwin 2003), Bahamas (Bennett et al. 2011), and Australia and Tasmania (Hartwick 1991, Gerswhin 2005, Kingsford et al. 2012, Schlaefer et al. 2020, 2021).

Although Stiasny described one specimen from Thailand in 1922 that morphologically resembles *C. sivickisi* (with little differences according to him), it was not until 4 years later that he named the species based on a jellyfish he found in Mindoro, Philippines. Therefore, although found later, the official holotype of *C. sivickisi* is the specimen from the Philippines, which was deposited in the Natural History Museum of London. But the holotype had been on loan since 1964 and was never returned. The only hint for the search of the holotype was the name "Dr. Ralph" noted on a slip in the empty sample vessel. With the help of Miranda Lowe (Principal Curator for Crustacea and Cnidaria at the London Natural History Museum) and Alan Hoverd (former Technical Team Leader at the School of Biological Sciences of Victoria University, Wellington, New Zealand), the missing holotype was recovered after almost sixty years in a collection of Wellington University, New Zealand and returned to London in 2020. A detailed description of this curious search will be given in the review of the genus *Copula* underway in our laboratories.

In this study, after a morphological comparison with the recovered holotype (full description will be given in the review of the genus) and a genetic analysis along with specimens sampled from all over the world, we present the description of a new species of cubozoan, *Copula lucentia* sp. nov., based on specimens collected in El Campello, Alicante, Spain (Western Mediterranean Sea).

MATERIAL AND METHODS

Medusae and environmental variables sampling

Copula lucentia medusae were located and sampled by using waterproof LED lights (CE RoHS IP65, 50W) at both sides of Illeta dels Banyets, El Campello (NE Spain, 38º25'56.01"N 0º22'57.75"W) (Fig. 1), within the SCI ESZZ16008 Espacio Marino del Cabo de les Hortes. On sampling days, two lights were mounted at dusk, 15 meters from the shoreline and 1 meter deep. When conditions were appropriate (sea surface very calm and transparent waters), first cubozoans appeared after about one hour of light exposure and during the following two hours they were gathered using plastic beakers, put into a large can with filtered seawater and transported to the Marine Laboratory UA-Dénia for initial identification.

For three consecutive years (2016-2018), from August to November, we carried out 21 nocturnal samplings. For genetic analyses we used specimens collected on 22 September 2016 and for taxonomy, on 17 October 2018. Genetic and morphological samples were preserved in 90% ethanol and 4% formalin buffered with sodium tetraborate, respectively. Holotype and paratypes are deposited in the National Museum of Natural Sciences (Museo Nacional de Ciencias Naturales). DNA samples and additional studied material are deposited in LEDALab DNA collection (UNESP-Bauru).

Environmental variables (temperature and salinity) were measured using a data logger Infinity-CT JFE Advantech. Coordinates of the sampling locations were recorded with a GPS device (Garmin 72H).

Morphological analysis

Morphology of *Copula lucentia* specimens was studied under a stereomicroscope (Leica S8APO). Taxonomic observations and measurements were made on preserved specimens unless otherwise indicated. Standard measurements were used (Gershwin 2005, Straehler-Pohl 2014, 2017, 2019, Acevedo 2019): bell height (BH) = measured from the apex of the bell to velarial turnover; diagonal bell width (DBW) = distance between opposite pedalia at level of pedalia joining bell; interpedial diameter (IPD) = distance between opposite pedalia (outer pedalial wing edges) at the level of the bell turn-over; pedalial length (PL) = distance from attachment to the bell to the tentacle insertion, as proportion in relation to bell height; pedalial width (PW) at the widest diagonal level, as proportion in relation to pedalial length. Photographs were taken with a digital camera Nikon D300S. Morphology of *Copula sivickisi* holotype was studied under a stereomicroscope (Bresser 5804000), photos were taken with digital cameras Canon EOS 550D and Bresser MikrOkular Full HD.

For nematocysts analysis, pieces of different preserved tissues (tentacles, gastric phacellae, umbrella and pedalia) were placed onto glass slides and covered with cover slips, pressing firmly. Nematocysts were examined and measured with a Leica ICC50 camera connected to a Leica DM500 microscope using LAS EZ software v. 3.4.0. Observations were made through a 40x objective (i.e. 400x magnification). Nematocyst were identified following Östman 2000 and Gershwin 2006.

Molecular analysis

Three specimens collected in September 2016 were used to obtain molecular markers (16S and COI) for comparison with specimens from other regions, especially Asiatic Pacific. The DNA was extracted with DNAdvance (magnetic beads), PCR reactions and conditions followed under predefined conditions, see Acevedo et al. (2019) for 16S and LCO1490 (GGTCAACAAATCATAAAGATATTGG) and HCO2198 (TAAACTTCAGGGTGACCAAAAAATCA) and Folmer et al. (1994) to amplify part of the COI gene (expected fragment of 670 to 804 bp) (mitochondrial markers, ribosomal and protein coding genes respectively). Amplicons were purified using AMPureH kit following manufacturer's instructions, and made ready for sequencing using the BigDyeH Terminator v3.1 kit same primers and Tm temperature conditions as in PCR reactions). Sequencing was carried out on an ABI BRISMIH3100 genetic analyzer (Hitachi), and resulting sequences were assembled and edited using Geneious 9 (removing ambiguous base calls and primer sequences). New sequences were submitted to NCBI's GenBank (COI - OQ857826 - OQ857828; 16S - OQ869612 - OQ869614). Evolutionary distances of mitochondrial (16S rDNA and COI) genes from Spanish Cubozoan were analyzed within other tiny Cubozoan from different areas in MEGA X (p-distance) and ML reconstruction were performed in Geneious 9 (PhyML) with bootstrap test with 100x replicates.

RESULTS

Systematics

Phylum Cnidaria Verrill, 1865

Subphylum Medusozoa Petersen, 1979

Class Cubozoa Werner, 1973

Order Carybdeida Gegenbaur, 1856

Family Tripedaliidae Conant, 1897

Genus *Copula* Bentlage, Cartwright , Yanagihara, Lewis, Richards & Collins 2010

***Copula lucentia*, sp. nov**

Zoobank: urn:lsid:zoobank.org:act:B314438E-D3B1-484C-93BE-FA1517190B81

Tables 1-2; Fig. 2,4.

Copula lucentia Fonfría, Straehler-Pohl, Stampar & Bordehore

Type material

Holotype: Spain: National Museum of Natural Sciences (Museo Nacional de Ciencias Naturales): 1 specimen, female, (MNCN 2.02/1) (in-live: 6.1 mm BH, 6.3 mm DBW), Illeta dels Banyets, El Campello, Spain (38º25'56.01"N 0º22'57.75"O), coll. by E. S. Fonfria, 17 October 2018.

Paratypes: Spain: National Museum of Natural Sciences (Museo Nacional de Ciencias Naturales): 1 specimen, female, (MNCN 2.02/2)(in live: 5.0 mm BH, 5.4 mm DBW), Illeta dels Banyets, El Campello, Spain, 38º25'56.01"N 0º22'57.75"O coll. by ES. Fonfria, 17 October 2018; 1 specimen, female (MNCN 2.02/3)(in live: 5.5 mm BH, 5.9 mm DB), Illeta dels Banyets, El Campello, Spain, 38º25'56.01"N 0º22'57.75"O coll. by ES. Fonfria, 17 October 2018; 1 specimen, female (MNCN 2.02/4)(in live: 4.9 mm BH, 5.2 mm DBW), Illeta dels Banyets, El Campello, Spain, 38º25'56.01"N 0º22'57.75"O coll. by ES. Fonfria, 17 October 2018.

Type locality: Illeta dels Banyets, El Campello, Spain (38º25'56.01"N, 0º22'57.75"O) (Western Mediterranean Sea).

Etymology: The species is based on "Lucentum", Latin name of Alicante, Spanish province where the type locality of El Campello is located.

Synonyms: none.

Diagnosis

Copula species with 4 velarial canal roots per quadrant which taper towards the velarial rim. Each root bears 1-2 unbranched, narrow and triangular velarial canals with sharp tips.

Description (from holotype and paratypes)

Bell blunt pyramidal, slightly wider than high, slightly flattened apex, rigid, highly transparent, colourless, with minute to small (0.1 mm) round white nematocyst warts scattered from apex to bell turnover (Fig. 2a-b), no size differences in small nematocyst warts on apex and rest of umbrella (Fig. 2o). Apex markedly sculptured with 4 rounded triangular to hexagonal adhesive pads (practically unnoticeable in live specimens, but clearly visible –opaque- in preserved ones) located above the four gastric phacellae (Fig. 2c-d). Bell height up to 6.3 mm and bell width up to 6.9 mm (IPD), up to 6.1 mm (DBW) in live specimens.

Pedalium, single, unbranched, flattened, blade-shaped, intermediate in length (PL:<30% BH, PW:<40 PL), located at the four interradial corners of the bell margin, with 4-6 horizontal rectangular white, round nematocysts warts to longish oval, horizontal, nematocyst bands on outer pedalial keel (Fig. 2f-g). Pedalium carrying single tentacle, round in cross-section, flaring at base, showing a striped pattern of broad whitish to yellow and very narrow orange to brown bands in live specimens (Fig. 2e). Pedalial canal with rounded knee bend without any hook or thorn appended to outer knee bend (Fig. 2f-g).

Rhopalium located inside a spherical to upside-down egg-shaped rhopalial niche cavity with a vertical key-hole shaped ostium which is closed at the base without any covering scales, ca. 5-8% BH up from bell margin. Rhopalium with 6 eyes (2 median lens eyes + 2 lateral slit eyes + 2 lateral pit eyes) and a sausage-shaped statolith. Rhopalial horns narrow and long (ca. 1/2 of niche cavity height)(Fig. 2k,m).

Velarium, narrow (<33% BH) without nematocysts warts, containing 2 velarial canal roots per octant which taper towards the velarial rim, each root bears 1 - (mostly) 2 unbranched, narrow triangular velarial canals with sharp tips. If two canals, beak-like shape: both triangular canals are separated by a narrow gap, outer canals (facing frenulum or pedalium) slightly shorter than inner canals, no additional secondary canals or branches. Absence of perradial, adradial or interradial lappets. No velarial spots observed as specimens were not mature (Fig. 2i-j). Frenulae, 4, one in each perradius, comprising a single sheet extending from the lower half of the rhopalial niche to the velarial margin (Fig. 2n).

Manubrium, four-lobed, cruciform, free of nematocysts warts. Gastric phacellae, 4, concave-shaped horizontal rows of vertically stacked, simple, short, unbranched, multiple rooted whitish to yellow gastric filaments (ca. 50 filaments per phacellum). Subgastral sacs, 8, rounded, two flanking each gastric phacellum, orange-yellow in colour (Fig. 2d-h).

Gonads, 4 pairs, hemigonads, butterfly-shaped. All specimens collected were female: single wing oblong leaf-shaped, located in the uppermost part of interradial septa, not fully developed yet (Fig. 2l).

Nematocysts: (after to Östman 2000, Gershwin 2006)

Four different nematocyst types were identified and measured in the paratype MNCN 2.02/4 (table 1); spherical holotrichous isorhizas (tentacle, exumbrellar warts and pedalium), oval beehive isorhiza (tentacle), medium-sized lemon-shaped microbasic euryteles (tentacle), tiny microbasic euryteles (gastric cirri and subgastral sacs). Manubrium lacking nematocysts.

Sexual dimorphism: No data are available yet; males have yet to sampled to assess dimorphism. However, sexual dimorphism is expected, as in other Tripedaliidae (Hartwick 1991, Lewis et al. 2008, Bentlage et al. 2010, Straehler-Pohl et al. 2014).

Mating behaviour, fertilization, polyps and asexual reproduction: No data are available yet. Only immature specimens were sampled to date.

Habitat and ecology: *Copula lucentia* were found in shallow waters (< 1.5 m in depth). Illeta dels Banyets is a rocky peninsula of 10000 square meters surrounded by sandy bottoms with patchy *Posidonia oceanica* meadows and areas covered by the green alga *Caulerpa prolifera*. At south it limits with the harbor of El Campello and at north it is open to a small bay. Individuals of *C. lucentia* have been collected at both sides of the peninsula (Fig. 1).

During the three years of nocturnal samplings between August and November, *Copula lucentia* individuals were collected mainly in September (154 specimens, 6 samplings) and October (368 specimens, 9 samplings), with anecdotal catches in November (2 specimens, 5 samplings) and none in August (0 specimens, 1 sampling; Fig. 3). From them, 239 jellyfish were measured. Maximum and minimum DBW and BH obtained were 6.3 and 1.2 mm, and 5.8 and 0.9 mm, respectively. Mean size was 2.5 ± 1.0 mm in DBW and 2.2 ± 0.9 mm in BH (mean ± standard deviation).

In the 3 samplings carried out in September 2016, we also found a total of 30 specimens of *Carybdea marsupialis.* In the following 18 samplings, however, their presence was not detected.

All the specimens were found at warmer seawater temperatures (from 22.3 ºC to 28.0 ºC), with only two individuals collected below this range; one at 21.6 ºC and other at 17.8 ºC (both in October 2017, on day 3 and 21). Salinity range was from 36.9 to 37.6.

A publication on their diet (based on the analysis of stomach contents) is under way in our laboratories.

Distribution: This species is currently known only from the type locality (Spain, Western Mediterranean Sea; Fig. 1).

Hazardousness: No stinging activity was noted during sampling and handling of *C. lucentia,* although envenomation cannot be completely discarded until specific studies are performed. No stinging events in the area have been attributable to this species to date.

Molecular data

Results indicate that the specimens from Mediterranean Spain belong to the genus *Copula*, although present differences of approximately 20% (p-distance) in relation to specimens from Japan and Australia based on COI. The sequences recovered from the 16S amplified sector are exactly the same among the samples from Spain, however, they present significant divergence in relation to the material from

Australia, for example. The specimen USNM1124561 (GQ849113) from Double Island, Cairns, Australia has 57 transitions and 20 transversions in relation to the Spanish material in a 397 bp alignment.

The ML reconstruction shows a “stand-alone” pattern to the specimens of *Copula* from Spanish coast (Fig. 4) in both mitochondrial markers. Based on this, it can be concluded that the Mediterranean specimens found are not *C. sivickisi* and consequently a new species to the genus.

DISCUSSION

The genus *Copula* included hitherto a single valid species, *C. sivikisi* (Stiasny, 1926), reported across the Pacific and in the Indian and Atlantic Oceans. The species described here, therefore, is not only the first report of the genus in the Mediterranean Sea (being the second box-jellyfish species truly detected in this area after *Carybdea marsupialis* according to Acevedo et al. 2019) but also the northernmost distributional place for the genus *Copula* reported so far (Morandini et al. 2014).

Differential Diagnosis: *Copula sivickisi* from the Philippines vs. *Copula* specimens from the Spanish coast of the Mediterranean Sea (Table 2)

The genus *Copula*, designated by Bentlage et al. (2010), can be differentiated from all other cubozoan genera by their possession of keyhole-shaped rhopalial niche openings and adhesive pads on the exumbrellar apex with which they attach themselves to different substrates when resting (Hartwick 1991). The specimens found in NW Mediterranean Sea meet these requirements and resemble *C. sivickisi* in many morphological characteristics, but differ significantly in several key structures (Table 2) defined e.g. by Gershwin (2005), Straehler-Pohl et al. (2017) and Acevedo et al. (2019):

The bell shape of the Mediterranean specimens and of *C. sivickisi* is quite equal in shape but the Mediterranean specimens are much smaller (6.1 mm BH), about half the size of the Philippine specimens (10-13 mm BH; Stiasny 1926, present study).

The umbrellar nematocyst warts of *C. sivickisi* are very large, roundish, oval to biscuit-shaped while the bell nematocyst warts of the European *Copula* specimens are small and round.

Differences are also found in the length and shape of the rhopaliar horns originating from the top of the rhopalial niche cavity, growing vertically upwards. The ones found in *C. sivickisi* are outwardly curved, as long as the rhopalial niche cavity and equally wide throughout its length until its distal end (present study) while the ones in the Mediterranean specimens are curved towards each other, only half as long as the rhopalial niche cavity and are slightly flared at their distal ends.

Most obvious are the differences in the velarial canal pattern: While the canals in *C. sivickisi* are quite complex (canals are split into 3-6 parallel aligned secondary canals) and show a paw-like pattern (Stiasny 1926, Gershwin 2005, present study: Fig. 5i), the velarial canals in the European *Copula* specimens are more simple (triangular) and resemble, when 2 canals of one root are combined, a bird-beak (Fig. 5j). For more details on additional minor, morphological differences see Table 2 and Figure 5.

Based on above listed differences in morphological characters, it can be concluded that the Spanish specimens are members of the genus *Copula,* but they do not belong to the Philippine species *Copula sivickisi*, instead representing a new species, for which we provide the name *Copula lucentia*, sp. nov.

Molecular analysis

Molecular analysis is not possible for the type material of *C. sivickisi*, as the holotype and its paratype were preserved in formol. Our molecular phylogenetic analyses on Pacific specimens and *C. lucentia* (Fig. 4) show that there are more than two *Copula species* united under the name *Copula sivickisi* as other populations from other oceans differ from *C. lucentia*. Also, we can state that the divergence between the Pacific materials and the specimens reported in this study is too great to be an introduced species with accumulated mutations over isolation during the Anthropocene. Some studies (eg. Morandini et al., 2017) reported some evidence of non-recent invasions, but still during the Anthropocene. However, the molecular divergence of this kind of invasive species in relation to the putative original population is not very high, as is the case with our samples of *Copula*.

Biology and ecological data

Copula lucentia was found actively swimming in El Campello in autumn with temperatures between 17.8 and 28.0 ºC. These results are similar to those from Lewis & Long (2005) and Toshino et al. (2014), who collected *Copula* sp. specimens around Okinawa (SW Japan) when water temperature was 23-28ºC and Schlaefer et al. (2020) who sampled *Copula* sp. specimens in Townsville (NE Australia) with an average water temperature of 26. ºC. In the lower end of the range it also coincides with the data recorded by Hoverd (1985) in New Zealand, where *Copula* sp. specimens, although present all year around, were mainly observed in February when temperatures were 17-19ºC.

During three consecutive years (2016-2018) we found hundreds of *C. lucentia* individuals in El Campello, with a DBW and BH average size of 2.5 ± 1.0 mm and 2.2 ± 0.9 mm (mean ± SD, n=239), respectively. According to Toshino et al. (2014), polyps of supposed Japanese *C. sivickisi* transformed into a single medusa without leaving any regenerative remnants, and newly detached medusae were about 1.2 mm in umbrella height, growing up to 3.4 mm when they were 13 days old. If both *Copula* species develop similarly, and considering the small size of the specimens and the high abundance recorded, we can assume that the population is well-established in the area. However, few mature specimens were gathered during samplings, for unknown reasons. Juveniles and adults of the cubozoan *C. marsupialis* are also usually found in different areas some kilometers apart (Bordehore et al. 2020). In that case, the main hypothesis is that it could be related to the swimming capacity of the different medusae stages, with adults able to swim against currents and select the most favorable habitats (Bordehore et al. 2020). Schlaefer et al. 2020 demonstrated strong swimming abilities for *C. sivickisi* specimens with IPD (inter pedalial diameter, defined as distance between opposite pedalia –outer pedalial wing edges- at the level of the bell turn over according to Straehler-Pohl 2014) greater than 4 mm, but did not assess the swimming capability of newly metamorphosed medusae. Several authors reported that swimming performance increases with size (Shorten et al. 2005, Garm et al. 2007, Colin et al. 2013, Bordehore et al.

2020), so the smallest ones may not have the ability to swim countercurrent and would be affected by current advection. Nevertheless, a broader spatial and temporal grid sampling should be implemented to verify if this hypothesis is applicable to *C. lucentia* and to know its distribution throughout the year.

In the first three samplings of 2016, several specimens of *C. marsupialis* were found together with *C. lucentia*, but this did not happen again in the 18 subsequent samplings. Although it is not unusual to find different species of cubozoa co-inhabiting in the same area (e.g. *C. sivickisi* and *Carybdea cuboides*, formerly named *Carybdea arborifera*, according to Straehler-Phol 2020, and *Alatina morandinii* and *Tripedalia binata*, Toshino et al. 2019), the ecological implications of this presence-prolonged absence need to be studied.

No stinging activity was noted during sampling and handling of *C. lucentia,* although it would not be completely discarded until specific studies are performed. In Australia, *C. sivickisi* was reported to cause painful stings to the exposed skin of divers, with erythema and blisters persisting for hours but without systemic effects (Hartwick 1991) but, on the contrary, an accidental encounter of a small Japanese boy with dozens of specimens in Akajima, Okinawa Prefecture, Japan turned out to be harmless (Straehler-Pohl 2019, personal experience).

The record of this jellyfish is a clear indication that the Mediterranean Sea still requires studies on planktonic organisms. Taking into account that they live within a Site of Community Importance (ESZZZ16008, Espacio marino del Cabo de les Hortes) located in a sun and beach tourist municipality, further ecological and toxicological studies would be necessary to determine its role within the ecosystem and its public health implications.

Moreover, considering that many marine species consist of cryptic species, that several morphological differences between specimens of *C. sivickisi* from different locations have already been mentioned (Stiasny 1926, Straehler-Pohl et al. 2014) and that a recent behavioral study suggest a poor dispersal potential for this species (Schlaefer et al. 2020), a comprehensive revision for the genus *Copula* is required as a deeper molecular study (Fig. 4) hints to the fact that several undescribed new species are united under the name of *Copula sivickisi*.

Declarations

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Conflict of interest. The authors declare that they have no conflict of interest.

Ethical approval. All applicable international, national and/or institutional guidelines for the care and use of animals were followed by the authors.

Sampling and field studies. All necessary permits for sampling and observational field studies have been obtained by the authors from the competent authorities and are mentioned in the acknowledgements, if applicable. The study is compliant with CBD and Nagoya protocols.

Data availability. The sequence data generated for the new species are available in GenBank repository, https://www.ncbi.nlm.nih.gov/nucleotide. Genbank accession numbers are provided in the text.

Authors' contribution. ESF and CB carried out samplings. ESF carried out the morphometric study of the new species. ISP reviewed morphometric studies and redescribed the holotype. SNS did phylogenetic analyses. AC provided genetic sequences for molecular analysis. AH moved heaven and earth to find the missing holotype. CB obtained the funding for the research. ESF, ISP and SNS wrote the original draft. All authors reviewed and approved the manuscript.

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Tables

Table 1. Cnidome of *Copula lucentia* sp. nov. L= length of undischarged capsule in μm, W= width of undischarged capsule at widest point in μm, SD = standard deviation, N= number of nematocysts measured.

Part	Nematocyst type	Measure	Min	Max	Mean	SD	N
Tentacles	Spherical holotrichous isorhizas	W	11.4	14.4	12.7	0.8	50
		L	11.7	14.9	13.2	0.9	50
	Oval beehive isorhiza	W	10.0	12.4	10.9	0.6	50
		L	14.0	18.0	15.9	1.0	50
	Lemon-shaped microbasic eurytele	W	9.9	11.9	10.8	0.5	50
		L	13.0	18.6	16.5	1.3	50
Exumbrella warts	Spherical holotrichous isorhizas	W	11.0	15.0	13.0	1.0	50
		L	11.2	15.3	13.6	1.1	50
Pedalia	Spherical holotrichous isorhizas	W	12.0	16.2	13.6	1.1	50
		L	12.3	16.3	14.2	1.0	50
Gastric cirri - Subgastral sacs	Tiny microbasic eurytele	W	6.6	9.3	8.1	0.5	50
		L	7.3	9.7	8.6	0.5	50

Table 2. Comparison of morphological characters of *Copula sivickisi* and *Copula lucentia.* BH = Bell height, IPD = Interpedial diameter, PL = Pedalial lentgh, StL = Stalk length, PW = pedalial width.

Character	*Copula sivickisi*	*Copula lucentia*
Bell shape	- blunt pyramidal	- blunt pyramidal
size (BH, IPD, IPD/BH)	- 10-13 mm, 12-16 mm, 1:1.23	- 6.1 mm, 6.9 mm, 1:1.10 mm
nematocyst warts	- **very large**, whitish, **roundish, oval to biscuit-shaped**, covering bell **from below apex** to bell-turnover	- **minute to small**, whitish, **round**, covering bell **from apex** to bell turnover
Rhopalial niches	- **14% BH up from margin, cavity: spherical**	- **5-8% BH up from bell margin, cavity: spherical to upside-down egg-shaped**
niche opening	- keyhole-shaped	- keyhole-shaped
rhopalial horns	- long (**≥ height of rhopalial niche),** very narrow, vertical, slightly **curved apart from each other**, antenna-shaped **without knob at distal end**	- intermediate in length (**≤50% of rhopalial niche height),** very narrow, vertical, **curved towards each other**, antenna-shaped **slightly flared at distal end**
Pedalia	- unbranched, **long (PL:PL: ≤50% BH, StL: none; PW: ≤50% PL)**, slender knife-blade-shaped	- unbranched, intermediate in length (PL: **≤30% BH; StL: none; PW: ≤40% PL),** slender knife-blade-shaped
canals	- oval in cross-section, straight, equal breadth from proximal to distal end	- oval in cross-section, straight, nearly equal breadth from proximal to distal end
knee bend	- rounded, no appendage	- rounded, no appendage
tentacles	- simple, filiform, homogeneously banded with nematocyst battery rings	- simple, filiform, homogeneously banded with nematocyst battery rings
nematocyst warts	- **outer wing with 3–7 broad, rectangular nematocyst bands** on outer keel of pedalium, inner wing free of nematocyst warts	- **outer wing with 4-6 broad, round warts to horizontal longish oval nematocyst bands** on outer keel of pedalium, inner wing free of nematocyst warts
Velarium	- narrow, <33% BW	- narrow, <33% BW
roots	- 2 / octant	- 2 / octant
canals	- **2 / root, 2/3 split into 3-6 simple, finger-like, straight to curved secondary canals**	- **1-(mostly)2 / root, both triangular canals of one root are separated by a narrow gap, showing a broad base, tapering into sharp tips, outer canals (facing frenulum or pedalium) slightly shorter than inner canals, no additional secondary canals or branches**
pattern	- **paw-shaped**	- **triangular if 1 canal, beak-like if 2 canals**

Gastric phacellae	- horizontal, slightly concave/crescent-shaped rows, lining four stomach corners	- horizontal, slightly concave/crescent-shaped rows, lining four stomach corners
gastric filaments	- multiple rooted, **ca. 40**, simple, unbranched, vertically stacked	- multiple rooted, **ca. 50**, simple, unbranched, vertically stacked,
stomach	- flat, shallow	- flat, shallow
manubrium	- short (30% BH), fourlobed, short moutharms	- short (30% BH), fourlobed, short moutharms
Lateral gonads	- (holotype, female) 4 butterfly-shaped pairs, hemigonads, **single wings biscuit-shaped**, brown (fertilized); sexes supposed dimorph	- (female) 4 butterfly-shaped pairs, hemigonads, **single wings leaf-shaped**, transparent to whitish (immature); sexes supposed dimorph

Figures

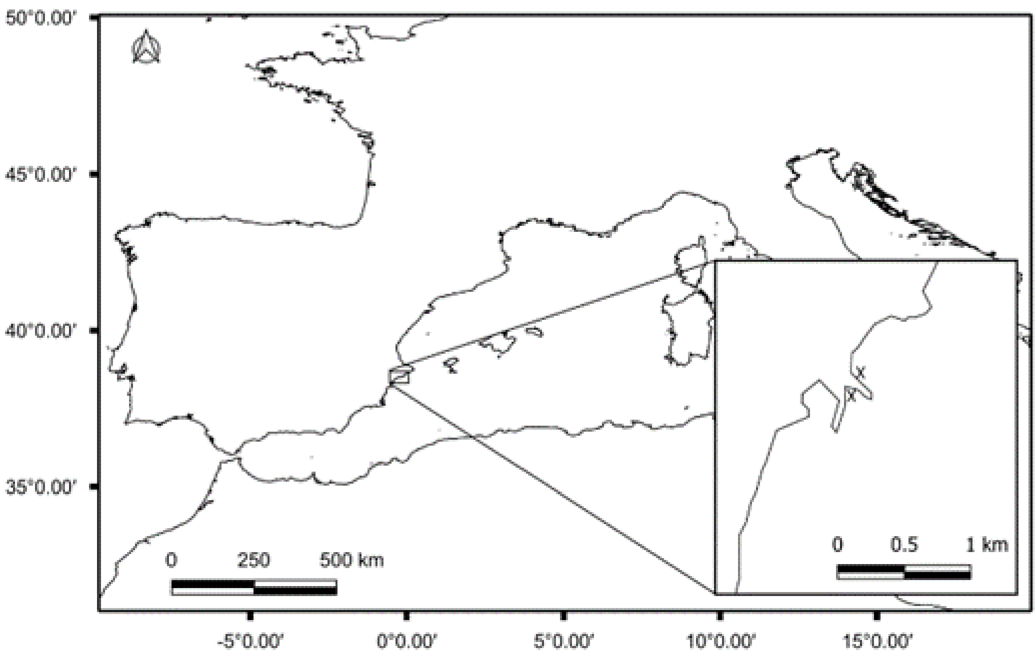

Figure 1

Map of the sampling sites (X), located in the Western Mediterranean.

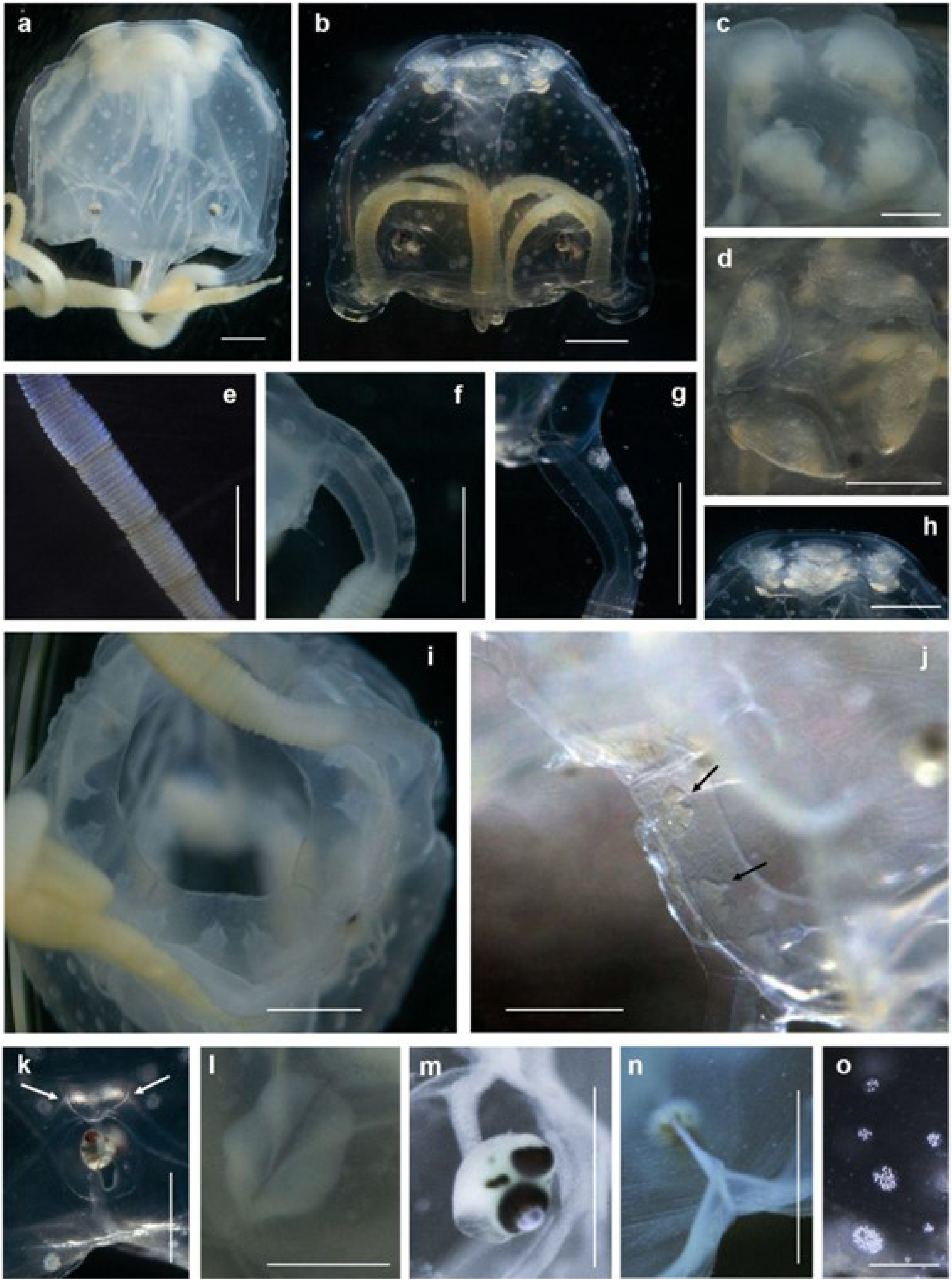

Figure 2

Copula lucentia sp. nov. holotype (pictures a,c,e,f,i,l,o; preserved specimen except in e) and paratype MNCN 2.02/2 (pictures b,d,g,h,j,k,m,n; live specimen except in n). **a,b**: Lateral view. **c,d**: Adhesive pads on apex. **e**: tentacle. **f,g**: pedalium. **h**: gastric cirri and subgastral sacs. **i,j**: velarium and velarial canals (arrows). **k**: rhopalial niche and rhopalial horns (arrows). **l**: immature gonads. **m**: rhopalia. **n**: frenulum. **o**: bell nematocyst warts. Scales: 1 mm.

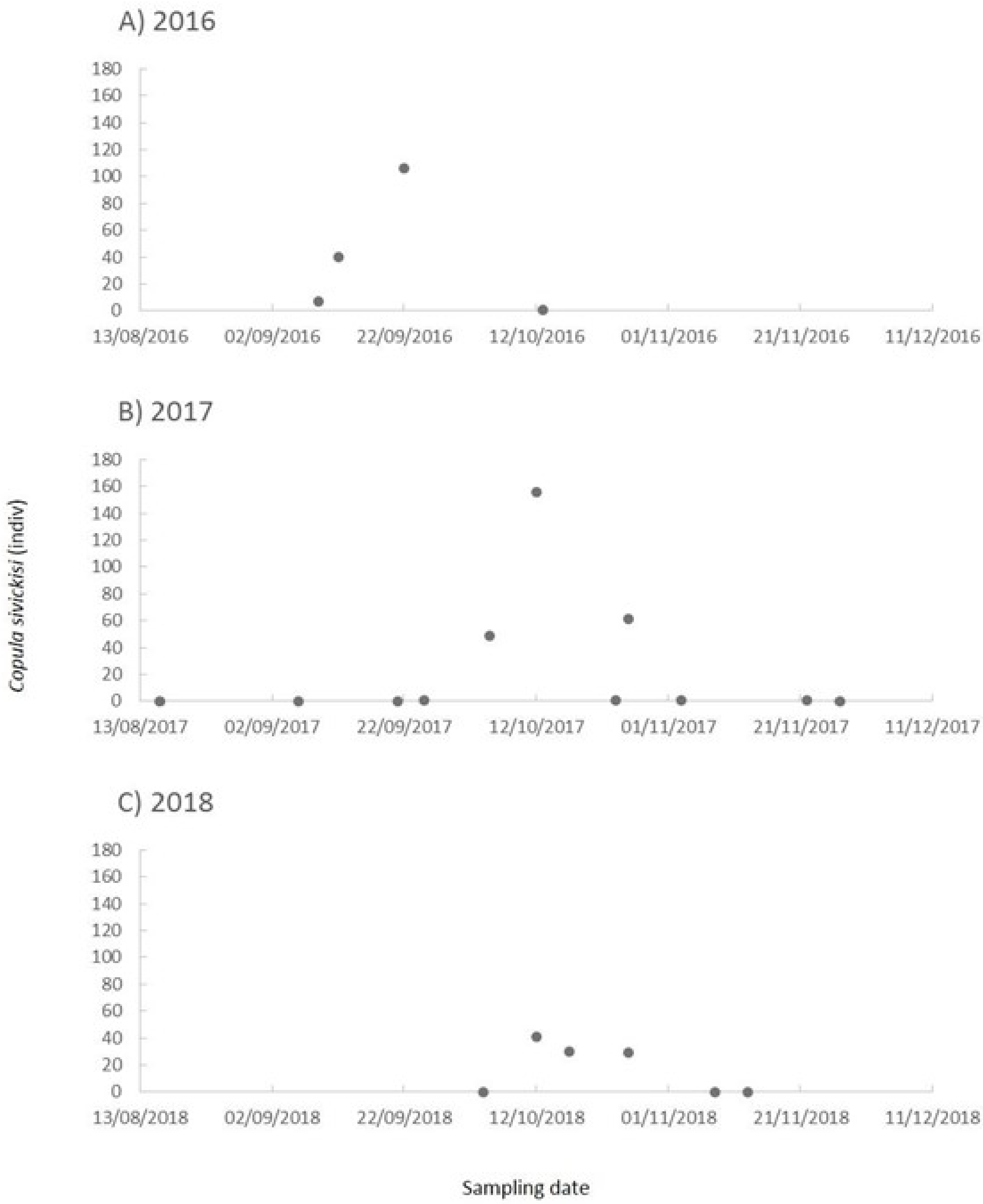

Figure 3

Copula sivickisi individuals captured by sampling dates during ~3 hours of light exposure. Years: A) 2016, B) 2017 and C) 2018.

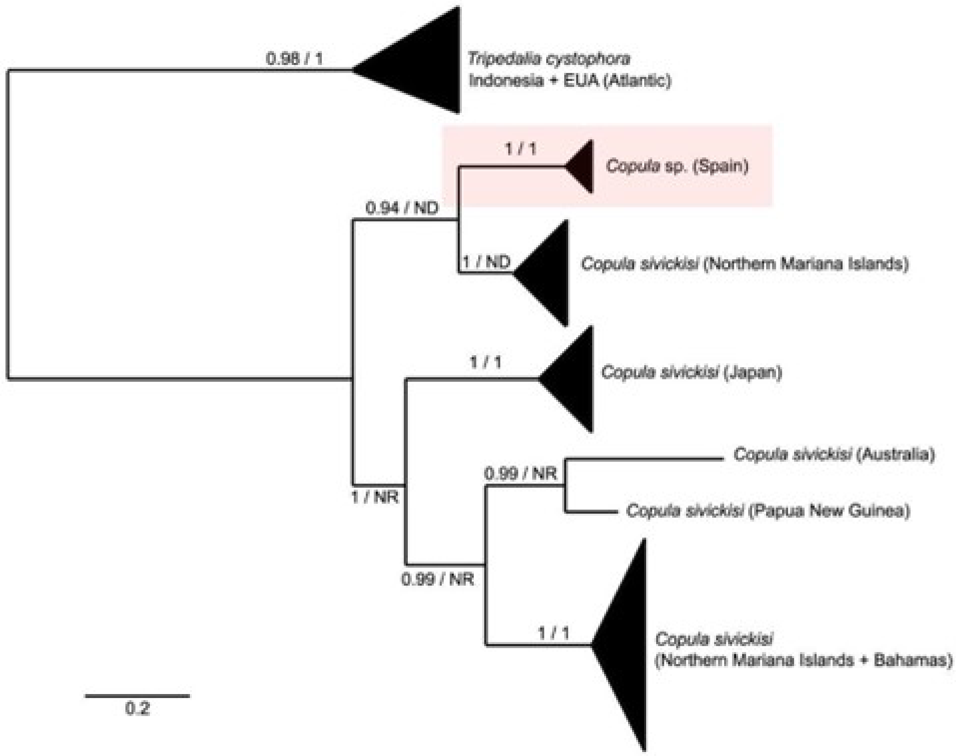

Figure 4

Maximum Likelihood of specimens of the genus *Copula*from different regions of the World based on mitochondrial marker 16S. Bootstrap values (16S/COI) are indicated in each branch. NR: Not recovered; ND: No data.

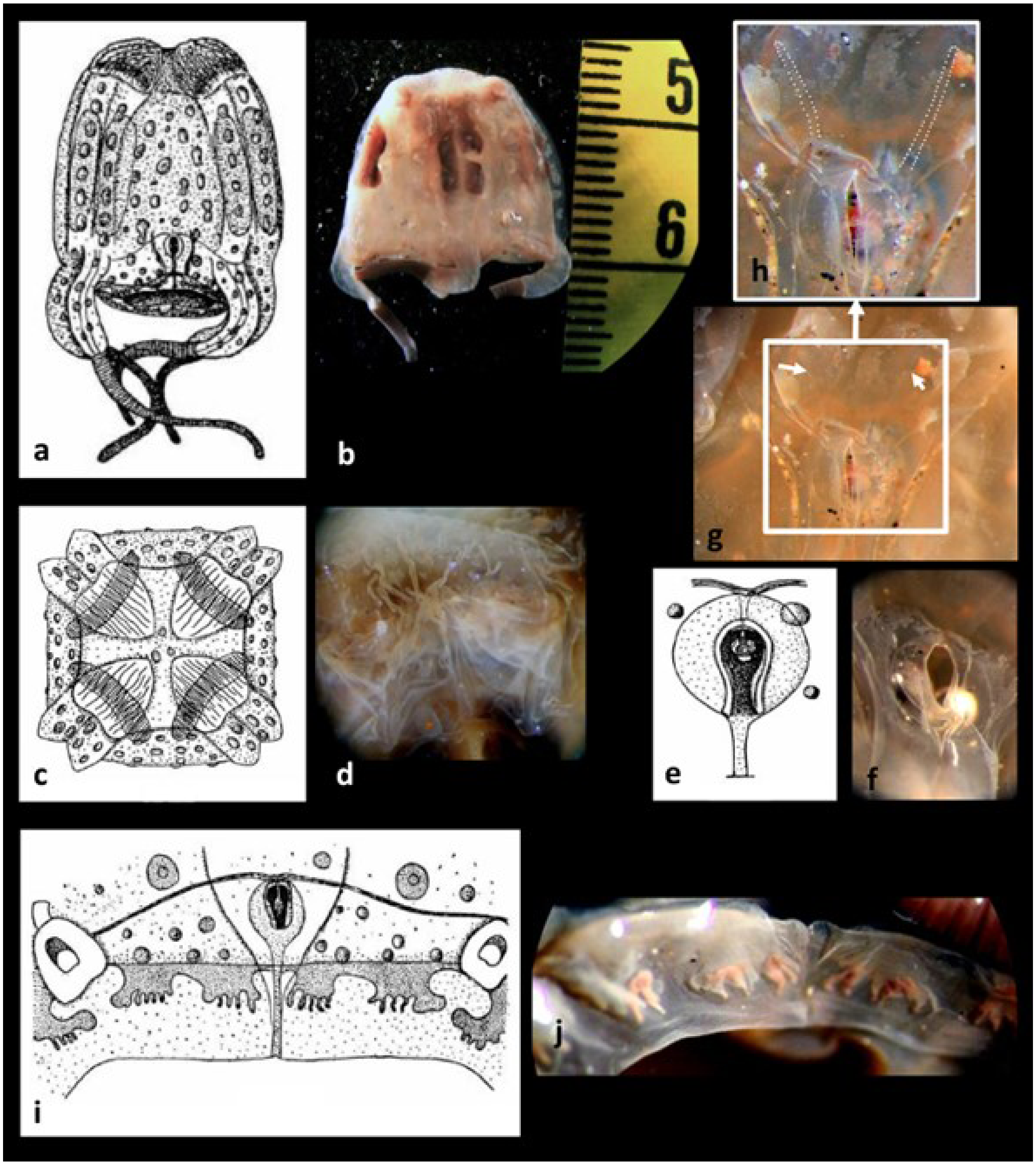

Figure 5

Copula siviski line drawings by Stiasny (a, c, e, i: Stiasny 1926: Figs. 1-4) and images of holotype (b, d, f, g, h, j: preserved specimen). **a, b**: Habitus, lateral view. **c, d**: Adhesive pads on apex and gastric filaments in stomach. **e, f**: rhopalial niche. **g, h**: rhopalial niche with rhopalial horns highlighted (g: white arrows, h: dotted line). **i, j**: velarium with “paw”-shaped velarial canals.