



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, 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 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* sp. nov., the velarial canal roots taper towards the velarial rim and each root bears one to two narrow triangular canals with sharp tips, resembling a bird beak. In contrast, the velarial canal roots of *C. sivickisi* bear two short, broad canals that increase breadth towards the velarial rim and split up into three- to six-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 confirm the distinction of the new species and 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 but that a revision of the genus *Copula* is necessary.

Keywords Tripedaliidae · Taxonomy · Morphology · New species · Ecology

Introduction

Cubozoa (Werner, 1973), also known as cubomedusae or box jellyfish, is the smallest cnidarian class with 50 currently accepted species, half of them described in the past two decades (Collins 2024). 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),

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ability to swim actively and horizontally against currents that defines them as nekton and not as plankton which drifts passively (Haeckel 1890, Gordon and Seymour 2009, Garm et al. 2007, Colin et al. 2013, Schlaefer et al. 2020, 2021; Bordehore et al. 2024), complex mating behavior (Lewis and Long 2005; Lewis et al. 2008), associative learning (Bielecki et al. 2023), and powerful venoms (Kintner et al. 2005; Yanagihara and Shohet 2012; Jouiaei et al. 2015) that can even result in fatalities (Fenner and Hadok 2002; Tibballs 2006; Carrete and Seymour 2013; Sucharitakul et al. 2017). Because of these fascinating traits, box jellyfish garner a great deal of attention from both the scientific community and the general public.

Among this class, the genus *Copula* (Bentlage, Cartwright, Yanagihara, Lewis, Richards & Collins, 2010) was recently introduced 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 and Long 2005; Garm et al. 2012; Morandini et al. 2014; Straehler-Pohl et al. 2014; Toshino et al. 2014), Hawaii (Matsumoto et al. 2002; Crow et al. 2006), Guam (Gershwin 2003), Bahamas (Bennett et al. 2011), and Australia and Tasmania (Hartwick 1991; Gershwin 2005, Kingford 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* (NHM 1932.2.6.3) is the

specimen from the Philippines, which was deposited in the Natural History Museum of London. On loan since 1964 and never returned, we recovered the missing holotype after almost 60 years in a collection of Wellington University, New Zealand, and returned it 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).

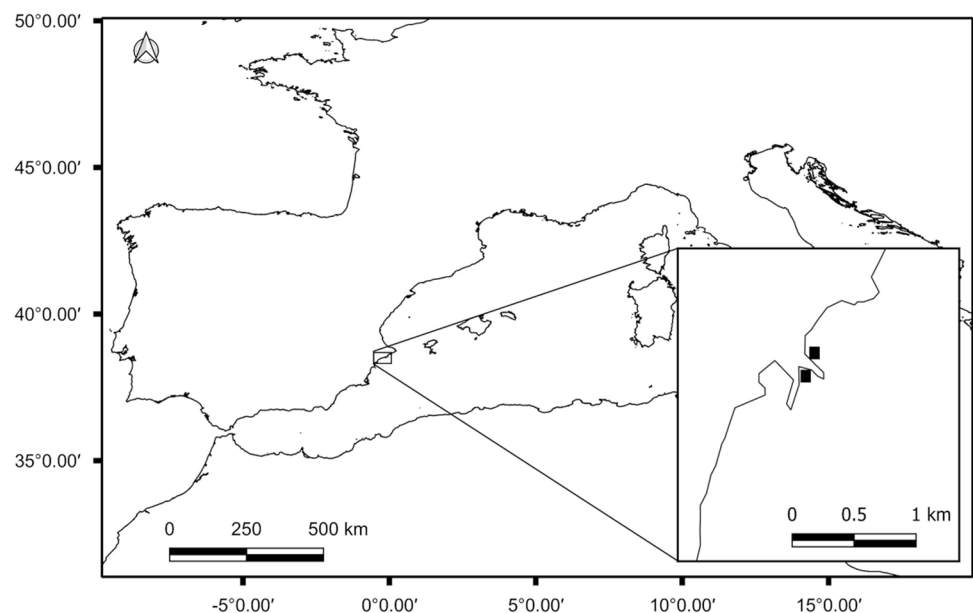
In this study, after a morphological comparison with the recovered holotype and a genetic analysis along with specimens sampled in different locations around the world, we present the description of a new species of cubozoan, *Copula lucentia* sp. nov., based on specimens collected in El Campello, Alicante, Spain (Western Mediterranean Sea).

Material and methods

Medusae and environmental variable sampling

Copula lucentia sp. nov. 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, 38° 25' 56.01" N 0° 22' 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 m from the shoreline and 1 m deep. When conditions were appropriate (sea surface very calm and transparent waters), first cubozoans appeared after about 1 h of light exposure and during the following 2 h, 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.

Fig. 1 Map of the sampling sites (marked with black squares), located in the Western Mediterranean



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 were deposited in the National Museum of Natural Sciences (Museo Nacional de Ciencias Naturales). DNA samples and additional studied material were deposited in UNESP-LEDALab-Sao Paulo State University-Evolution and Aquatic Diversity Lab (tissue collection).

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* sp. nov. 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 et al. 2014, 2017; Straehler-Pohl 2019; Acevedo et al. 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 turnover; 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 (NHM 1932.2.6.3) was studied under a stereomicroscope (Bresser 5804000); photos were taken with digital cameras Canon EOS 550D and Bresser MikrOkular Full HD.

For nematocyst 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 $\times 40$ objective (i.e., $\times 400$ magnification). Nematocysts 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 (IZ–Smithsonian Molecular database) (Table 1). The DNA was extracted with Agencourt DNAdvance (magnetic beads), PCR reactions, and conditions followed under predefined conditions (see Acevedo et al. (2019) for 16S and LCO1490 (GGTCAACAAATCATAAAGATATTGG) and HCO2198 (TAAACTTCAGGGTGACCAAAAATCA) 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 AMPure XP (Agencourt) kit following manufacturer's instructions and made ready for sequencing using the BigDyeH Terminator v3.1 (Applied Biosystems) kit, the same primers, and Tm temperature conditions as in PCR reactions. Sequencing was carried out on an ABI PRISM@3100 genetic analyzer (Hitachi), and resulting sequences were assembled and edited using Geneious 9 (Biomatters Ltd) (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 cubozoans from different areas in MEGA X (*p*-distance), and ML reconstruction was performed in Geneious 9 (PhyML) with bootstrap test with 100 \times replicates.

p-distance model (GTR) of base substitution was used to calculate genetic distances in MEGA XI software (Tamura et al. 2021).

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

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<https://zoobank.org/B314438E-D3B1-484C-93BE-FA1517190B81>

Tables 2 and 3; Figs. 2, 4, and 6.

Copula lucentia

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),

Table 1 GenBank record of the new material sequenced in this study. More details in each GenBank record

Species	Locality	COI	16S
<i>Tripedalia cystophora</i>	Florida, USA	PP352508-PP352518	PP352034-PP352038
<i>Copula sivickisi</i>	Bahamas	PP352496-PP352503; PP352506-PP352507	PP352027-PP352031
<i>Copula sivickisi</i>	Papua New Guinea	PP352504-PP352505	PP352032-PP352033
<i>Copula sivickisi</i>	Japan	PP352519-PP352521	PP352039-PP352042
<i>Copula sivickisi</i>	Saipan, Northern Mariana Islands	–	PP352043-PP352054
<i>Copula lucentia</i> sp. nov.	Spain	OQ857826-OQ857828	OQ869612-OQ869614

Illeta dels Banyets, El Campello, Spain (38° 25' 56.01" N 0° 22' 57.75" W), 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, 38° 25' 56.01" N 0° 22' 57.75" W 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, 38° 25' 56.01" N 0° 22' 57.75" W 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, 38° 25' 56.01" N 0° 22' 57.75" W coll. by ES. Fonfria, 17 October 2018.

Type locality: Illeta dels Banyets, El Campello, Spain (38° 25' 56.01" N, 0° 22' 57.75" W) (Western Mediterranean Sea).

Etymology: The species name derived from “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, colorless, 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.

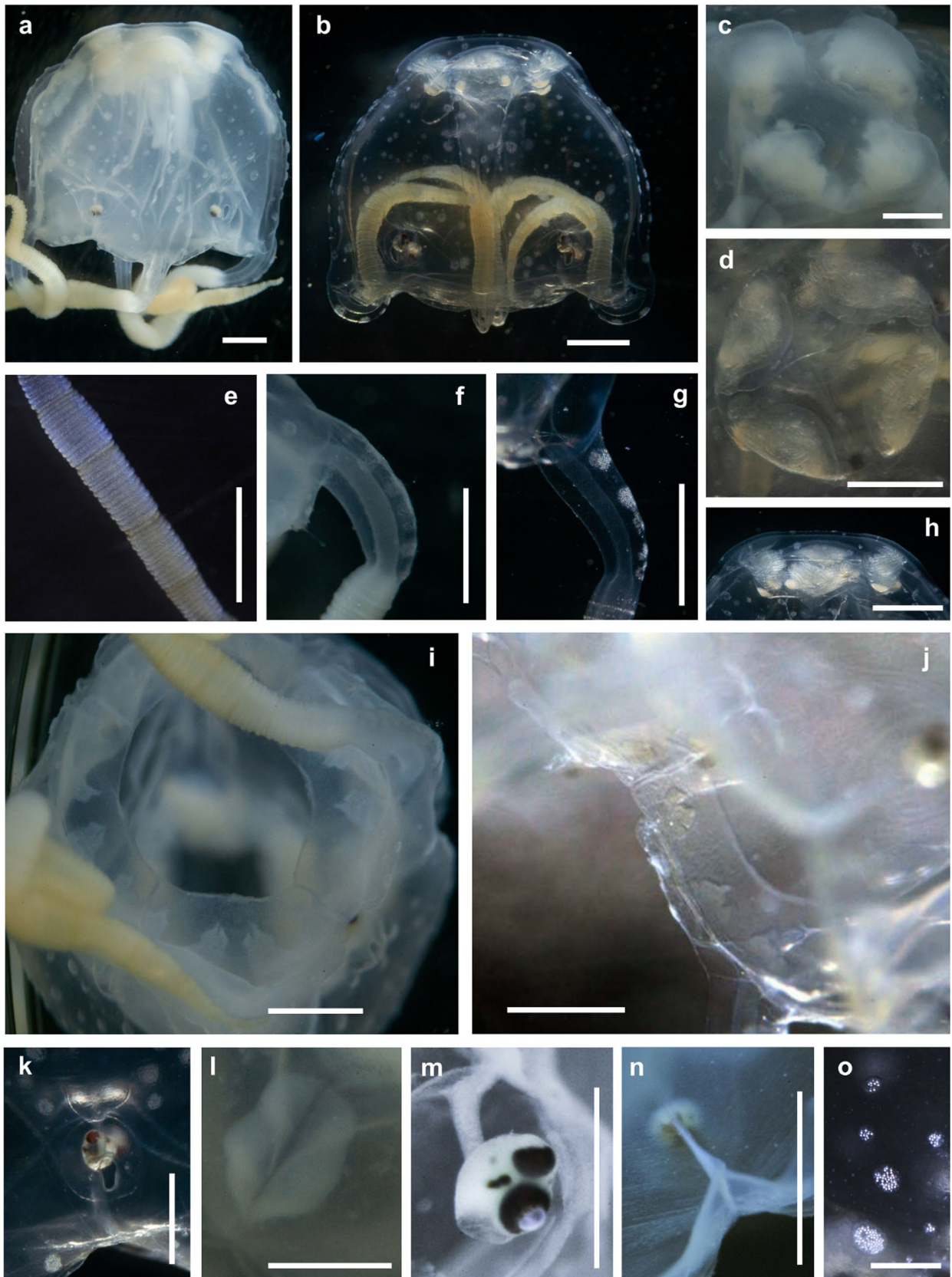
Pedaliium, single, unbranched, flattened, blade-shaped, intermediate in length (PL: <30% BH, PW: <40 PL), located at the four interradial corners of the bell margin, with 4–6 horizontal rectangular white, round nematocyst warts to longish oval, horizontal, nematocyst bands on outer pedaliial keel (Fig. 2f, g). Pedaliium 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

Table 2 Cnidome of *Copula lucentia* sp. nov. *L* length of undischarged capsule in micrometer, *W* width of undischarged capsule at widest point in micrometer, *SD* standard deviation, *N* number of nematocysts measured

Part	Nematocyst type	Measure	Min	Max	Mean	SD	<i>N</i>
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 3 Comparison of morphological characters of *Copula sivickisi* and *Copula lucentia* sp. nov. *BH* bell height, *IPD* interpedalial diameter, *PL* pedalial length, *StL* stalk length, *PW* pedalial width

Character	<i>Copula sivickisi</i>	<i>Copula lucentia</i> sp. nov.
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 (\geq height of rhopalial niche), very narrow, vertical, slightly curved apart from each other , antenna-shaped without knob at distal end	- Intermediate in length (\leq 50% of rhopalial niche height), very narrow, vertical, curved towards each other , antenna-shaped slightly flared at distal end
Pedalia	- Unbranched, long (PL: \leq 50% BH, StL: none; PW: \leq 50% PL) , slender knife-blade-shaped	- Unbranched, intermediate in length (PL: \leq 30% BH; StL: none; PW: \leq 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), four-lobed, short moutharms	- Short (30% BH), four-lobed, short moutharms
Lateral gonads	- (Holotype, female) 4 butterfly-shaped pairs, hemigonads, single wing biscuit-shaped , brown (fertilized); sexes supposed dimorph	- (Female) 4 butterfly-shaped pairs, hemigonads, single wing leaf-shaped , transparent to whitish (immature); sexes supposed dimorph



◀ **Fig. 2** *Copula lucentia* sp. nov. holotype (MNCN 2.02/1) (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** rhopalialia; **n** frenulum; **o** bell nematocyst warts. Scales, 1 mm

(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 nematocyst 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 interradiol 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 nematocyst 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 color (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 interradiol septa, not fully developed yet (Fig. 2l).

Nematocysts: (after Östman 2000; Gershwin 2006)

Four different nematocyst types were identified and measured in the paratype MNCN 2.02/4 (Table 2); 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 be 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 behavior, fertilization, polyps, and asexual reproduction: No data are available yet. Only immature female specimens were sampled to date.

Habitat and ecology: *Copula lucentia* sp. nov. were found in shallow waters (< 1.5 m in depth). Illeta dels Banyets is a rocky peninsula of 10,000 m² 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* sp. nov. have been collected at both sides of the peninsula (Fig. 1).

During the 3 years of nocturnal samplings between August and November, *Copula lucentia* sp. nov. 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 \pm 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 to 28.0 °C), with only two individuals collected below this range; one at 21.6 °C and other at 17.8 °C (both in October 2017, on days 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 underway 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* sp. nov., 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 they present differences of approximately 20% (*p*-distance) in relation to specimens from Japan and Australia based on COI (Table 4). The sequences recovered from the 16S amplified sector are exactly the same among the samples from Spain; however, they present significant divergence

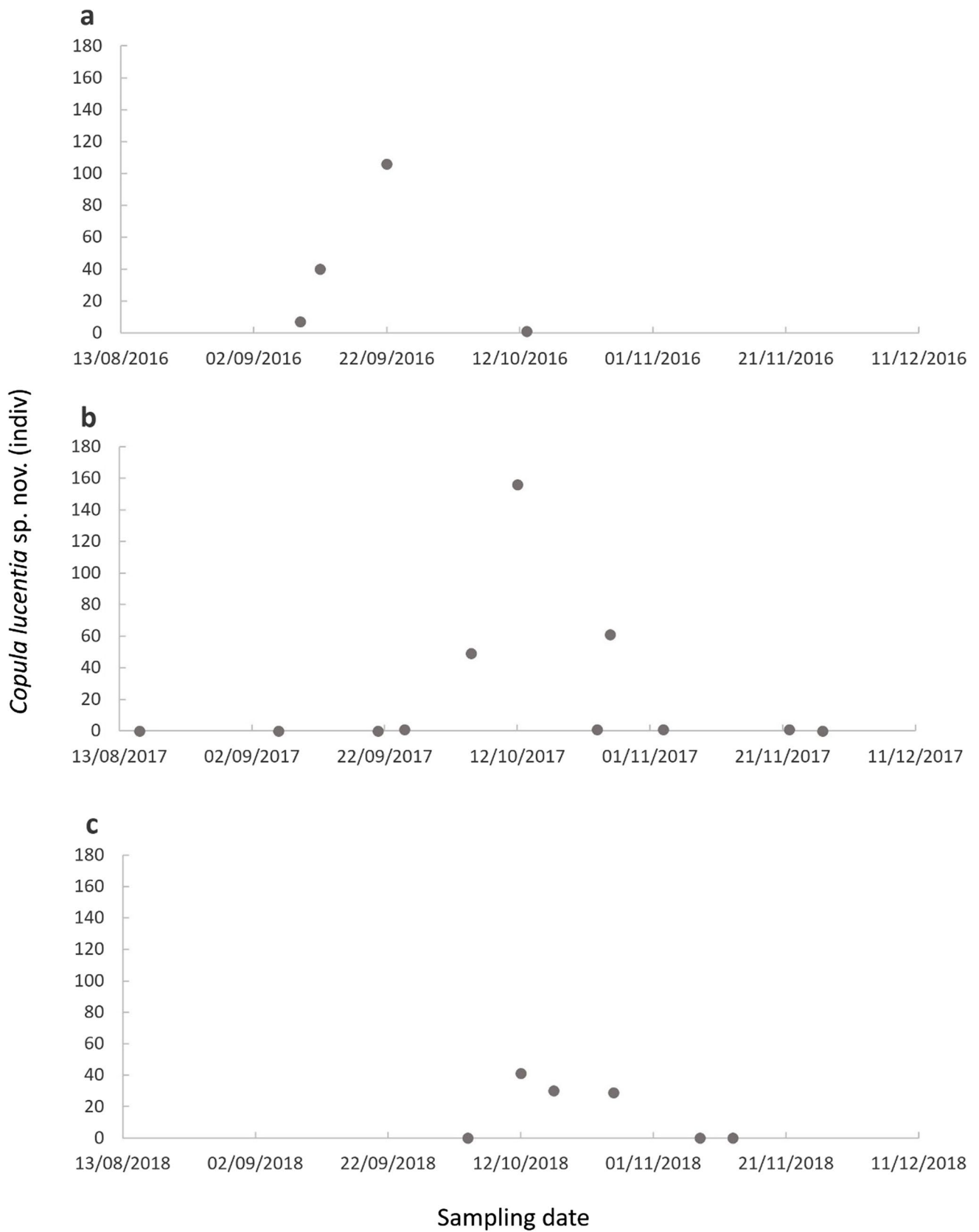


Fig. 3 *Copula lucentia* sp. nov. individuals captured by sampling dates during ~3 h of light exposure. Years, **a** 2016; **b** 2017; **c** 2018

Table 4 Estimates of evolutionary divergence (*p*-distance value) between barcode sector (COI) of *Copula* matrix and *Tripedalia cystophora*

	<i>T. cystophora</i>	<i>Copula</i> – Bahamas + Northern Mariana Island	<i>Copula</i> – Japan	<i>Copula</i> - Australia	<i>Copula</i> – Papua N. Guinea	<i>Copula</i> - Northern Mariana Island	<i>Copula</i> - Spain
<i>T. cystophora</i>	0.002-0.006						
<i>Copula</i> – Bahamas + Northern Mariana Island	0.232 (0.227-0.233)	0					
<i>Copula</i> – Japan	0.231 (0.229-0.231)	0.2106 (0.207-0.213)	0.0-0.005				
<i>Copula</i> - Australia	0.23 (0.229-0.232)	0.227	0.228 (0.225-0.231)	-			
<i>Copula</i> – Papua N. Guinea	0.201 (0.196-0.202)	0.217	0.191 (0.189-0.20)	0.21 (0.209-0.211)	0.002		
<i>Copula</i> - Northern Mariana Island	0.222 (0.208-0.236)	0.238 (0.225-0.251)	0.233 (0.229-0.243)	0.218 (0.217-0.219)	0.217 (0.217-0.219)	0-0.05	
<i>Copula</i> - Spain	0.216 (0.213-0.219)	0.201	0.199 (0.196-0.202)	0.204	0.178	0.204 (0.202-0.206)	0

in relation to the material from Australia (Table 5). 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. sivickisi* (Stiasny, 1926), reported mainly across the Pacific and also 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 lucentia* sp. nov. from the Spanish coast of the Mediterranean Sea (Table 3)

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*

Table 5 Estimates of evolutionary divergence (*p*-distance value) between 16S sector of *Copula* matrix and *Tripedalia cystophora*

	<i>T. cystophora</i>	<i>Copula</i> – Bahamas + Northern Mariana Island	<i>Copula</i> – Japan	<i>Copula</i> - Australia	<i>Copula</i> – Papua N. Guinea	<i>Copula</i> - Northern Mariana Island	<i>Copula</i> - Spain
<i>T. cystophora</i>	0.007-0.03						
<i>Copula</i> – Bahamas + Northern Mariana Island	0.315 (0.312- 0.322)	0-0.02					
<i>Copula</i> – Japan	0.307 (0.306- 0.308)	0.2104	0.0				
<i>Copula</i> - Australia	0.33 (0.327-0.35)	0.263	0.2935	-			
<i>Copula</i> – Papua N. Guinea	0.35 (0.327-0.372)	0.20 (0.182- 0.224)	0.241 (0.223- 0.259)	0.26 (0.244- 0.276)	0.02		
<i>Copula</i> - Northern Mariana Island	0.286 (0.277- 0.298)	0.251 (0.248- 0.265)	0.248 (0.246- 0.25)	0.26 (0.256- 0.272)	0.257 (0.233- 0.293)	0-0.05	
<i>Copula</i> - Spain	0.316 (0.311-0.32)	0.255	0.274	0.264	0.266 (0.246- 0.286)	0.236	0

in many morphological characteristics, but differ significantly in several key structures (Table 3) defined, e.g., by Gershwin (2005), Straehler-Pohl et al. (2017), and Acevedo et al. (2019).

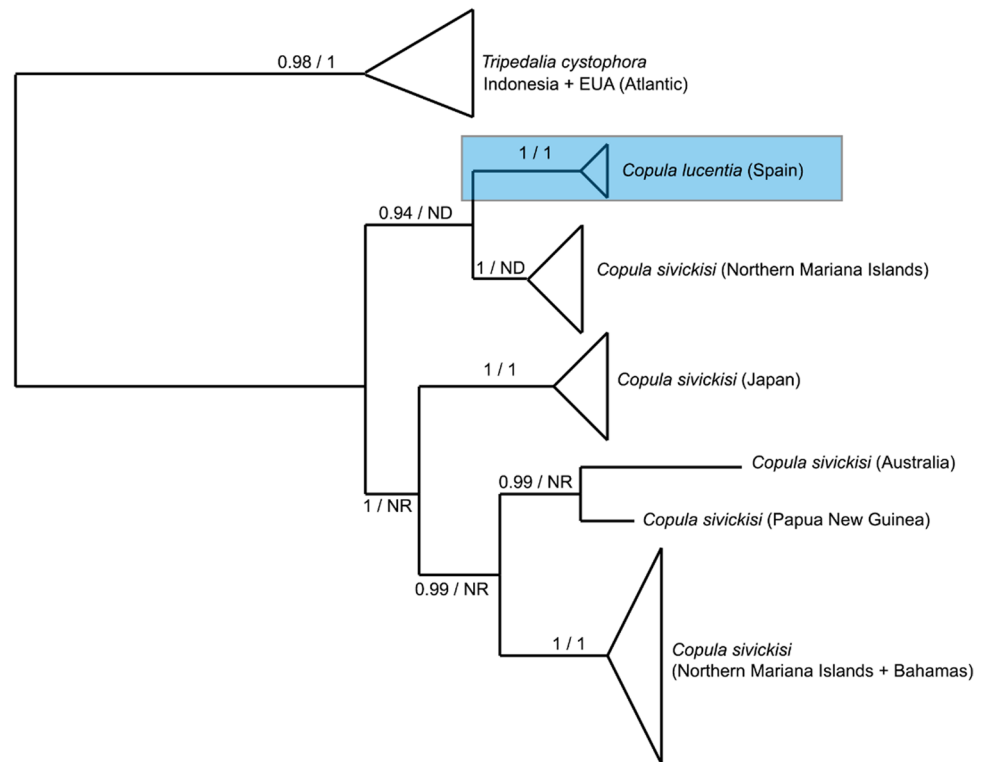
The bell shape of the Mediterranean specimens and of *C. sivickisi* is quite equal in shape but the Mediterranean specimens are much smaller (6.1 mm BH, present study: Fig. 6a, d), about half the size of the Philippine specimens (10–13 mm BH; Stiasny 1926, present study: Fig. 6a, d). The Mediterranean specimens are not yet mature but grown (gonads are developed but eggs are not yet ripe). The medusae meet the size of fully grown specimens from Japan (5.5–7.0 mm; Straehler-Pohl 2011, Figs. 3, 6, 7, and 8; Straehler-Pohl et al. 2014, Fig. 2a, h) but show a more complex velarial canal pattern (Straehler-Pohl et al. 2014, Fig. 2c, j); therefore, we conclude that this species is not the one from Japan but as small sized in maturity.

The umbrellar nematocyst warts of *C. sivickisi* are very large, roundish, oval to biscuit-shaped (Fig. 5a, b) while the bell nematocyst warts of the European *Copula* specimens are small and round (Fig. 2a, b, o).

Differences are also found in the length and shape of the rhopalial 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: Figs. 5g, h and 6e) 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 (Figs. 2k and 6b).

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

Fig. 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



study: Figs. 5i and 6c), 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 (Figs. 2j and 6f). For more details on additional minor morphological differences, see Table 3 and Figs. 2 and 5.

Based on the 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* (NHM 1932.2.6.3), 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* sp. nov. (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* sp. nov. 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 (e.g., 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 sp. nov. was found actively swimming in El Campello in autumn with temperatures between 17.8 and 28.0 °C. These results are similar to those from Lewis and Long (2005) and Toshino et al. (2014), who collected *Copula sivickisi* specimens around Okinawa (SW Japan) when water temperature was 23–28 °C and Schlaefer et al. (2020, 2021) who sampled *Copula sivickisi* individuals in Townsville (NE Australia) with an average water temperature of 26 °C. In the lower end of the range, it also coincides with the data recorded by Hoverd (1985) in New Zealand, where *Copula sivickisi* specimens, although present all year around, were mainly observed in February when temperatures were 17–19 °C.

During three consecutive years (2016–2018), we found hundreds of *C. lucentia* sp. nov. individuals in El Campello, with a DBW and BH average size of 2.5 ± 1.0 mm and 2.2 ± 0.9 mm (mean \pm 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,

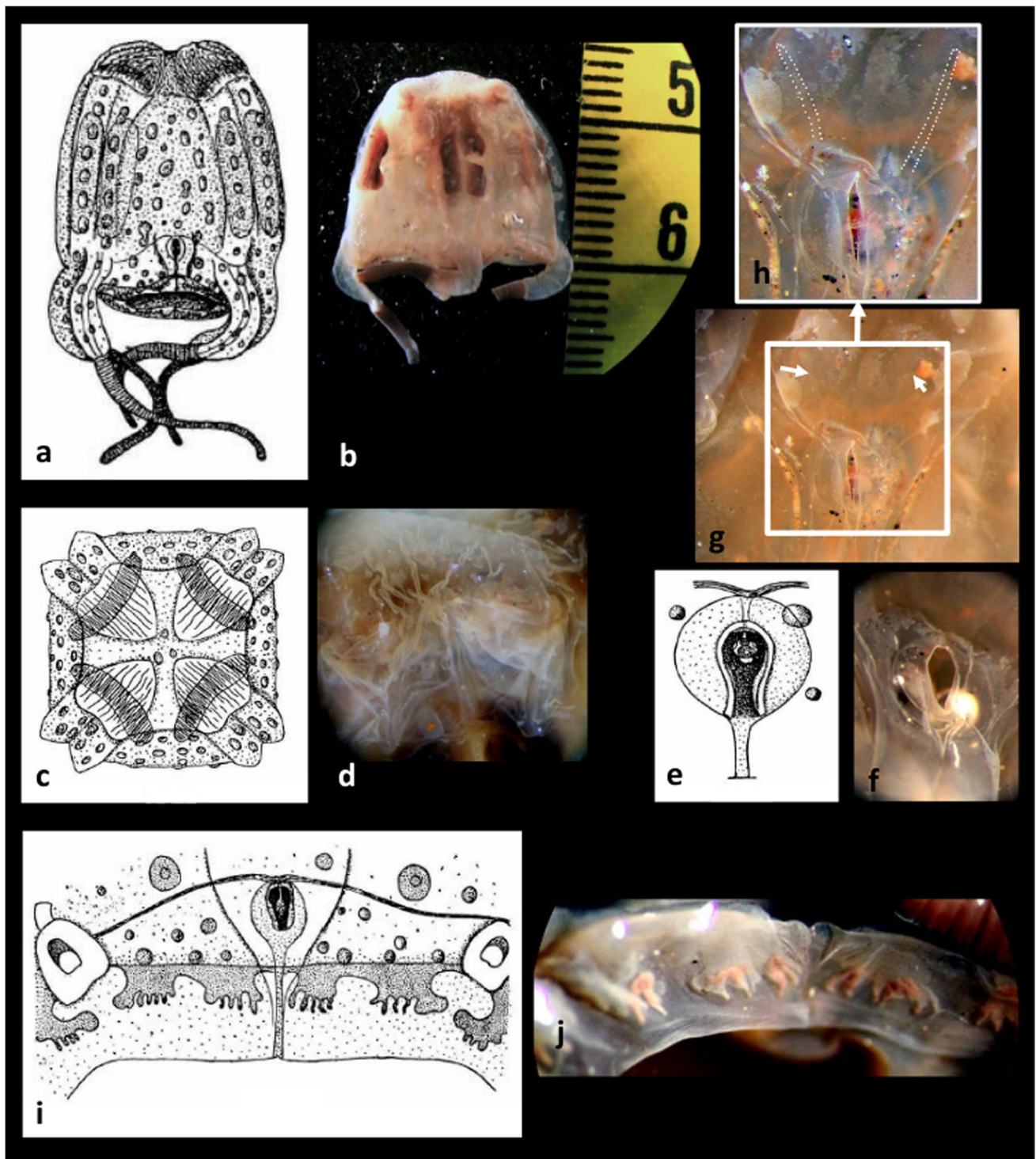


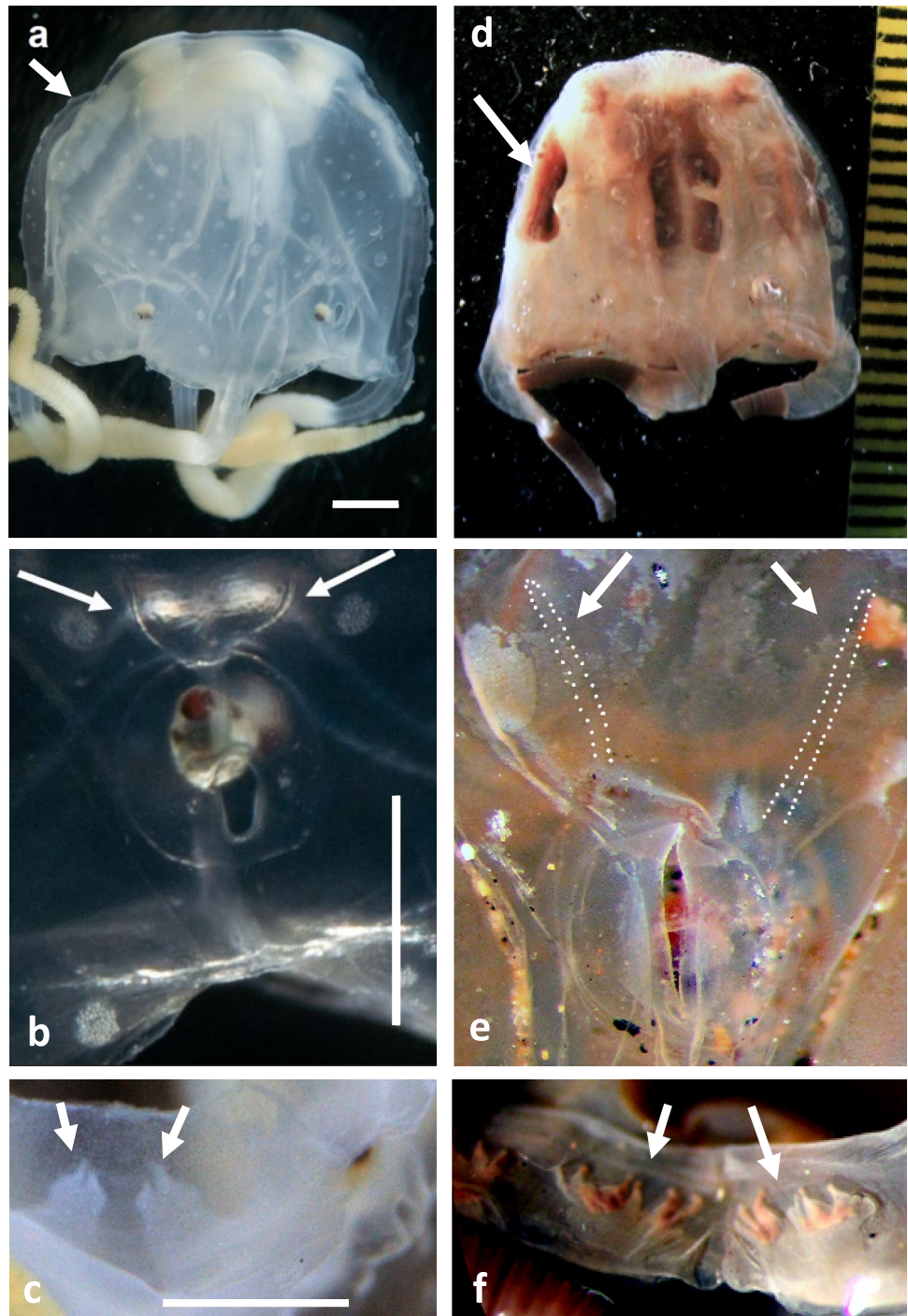
Fig. 5 *Copula sivickisi* line drawings by Stiasny (a, c, e, i Stiasny 1926: Figs. 1, 2, 3, and 4) and images of holotype (NHM 1932.2.6.3) (b, d, f, g, h, j preserved specimen). a, b Habitus, lateral view; c, d adhesive pads on apex and gastric filaments in the stomach; e, f rho-

pial niche; g, h rhopalial niche with rhopalial horns highlighted (g white arrows, h dotted line); i, j velarium with “paw”-shaped velarial canals

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 although

we found only females and young. However, few mature specimens were gathered during samplings, for unknown reasons. Juveniles and adults of the cubozoan

Fig. 6 Comparison of type material of *Copula lucentia* sp. nov. (a–c) and *Copula sivickisi* (d–f). The specimens of both species are adult females, the one of *C. lucentia* sp. nov. is not yet gravid (a, c); the one of *C. sivickisi* is gravid (d, f). *C. lucentia* sp. nov. is about 6 mm in bell height (a); *C. sivickisi* is 13 mm in bell height (d). The rhopalial horns of *C. lucentia* sp. nov. (b) are bent inwards, very narrow, and much smaller than the broad, outward bent rhopalial horns of *C. sivickisi* (e). The velarial canals of *C. lucentia* sp. nov. are quite narrow and bear two narrow, parallel arranged secondary canals per root that give the velarial canal a bird-head silhouette with the secondary canals forming its beak (b). The velarial canals of *C. sivickisi* are very broad and paw-shaped with several claw-shaped secondary canals (f)



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 medusa stages, with adults able to swim against currents and select the most favorable habitats (Bordehore et al. 2020, 2024). Schlaefer et al. (2020, 2021) demonstrated strong swimming abilities for *C. sivickisi* specimens with IPD (interpedalial diameter, defined as distance between opposite

pedalia—outer pedalial wing edges—at the level of the bell turnover according to Straehler-Pohl et al. 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* sp. nov. 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* sp. nov., 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-Pohl 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* sp. nov., 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 suggests a poor dispersal potential for this species (Schlaefer et al. 2020, 2021), a comprehensive revision of the genus *Copula* is required as a deeper molecular study (Fig. 4) hints to the fact that several undescribed species are united under the name of *Copula sivickisi*.

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Declarations

Conflict of interest The authors declare no competing interests.

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.

Author 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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