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A new species of pygmy *Paroctopus* Naef, 1923 (Cephalopoda: Octopodidae): the smallest southwestern Atlantic octopod, found in sea debris

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Abstract

The new species, *Paroctopus cthulu* sp. nov. Leite, Lima, Lima and Haimovici was recorded from shallow coastal waters of south and southeastern Brazil, where most specimens were found sheltered in marine debris. It is a small octopus; adults are less than 35 mm mantle length (ML) and weight around 15 g. It has short- to medium-sized arms, enlarged suckers on the arms of both males and females, a relatively large beak (9% ML) and medium to large mature eggs (3.5 to > 9 mm). The characteristics of hatchlings of two brooding females, some of their anatomical features, and in situ observations of their behavior are a clue to the life history of it and closely related pygmy octopuses. The Bayesian phylogenetic analysis showed that *Paroctopus cthulu* sp. nov. is grouped in a well-supported clade of *Paroctopus* Naef, 1923 species, clearly distinct from *Octopus joubini* Robson, 1929 and *Paroctopus mercatoris* (Adam, 1937) from the Northwestern Atlantic. The description of this new species, living in habitat altered by humans, debris in shallow water off Brazil, offered an opportunity not only to evaluate the relationship among the small octopuses of the western Atlantic, Caribbean and eastern Pacific, but also their adaptation to the Anthropocene period.

Keywords Anthropocene · Marine debris · Octopus · Integrative taxonomy · Phylogeny · Brazilian coast

Introduction

The pygmy octopuses are small bodied species of Octopodidae d'Orbigny, 1840 in Férussac and d'Orbigny 1835 some of which mature as small as a 20-mm dorsal mantle length (around 100 mm of total length). Most of them are currently placed in the

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genus *Paroctopus* Naef, 1923, which was originally proposed by Naef (1923) based on the relatively large size of the eggs of *Paroctopus digueti* Perrier & Rochebrune, 1894 (capsule length 10 mm). Two years later, Grimpe (1925) erected the genus *Pseudoctopus* Grimpe, 1925 based on the same morphotype species, citing the single attachment of eggs, as well as the egg size. Robson (1929), in an attempt to validate Naef's genus, amplified the diagnosis with several additional characters, namely (1) possession of relatively long copulatory organ (Ligula length index LLI 7–20); (2) short arms; and (3) squat, bursiform body. Pickford (1945, 1946) initially accepted the validity of the genus in her evaluation of the Octopodidae fauna of the western Atlantic. However, she later rejected the name when discussing the generic placement of the large egg species, *Octopus bimaculoides* Pickford & McConnaughey, 1949.

As currently understood, the genus is represented by a transisthmian geminate species complex endemic to tropical and subtropical waters in the Americas (see Berry 1953, Nesis 1978, Lima et al. 2020). This complex includes *Paroctopus digueti* (type species) along the tropical eastern Pacific, and two morphotypes: one with smaller eggs (< 4 mm) and the other with larger eggs (> 5 mm) in the Northwestern Atlantic, Caribbean Sea and Gulf of Mexico (Forsythe and Hanlon 1980; Tiffany et al. 2006). Due to their small size, the pygmy octopuses have been used in laboratory experiments on mating behavior (Mather 1978; Cigliano 1995); reproductive biology and growth (Opresko and Thomas 1975) and ontogeny of behavior, habitat use and distribution (Mather 1980a, b, 1982a, b, 1984).

When it comes to the Northwestern Atlantic and Caribbean Sea, the pygmy taxonomy is confusing. In the Caribbean Sea (St. Thomas/ Virgin Island), the small egged species was described as *Octopus joubini* Robson, 1929 and a broad literature citing this species is available (Pickford 1945; Boletzky and Boletzky 1969; Forsythe 1984). However, some important publications cited the large-egged species also under the name *O. joubini* (Opresko and Thomas 1975; Hanlon 1983). Other studies refer to a pygmy octopus with large eggs from the Gulf of Mexico (Dry Tortugas and Tampa Bay) as *Paroctopus mercatoris* (Adam, 1937) (Forsythe and Hanlon 1980; Forsythe and Toll 1991; Tiffany et al. 2006), despite the fact that the holotype of *P. mercatoris* is a female bearing relatively small eggs (3 mm) (Voss and Toll 1998). In fact, some authors considered the small egged species, *O. joubini* and *P. mercatoris*, as possible *Paroctopus* (Voss and Toll 1998; Jereb et al. 2014; Lima et al. 2020), while the large-egged species is still undescribed.

The available literature on pygmy octopuses from the Southwestern Atlantic is scarce and not less confusing. Haimovici (1985) registered as *O. joubini* a small juvenile collected in a tide pool off Vitória, Espírito Santo State. Perez and Haimovici (1991) designated as *O. joubini* a lot of small octopods (MZUSP 27,028) collected in São Paulo

State (23° 30′ S) in their list of cephalopod species deposited at the Museu de Zoologia da Universidade de São Paulo. In recent years, several small adult octopuses with stocky bodies and medium-sized arms, some of them bearing the enlarged suckers and a medium size ligula, which fit the description of the *Paroctopus* type species, were collected in shallow waters of Santa Catarina and Rio de Janeiro states, along the warm temperate Brazilian coast. Live specimens were observed using human garbage as shelters. They included two brooding females with medium to relatively large eggs, which enabled the descriptions of eggs, embryos, and hatchlings, and thus provided biological and ecological information on the early stages of the life cycle. Morphological features and body proportions of eggs and hatchlings in relation to the adults are important for inferences about the developmental mode—planktonic or benthic—of octopus hatchlings, providing key information on life history traits.

Molecular and morphological characterization of these specimens does not fit with the available information on the valid species of the genus and support their description as a new species. Additional images provided valuable information on their behavior. Herein, we provide a detailed and integrated description of a new species of *Paroctopus* collected from sea garbage, including descriptions of adult males and females, eggs, embryos, and hatchlings, along with molecular data and analyses.

Material and methods

Collection samples

A total of 12 specimens (six adult males, three adult females, and three juveniles) was collected in the shallow coastal waters of Rio de Janeiro municipality and Ilha Grande continental island (Angra dos Reis municipality) in Rio de Janeiro (RJ) State, and in Porto Belo municipality in Santa Catarina (SC) State, Southeastern and Southern Brazil (Fig. 1). Most of the RJ specimens were collected at depths shallower than 5 m by hand, during snorkeling or SCUBA diving on rubble or sandy bottoms near the rocky coast. The sea temperature varied from 19 to 26° C. The specimens were collected by sorting solid garbage found on the sea bottom, including metal cans, glass bottles, and plastic objects such as snorkel mouthpieces. No specimen was found inside empty shells, although we also looked for them during the dives. Two females with eggs were found, one spawned inside a snorkeling mouthpiece, and the other in a metal beer can.

Molecular data and analyses

Tissue samples of the mantle or arms of three specimens (CRT4863 from MORG 52,777; CRT4864 from
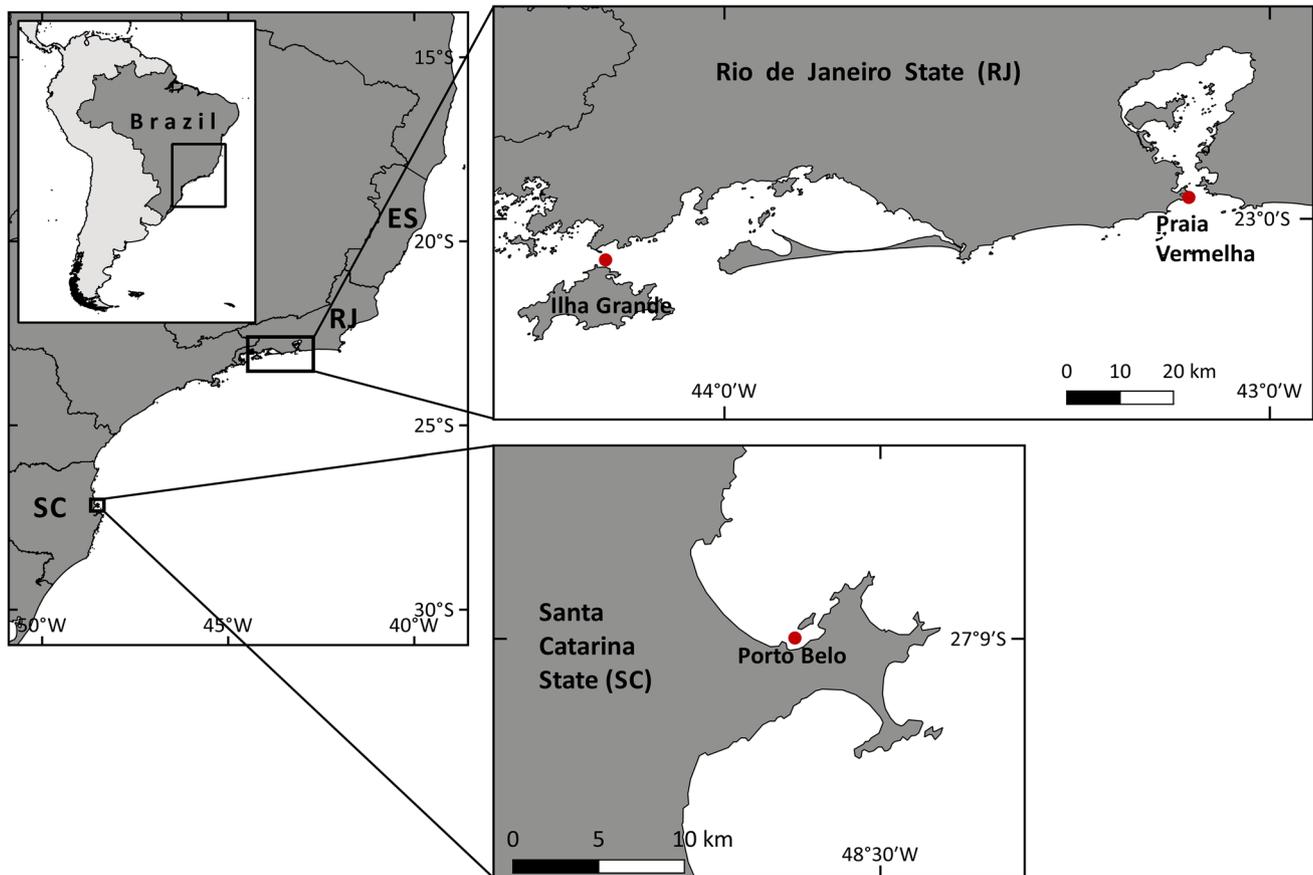
Fig. 1 *Paroctopus cthulu* sp. nov. distribution (Espírito Santo (ES) to Santa Catarina (SC)) and collection area map (Rio de Janeiro (RJ) and Santa Catarina (SC))

MORG52779; CRT4867 from MORG 52,780; GenBank accession numbers: MN933645, MN933646, MWI96228) were preserved in 99% ethanol from which genomic DNA was extracted using the GF-1 Nucleic Acid Extraction kit (Vivantis, Malaysia) according to the manufacturer's instructions. Sequences of 33 species were also retrieved from GenBank (Table 1). Fragments of the mitochondrial cytochrome oxidase subunit I (COI) gene were amplified by using the universal primers LCO1490 and HCO2198 (Folmer et al. 1994). The PCR amplification reactions were conducted in a final volume of 25 µL containing 1 µL forward primer, 1 µL reverse primer (10 mM), 12.5 µL Taq DNA Polymerase Master Mix (Ampliqon A/S) or MyTaq RedMix (Bioline), 8.5 µL H_2O, and 2 µL DNA (20–40 ng/µl). Amplification PCR cycle parameters were 3 min at 95 °C for denaturation, followed by 35 cycles of 1 min at 94 °C, 1 min at 45 °C for annealing, 1.5 min at 72 °C for extension, and a final extension step of 4 min at 72 °C. The PCR products were purified and sequenced by Macrogen Inc, Seoul, Korea. Electropherograms were edited with Geneious 9.0.2 (Kearse et al. 2012) and sequences were aligned by Clustal W using Mega 6 (Tamura et al. 2013). The substitution model GTR + G was chosen using the software jModeltest (Posada 2008).

Bayesian phylogenetic inference was carried out in BEAST 1.8.4 (Drummond et al. 2012). An uncorrelated lognormal relaxed clock model was used. Monte Carlo Markov Chain (MCMC) runs were performed for 1×10^8 generations, sampling one tree each 1×10^4 runs. The convergence of MCMC runs, effective sample size, and the correct "burn–in" for the analysis were assessed using Tracer v1.6 (Rambaut et al. 2014). A consensus tree accessing the posterior probability values of each clade was generated using TreeAnnotator 1.8.3 (Drummond et al. 2012) and displayed using FigTree 1.4.3. Pairwise genetic distances of *Paroctopus* species were calculated using K2P (Kymura 2-parameter) distance in MEGA 6 (Tamura et al. 2013) (Table 1 provides information on octopod specimens used to construct the Bayesian phylogenetic tree, including Genbank accession number).

Institutional abbreviations: CRT: Aquatic Fauna Tissue Collection/ Universidade Federal do Rio Grande do Norte.

Morphological data

Most specimens were fixed in 10% formalin and preserved in 70% ethanol. Measurements, counts, and indices

Table 1 Octopod specimens used to construct the Bayesian phylogenetic tree, including Genbank accession numbers and references. Asterisks indicate species of the *Paroctopus* clade

Species	GenBank	Reference
Amphioctopus fangsiao	HQ846155	Dai et al. 2012
Amphioctopus marginatus	KP976308	Shen et al. 2016
Amphioctopus burryi	MG778074	Ritschard et al. 2019
Amphioctopus kagoshimensis	MK185892	Braid and Bolstad 2019
'Octopus' laqueus	AB430543	Kaneko et al. 2011
Octopus incella	AB430542	Kaneko et al. 2011
Octopus micropyrsus	MK649805	Díaz–Santana–Iturrios et al. 2019
Octopus bimaculatus	KT335828	Pliego-Cárdenas et al. 2014
Octopus bimaculoides	KF225006	Pliego-Cárdenas et al. 2014
Octopus briareus	MN933636	Lima et al. 2020
Octopus hubbsorum	KF225002	Pliego-Cárdenas et al. 2014
Octopus hummelincki	MN508071	Lima et al. 2020
Octopus insularis	MN508072, MN508073	Lima et al. 2020
Octopus maya	MN508077	Lima et al. 2020
Octopus mimus	KT335830	Pliego-Cárdenas et al. 2014
Octopus oculifer	KT335831	Pliego-Cárdenas et al. 2014
Octopus tetricus	KJ605260	Amor et al. 2014
Octopus vulgaris (sensu stricto)	AJ252778	Hudelot (unpubl.)
Octopus americanus	MN933649	Lima et al. 2020
Octopus americanus	MN933651	Lima et al. 2020
'Octopus' tehuelchus *	GU355934	Acosta-Jofré et al. 2012
'Octopus' alecto *	MK649785	Díaz–Santana–Iturrios et al. 2019
'Octopus' mercatoris *	GQ900743	Huffard et al. 2010
Paroctopus digueti *	KT335833	Pliego-Cárdenas et al. 2014
Paroctopus sp. new species *	MN933645, MN933646, MW796228	This study
'Octopus' joubini *	AY377732	Okusu et al. 2003
'Octopus' fitchi *	KT335832	Pliego-Cárdenas et al. 2014
Callistoctopus macropus	MN933632, MN933633	Lima et al. 2020
Callistoctopus ornatus	HM104257	Strugnell et al. 2013
Macrotritopus defilippi	MN933638	Lima et al. 2020
Octopodidae sp. (White V)	GQ900737	Huffard et al. 2010
Thaumoctopus mimicus	GQ900746	Huffard et al. 2010
Tremoctopus violaceus	AF377978	Carlini et al. 2001
Loligo vulgaris	KM517928	Gebhardt and Knebelsberger 2015

followed Roper and Voss (1983) and Huffard and Hochberg (2005). Sucker counts included all suckers in each arm. The following abbreviations are used for measurements and indices:

AFL, left arm formula; AFR, right arm formula; ALI, arm length index (arm length/ML × 100); ASC, arm sucker count (number of suckers of each designated arm); AWI, arm width index (arm width at the widest point on stoutest arm/ML × 100); CaLI, calamus length index (calamus length/LL × 100); FLI, funnel length index (funnel length/ML × 100); FFLI, free funnel length index (free funnel length/funnel length × 100); GiLC, gill lamellae count per demibranch; HWI, head width index (head width/ML × 100); LLI, ligula length index (ligula length/ hectocotylized arm length × 100); ML, dorsal mantle length; MWI, mantle width index (mantle width/ML × 100); OAI, opposite arm length index (hectocotylized arm length/normal third arm length × 100); PLI, terminal organ (penis) length index (terminal organ length/ML × 100); SDeI, enlarged sucker diameter index (enlarged sucker diameter/ ML × 100); SDnI, largest normal sucker diameter index (largest normal sucker diameter/ML × 100); SpLI, spermatophore length index (spermatophore length/ ML × 100); SpRLI, sperm reservoir length index (sperm reservoir length/spermatophore length × 100); SpWI, spermatophore width index (spermatophore width/spermatophore length × 100); TL, total length; WDI, web depth index (web more depth/mantle × 100); WF, web formula; WT, total

preserved weight. For the species diagnosis and description, we present the index ranges with the minimum and maximum interval, separate by a hyphen (xx–xx).

Unless stated otherwise, all measurements are in mm and weights in *g*. Small structures such as the ligula, calamus, spermatophores, eggs, and radula were measured with an ocular micrometer in a binocular microscope.

The description was based on adult males with fully formed spermatophores, ligula and enlarged suckers; adult or spawned females with developing oocytes or spawned eggs; and some subadult specimens in which the sex could not be determined. All the specimens evaluated for this paper were deposited in the mollusk collections of the MORG and MCPUCRS.

Body patterns and behavioral postures were photographed and filmed during dives or inside an aquarium. The chromatic, skin texture, and body patterns components were described following Mather (1972) and Hanlon (1988).

Eggs, embryos, and hatchlings were described after collection of a brooding female inside an aluminum can in Rio de Janeiro (Praia Vermelha Beach). Live eggs and hatchlings were filmed, fixed in alcohol 70% and then sent to the Cephalopod Early Life Stages Laboratory at the University of Parana, PR, Brazil. The eggs and hatchlings were analyzed and measured under a stereo microscope and their morphology and chromatophore patterns described. The following indices were obtained for the descriptions of eggs and hatchlings: egg index = egg length × 100/brooding female ML), hatchling size index = hatchling ML × 100/brooding female ML), hatchling AL index = AL of hatchling × 100/ML hatchlings, according to Boletzky (1974), Boletzky et al. (2002) and Hochberg et al. (1992).

Institutional abbreviations: Oceanographic Museum "Prof. Eliézer de Carvalho Rios," Universidade Federal do Rio Grande, Brazil (MORG) and the Sciences Museum of the Pontifícia Universidade Católica do Rio Grande do Sul, Brazil (MCPUCRS), Zoology Museum of the University of São Paulo, Brazil (MZUSP), Santa Barbara Museum (SBNHM) and National Museum of Natural History (NMNH – Smithsonian), British Museum National, History, England (BMNH).

Results

Molecular analyses

Fragments of 564 bp of COI gene were used to infer phylogenetic relationships among some genera of octopod species. The Bayesian phylogenetic analyses showed that *Paroctopus* sp. nov. specimens grouped in a clade composed of *Paroctopus* species, including the type species *P. digueti*, but clearly separated from other Western Atlantic pygmy species. The new species is closely related to *O. joubini* and *P. mercatoris* sequences, retrieved from GenBank (Posterior probability [PP] = 1). The genetic analyses using the mitochondrial gene COI showed 9% of genetic distance between the *Paroctopus* sp. n. and *O. joubini* (AY377732), and between *Paroctopus* sp. n. and *P. mercatoris* (GQ900743). However, the sequences of *O. joubini* (AY377732) and *P. mercatoris* (GQ900743) deposited at GenBank are identical, suggesting a misidentification or species synonymy (see Table 2, Fig. 2).

The clade including *Paroctopus* species is well-supported (PP = 0.96) and indicated three other small species currently assigned to *Octopus* genus that grouped in the *Paroctopus* clade: *Octopus tehuelchus* d'Orbigny, 1840 in Férussac and d'Orbigny 1835 (Southwest Atlantic from southern Brazil to northern Patagonia), *Octopus alecto* Berry, 1953 and *Octopus fitchi* Berry, 1953 (both from Gulf of California, Mexico to Ecuador) (Fig. 2).

Systematic and description

Family Octopodidae d'Orbigny, 1840 in Férussac and d'Orbigny, 1835.

Subfamily Octopodinae d'Orbigny, 1840 in Férussac and d'Orbigny, 1835.

Genus *Paroctopus* Naef, 1923

Frequent Synonyms: Pseudoctopus Grimpe, 1925*; Octopus joubini* (Haimovici 1985; Perez and Haimovici 1991) *Paroctopus* cf. *joubini* (Lima et al. 2020).

Table 2 Genetic distances (Kimura 2_parameter) using fragments of COI mitochondrial genes among species assigned to the genus *Paroctopus* and related species. Genetic distances among the *Paroctopus* sp. nov. and *O. joubini* and *O. mercatoris* are shown in bold

| Species	1	2	3	4	5	6	7	8
1. *Paroctopus* sp. nov								
2. *Paroctopus* sp. nov	0.000							
3. *Paroctopus* sp. nov	0.000	0.000						
4. '*Octopus*' joubini	**0.093**	**0.093**	**0.093**					
5. '*Octopus*' mercatoris	**0.093**	**0.093**	**0.093**	0.000				
6. *Paroctopus digueti*	0.110	0.110	0.110	0.132	0.126			
7. '*Octopus*' tehuelchus	0.129	0.129	0.129	0.131	0.131	0.140		
8. '*Octopus*' alecto	0.134	0.134	0.134	0.157	0.157	0.030	0.163	
9. '*Octopus*' fitchi	0.138	0.138	0.138	0.144	0.145	0.128	0.149	0.154
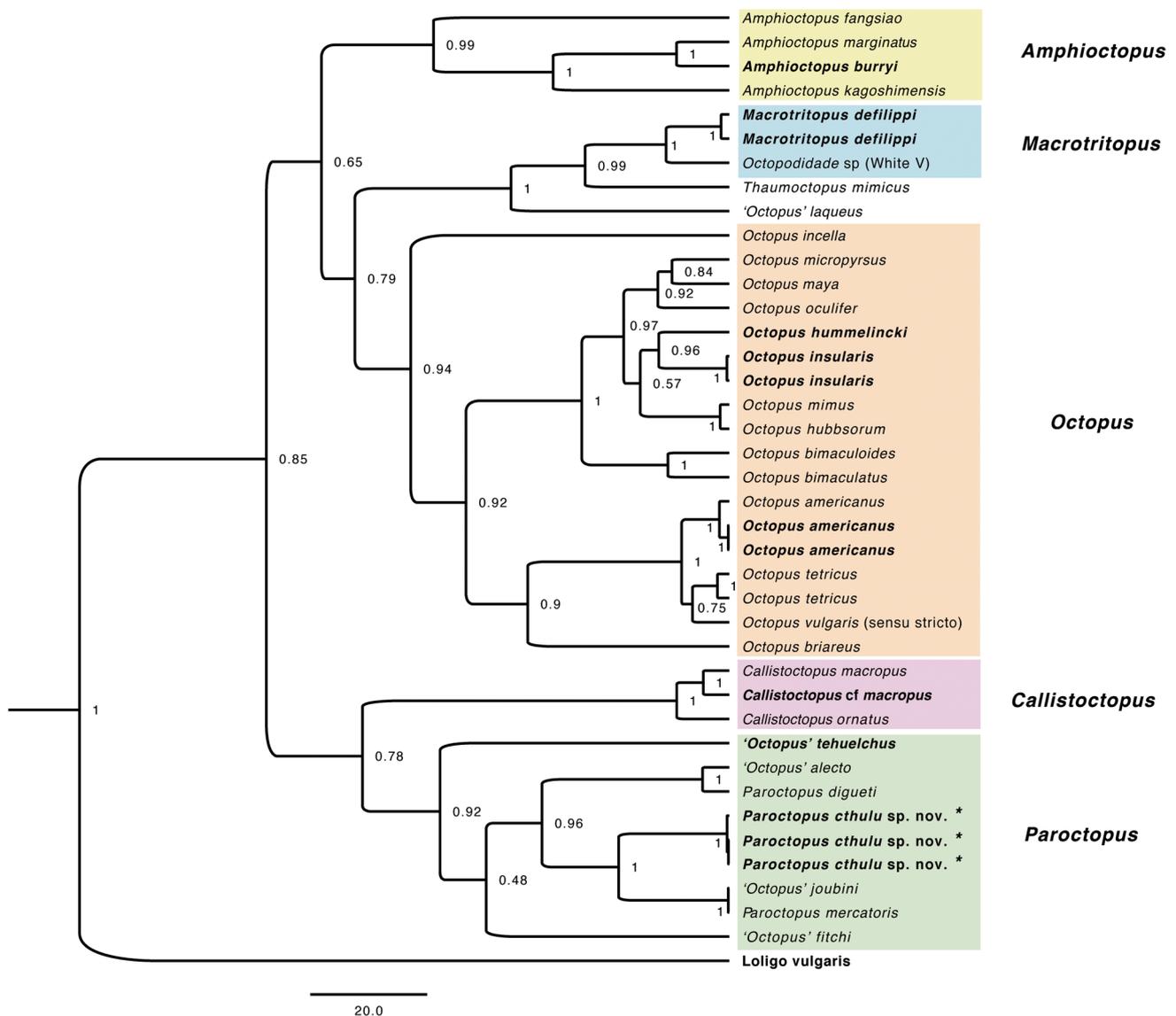
Fig. 2 Bayesian phylogenetic tree based on COI showing some genera of Octopod species. Species provisionally placed in the genus Octopus are highlighted with single quotes. The Bayesian posterior probabilities of the clades are shown on the nodes. The new species described in this study is marked with an asterisk in the *Paroctopus* clade (green). In bold are Brazilian octopod species

Type Species: Paroctopus digueti (Perrier & Rochebrune, 1894).

Genus amplified diagnostic features: Small-bodied; mantle short and wide, with large eggs. Stylets present, non-mineralized. Arms short- to medium-sized, stocky, 3 to 4 times mantle length. One to three enlarged suckers on the arms of males. Copulatory organ (ligula) medium size; calamus short. Gills with 5–8 lamellae per outer demibranch. Oviducal glands without braiding chambers. Spawned eggs (4.2 to 9 mm total size), attached singly with stalks to shells, hard bottom or objects in small clusters. In living specimens body uniformly colored with little pattern variability; patch and groove system absent; white spots absent from dorsal mantle and large arm base; frontal white spot complex present but faint. Skin without large primary papillae.

Paroctopus cthulu Leite, Lima, Lima & Haimovici sp. nov.

http://zoobank.org/03EFA7CC-4797-4244-A595-D87DCEDC7E72

Holotype: male (mature) 18.3 mm ML found on sandy bottom next to rocky reefs at a 5-m depth inside an aluminum beer can. Ilha Grande, Rio de Janeiro State (RJ), Brazil (23° 05′ S; 44° 14′ W); collected by Ricardo Dias, by hand during SCUBA dive in February/2005; MORG 52,754 (Fig. 3a).

Fig. 3 *Paroctopus cthulu* sp. nov. new species; **a** Dorsal view (holotype drawing by Leticia Cavole); **b** Stylet; **c** Hectocotylized arm with ligula and calamus; **d** Funnel organ. (a and c male holotype (ML 18.3 mm). b and d: mature female specimen (ML 32 mm)). (Scale bars: a: 10 mm; b–d: 2 mm. ML, mantle length)
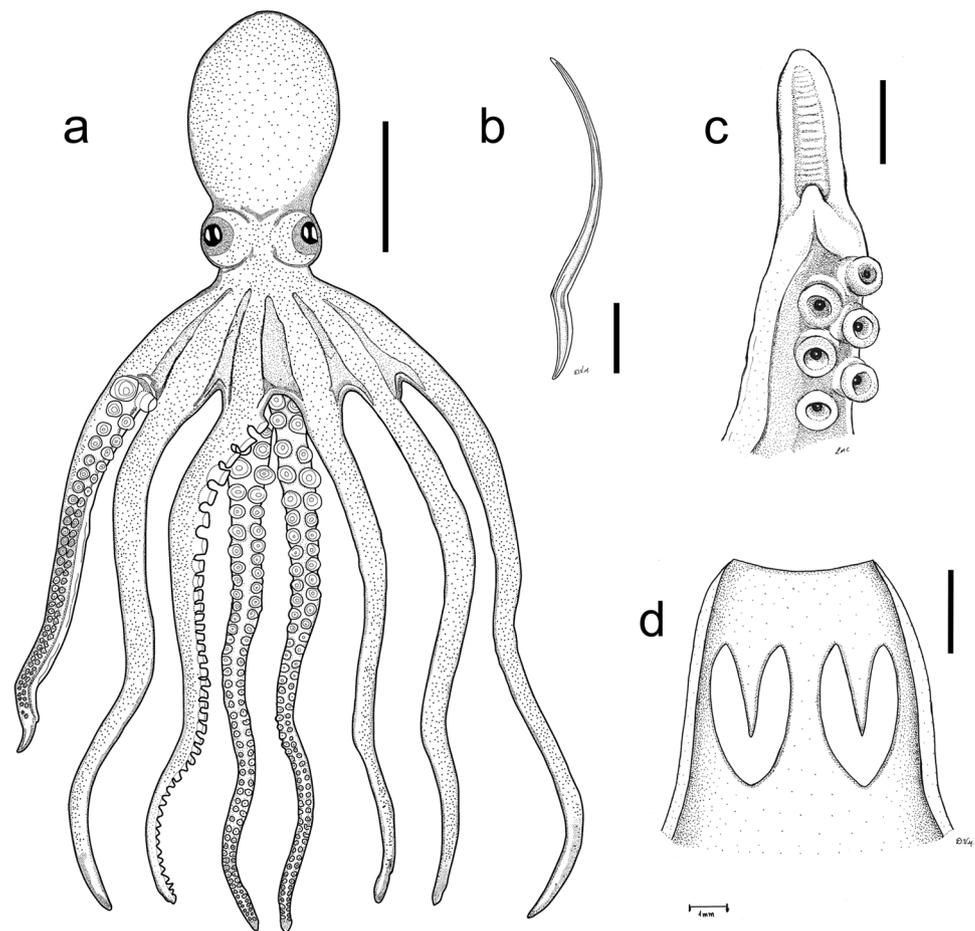
Paratypes: Adult male 29.0 mm ML collected in shallow waters in Porto Belo, Santa Catarina State (SC), Brazil (27° 09′ S; 48° 33′ W) in 1971 (MCPUCRS 3398); adult male 32.7 mm ML collected in shallow waters in Porto Belo/SC, Brazil (27° 09′ S; 48° 33′ W) in (1972 MCPUCRS 2686); adult male 24.0 mm ML collected by SCUBA diving in 1st July 1966 in Ilha Grande/RJ (MORG 52,778), collected by Ricardo Dias; adult male 14.0 mm ML collected by SCUBA diving with debris in February 2015 at less than 5 m depth, in Ilha Grande (23° 05′ S; 44° 14′ W) (MORG 52,777); adult male 19.7 mm ML collected by SCUBA diving with debris in February 2015 at less than 5 m depth, in Ilha Grande/RJ (23° 05′ S; 44° 14′ W) (MORG 52,767); adult female 32.0 mm ML, with eggs inside mantle, collected on 13 September 2007 in unknown depth and position in southern Brazil 2007 (MORG 52,778); spawned female with eggs 25.6 mm ML collected by SCUBA diving at debris in 2015 at less than 5 m depth, in Ilha Grande/RJ (23° 05′ S; 44° 14′ W) (MORG 52,780; DNA); adult female 21.3 mm ML collected by SCUBA diving with debris in 2015 at less than 5 m depth, in Ilha Grande/RJ (23° 05′ S; 44° 14′ W) (MORG 52,779); subadult male 11.0 mm ML collected by SCUBA diving with debris in February 2015 at less than 5 m depth, in Ilha Grande/RJ (23° 05′ S; 44° 14′ W) (MORG 52,781 DNA); subadult female 14.0 mm ML collected by SCUBA divers examining debris in 2014 at less than 5 m depth, in Ilha Grande/RJ (23° 05′ S; 44° 14′ W) (MORG 52,782); subadult male 9.5 mm ML collected by SCUBA diving with debris in 2014 at depths shallower than 5 m, in Ilha Grande/RJ (23° 05′ S; 44° 14′ W) (MORG 52,783).

Etymology: The name *cthulu* is a term with a dual allusion. First, it is an irony due to the small size of the new octopus species, compared to the giant fictional entity "Cthulhu," created by Lovecraft (1984) and described as resembling an octopus, a dragon and a human caricature. Second, it refers to the proposal of Donna Haraway of the Chthulucene as a diverse Earth-wide tentacular power of symbiosis. Chthulucene proposes a holistic and biocentric coexistence that will integrate and transform the far less optimistic view of the Anthropocene (Haraway 2015). Most *P. cthulu* sp. nov. specimens were found in metal and plastic debris, suggesting that the species is utilizing the garbage in oceans, as an alert to this increasing global threat to the marine biota.

Distribution: Paroctopus cthulu sp. nov. was found in shallow waters of Ilha Grande (23° 05′ S; 44° 14′ W). Females with spawned eggs were collected in Praia

Vermelha/Urca in Rio de Janeiro State (22° 57′ S; 43° 09′ W), also in Porto Belo, Santa Catarina State (27° 09′ S; 48° 33′ W), and in an unreported position along the south Brazil coast. Based on these collection sites, the present known distribution for *P. cthulu* sp. nov. is southeastern-southern Brazil (Fig. 1).

Diagnosis: adults small-sized (ML 14.0 to 33.0 mm), mantle and head wide with large and prominent eyes. One to three cirri over the eye and one below. Shallow web and thick arms subequal in length, three and a half times the ML. One to three enlarged suckers located on the 5th or 6th row in some or all arms of all adult males and one adult female. Third arm of the males hectocotylized with a moderate calamus and small ligula, two-thirds of the length of the opposite arm (Fig. 3c). Gills with 5 to 6 lamellae per hemibranch, usually 6. Adult females with medium to large oocytes (4.7 to 9.0 mm length). Spawned eggs attached with stalks to objects singly in small clusters. Hatchlings have 5.0 to 5.2 mm total length and 2.5 mm ML, arms with 14 to 16 large suckers. Body color of adult animals in the environment varies from yellow to reddish brown. Ventral surface of mantle, head and web with small well-spaced papillae, dorsal mantle, and head with larger papillae. Brownish red smooth dorsal mantle surface in preserved specimens.

Description: The following description is based on the holotype (male) and 8 mature paratypes (3 females and 5 males) and 3 subadults morphotypes (1 female, 2 males) (Tables 3, 4, and 5). Small size adults (ML up to 32.7 mm; 13 g total in preserved specimens). Broad mantle with a muscular wall (MWI 55–80) (min–max) (Fig. 4a); posterior mantle with a bilobed shape ventrally (Fig. 4b); head wide (47–72); funnel tubular (FLI 19–54), with almost half of it free (FFLI 39–63). UU-shaped funnel organ with similar size (Fig. 3d). Normal arms similar in length (ALI 270–450, mean 355), no consistent arm formulae; arm width (AWI 11–20). Total number of suckers on the normal arms 112–174, mean 150. Normal sucker diameter moderate (SDnI 8–13). One to three conspicuous enlarged suckers (SDeI 10–23, mean 0.15S), present in two to four arms of all examined adult males and one adult female, located at 5th or 6th row) (Fig. 4c and d). Third right arm of males hectocotylized goes to shorter to moderately shorter than the opposite arm (OAI 45–73), bearing 86–94 suckers; hectocotylus with a well-defined spermatophoric groove, small ligula (LLI 3.7–7.3) with clear transverse ridges and a moderately long calamus (Fig. 3c) (CLI 20–42). Web shallow (WDI 20–72), with no consistent web formulae differences among the evaluated individuals, however the sectors A and E usually are shallower. Demibranchs have stout lamellae, with 5 to 6 lamellae per demibranch, usually 6. Stylets medium-sized 5.4 to 6.7 mm (before preservation), with the posterior part longer and thinner (Fig. 3b).

Digestive tract: a dissected adult female (32.0 mm ML MORG 52,768) presented a typical *Octopus* digestive tract (Fig. 5a), with few peculiarities. Large buccal mass (6 mm; 19% of ML); pair of flattened, medium-sized anterior salivary glands (1.8 mm, 5.30%ML), and large posterior salivary glands triangular (8.0 mm; 25% of ML) joined by ducts to the buccal mass. Narrow esophagus followed by crop diverticulum and a wide stomach. Spiral caecum connected by two ducts to large digestive gland (12.0 mm, 37% of ML); ink sac embedded in digestive gland surface. Intestine relatively short and curved with a loop, ending in muscular rectum with anal flaps. Beak, relatively large if compared with the species size, 1.7 mm of upper hood length (9% of ML); prominent rostrum and sharp rostral tip (upper rostral length 0.5 mm), with narrow wings (Fig. 5b–d beak). Radula with rachidian tooth and two lateral teeth, one marginal tooth, one marginal plate, one lateral cusp on each side of rachidian tooth with a symmetric seriation, the position of the cusp shifts from the base to the middle of the tooth every one or two teeth (A 1–2); cusp on outer margin of first lateral tooth; second lateral tooth triangular, almost symmetrical; marginal tooth thin, curved; marginal plate small, flat (Fig. 5e).

Female reproductive system. The mature female (32.0 mm ML) has a very large round ovary (22.0 mm wide), occupying almost the whole posterior portion of the mantle, two short proximal oviducts (5.7 mm), two small spherical oviducal glands (3.7 mm), reddish orange in color, and a medium size distal oviducts (10.7 mm) (Fig. 6a). We counted a total of 30 oocytes inside the ovary. The three mature females (21.3 to 32.0 mm) showed oocytes varying from medium to large size (from 4.7 to 9.0 mm) (Fig. 6b and c).

Male reproductive system: The holotype mature male (18.3 mm ML) had a testis of 4.5 mm length, which is relatively large in the system; vas deferens narrow, with turns and wrapped in a membranous sac. Vas deferens opening into a spermatophore gland, long and curved accessory gland, both opening in an atrium linked to a long and wide Needham's sac, with almost the same size as the testis; small terminal organ tubular (PLI 10–18) diverticulum not clearly differentiated from the terminal organ (Fig. 6d). Spermatophores medium-sized (SpLI 39.9–43.7), narrow, without swelling (SpWI 3.5); medium-sized sperm masses (SpRLI 52.1), 19–20 turns on the sperm mass (Fig. 6e). The maximum number of spermatophores counted in the Needham's sac was 13.

Brooded eggs, embryos, and hatchlings: a female with more than 30 eggs individually attached to the snorkel mouthpiece was found at Ilha Grande (Fig. 7a). Another female was found with 124 eggs attached individually to an aluminum can by a thin chorion stalk (2.57 ± 0.18 mm, $n = 25$), along with empty chorions, as many individuals had hatched. These eggs were medium-sized, elongated to pear shape with a mean length of 4.61 ± 0.35 mm and

Table 3 *Paroctopus chula* sp. nov. Counts and measurements (mm), weight (g). R = right, L = left, I = inner, O = outer, 1 to 4 arm numbers, A to E web sectors depth

Deposit institution	MORG	MCPUCRS	MCPUCRS	MORG	MORG	MORG	MORG	MORG	MORG	MORG	MORG	MORG
Catalog number	52,778	3398	2686	52,754	52,777	52,767	52,768	52,780	52,779	52,781	52,782	52,783
Status	Paratype	Paratype	Paratype	Holotype	Paratype	Paratype	Paratype	Paratype	Paratype	Paratype	Paratype	Paratype
Collection date	1966	1971	1972	2015	2015	2015 fev	2007	2015	2015	2015 fev	2014	2014
Sex	Male	Male	Male	Male	Male	male	Female	Female	Female	Male	Female	Male
Maturity	Adult	Adult	Adult	Adult	Adult	Adult	Adult	Adult	Adult	Subadult	Subadult	Subadult
Total fixed weight (g)	9.2	14.3	13.5	3.3	2.4	3.6	ca 12.0	13.3	5.4	1.6	2.2	0.9
Total length	120	150	164	70	55	85	sd	105	73	53	73	54
Dorsal mantle length	24	29	32.7	18.3	14	19.7	32	25.6	21.3	11	14	9.5
Mantle width	15	24	19	14.4	11.1	12.3	24	21.4	18.2	6.9	10.4	7.1
Head width	17.3	15.7	15.4	12.5	10	10.3	16.7	12.8	13	8	11.1	7.4
Funnel length	10.9	13.6	10.4	7.8	7.6	6	10.5	8.1	4	4.7	7	3.9
Free funnel length	6.9	5.3	6.5	3.3	4.8	3.5	5.5	5	2	2.2	3.4	2.7
Ligula length	4	2.6	2	2.2	1.9	2				1.2		
Calamus length	1.5	1.1	0.8	sd	0.5	0.4				0.6		
Gill lamellae count (I/O)	5/5–5/5	6/6–6/6	5/5–5/5	6/6–6/5	6/5–/-	6/6–6/6	5/5–5/5	6/6–/-	5/5–5/-	6/6–6/6	6/6–6/6	6/6–6/6
Gill length (mean)	8.5	10	sd	4	sd	6.5	6.45	3.1	sd	3.5	4	2.55
Arm lengths 1 (R-L)	108–85		107–102	51–56						41–42	55–56	31–32
Arm lengths 2	90 R	105–111	103–90	–55	–46	78–	–79		–46	41–43	55–55	33–32
Arm lengths 3	55–	70–100	50–111	36–57	33–45	45–				32–42	53–50	31–32
Arm lengths 4	83–85	94–	99–124	53–56	–44	69–70			56–	41–41	54–48	29–29
Arm width (mean)	3.2	3.9	4.5	3.01	2.1	2.2	6.16	3.92	3.5	1.38	2.23	1.35
Web depth A (dorsal)	11	sd	10	7	7	sd	sd	8	10	7	6	4
Web depth B	10–10	–13	11–13	8–8	8–8			8–10	10–9	6–6	6–7	4–5
Web depth C	10–9	19–17	6–13	9–9	9–9			12–11	12–12	5–7	6–8	5–4
Web depth D	0	21–18	11–12	8–	9–9			12–12	11–11	5–7	8–7	5–3
Web depth E (ventral)	9	17	13	9	9	0	0	11	12	7	8	4
Number of suckers arm 1 (R-L)	–174		139–118	147–146		–174	–102			13–132	167–165	147–131
Number of suckers arm 2	157–	154–	145–112	154–148	157–	157–				144–139	165–169	141–149
Number of suckers arm 3	86–	86–144	56–126	85–160	93–150	86–			142–131	89–141	160–169	94–151
Number of suckers arm 4	174–	118–138	118–138	146–152	–151	174–	102–			132–142	165–	131–140
Larger normal sucker arm 1 (R-L)	2.3–2.3	–3.8	2.5–2.5	1.6–1.6	1.1–1.1	2–2		3–3		1.3–1.3	1.35–1.35	1.2–1.2
Larger normal sucker arm 2	2.3–2.3	3.8–3.8	2.5–2.5	1.6–1.6	1.1–1.1	2–2		3–3		1.3–1.3	1.35–1.35	1.2–1.2
Larger normal sucker arm 3	2.3–2.3	3.8–3.9	2.5–2.5	1.6–1.6	1.1–1.1	2–2	3.2-	3–3		1.3–1.3	1.35–1.35	1.2–1.2
Larger normal sucker arm 4	2.3–2.3	3.3–3.3	2.5–2.5	1.6–1.6	1.1–1.1	2–2	3.2–3.5	3–3		1.3–1.3	1.35–1.35	1.2–1.2
Larger enlarged sucker arm 1 (R-L)	3.2–3.2	–3.8		3.4–3.2				–3.4		1.9–2		
Larger enlarged sucker arm 2	3.2–3.2	3.8–3.8		3.4–3.2	3.2-	2.6–2.6	3.8–	–4.2		1.7–1.9		
Larger enlarged sucker arm 3	3.7–3.7	3.8–3.9		3.4–3.2	3.2–3.2	3–3	4.4–	–3		1.7–		
Larger enlarged sucker arm 4		3.3–3.3			–3.2	2.6–2.6	4.5–					

largest mean width of 2.3 ± 0.14 mm ($n = 25$). (Fig. 7b) The egg index was 23. The eggs have a transparent chorion and were not enclosed in capsules. The eggs were at different developmental stages, all of them before the second embryo inversion (stages XII.1–XIX.1, Deryckere et al. (2020)), indicating that spawning took place over several days. Late-stage embryos (stages XVIII–XIX.1) had large darkish eyes with a whitish retina and a mean eye diameter of 0.42 ± 0.1 mm (Fig. 7b). All the eight arms were well-developed and similar sizes, having from 10 to 12 suckers; from the buccal mass up to the web close to the bases of the arms there were two to three suckers distributed in a single series and from this point on suckers were in a zigzag double series along the length of the arms. Around the buccal mass, there was a single to double sucker ring formed by the single row of suckers up to the base of each arm. The funnel was long, wide and conspicuous, reaching the base of the ventral arms.

Embryo chromatophores: the preserved embryo has a large number of dark chromatophores. *Dorsal view*: On the arms there are from 12 to14 chromatophores, two at the base in a single series and the others in a zigzag series. On the head, there are about 20 to 24, both large extra-tegumental and small tegumental chromatophores interposed and sometimes superimposed. On the mantle, there are from 18 to 22 extra-tegumental chromatophores distributed in the central area. *Ventral view*: On the head, there are four, two very large extra-tegumental chromatophores on the lateral sides of the funnel; over the mantle there are from 61 to 72 brownish large chromatophores that seems to be distributed in 8–10 horizontal series, but when expanded cover the entire surface of the mantle. When the chromatophores are all expanded, the embryo has a dark coloration (Fig. 7c).

Hatchlings: Total lengths of hatchlings are 5.0 to 5.4 mm and the ML is 3.3 mm (Fig. 7d and Fig. 8a−c). The dorsal mantle edge is clearly visible at the base of the head, thus the mantle measures 2.5 mm from the mantle tip to the mantle edge. The mantle is roundish with a width of 1.9 mm. The head is wider (100% ML) than long (50% ML), with a somewhat concave shape posteriorly. The eyes are large and prominent (28% ML) and anteriorly oriented (Fig. 8a and b). The arms are long relative to the mantle (80% ML) and robust, with 14 to 16 suckers each. A conspicuous web is present at the base of all arms. The suckers are arranged in a biserial zigzag series, as in the embryo, and the size of the suckers decreases from the base towards the tips of the arms. The base of the arms occupies a narrow area in relation to the width of the head, leaving an empty space between the head and the arms, which gives the appearance of a short arm crown stalk (Fig. 8a and b). The body of the whole animal is covered by an unpigmented transparent skin "film," with the exception of the aboral surface of the arms, which is filled with suckers. This transparent film is likely

the epidermis and seems to be continuous over the whole body and the only apertures are found ventrally, at the mantle edge and at the funnel orifice. This is particularly evident in a lateral view (Fig. 8c).

Particularly on the mantle the skin is densely covered by Kölliker's organs, which gives a rough appearance. The skin film is conspicuous around the arms and head, giving the whole animal a transparent to whitish color when the chromatophores are contracted. When all the chromatophores are expanded, however, the hatchling acquires a very dark pigmentation as described below.

Hatchlings chromatophore pattern: on dorsal view, the chromatophore pattern on the arms seems to follow the disposition and number of suckers: there are from 10 to 14 chromatophores on each arm, one to two large ones at the base and the others distributed in a zigzag row from the base toward the tips of each arm. On the head, there are 24 chromatophores, 17 darkish brown, and seven yellow. In the anterior region, close to the base of the arms, there are four distributed in a rhombus shape, three yellow and a dark one; two larger dark ones between the eyes; six dark ones forming a row on midhead; four large dark ones at the base of the head and four close to the eyes (two dark ones interposed by two yellow ones). On the mantle only dark chromatophores were observed. There is a double row of chromatophores around the whole mantle edge with about 12 to 16 chromatophores each and the same patterns is seen on the posterior mantle, where each row has about 12 to 14 chromatophores. Many other small chromatophores are found scattered over the whole mantle without a particular pattern. There are from 8 to 11 extra-tegumental chromatophores covering the viscera, arranged in an oval shape (Fig. 8a). *Ventral view:* The distribution of chromatophores on the arms is the same as described for the dorsal view. The head has eight chromatophores, two yellow ones on the sides of each eye, two dark ones between the eyes and two very large dark ones on the sides of the funnel. Over the funnel there are six. The whole mantle is densely covered by approximately 70 to 80 chromatophores, which are distributed in 8–10 irregular rows. When all the chromatophores are expanded, the mantle is entirely dark (Fig. 8b). *Lateral view:* Over the head, there are two other chromatophores underneath the eyes, a small dark one and a large yellow chromatophore close to the mantle edge. On the ventral mantle, the single row of large chromatophores around the mantle edge is clearly seen (Fig. 8c).

Adult Body Pattern: fixed specimens (without previous freezing) had smooth skin on the dorsal surface (Fig. 4). Color in fixed specimens varied from light brown to light reddish, darker around the eyes on the dorsal surface and clearer cream color on the ventral, with fewer chromatophores. The dorsal mantle with small papillae around the eyes was visible only in few specimens (Fig. 4a and b).

Table 4 *Paroctopus cthula* sp. nov.: index. R = right, L = left, I = inner, O = outer, 1 to 4 arm numbers, A to E web sectors depth

	MORG	MCPUCRS	MCPUCRS	MORG	MORG	MORG	MORG	MORG	MORG	MORG	MORG	MORG
Catalog number	52,778	3398	2686	52,754	52,754	52,767	52,768	52,754	52,754	52,767	52,780	52,780
Status	Paratype	Paratype	Paratype	**Holotype**	Paratype	Paratype	Paratype	Paratype	Paratype	Paratype	Paratype	Paratype
Collection date	1966	1971	1972	2015	2015	2015	2007	2015	2015	2015	2014	2014
Sex and maturity	adult male	adult male	adult male	adult male	adult male	adult male	adult female	spawned female	adult female	subadult male	subadult female	subadult male
Dorsal mantle length (mm)	24.0	29.0	32.7	18.3	14.0	19.7	32.0	25.6	21.3	11.0	14.0	9.5
Mantle width index	80	60	58	64	75	62	75	55	70	75	82	78
Head width index	72	54	47	68	71	52	52	50	61	73	79	78
Gill length index	33	34	sd	22	sd	33	20	24	sd	32	29	27
Funnel length index	45	47	32	43	54	30	33	32	19	43	50	0
Free funnel length index	63	39	63	42	63	58	52	62	50	47	49	0
Ligula length index	7	4	4	6	6	4	–	–	–	11	–	–
Calamus length index	38	42	40	–	26	20	–	–	–	50	–	–
Hectocotilized arm length index	–	70	45	63	73	–	–	–	–	76	–	–
Arm 1 length index	4.5–3.5	–	3.2–3.1	2.7–3				4.5–3.5		3.7–3.8	3.9–4.0	3.2–3.3
Arm 2 length index	3.8 R	3.6–3.8	3.1–2.7	3.0L	3.2 R	3.9 R		3.8 R	2.1 R	3.7–3.9	3.9–3.9	3.2–3.3
Arm 3 length index	2.3 R	2.4–3.4	1.5–3.3	1.9–3.1	2.3–3.2	2.2 R		2.3 R		2.9–3.8	3.7–3.5	3.0–3.0
Arm 4 length index	3.5–3.5	3.2 R	3.0–3.7	2.8–3	3.1L	3.5–3.5		3.5–3.5	2.6 R	3.7–3.7	3.8–3.4	3.0–3.0
Arm width index	14	14	14	16	15	11	20	15	16	13	16	14
Web depth (A = dorsal) index	46	–	31	38	50	–	–	31	47	64	43	42
Web depth (B) index	41–41	44L	33–39	43–43	57–57			31–39	46–42	54–54	42–50	42–52
Web depth (C) index	41–37	65–58	39L	49–49	64–64			46–42	56–56	45–63	42–57	52–42
Web depth (D) index	29 R	72–62	33–36	43–0	64–64			46–46	51–51	45–63	57–50	52–31
Web depth (E = ventral) index	21	55	28	36	57			43	52	27	50	42
Largest normal sucker index	12	13	–	9	8	10	10	12	–	12	10	13
Largest enlarged sucker index	17	13	–	18	23	10	13	14	–	17	–	–
Enlarged suckers row	5th	5th	–	6th	5th	5th	5th	–	–	5th	–	–

Table 5 Morphological comparison between *Paroctopus cthulu* sp. nov and *O. joubini* holotype, *O. mercatoris* syntype and other *O. joubini* morphotypes deposited at different museums. The specimens were evaluated by T.L. and by Dr. E. Hochberg

	P. cthulu sp. nov	*O joubini*	*P. mercatoris*	*O. joubini*	*O. joubini*	*O. joubini*	*O. joubini*
Reference and type material	New species Southern Brazil Small eggs morphotype (4F, 8 M) This study	Robson, 1929, Holotype, BMNH 1889.4.24.30 one female Caribbean, St Thomas. Small eggs morphotype	Adam, 1937 Syntype 1F, 1 M; Gulf of México, Florida. Small egg morphotype	SBMNH (3F, 3 M) Caribbean/Belize Small eggs morphotype	Pickford, 1945 Gulf of Mexico, Florida Big eggs morphotype	Forsythe, 1977 NMNH (3F, 1 M) Reared Gulf of Mexico Big eggs morphotype	Arocha & Urosa, 1982 (11F, 4 M) Southern Caribe, Venezuela
Dorsal mantle length female (ML)	21.3–32	16	18	12–15.5	25–54	25–26	12.9–35.9
Dorsal mantle length male (ML)	14–32.7		16	12–15.5	35–36	23	13.3–26.6
Head width index (HWI)	47–72	57	75–83	58–91	37–72	38–70	41–57
ALI: arm length index	2–4.5	67–69% TL	4	3 to 6	3 to 5	4 to 5	70–76%TL
Sucker normal index (SDnI)	8 13	9	6.3–11	6.5–8	7–11	6.4–12.7	4.7–11.0
Enlarged sucker index (SDeI)	10 23		12.5	16–20	12.5–13.3	13–22	15.9–19.3
Female or male enlarged sucker	M and some F	Absent	Only M	Only M	Only M	M and 1F	Only M
Normal arm sucker count (ASC)	102–174	79	126–134	58–94		69–99	
Hectocotylized arm sucker count (HASC)	56–93		70	45–70		45	42.7–59.0
Web depth index (WDI)	20–72	28	25–56	32–71	19–27.5	35–69	7.4–12.2
Web formula	Larger A, variable	DB=C=E> A	CBDAE	CD > AE	CDBAE or DCBEA	Larger A	42.7–59.0
Opposite arm index (OAI)	60–70			64–71		78	4.5–17.7
Ligula length index (LLI)	3.7–7.3		7.9	5.6–7.7	6.3–6.9	5	20.0–36.8
Calamus length index (CaLI)	20–42		18	33–33	21–22	31.6	24–31
Spermatophore length index (SpLI)	40–43				55.5		11.2–16.7
Penis index	– 10 to 18		12.5	16	22–22.5		5–7
Number of gills lamellae	5–6	4	5–6	4–6	5–6.5	5–6	~10
Eggs larger diameter (mm)	4.7–9	3.2	2.7–3	0.6–2.8	7.5–8.4	4–6.5	

We observed five main body patterns in living animals (Fig. 9): (1) uniform reddish with dark eyes (a); (2) uniform dark brownish (b); (3) uniform light brown with white dots (c); (4) mottle with yellow blotch and white spots (d); (5) brown with white stripes and blotches on arms and mantle (e). We only observed a patch and groove trellis arrangement on the dorsal mantle during the patterns Uniform light brown with white dots and Brown and white stripes. Three chromatophore colors were identified (red, brown, and yellow). The brown and red colors could be widespread throughout the whole body (Fig. 9a, b), while white (no chromatophores expanded) and yellow were concentrated in localized areas: yellow appeared as blotches on the dorsal mantle, head, and proximal arms areas (Fig. 9c); while small white dots were spread across the whole body (9d), and as two frontal white circles. The skin texture was characterized by three primary papillae around the eyes and smaller ones spread throughout outside mantle, head, and proximo-distally on the first arms.

Distinguishing postures: We observed three stereotyped postures: sitting with curled arms pointed, (9a, c); sitting with eyes raised (9e) and the first pair of dorsal arms up showing the larger suckers on the aboral surface of the arms (Fig. 9f).

Remarks: As noted above in the introduction, *O. joubini* is the name used most frequently for the small egged pygmy species from the North Atlantic Ocean, Caribbean Sea, and Mexican Gulf (Jereb et al. 2014). The holotype of this species (BMNH 1889.4.24.30) is 16 mm ML, a female bearing medium ripe eggs measuring 3.2 mm in length (see Table 5). Voss and Toll (1998) further described the species based not only on the holotype, but also on specimens examined by Forsythe and Toll (1991). These last authors observed mature females with 150 to 3000 ripe eggs of 2.3 to 4.8 mm in length. Compared to the description of Forsythe and Toll (1991) for *O. joubini, P. cthulu* sp. nov. has larger eggs (4.2 to 9.0 mm), a deeper web (WDI 20–72 vs. 28) and more arm suckers (ASC 102 to 178 vs. 79) (see Table 5). The sole criterion that Norman et al. (2014) used to consider *O. joubini* a member of the *Octopus* genus was the "small" size of its eggs. However, considering our genetic and morphological results, we suggest that this criterion needs to be reevaluated.

Compared with the small egged morphotypes collected from Belize in the Caribbean and deposited in the Santa Barbara Museum (see Table 5), *P. cthulu* sp. nov. also showed a larger normal sucker index (SDnI 8–13 vs. 6–11) and enlarged sucker index (SDeI 10–23 vs. 12.5). Another important morphological feature of *P. cthulu* sp. nov. is the presence of enlarged suckers in two out of three females evaluated, while the morphotype of *O. joubini* only had enlarged suckers in male specimens. *Paroctopus cthulu* sp. nov. had more suckers on normal arms (ASC 102–174 vs. 58–94) and also on hectocolized arm (ASCH 56–93 vs.

45–70) compared with Belize forms. It also had more gill lamellae (5–6 vs. 4), and bigger eggs (4.7–9 vs. 3.2).

The southernmost record of *O. joubini* is the northeast islands near Venezuela (Arocha and Urosa 1982). Compared to the *O. joubini* specimens from the Venezuelan Caribbean, *P. cthulu* sp. nov. showed a distinct funnel organ (UU×W), deeper interbrachial membrane (WDI 29–72 vs. 7.4–12.2), shorter ligula (LLI 4–7 vs. 4.5–17.7), shorter penis (PLI 1.8–6 vs. 11.2–16.7), longer spermatophores (SpLI 39.9–43.7 vs. 23–26), and lower number of turns on the spermatic mass (19–20 vs. 50). Except for the eggs size and radula seriation (A2–3 vs. A4–6), similar to that described by Adam (1941), Arocha and Urosa (1982) suggested that all 15 specimens fitted into the *O. joubini* description by Robson (1929) and Pickford (1945).

Another name used for the pygmy octopus from the North Atlantic and Gulf of Mexico is *P. mercatoris.* Pickford (1945) compared *P. mercatoris* and *O. joubini* by morphometric indices, and considered the former species as a synonymy of *O. joubini.* However, Forsythe and Toll (1991) after rearing the two forms of *O. joubini* (large and small egged) concluded that they are in fact two different species. Their conclusion was based on the hatchling size, as while the small egged specimen produced planktonic paralarvae, the large-egged individuals produced benthic juveniles. For these authors, the small egged pygmy species is conspecific with the holotype of *O. joubini*, and not the widely studied and better known large-egged species, although both species occur in the Caribbean Sea and the Gulf of Mexico. For these authors, the taxonomy of the large-egged pygmy species from the northwestern Atlantic is still not clarified.

Besides the available holotypes and syntypes, we also compared the new species with large-egged specimens deposited at the National Museum of Natural History (NMNH–Smithsonian) from different localities (see Table 5). Our specimens had a larger calamus index (CLI 20–42 vs. 21–31.6), shorter spermatophore index (SpLI 40–43 vs. 55.5), more suckers on normal arms (ASC 102–174 vs. 69–99), and also on the hectocotylyzed arm (ASCH 56–93 vs. 45) when compared with the large-egged morphotypes from South Florida (see Pickford 1945), and from those in the experiments conducted at the National Resource Center for Cephalopods in Texas (see Forsythe and Hanlon 1980) (Table 5).

In addition, the body pattern when compared to both *O. joubini* morphotypes is quite different. *Paroctopus cthulu* sp. nov. species has a characteristic reddish orange coloration, but with variable body patterns that includes also use of the yellow, white and black chromatophores and papillae all across the body. In contrast, *O. joubini* (small egged) have a dark brownish tone, also described in the large-egged morphotype (Forsythe and Toll 1991) with no ability to modify skin texture other than 3 to 4

Fig. 4 Fixed *Paroctopus cthulu* sp. nov.; **a** Dorsal view; **b** Ventral view; **c** Male with enlarged suckers; **d** Female with enlarged sucker. Scale bars: a-d, 10 mm
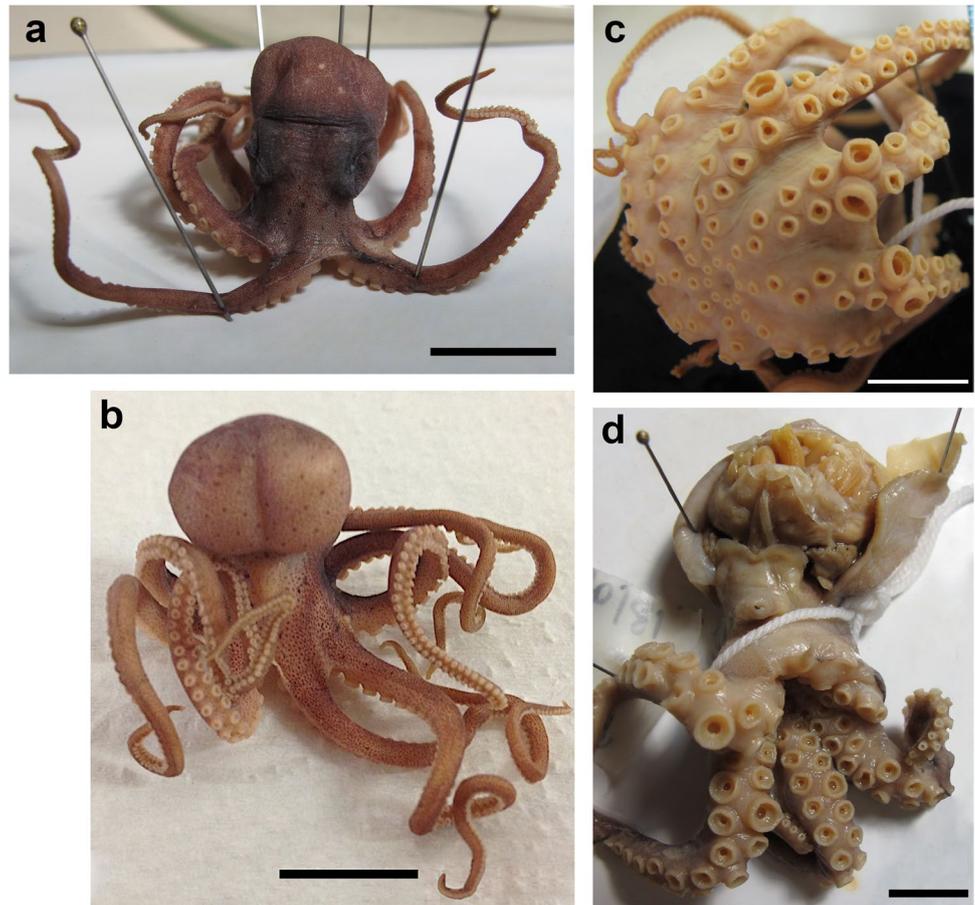
papillae. Mather (1984) also indicated that the *O. joubini* large-egged morphotype became strongly nocturnal after the third week of life, which is compatible with its drab skin and few body patterns, most of them reddish or dark colors.

As the new species is distinct from *O. joubini* sensu stricto and the large-egged morphotype, it must also be compared with other Octopodidae from the southwestern Atlantic, described by Palacio (1977), and more recently by Leite and Haimovici (2006), Vaske –Jr and Costa (2011), Haimovici et al. (2009). *Paroctopus cthulu* sp. nov. has the smallest adult size, when compared to all described southwestern Atlantic octopod species (32 mm ML vs. 70 mm ML to *Amphioctopus burryi* (Voss, 1950); up to 250 mm ML (Voss, 1951), *Octopus americanus* Montfort 1802 (Avendaño et al. 2020), confirming that it is the smallest octopod species from southwest Atlantic.

Octopus hummelincki Adam, 1936 has a larger adult size (70 mm ML) and has ocelli on the web under the eyes, has dissimilar spermatophores, ligula, radula, and skin color and textures (Burgess 1966; Leite and Haimovici 2006). *Amphioctopus burryi* is another small octopus that uses gastropod shells and debris as shelters (Hanlon and Hixon 1980). This species has a complex body pattern, with a grainy skin and a conspicuous purplish brown stripe along the entire leading edge of the arm pairs I to III, which makes its recognition easy. *Octopus tehuelchus* has a larger adult size (90 mm ML), longer arms with fewer suckers (about 100), and females bear larger eggs up to 18 mm in diameter (Palacio 1977; Voss and Toll 1998). *Callistoctopus furvus* (Gould, 1852) has a distinctly larger adult size (190 mm ML, with a distinctly red and white coloration on body and arms (Jesus et al. 2021)). *Macrotritopus* cf *defilippi* (Verany, 1851) has larger adult size, and, longer and thinner arms, with a skin with pallid color (Mangold 1998), while *O. americanus* (Montfort, 1802) (Avendaño et al. 2020) and *Octopus insularis* Leite & Haimovici, 2008 are bigger animals with larger adult size (Leite et al. 2008).

Habitat and in vivo observations

There is no information on the habitat of the four specimens deposited in the MORG and MCPUCRS collections.
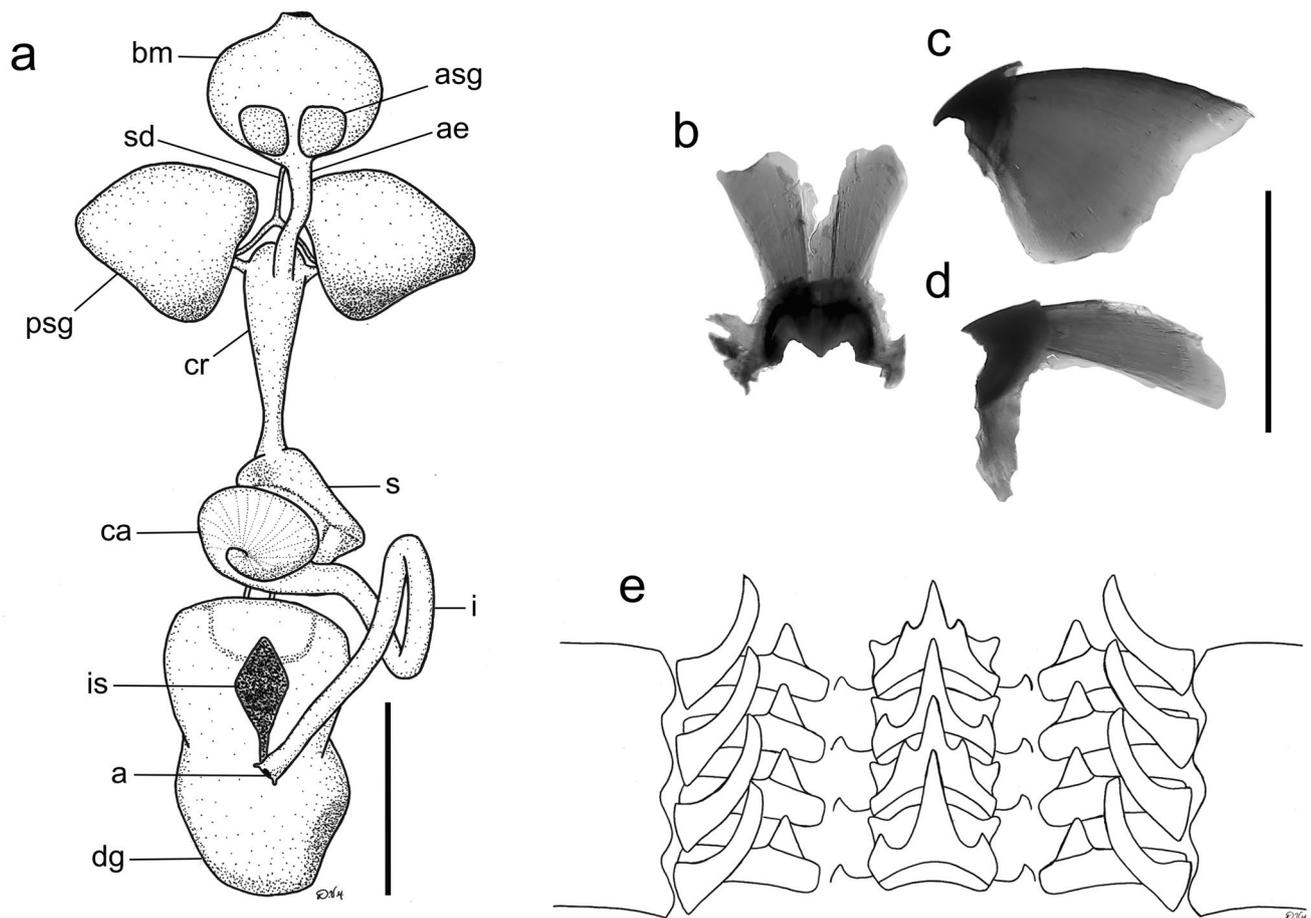
Fig. 5 *Paroctopus cthulu* sp. nov.; **a** digestive tract (a, anus; ae, anterior esophagus; asg, anterior salivary gland; bm, buccal mass; ca, caecum; cr, crop; dg, digestive gland; i, intestine; is, ink sac; psg, posterior salivary gland; s, stomach; sd, salivary duct); **b** frontal view of lower beak; **c** lateral view of upper beak; **d** Lateral view of lower beak; **e** Radula. (Mature female specimen (ML 32 mm). Scale bars: a. 10 mm; b–c–d 5 mm)

Those collected in 2014 and 2015 in Rio de Janeiro were found during the daytime at 0.5 to 5 m depth, on sandy or muddy bottoms near rocky shores inside metallic cans, plastic objects, or glass bottles (Figs. 7a and 10). The specimens came out of the debris as soon as they were taken out of the water. No specimen was collected from shells.

The debris occupied varied in preservation, some of the cans were fragmented and rusty (Fig. 10a and b), others were intact with some biological encrustation, and few were intact and well preserved. Two spawned females were observed in vivo in their habitat. One, among the collections in February 2015 at Ilha Grande, was found inside a plastic snorkel mouthpiece with eggs attached singly in small clusters (Fig. 7a) at 6 m depth, and a sea water temperature around 22 °C, during summer time. The second female was followed for three weeks at Praia da Urca, Rio de Janeiro. She was found inside an aluminum can, at a shallow depth (2 m), and sea water temperatures around 25 °C, from February to March.

Discussion

Our study identified and described a new species of the genus *Paroctopus*, the first pygmy octopus of the Southwestern Atlantic, that was misidentified in previous studies (Haimovici 1985; Perez and Haimovici 1991; Lima et al. 2020), probably due to the confused taxonomy of the group, including egg morphology usually not available in preserved museum material. Both morphological and molecular analyses corroborate the great divergence of *P. cthulu* sp. nov. from the North Atlantic complex of pygmy octopuses, whose taxonomy is still not solved (Jereb et al. 2014).

The genetic distances between *P. cthulu* sp. nov. and *O. joubini/P. mercatoris* are large enough (around 9%) to claim that the linage from Brazil is a different species of pygmy octopus from those in FL, USA. Additionally, the COI sequences *from O. joubini* and *P. mercatoris* are identical, which means either a misidentification problem or the species are synonymous. Misidentifications in other Atlantic

Fig. 6 *Paroctopus cthulu* sp. nov.; **a** Female reproductive system (do, distal oviduct; o, ovary; og, oviductal gland; po, proximal oviduct); **b** Open ovary full of mature oocytes; **c** Oocytes; **d** Male reproductive system. (ag, accessory gland; d, diverticulum; ns, Needham's sac; p, penis or terminal organ; sg, spermatophoric gland; t, testis; vd, vas deferens); **e** Spermatophore (cb, cement body; ea, ejaculatory apparatus; f, filament; oc, oral cap; SpR, sperm reservoir). (a–c: mature female specimen (ML 32 mm); d–e: male holotype specimen (ML 18.3 mm). Scale bars: a–d, 5 mm. e, 1 mm)
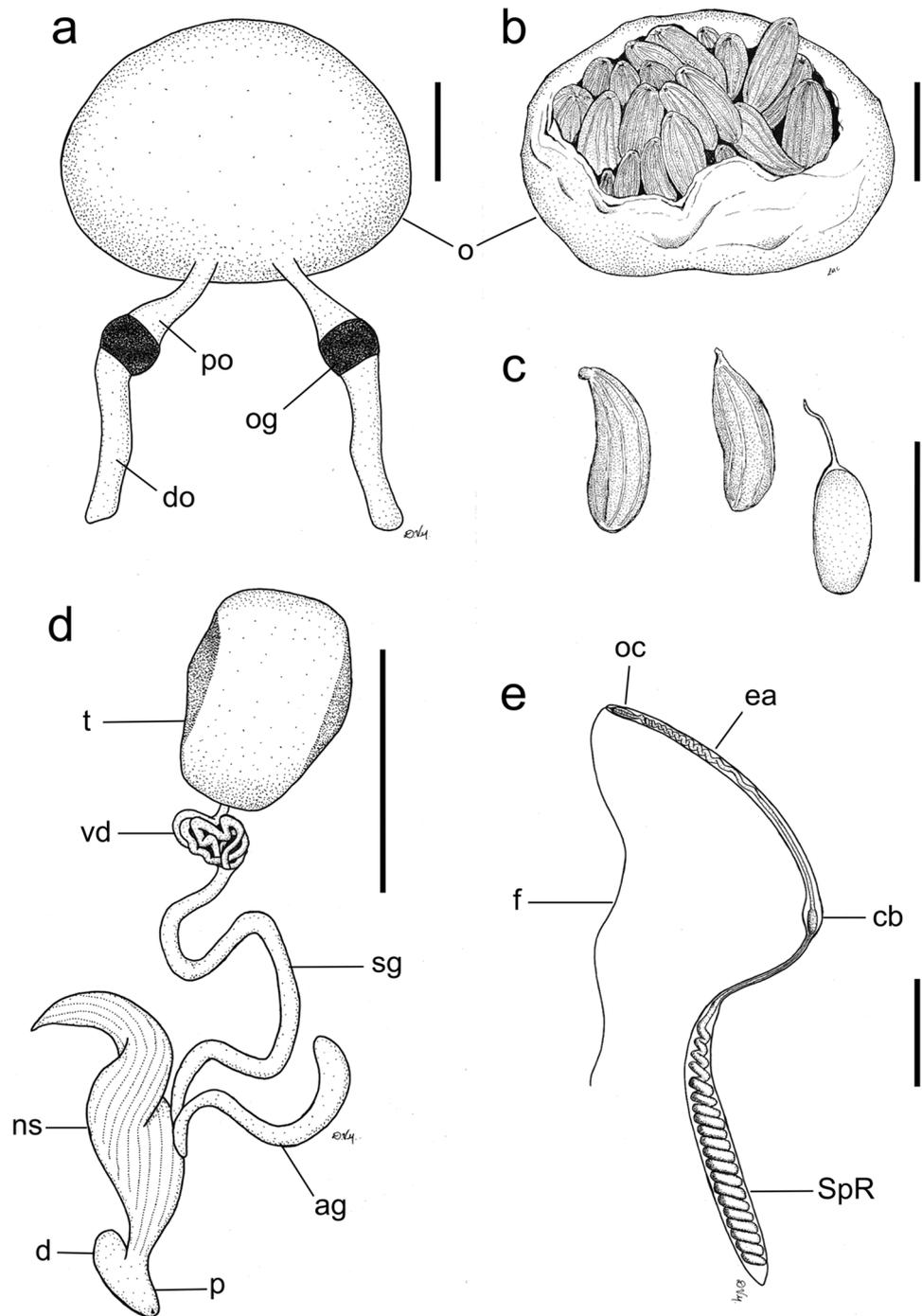
octopod species were found previously, and coupling morphological, molecular, and ecological data, have been useful to address taxonomic uncertainties (Lima et al. 2017).

Based on taxonomic arrangement and molecular data, from now on we will consider *O. joubini* part of the *Paroctopus* genus. Besides *P. joubini*, the phylogeny indicated three other small species assigned to *Octopus* grouped in the *Paroctopus* clade, suggesting they belong to this genus. The first species is *O. tehuelchus*, a small octopus (200 mm ML) with large eggs distributed from southern Brazil to northern Patagonia in

Argentina (Jereb et al. 2014). The second is *Octopus alecto*, a Pacific pygmy species found in the Gulf of California from Mexico to Ecuador. The third species assigned to the *Paroctopus* genus is *Octopus fitchi*, another Pacific pygmy species found in shallow waters (down to 30 m) in sandy and muddy substrates from the Gulf of California and Mexico to Ecuador (Jereb et al. 2014).

A recent study using molecular analysis of partial COI gene sequences and traditional morphometry data suggested that *O. alecto* should be considered *Paroctopus*
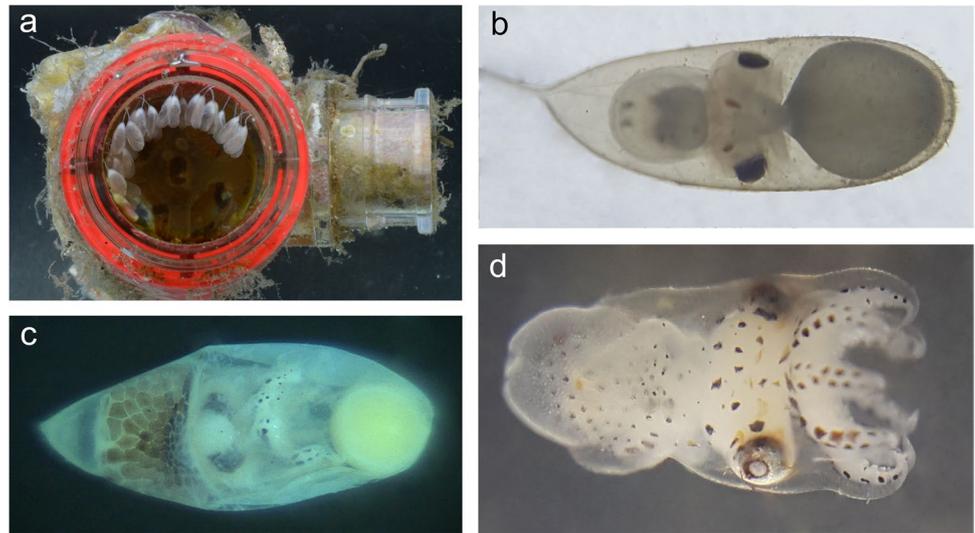
(Díaz–Santana–Iturrios et al. 2019). Additionally, Magallón-Gayón et al. (2019) sequenced the complete mitochondrial genome of *O. fitchi* and pointed out that this species does not belong to the genus *Octopus*, suggesting that it seems closer to the *Paroctopus* group. The three species have small size, medium to large size eggs, and inhabit shallow waters. Although these studies suggest that these species could be allocated in the genus *Paroctopus*, it is worth noting that the single use of the mitochondrial gene COI is not enough to clarify their phylogenetic relationships. Therefore, further systematics studies using both morphological information and careful observation of the type series, and molecular data with more loci, including nuclear markers or a genomic approach should be conducted to place correctly these species within the Octopods phylogeny.

The Western Atlantic pygmy octopuses probably shared a common ancestor before the uplift of the Isthmus of Panama, which is evidenced by their close relationship with *P. digueti* and *Octopus alecto* from the East Pacific (Lima et al. 2020). *Paroctopus cthulu* may have arrived in the Southwest Atlantic via shallow water of the continental shelf linking South and Central America, before the effects of Amazon river discharge in the Atlantic Ocean around 10 million years ago (Mya) (Hoorn 1994), which became a low salinity barrier for many marine species (Muss et al. 2001; Rocha 2003; Gleadall 2013). This event coincided with the split between *P. cthulu* sp. nov. and *O. joubini* (mean 9.4 Mya) according to Lima et al. (2020). The Brazilian pygmy octopus probably settled in the Southeast and South of Brazil due to its preference for subtropical waters. Until now, we only have
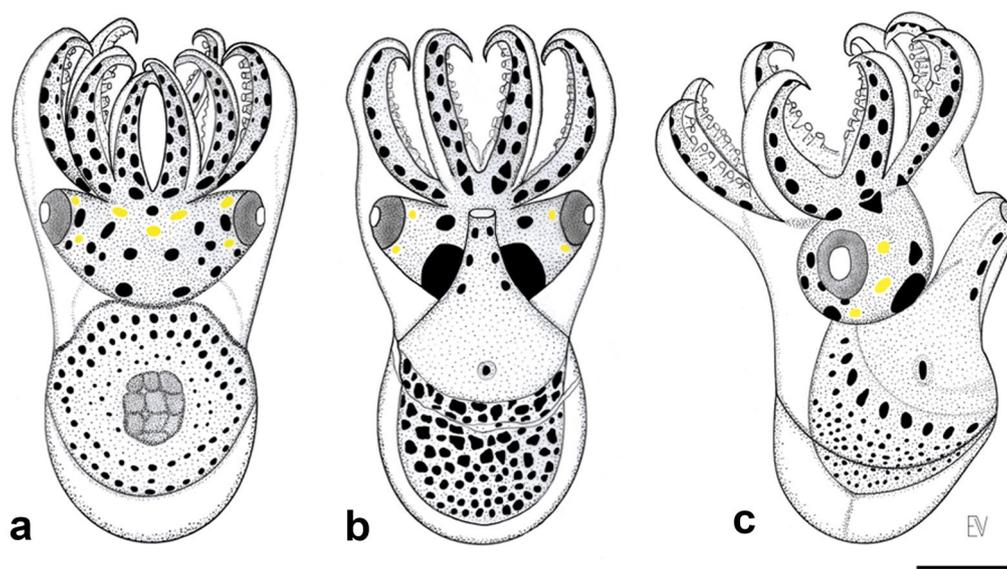

Fig. 8 *Paroctopus cthulu* sp. nov. Newly hatched paralarva drawings; **a** Dorsal view; **b** Ventral view; **c** Lateral view. (Scale bar = 1 mm)

Fig. 9 *Paroctopus cthulu* sp.
nov. Body patterns and pos-
tures of juveniles and adults;
a Uniform reddish with dark
eyes; **b** Uniform dark brown-
ish; **c** Uniform light brown
with white dots; **d** Mottle with
yellow blotch and white spots;
e Brown with white stripes and
blotches on arms and mantle;
f Stereotyped postures: with first
pair of dorsal arms up showing
the larger suckers on the aboral
surface of the arms
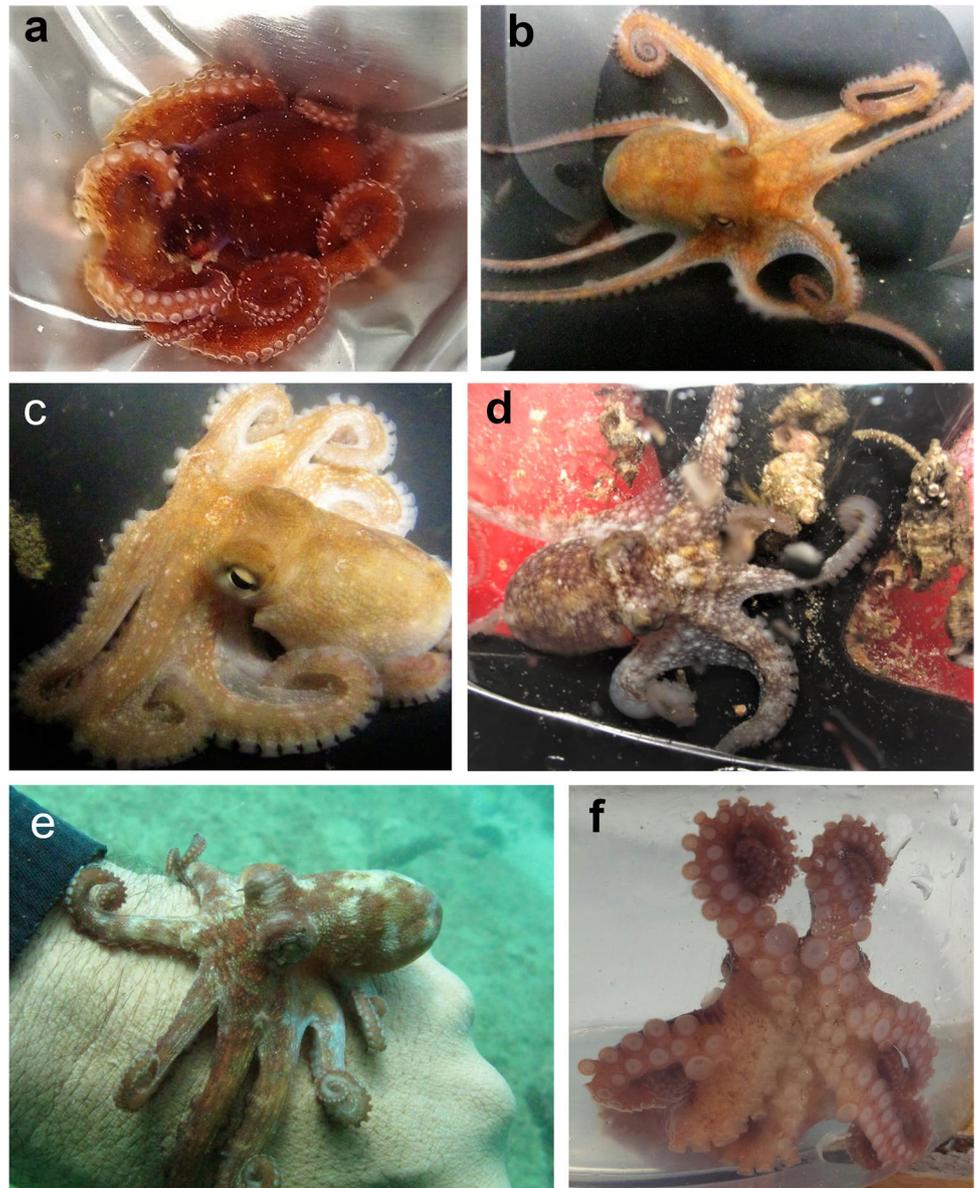
recorded it from Espirito Santo (20° 19′ 09′ S and 40° 20′ 50′
W) to Santa Catarina (27° 16′ S: 44° 57′ W) states (Fig. 1).

   After evaluating the octopus species described by Arocha
and Urosa (1982) in the southernmost area of the Carib-
bean, and papers on the distribution and biogeography of
shallow octopus species along the American coast (Voight
1998, Gonçalez et al. unpublished observations), we real-
ized that the south Caribbean Sea is the distribution limit
for octopus species with large eggs described from North
to Central Atlantic and the Caribbean sea, including *O.
joubini*, *P. mercatoris*, *Octopus briareus* Robson, 1929 (egg
length 11–15 mm), and *Octopus zonatus* Voss, 1968 (egg
length 6.6–8.2 mm). These species were not recorded in the
Amazon reef system or in northeast Brazil (Leite and Hai-
movici 2006; deLuna Sales et al. 2019), probably because

they produce benthic juveniles, with limited dispersal range
(Voight 1998; Villanueva et al. 2016) across long distances
and salinity barriers. In addition, the Amazon River mouth
might act as a barrier to their dispersal, preventing passage
southwards.

Early life stages

The mode of development of octopus hatchlings—whether
planktonic or benthic—can often be inferred by morpho-
logical traits, involving the body proportions of hatchlings
and adults (Boletzky 1974; Boletzky et al. 2002). In gen-
eral, species producing eggs smaller than 10% of the adult
ML, which result in an egg index < 10, and smaller hatch-
lings (hatching size index > 5) with short arms (< 50%ML)

Fig. 10 Type of marine debris where the *Paroctopus cthulu* sp. nov. were found at Ilha Grande in Rio de Janeiro (RJ) State; **a** Aluminum can intact and well preserved, with no biological encrustation or fragmentation, the arrow is pointing to the *P. cthulu* sp. nov.; **b** Aluminum can with biological encrustation and fragmentation, with a *P. cthulu* sp. nov. inside (arrow)
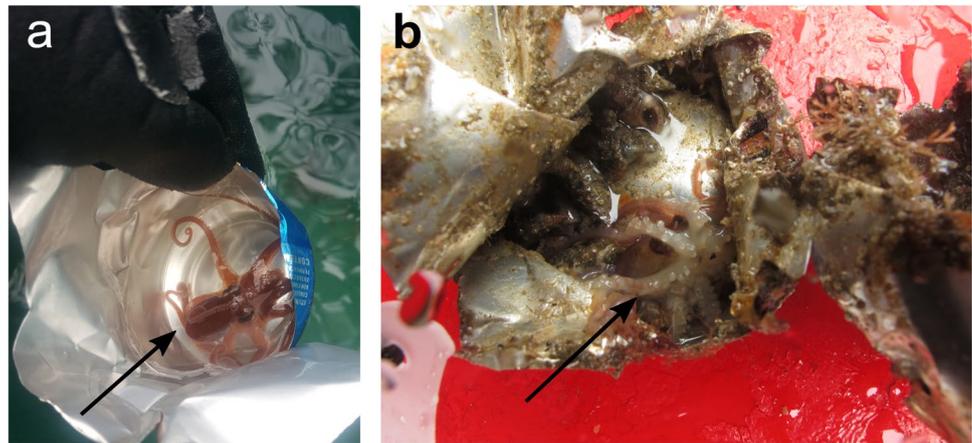
produce planktonic offspring, while species with large eggs (> 10 mm, egg index > 10) and large hatchlings with long arms, produce benthic hatchlings. Intermediate-sized eggs (6–9 mm) can produce either planktonic or benthic hatchlings (Boletzky 1974; Boletzky et al. 2002; Hochberg et al. 1992).

In *P. cthulu* sp. nov., eggs ranged from 4.2 to 5.5 mm in length, but larger oocytes (9 mm) were found in mature females, producing an egg index from 14.7 to 28. The hatching size index ranged from 10 to 18, with a hatchling AL index of 80%. While the egg length suggests either planktonic or benthic hatchlings, the AL index suggests planktonic hatchlings, but the egg index and the hatchling size index strongly indicate benthic hatchlings. Thus, *P. cthulu* sp. nov. has morphological features and proportions that would fit both the planktonic and benthic mode of development.

The peculiar morphological features of *P. cthulu* sp. nov. hatchlings raise many questions on the nature of its habitat and behavior. Among these features are the large prominent eyes and the robust funnel. The body is fragile and transparent, particularly the arms, with a clear web and a skin film covering their entire length, and has large cavities formed both dorsally and ventrally by the skin film. As well there is a dense distribution of Kolliker organs on the mantle. These morphological features are typical of planktonic hatchlings instead of benthic ones (Villanueva and Norman 2018).

Octopus paralarvae and pelagic octopods have both a dorsal and a ventral mantle cavity. In the later, these cavities are believed to facilitate maneuverability, while squid paralarvae have only a ventral mantle cavity (Villanueva and Norman, 2018). The two mantle cavities in *Octopus* paralarvae might help to increase the hydrostatic pressure inside the mantle cavity, which in turn increases the propulsive jetting and thus displacement of paralarvae, perhaps to balance the lack of fins, which acts as propulsors in squid paralarvae (Vidal et al. 2018). *P. cthulu* sp. nov. hatchlings have very large cavities, suggesting that these cavities might help to increase propulsive jetting and thus swimming performance. Another

strong evidence for this reasoning is the large size of the funnel in relation to the ML of the hatchlings.

Ortiz et al. (2006) suggested that *Enteroctopus megalocyathus* (Gould, 1852) hatchlings could live in the suprabenthos for a short period of time. The suprabenthos includes bottom dependent animals, such as mysids, isopods, and amphipods, living in the water layer just above the sea floor and performing vertical migrations above the bottom (Brunel et al. 1978). Another study on activity, locomotion and behavior of *O. joubini* has reported that during the first week after hatching, the young animals are active during the day and in their first month of life displayed a "semi–benthic" behavior, involving moving to higher spots (rocks or edges of the aquaria) and swimming in the open water, often drifting with spread arms in the water column (Mather, 1984). Such behavior of drifting in the water column with spread arms described for young *O. joubini* would seems also reasonable for *P. cthulu* sp. nov. hatchlings. That would explain the need for the protuberant eyes and funnel, the arm webs; and their lateral extensions, besides the large cavities formed by its conspicuous skin film. The possibility that *P. cthulu* sp. nov. hatchlings could be temporarily planktonic or suprabenthic, prior to settling to the benthos, is indeed intriguing, as it would indicate a plastic mode of development for octopods, which would combine the advantages of dispersal and large offspring size, and explain the peculiar morphology of *P. cthulu* sp. nov. hatchlings. This possibility remains open for future behavioral studies.

Habitat and in situ behavior

The specimens collected at this study were found inside debris on sand/muddy substrate, usually hidden below foliage and branches of terrestrial origin, but not in seagrass habitats, as *O. joubini* does in the Caribbean and North Atlantic (Eidemiller 1972; Arocha and Urosa 1982; Mather 1982b; Tiffany et al. 2006).

Paroctopus or pygmy species have been reported using gastropod or bivalve shells as their main refuge (Mather 1982a, b; Voight 1990; Iribarne 1990), with eventual use of artificial dens as shelter (Voight 1988). The type, size, and availability of these shells influenced the octopus' abundance and possibly fecundity (Mather 1984; Iribarne 1990; Voight 1992). Empty gastropod shells are an important resource for many animals, including octopuses, in shallow benthic marine communities and this dynamic could shape a benthic community structure (McLean 1983). Natural seashells are becoming increasingly scarce in shallow clear and warm waters due to tourism and collection for craftwork and decoration (Alves et al. 2006; Kowalewski et al. 2014), while marine debris is increasingly available due to pollution by debris in the oceans (Jambeck et al. 2015). Since we found all octopuses evaluated in this study only inside marine debris, with different sizes, sexes, and reproduction stages, including brooding females, it is quite possible that *P. cthulu* sp. nov. find in this debris an alternative shelter along the beaches of Ilha Grande frequented by tourists.

Considering the consequences of a successful habitat choice for benthic octopuses and the various negative impacts of solid waste on marine ecosystems, it is interesting to see debris as conveying an advantage (see also Anderson et al.1999; Katsanevakis and Verriopoulos 2004). This choice of trash has also been observed for other invertebrate species such as hermit crabs (Zulueta 2019) and sea urchins (Barros et al. 2020). This may demonstrate the plasticity that cephalopods have (Hochner et al. 2006; Albertin et al. 2015) and show that the octopuses are adapting to human impact. More studies are being carried out by our research group to clarify this ecology, which may be important for the conservation of the new species.

The description of this new species, *P. cthulu* sp. nov. living in an altered habitat of human debris in shallow water of Brazil, offers an opportunity not only to evaluate the relationship among the small octopuses of the western Atlantic, Caribbean and eastern Pacific, but also their adaptation to the Anthropocene period. In addition, the fairly large eggs of this species allow us to speculate about the possible benthopelagic lifestyle of hatchlings of this genus.

Declarations

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

Ethics approval International and national guidelines for the care and use of cephalopods 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 appropriate authorities (Instituto Chico Mendes de Conservação da Biodiversidade, ICMBio: License number 304841).

Data availability The sequences generated and analyzed during the current study were submitted to GenBank repository. GenBank accession numbers are provided in the text.

Authors' contributions TL and MH conceived the ideas; TL, MH, SM and FL designed the methodology and analyzed the data; TL SM, FL, RD and GG collected the data and described the habitat and living behaviors; TL, MH, DV described the species morphologically and worked on figures and drawings; FL and SL, provided molecular data and analyses; EV analyzed eggs and hatchlings data, described the early life stages and prepared their drawings; JM participated in forming ideas, writing and reviewed all manuscript components. All authors contributed to writing of the drafts and final manuscript submitted for publication.

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