

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

Tatiana S. Leite (✉ tati.polvo@gmail.com)

Universidade Federal de Santa Catarina Centro de Ciencias Biologicas <https://orcid.org/0000-0001-9117-9648>

Erica A.G. Vidal

Universidade Federal do Parana Setor de Ciencias da Terra

Françoise Dantas Lima

Universidade Federal do Rio Grande do Norte Centro de Biociencias

Sergio M.Q. Lima

Universidade Federal do Rio Grande do Norte Centro de Biociencias

Ricardo M Dias

Universidade Federal do Sul da Bahia

Giulia A. Giuberti

Universidade Federal do Estado do Rio de Janeiro

Davi De Vasconcellos

Universidade Federal do Rio Grande

Jennifer A. Mather

University of Lethbridge

Manuel Haimovici

Universidade Federal do Rio Grande

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Abstract

The new species, *Paroctopus cthulu* sp. nov. Leite, Haimovici, Lima and Lima, was recorded from very shallow coastal waters on sandy/muddy and shelter-poor bottoms with natural and human-origin debris. It is a small octopus, adults are less than 35 mm mantle length (ML) and weigh around 15 g. It has short to medium sized arms, enlarged suckers on the arms of both males and females, large posterior salivary glands (25 %ML), 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 behaviour are a clue to the life history of it and closely related pygmy octopuses. The Bayesian phylogenetic analysis showed that *Paroctopus cthulu* sp.nov. specimens grouped in a well-supported clade of *Paroctopus* species, separate from *P. joubini* and *P. cf. mercatoris* from the Northwestern Atlantic. The description of this new species, living in a novel habitat of human 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.

Introduction

The pygmy octopuses are small-bodied species of Octopodidae, some of which mature as small as 20 mm dorsal mantle length (around 100 mm total length). Most of them are currently placed in the genus *Paroctopus*. This genus 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 *Pseudooctopus* based on the same morphotype species, citing the single attachment of eggs, as well as the egg size. Naef discarded the genus name *Paroctopus* in 1928, apparently because he felt it was not valid, although he did not discuss the reasons for such a decision. Robson (1929) resurrected the name, but had reservations about erecting a new genus based solely on egg size. In an attempt to validate Naef's genus, he 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 octopodine 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 trans-isthmian geminate species complex endemic to tropical and subtropical waters in the Americas (see Berry 1953, Nesis, 1975, 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 Northeastern 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, 1980b, 1982a, 1982b, 1984).

When it comes to the Northwestern Atlantic and Caribbean Sea, the pygmy taxonomy is mixed up. In the Caribbean Sea (St. Thomas/ Virgin Island), the small-egged species was described as *Octopus joubini* Robson 1929 (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 *Octopus mercatoris* Adam, 1937 (Forsythe and Hanlon 1980, Forsythe and Toll 1991, Tiffany et al. 2006), despite the fact that the holotype of *O. 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 *O. 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 27028) collected in São Paulo State (23° 30' S) in their list of cephalopod species deposited at the Zoology Museum of the University of 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, that fits the description of the *Paroctopus* type species, were collected in shallow waters of Santa Catarina and Rio de Janeiro states, along the 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 those of the adults are important for inferences about the developmental mode – planktonic or benthic – of octopus hatchlings, providing key information on life history traits.

Genetic and morphological characterization of these specimens does not fit with the available information on the described species of the genus and support their description as a new species. Additional images and videos 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.

Materials 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, Central and Southern Brazil (Fig. 1a and b). 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 temperature varied from 19 to 26 °C. The specimens were collected by sorting solid garbage

found on the sea bottom, including metallic cans, glass bottles and plastic objects such as snorkeling mouthpieces, among others. No specimen was found inside empty shells, although we looked for them, also 9 divers. Two females with eggs were found, one spawned inside a snorkeling mouthpiece and the other in a metal beer can.

Morphological data

Most specimens were fixed in 10% formalin and preserved in 70% ethanol. Measurements, counts and indices followed Roper and Voss (1983) and Huffard and Hochberg (2005). Sucker counts included all suckers in each arm, rather than only those on the basal half. The following abbreviations are used for measurements and indices:

AFL: left arm formula, AFR: right arm formula, ALI: arm length index (arm length/ML \times 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 \times 100), CaLI: calamus length index (calamus length/LL \times 100), FLI: funnel length index (funnel length/ML \times 100), FFLI: Free funnel length index (free funnel length/funnel length \times 100); GiLC: gill lamellae count per demibranch, HLI: hectocotylus length index (hectocotylus length/hectocotylized arm length \times 100; HWI: head width index (head width/ML \times 100), LLI: ligula length index (ligula length/HL \times 100), ML: dorsal mantle length, MWI: mantle width index (mantle width/ML \times 100), OAI: opposite arm length index (hectocotylized arm/normal third arm length \times 100), PLI: terminal organ (penis) length index (terminal organ length/ML \times 100), SDeI: enlarged sucker diameter index (enlarged sucker diameter/ML \times 100), SDnI: largest normal sucker diameter index (largest normal sucker diameter/ML \times 100), SpLI: spermatophore length index (spermatophore length/ ML \times 100), SpRLI: sperm reservoir length index (sperm reservoir length/ spermatophore length \times 100), SpWI: spermatophore width index (spermatophore width/ spermatophore length \times 100), TL: total length, WDI: web depth index (web more depth/mantle \times 100), WF: web formula, WT: total preserved weight.

Unless stated otherwise, all measurements are in mm and weights in g. Small structures such as the ligula, calamus, spermatophores and eggs 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 \times 100/adult ML), hatchling size index = hatchling ML \times 100/ adult ML), hatchling AL index = AL of hatchling \times 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 \square Smithsonian), British Museum National, History, England (BMNH)

Molecular data and analyses

Small pieces of mantle or arms of three specimens were preserved in 99 % ethanol from which genomic DNA was extracted using the GF \square 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 μ L containing 1 μ L forward primer, 1 μ L reverse primer (10 mM), 12.5 μ L Taq DNA Polymerase Master Mix (Ampliqon A/S) or MyTaq RedMix (Bioline), 8.5 μ L H $_2$ O and 2 μ L DNA (20 – 40 ng/ μ L). 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 ClustalW 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) run 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 'burnin' 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 distance in MEGA 6 (Tamura et al. 2013) (see Table 1 of octopod specimens used to construct the Bayesian phylogenetic tree in this study).

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*, clearly separated from other Western Atlantic closely related pygmy species, and *P. joubini* and *P. cf. mercatoris* sequences, retrieved from GenBank (Posterior probability [PP] = 1). The genetic distance between the *Paroctopus* sp. nov. and *P. joubini* (AY377732), and between *Paroctopus* sp. n. and *P. cf. mercatoris* (GQ900743, Florida). However, the sequences of *P. joubini* (AY377732) and *P. cf. mercatoris* (GQ900743, Florida) 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 revealed three other small species assigned to the *Octopus* genus and grouped in the *Paroctopus* clade: *Octopus tehuelchus* d'Orbigny, 1834 (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).

Systematic and description

Family Octopodidae d'Orbigny, 1840

Subfamily Octopodinae d'Orbigny, 1840

Genus *Paroctopus* Naef, 1923

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

Type Species: *Octopus digueti* Perrier and Rochebrune, 1894.

Genus amplified diagnostic features: Small-bodied; mantle short and wide, with large eggs. Stylets present, non-mineralized. Arms short-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 with 4.2-9 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 presents but faint. Skin without large primary papillae.

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

urn:lsid:zoobank.org:act:03EFA7CC-4797-4244-A595-D87DCEDC7E72

Holotype: male (mature) 18.3 mm ML found on sandy bottom next to rocky reefs at 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. February/2005; MORG 52754 (Fig. 3)

Paratypes: Adult male 29 mm ML collected in shallow waters in Porto Belo/SC Brazil (27° 09' S; 48° 33' W) in 1971 (MCPUCRS 3398); adult male 32.70 mm ML collected in shallow waters in Porto Belo/Santa Catarina State (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 52778); 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 52777); 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 52767); 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 52778); 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 52780; DNA); adult female 21.30 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 52779); 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 52781 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 52782); 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 52783).

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 (1928) 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 southern Brazil (Fig. 1).

Diagnosis: adults small-sized (ML 14 to 33 mm), mantle and head wide with large and prominent eyes. One to three cirri over the eye and one above. 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 few adult females. Third arm of the males hectocotylized with a moderate calamus and small ligula, 1/3 of the length of the opposite arm (Fig. 3b). Gills with 5-6 lamellae per hemibranch, usually 6. Adult females with medium to large oocytes in the ovaries (4.7 to 9 mm length). Spawned eggs attached with stalks to objects singly in small clusters. Hatchlings have 5.0-5.2 mm total length and 2.52 mm ML, arms with 14-16 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). U-shaped funnel organ with similar size (Fig. 3c). Normal arms similar in length (ALI 270-450, in mean 355), no consistent arms formulae; arms width (AWI 11-20). Total number of suckers on the normal arms 112 to 174, mean 15. Normal sucker diameter moderate (SDnI 8-13 in mean 0.105). One to three conspicuous enlarged suckers (SDel 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. 3c and 3d). Third right arm of males hectocotylized, moderately shorter than the opposite arm (OAI 45-73), bearing 86-94 suckers; hectocotylus with a well-defined spermatophoric groove, relatively short hectocotylus (HLI 5-10), a moderately long calamus (CLI 20-42) and small ligula (LLI 3.7-7.3) with clear transverse trabeculae. Web shallow (WDI 20-72), with no consistent web formulae differences among the evaluated individuals, however the sectors A and E usually are shallower. Hemibranchs have stout gills, with 5-6 lamellae per hemibranch, usually 6. Stylets medium sized 5.4-6.7 mm (before preservation), with the posterior part longer and thinner

Digestive tract: a dissected adult female (32.0 mm ML MORG 52768) presented a typical *Octopus* digestive tract (Fig. 5a), with few particularities. 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 mm; 25% of ML) joined by ducts to the buccal mass. Narrow oesophagus followed by crop diverticulum and a wide stomach. Spiral caecum connected by two ducts to large digestive gland (12 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-c beak). Rachidian tooth on a half radula; two lateral teeth, one marginal tooth, one marginal plate, one lateral cusp on each side of rachidian tooth with a symmetric seriation, the cusp goes 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. 4d).

Female reproductive system. The mature female (32. mm ML) has a very large round ovary (22 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 compared to the mantle length (from 4.7 to 9 mm) (Fig. 6b).

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 1.8 - 6) diverticulum not clearly differentiated from the terminal organ (Fig. 6c). 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 spermatic mass (Fig. 6d). 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 were found attached individually to the snorkel mouthpiece (Fig. 7a). Another female with spawning were found with 124 eggs were found attached individually to the 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 largest mean width of 2.3 ± 0.14 mm (n=25). (Fig. 7b) The egg index was 23. They have a transparent chorion and were devoid of other 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 10-12 suckers distributed in a single row from the buccal mass up to the web close to the base of the arms and in a zigzag double row 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 12-14 chromatophores, two at the base in a single row and the others in a zigzag row. On the head, there are about 20-24, both large extra-tegmental and small tegumental chromatophores interpolated and sometimes superimposed. On the mantle, there are from 18 to 22 extra-tegmental chromatophores distributed in the central area. *Ventral view.* On the head, there are four, two very large extra-tegmental 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 rows, 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: Hatchlings have a total length of 5.0-5.4 mm (Fig. 7d and Fig. 8a-c). The mantle edge is clearly visible at the base of the head, indicating a ML of 2.52 mm, or 3.3 mm ML if the middle point between the eyes is considered instead. The mantle is roundish with a width of 1.9 mm. The head is wider (100% ML) than long (50% ML), with approximate concave shape. The eyes are large and prominent (28% ML) and anteriorly inserted (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 row, 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 skin film seems to be continuous over the whole dorsal side of the 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), where it is possible to notice that the skin film forms a large and continuous dorsal cavity from the mantle to the tips of the arms and also ventrally from the arms tips to the funnel.

Thus, the hatchling has a large continuum dorsal cavity and both ventral mantle and head cavities, the internal organs occupy a relatively small area inside the mantle. 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 less silhouette 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 that of the suckers. There are from 10–14 chromatophores on each arm, one to two large ones at the base (in the arm–crown stalk) 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 interpolated 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 12–16 chromatophores each and the same patterns is seen on the posterior mantle, where each row has about 12–14 chromatophores. Many small other chromatophores are found scattered over the whole mantle without a particular pattern. There are from 8 to 11 extra-tegumental chromatophores covering the visceral mass 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 70–80 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 it is possible to notice 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).

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, 9b), 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 mantle, head and proximo–dorsally on the first arms.

Distinguishing postures: We observed three stereotyped postures: sitting with curled arms pointed, (9a; 9c); 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)

Habitat and *in vivo* observations

There is no information on the habitat of the four specimens deposited in the MORG and MCPUCRS collections. 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 (Fig. 10), inside metallic cans, plastic objects or glass bottles. 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 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 temperatures around 25 °C, from February to March.

Discussion

Our study revealed a new species of the genus *Parocotopus*, the first pygmy octopus described for the Southwestern Atlantic. Both morphological and molecular analyses corroborate the great divergence of *P. ctulu* sp. nov. from the Northern Atlantic complex of pygmy octopuses, whose taxonomy is still not solved (Jereb et al.2014).

Molecular phylogeny and geographic distribution

The genetic distances between *P. ctulu* and *P. joubini*/*P. cf. mercatoris* are large enough (around 9%) to claim that the lineage from Brazil is a different species of pygmy octopus from those in Florida, USA. Additionally, the COI sequences from *P. joubini* and *P. cf. mercatoris* are identical, which means either a

misidentification problem or the species are synonymous. Misidentifications in other Atlantic octopod species were found previously, and coupling morphological, molecular, and ecological data, have been useful to address taxonomic uncertainties (Lima et al., 2017).

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* and *P. 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 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 Caribbean, and papers on the distribution and biogeography of shallow octopus species along the American coast (Voight 1998, González et al. unpublished observations), we realized 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 *P. 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 Haimovici 2006; de Luna Sales et al. 2019), probably because they produce benthic juveniles, with limited dispersal range (Voight 1998, Villaneuva 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.

The phylogeny indicated that three small species assigned to the *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* Berry, 1953, 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-Isturrios 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, median to large size eggs and inhabit shallow waters. Our analysis confirms and expands previous studies, suggesting that these species should be reviewed and moved to the *Paroctopus* genus.

Morphological comparison with related taxa

As noted above in the introduction, *Octopus joubini* is the name used most frequently for the small eggged 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 150–3000 ripe eggs of 2.3–4.8 mm in length. Compared to the description of Forsythe and Toll (1991) for *O. joubini*, *P. cthulu* sp. nov. has larger eggs (4.2–9.0 mm), a deeper web (20–72 vs 28) and more arm suckers (ASC 102–178 vs 79) (see Table 5). The sole criterion that Norman et al. (2016) 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 eggged species morphotypes collected from Belize in the Caribbean and deposited on 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 (SDel 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 *P. joubini* only had enlarged suckers in male specimens. *P. 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 *Octopus 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 x W), deeper interbranchial 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 spermatid mass (19–20 vs 50). See Fig. 3 in Arocha and Urosa, 1982, and the different radula seriation (A2–3 vs A4–6). Except for the eggs size and radula seriation, 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 North Atlantic and Gulf of Mexico is *O. mercatoris*. Pickford (1945) compared *O. 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 eggged) concluded that they are in fact two different species. Their conclusion was based on the hatchling size, as while the small eggged specimen produced planktonic paralarvae, the large eggged individuals produced benthic juveniles. For these authors, the small eggged pygmy species is conspecific with the holotype of *O. joubini*, and not the widely studied and better known large eggged species, although both species occur in the Caribbean Sea and the Gulf of Mexico. For these authors, the taxonomy of the large eggged pygmy species from the northwestern Atlantic is still not clarified.

Besides the available holotypes and syntypes, we also compared the new species with large eggged specimens deposited at 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 hectocolized arm (ASCH 56–93 vs 45) when compared with the large-egg 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 *P. 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-egg) have a dark brownish tone, also described in the large-egg morphotype (Forsythe and Toll, 1991) with no ability to modify skin texture other than 3–4 papillae. Mather (1984) also indicated that the *O. joubini* large-egg 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-egg 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). *P. cthulu* sp. nov. has the smallest adult size, when compared to all described southwestern Atlantic octopus 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 octopus 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 gastropods shells and debris as shelters (Hanlon and Hixon 1980). This species as having a complex body pattern, with a grainy skin and a conspicuous purplish brown stripe along the entire leading edge of the arms pairs I to III, which makes its recognition easy. *Octopus tehuelchus* d'Orbigny, 1835 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). *Pinnoctopus (Callistoctopus) macropus* (Risso, 1826) has a distinct larger adult size (150 mm ML, with a distinct red and white coloration on body and arms (Mangold 1998, Leite and Haimovici 2006). *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* (Avendaño et al, 2020) and *O. insularis* (Leite et al. 2008) are bigger animals with larger adult size.

Early life stages

The mode of development of octopus hatchlings – whether planktonic or benthic – can often be inferred by morphological traits, involving the body proportions of hatchlings and adults (Boletzky 1974; Boletzky et al. 2002). In general, species producing eggs smaller than 10% of the adult ML, which result in an egg index <10, and smaller hatchlings (hatching size index >5) with short arms (<50 %ML) 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. Hatchlings have a mean ML of 2.52, with a hatching size index ranging from 8.5 to 12.7 and 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, with 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 a large continuum dorsal cavity and also both mantle and head ventral cavities formed by the skin film, suggesting that these cavities might help to increase propulsive jetting and thus swimming performance, while also minimizing sinking. Another strong evidence for this reasoning is the large size of the funnel in relation to the ML of the hatchlings.

It was suggested that *E. megalocyathys* hatchlings could live in the suprabenthos for a short period of time, which would favor their dispersal before settling definitively to the benthos (Ortiz et al. 2006). This 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 and with good swimming ability (Brunel et al. 1978). Another study on activity, locomotion and behavior of *P. 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). The author also reported that the activity and swimming behavior of *P. joubini* changed slowly over a few weeks, and adults were only active at night and never moved far from the bottom.

Such behavior of drifting in the water column with spread arms described for young *P. joubini* would seem also reasonable for *P. cthulu* sp. nov. hatchlings. That would explain the need for the protuberant eyes and funnel, the arms web; and their lateral extensions, which look similar to swimming keels in the arms of squid paralarvae, 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* behaviour

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 phanerogam habitats, as *O. joubini* in the Caribbean and North Atlantic (Eidemiller 1972, Arousa and Urosa 1982, Mather 1982, Tiffany et al. 2006).

Paroctopus or pygmy species were reported using gastropod or bivalve shells as their main refuge (Mather 1982a, 1982b, 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 (Souto-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). 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 Verriopolis 2004). This choice of trash has also been observed for other invertebrate species such as hermit crabs (Zuleta 2019) and sea urchins (Barros et al. 2020). This may demonstrate the plasticity that cephalopods show (Hochner et al. 2006; Albertin et al. 2015) and shows the octopuses are adapting to human impact. More studies are being carried out by our research group to clarify this ecology, which can be important for the conservation of the new species.

The description of this new species, *Paroctopus cthulu* sp. nov., living in a novel 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

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Conflicts of interest/Competing interests The authors declare that they have no conflict of interest

Ethics approval All applicable international, national, and/or institutional guidelines for animal testing, animal care and use of animals were followed by the authors. The research was approved by the Instituto Chico Mendes de Conservação da Biodiversidade, ICMBIO (304841).

Consent to participate The authors declare that they are consent to participate of this research

Consent for publication The authors declare that they are consent for publication

Availability of data and material The datasets analysed during the current study are available on GenBank accession. Holotypes and paratypes were deposited at museum collection, and the numbers added at this manuscript.

Authors' contributions Tatiana Leite (TL) and Manuel Haimovici (MH) conceived the ideas; TL, MH, Sergio Maia (SM), Françoise Lima (FL) designed the methodology and analyzed the data; TL, SM, FL, Ricardo Dias (RD) and Giulia Giuberti (GG) collected the data and describe habitat and living behaviors; TL, MH and Davi de Vasconcellos (DV) described the species morphologically and worked on figures and drawings; FL and SL, did the molecular data and analyses; Erica Vidal described the early life stages; Jennifer Mather participated in forming ideas, writing and reviewed all manuscript components, as she had experience with pygmy octopod species from the Caribbean, and she is a native English speaker. All authors contributed to writing of the drafts and final manuscript submitted for publication.

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Tables

Table 1. Octopod specimens used to construct the Bayesian phylogenetic tree. AN = accession number.

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

Table 2: Genetic distances (Kimura 2 μ 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 *P. joubini* and *P. mercatoris* are shown in bold.

Species	1	2	3	4	5	6	7	8
1. <i>Paroctopus</i> sp. nov.								
2. <i>Paroctopus</i> sp. nov.	0.000							
3. <i>Paroctopus</i> sp. nov.	0.000	0.000						
4. <i>Paroctopus joubini</i>	0.093	0.093	0.093					
5. <i>Paroctopus mercatoris</i>	0.093	0.093	0.093	0.000				
6. <i>Paroctopus digueti</i>	0.110	0.110	0.110	0.132	0.126			
7. <i>Octopus tehuelchus</i>	0.129	0.129	0.129	0.131	0.131	0.140		
8. <i>Octopus alecto</i>	0.134	0.134	0.134	0.157	0.157	0.030	0.163	
9. <i>Octopus fitchi</i>	0.138	0.138	0.138	0.144	0.145	0.128	0.149	0.154

Table 3. *Paroctopus thulu* 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
Catalog number	52778	3398	2686	52754	52777	52767	52768	52780	52779	52781	52782
Status	Paratype	Paratype	Paratype	Holotype	Paratype	Paratype	Paratype	Paratype	Paratype	Paratype	Paraty
Collection date	1966	1971	1972	2015	2015	2015 fev	2007	2015	2015	2015 fev	2014
Sex	male	male	male	male	male	male	female	female	female	male	female
Maturity	adults	adults	adults	adults	adults	adults	adults	adults	adults	subadults	subad
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
Total length	120	150	164	70	55	85	sd	105	73	53	73
Dorsal mantle length	24	29	32.7	18.3	14	19.7	32	25.6	21.3	11	14
Mantle width	15	24	19	14.4	11.1	12.3	24	21.4	18.2	6.9	10.4
Head width	17.3	15.7	15.4	12.5	10	10.3	16.7	12.8	13	8	11.1
Funnel length	10.9	13.6	10.4	7.8	7.6	6	10.5	8.1	4	4.7	7
Free funnel length	6.9	5.3	6.5	3.3	4.8	3.5	5.5	5	2	2.2	3.4
Hectocotylus length	5.5	3.7	2.8		2.4	2.4				1.8	
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
Gill Length (mean)	8.5	10	sd	4	sd	6.5	6.45	3.1	sd	3.5	4
Arm lengths 1 (R - L)	108-85	-	107-102	51-56	-	-	-	-	-	41-42	55-56
Arm lengths 2	90 R	105-111	103-90	-55	-46	78-	-	-	-46	41-43	55-55
Arm lengths 3	55-	70-100	50-111	36-57	33-45	45-	-79	-	-	32-42	53-50
Arm lengths 4	83-85	94-	99-124	53-56	-44	69-70	-	-	56-	41-41	54-48
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
Web Depth A (dorsal)	11	sd	10	7	7	sd	sd	8	10	7	6
Web Depth B	10-10	-13	11-13	8-8	8-8	-	-	8-10	10-9	6-6	6-7
Web Depth C	10-9	19-17	6-13	9-9	9-9	-	-	12-11	12-12	5-7	6-8
Web Depth D	0	21-18	11-12	8-	9-9	-	-	12-12	11-11	5-7	8-7
Web Depth E (ventral)	9	17	13	9	9	0	0	11	12	7	8
Number of suckers arm 1 (R-L)	-174	-	139-118	147-146	-	-174	-102	-	-	138-132	167-16
Number of suckers arm 2	157-	154-	145-112	154-148	-	157-	-	-	-	144-139	165-16
Number of suckers arm 3	86-	86-144	56-126	85-160	93-150	86-	-	-	142-131	89-141	160-16
Number of suckers arm 4	174-	-	118-138	146-152	-151	174-	102-	-	-	132-142	165-
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
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
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
Larger normal	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

sucker arm 4											
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-	-	-	-	-

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

Catalog number	MORG 52778	MCPUCRS 3398	MCPUCRS 2686	MORG 52754	MORG 52754	MORG 52767	MORG 52768	MORG 52754	MORG 52754	MORG 52767	MO 527
Status	Paratype	Paratype	Paratype	Holotype	Paratype	Paratype	Paratype	Paratype	Paratype	Paratype	Par
Collection date	1966	1971	1972	2015	2015	2015	2007	2015	2015	2015	201
Sex and maturity	adult male	adult male	adult male	adult male	adult male	adult male	adult female	spawned female	adult female	subadult male	sub fer
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.
Mantle width index	80	60	58	64	75	62	75	55	70	75	82
Head width index	72	54	47	68	71	52	52	50	61	73	79
Gill length index	33	34	sd	22	sd	33	20	24	sd	32	29
Funnel length index	45	47	32	43	54	30	33	32	19	43	50
Free funnel length index	63	39	63	42	63	58	52	62	50	47	49
Ligula length index	7	4	4	6	6	4	-	-	-	11	-
Calamus length index	38	42	40	-	26	20	-	-	-	50	-
Hectocotylus length index	10	5	6	-	7	5	-	-	-	6	-
Hectocotylized 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
Arm 2 length index	3.8 R	3.6 - 3.8	3.1 - 2.7	3.0 L	3.2 R	3.9 R	-	3.8 R	2.1 R	3.7 - 3.9	
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
Arm 4 length index	3.5 - 3.5	3.2 R	3.0 - 3.7	2.8 - 3	3.1 L	3.5 - 3.5	-	3.5 - 3.5	2.6 R	3.7 - 3.7	3.8
Arm width index	14	14	14	16	15	11	20	15	16	13	16
Web depth (A=dorsal) index	46	-	31	38	50	-	-	31	47	64	43
Web depth (B) index	41 - 41	44 L	33 - 39	43 - 43	57 - 57	-	-	31 - 39	46 - 42	54 - 54	42
Web depth (C) index	41 - 37	65 - 58	39 L	49 - 49	64 - 64	-	-	46 - 42	56 - 56	45 - 63	42
Web depth (D) index	29 R	72 - 62	33 - 36	43 - 0	64 - 64	-	-	46 - 46	51 - 51	45 - 63	57
Web depth (E=ventral) index	21	55	28	36	57			43	52	27	50
Largest normal sucker index	12	13	-	9	8	10	10	12	-	12	10
Largest enlarged sucker index	17	13	-	18	23	10	13	14	-	17	-
Enlarged suckers row * females	5 th	5 th	-	6 th	5 th	5 th	5 th	-	-	5 th	-

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.

	<i>P. cthulu</i> <i>sp.nov.</i>	<i>O joubini</i>	<i>P. mercatoris</i>	<i>P. joubini</i>	<i>P. joubini</i>	<i>P. joubini</i>	<i>P. joubini</i>
Reference and type material	New species Southern Brazil Small eggs morphotype. (4F, 8M) This study	Robson, 1929. Holotype, BMNH 1889.4.24.30 one female. Caribbean, St Thomas. Small eggs morphotype	Adam, 1937. Syntype 1 F, 1 M); Gulf of México, Florida. Small egg morphotype	SBMNH (3 F, 3 M) Caribbean / Belize. Small eggs morphotype	Pickford, 1945. Gulf of Mexico, Florida. Big eggs morphotype	Forsythe, 1977 NMNH (3 F, 1M) Reared Gulf of Mexico Big eggs morphotype	Arocha & Urosa, 1982 (11 F, 4M). 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 & 1 F	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
Deep web 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	
Opposite arm index (OAI)	60 - 70			64 - 71		78	42.7-59.0
Ligula length index (LLI)	3.7 - 7.3		7.9	5.6 - 7.7	6.3 - 6.9	5	4.5-17.7
Calamus length index (CaLI)	20 - 42		18	33 - 33	21 - 22	31.6	20.0 - 36.8
Spermatophore length index (SpLI)	40 - 43				55.5		24 - 31
Penis index	1.8 - 6		12,5	16	22 - 22.5		11.2 - 16.7
Number of gills lamellae	5 - 6	4	5 - 6	4 - 6	5 - 6.5	5 - 6	5 - 7
Eggs larger diameter (mm)	4.7 - 9	3.2	2.7 - 3	0.6 - 2.8	7.5 - 8.4	4 - 6.5	-10

Figures

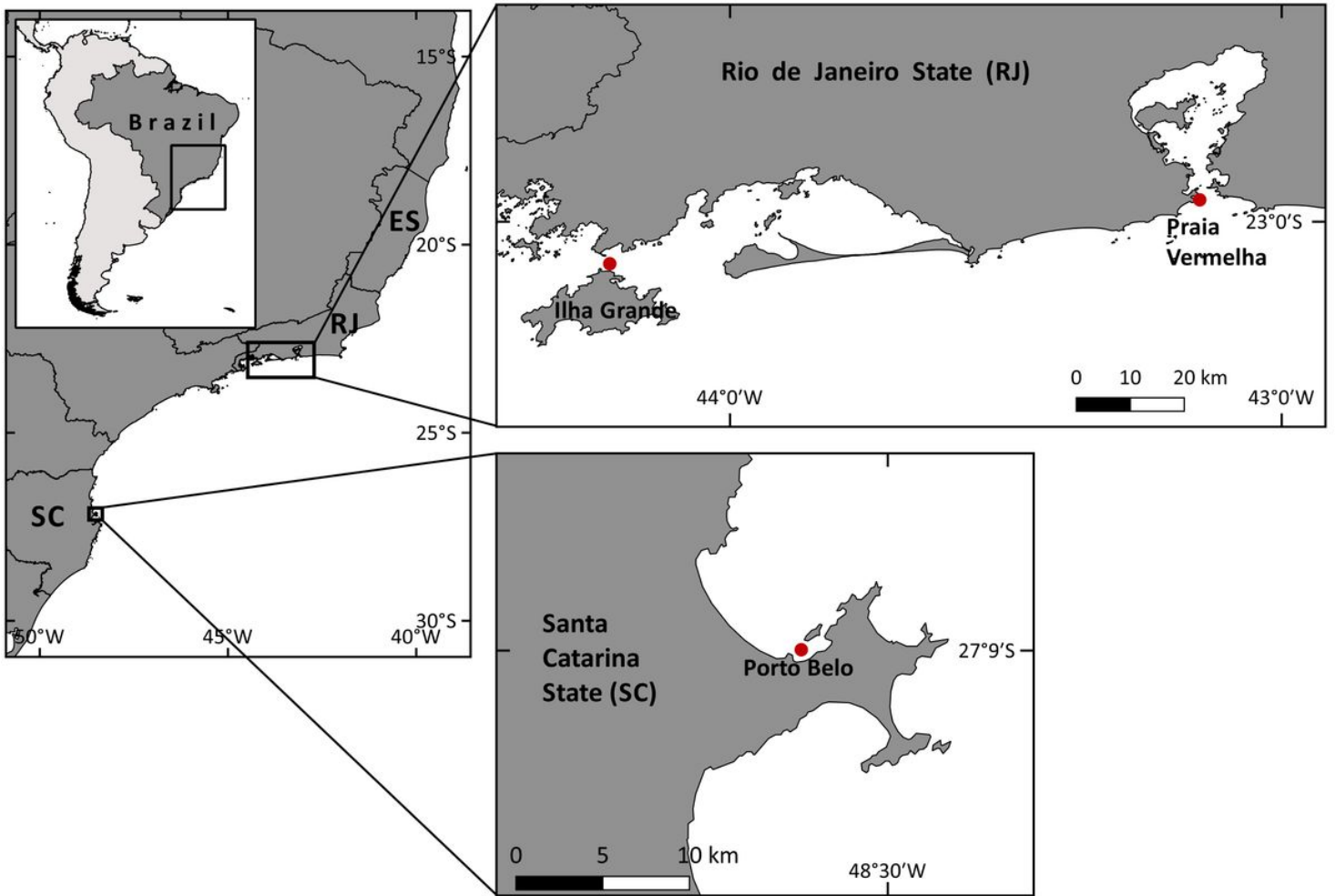


Figure 1
 Paroctopus cthulu sp. nov. distribution (Espírito Santo (ES) to Santa Catarina (SC)) and collection area map. (Rio de Janeiro/RJ and Santa Catarina/SC)

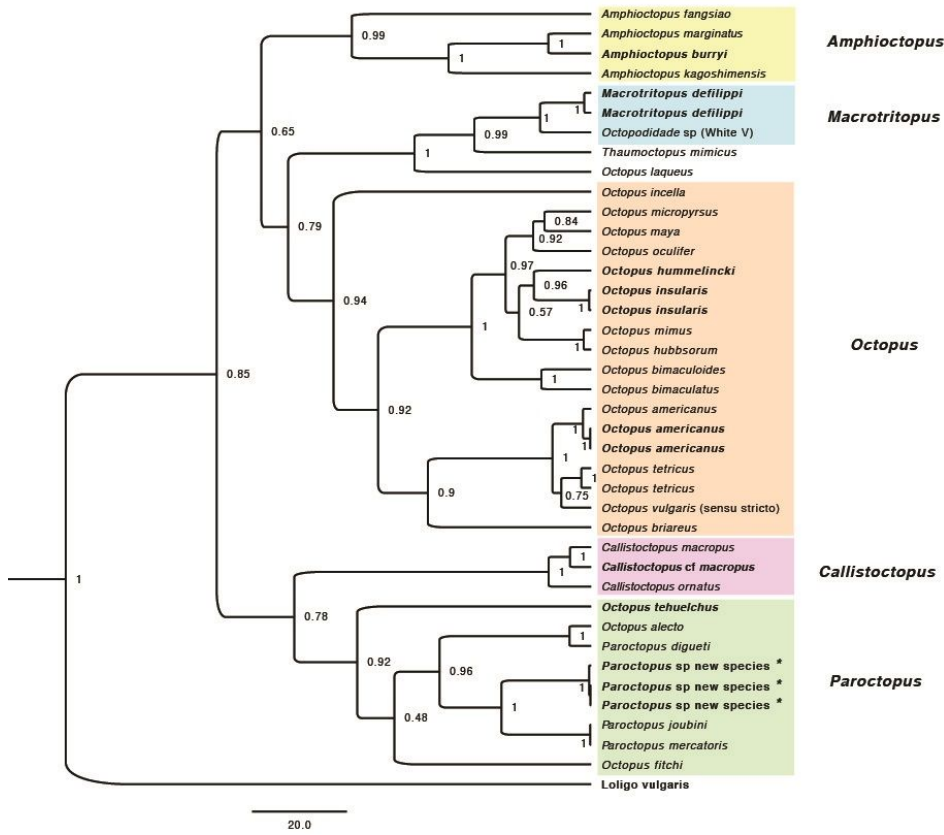


Figure 2

Bayesian phylogenetic tree showing some genera of Octopoda species. 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

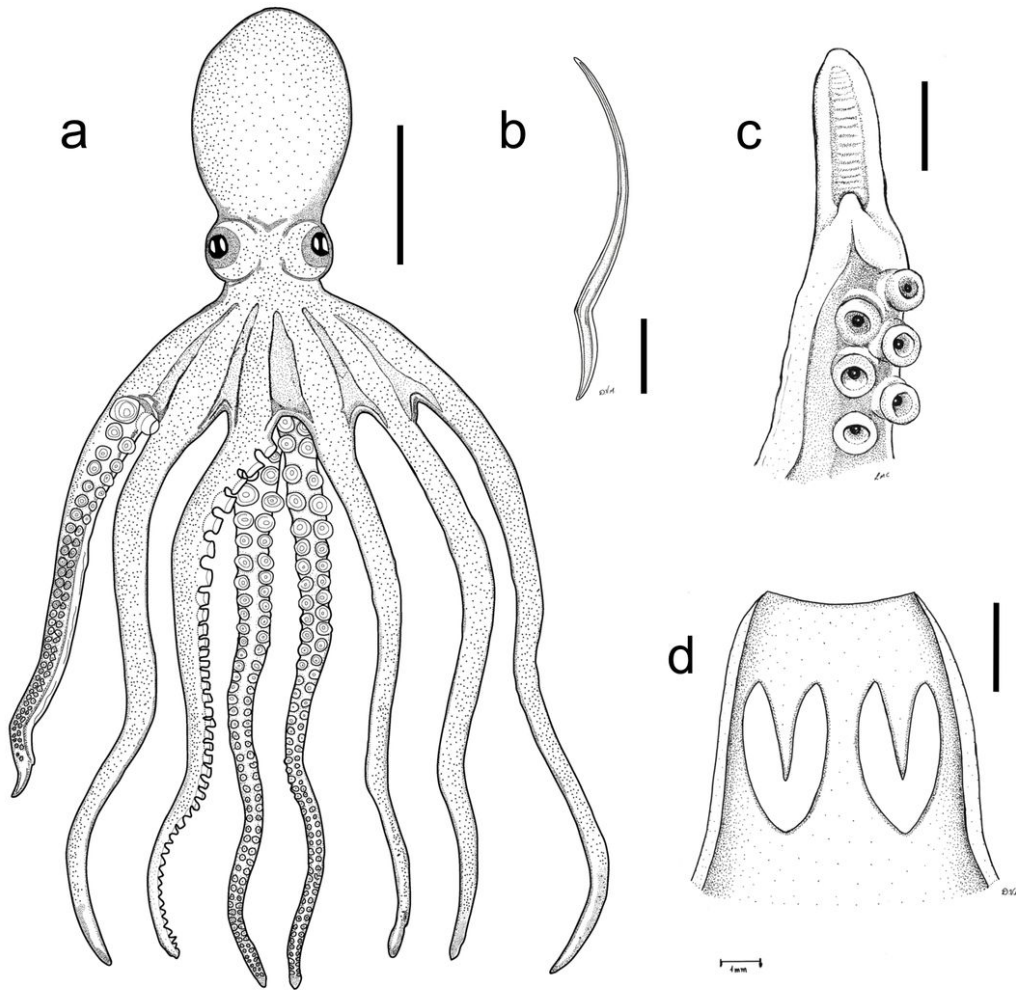


Figure 3

Paroctopus cthulu sp. nov. new species. a Dorsal view (@Leticia Cavole). b Stylet. c Hectocotylized arm with ligula and calamus. d Funnel organ. (a-c male holotype specimen (ML 18.3 mm). b-d: mature female specimen (ML 32 mm)). (Scale bars: a: 10 mm; b-d: 2 mm. ML, mantle length).

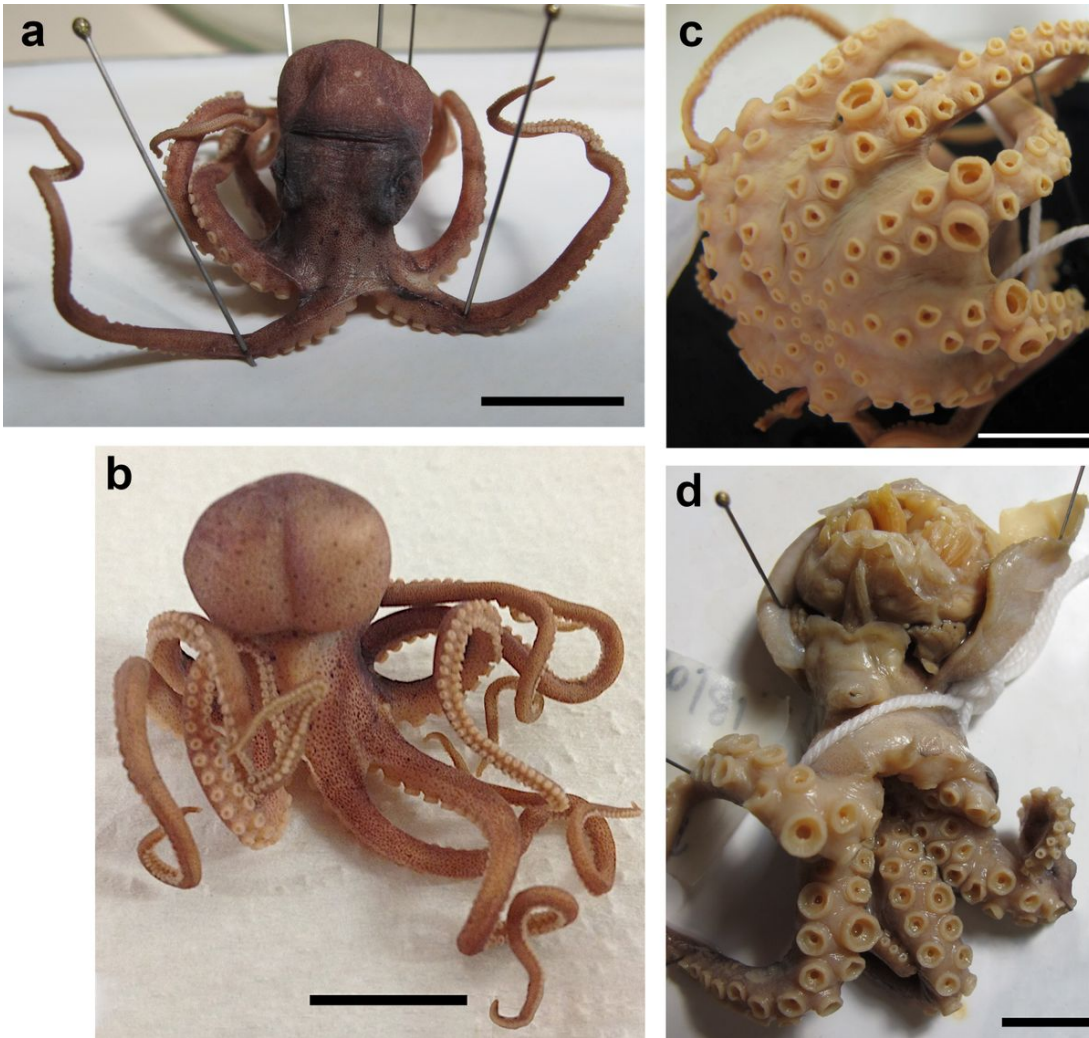


Figure 4

Fixed *Paroctopus cthulu* sp. nov. a dorsal view. b Ventral. c Male with enlarged suckers. d Female with enlarged sucker.

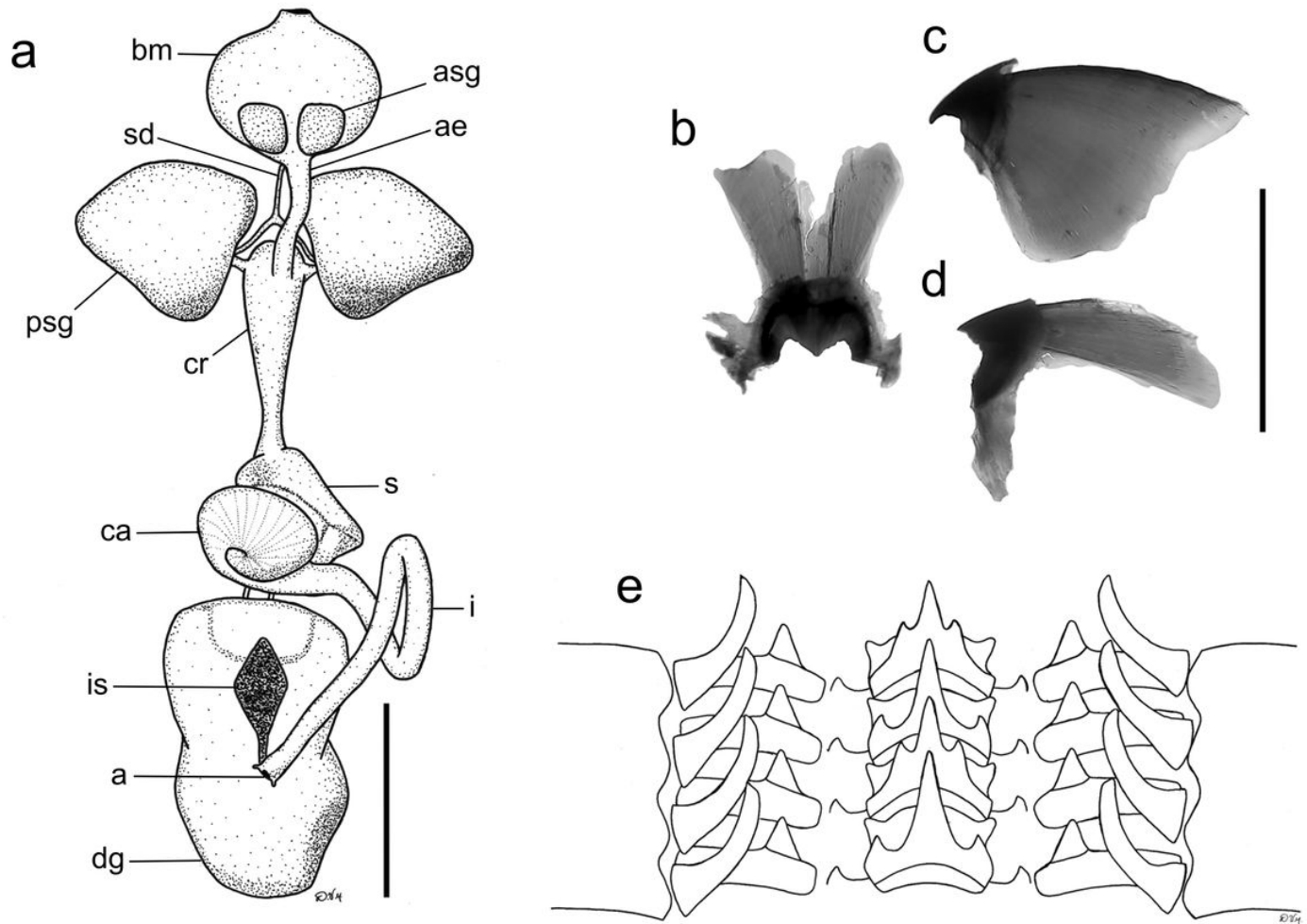


Figure 5
Paroctopus cthulu sp. nov. a digestive tract (abbreviations: a, anus; ae, anterior oesophagus; asg, anterior salivary gland; bm, buccal mass; ca, caecum; cr, crop; dg, digestive gland; i, intestine; is, ink sac; psg, posterior salivary gland; s, stomach; sd, salivary duct). b frontal view of 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).

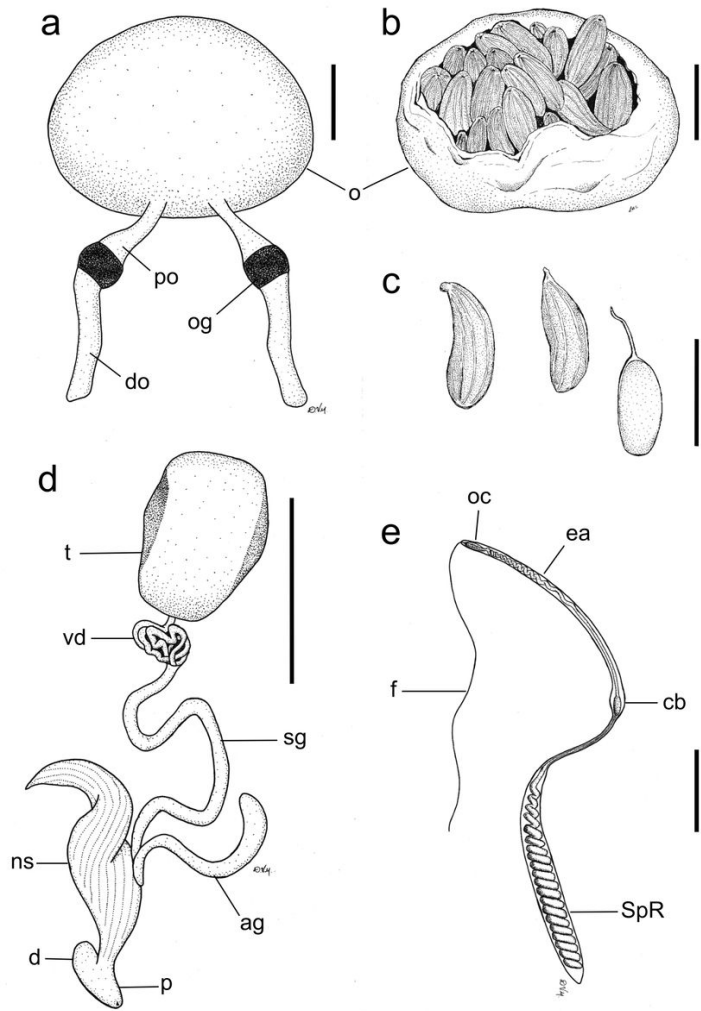


Figure 6
Paroctopus cthulu sp. nov. a Female reproductive system (abbreviations: do, distal oviduct; o, ovary; og, oviductal gland; po, proximal oviduct). b Open ovary full of mature oocytes. c Oocytes. d Male reproductive system. (abbreviations: ag, accessory gland; d, diverticulum; ns, Needham's sac; p, penis or terminal organ; sg, spermatophoric gland; t, testis; vd, vas deferens). e Spermatophore (abbreviations: 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).

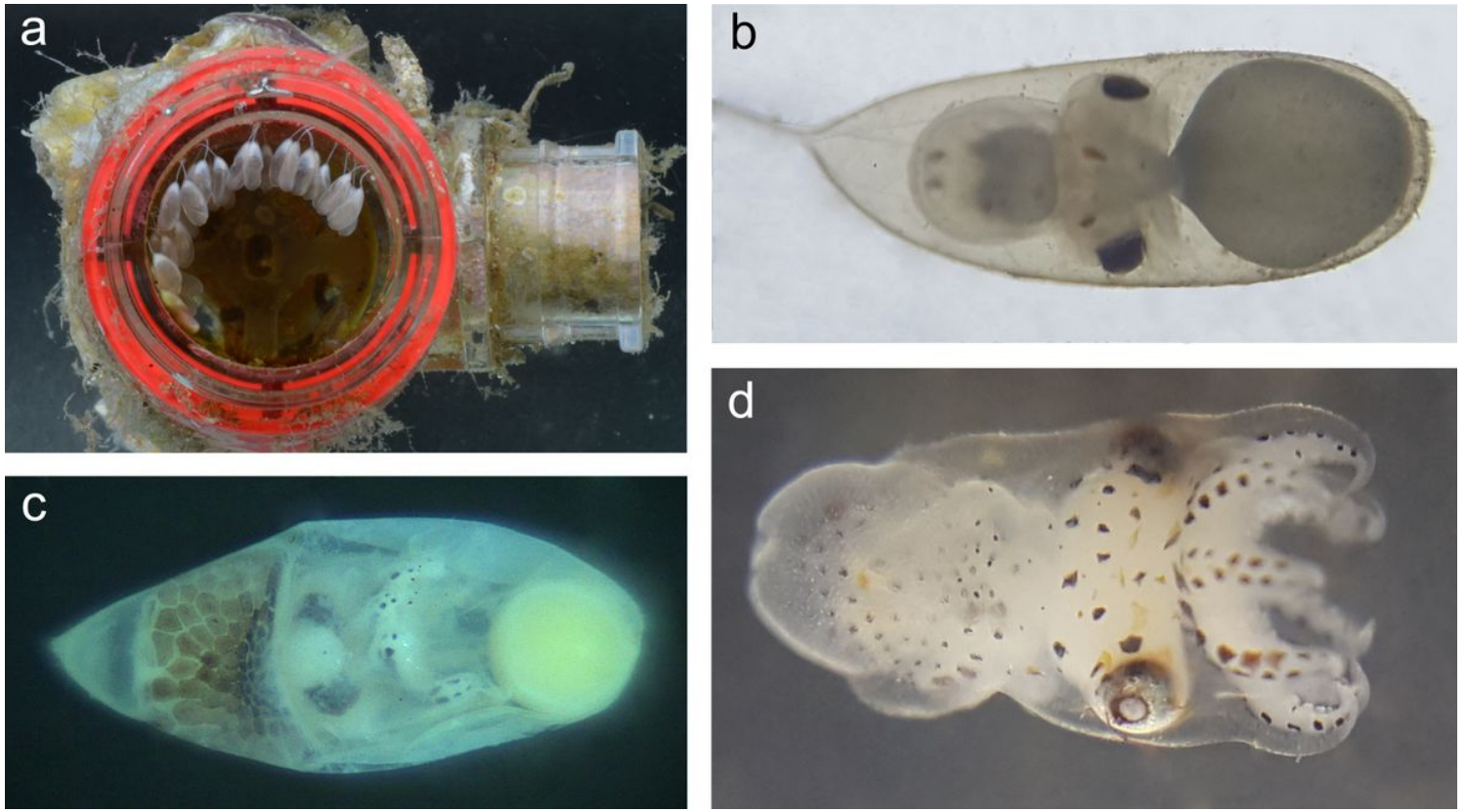


Figure 7
Paroctopus cthulu sp. nov. a Plastic snorkel mouthpiece with eggs attached singly in small cluster. b Late-stage embryo. c Late-stage embryo in ventral view with expanded chromatophores on the mantle. d New hatched paralarva.

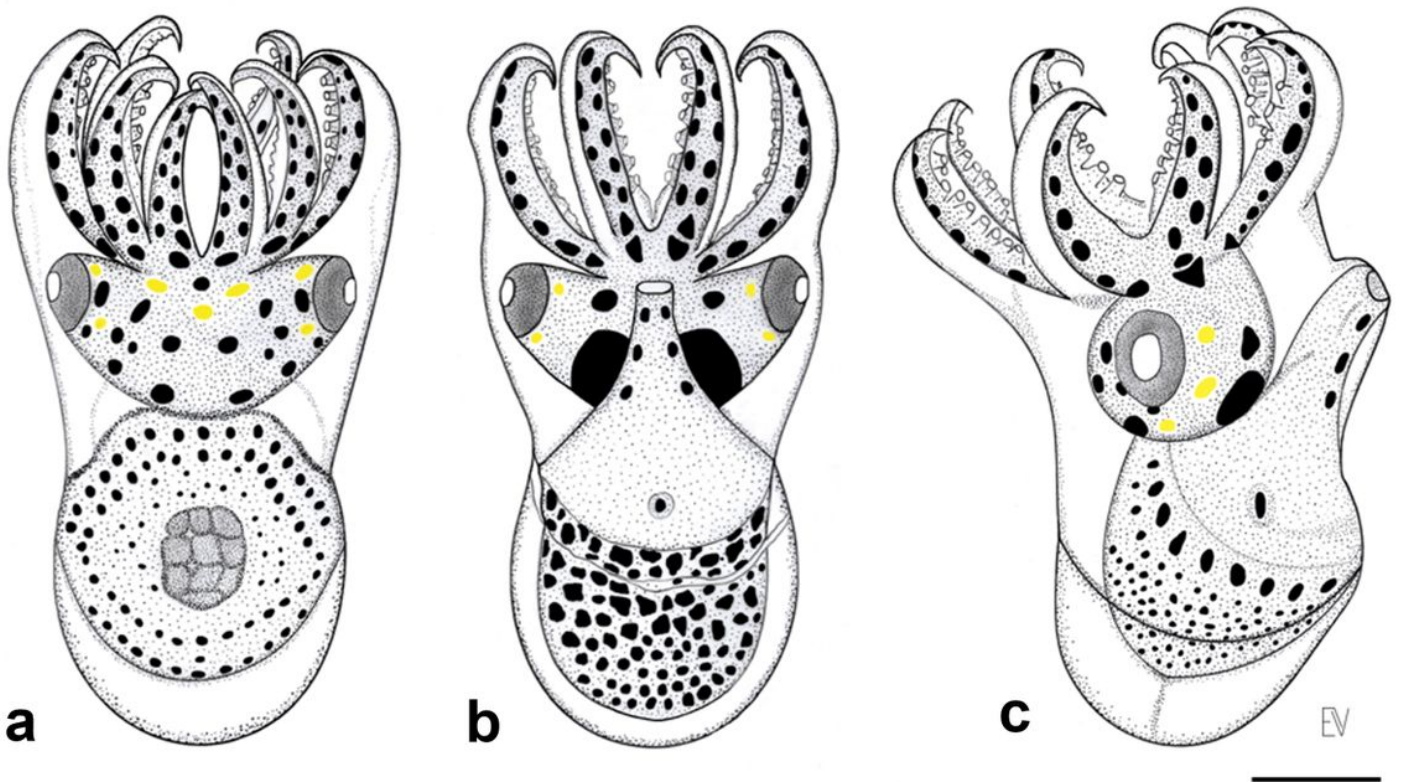


Figure 8

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

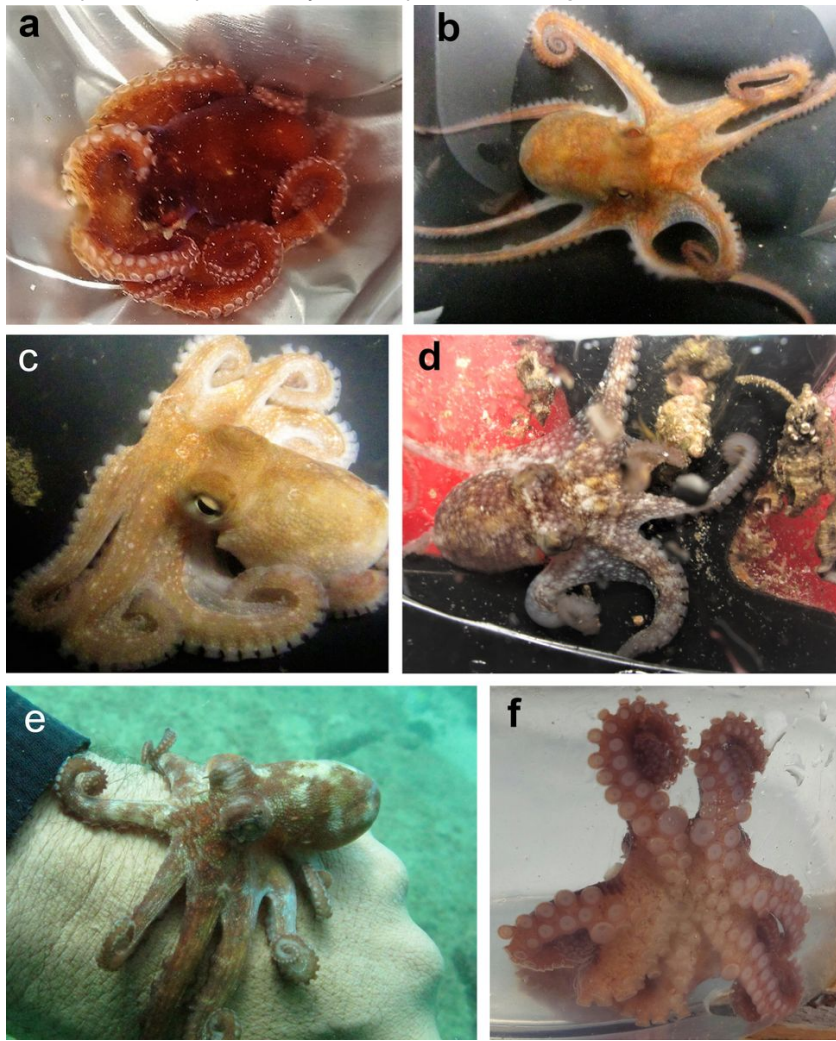


Figure 9
Paroctopus cthulu sp. nov. Body patterns and postures of juveniles and adults. a Uniform reddish with dark eyes. b Uniform dark brownish. c Uniform light brown with white dots. d Mottle with yellow blotch and white spots. e Brown with white stripes and blotches on arms and mantle. f Stereotyped postures: with first pair of dorsal arms up showing the larger suckers on the aboral surface of the arms.

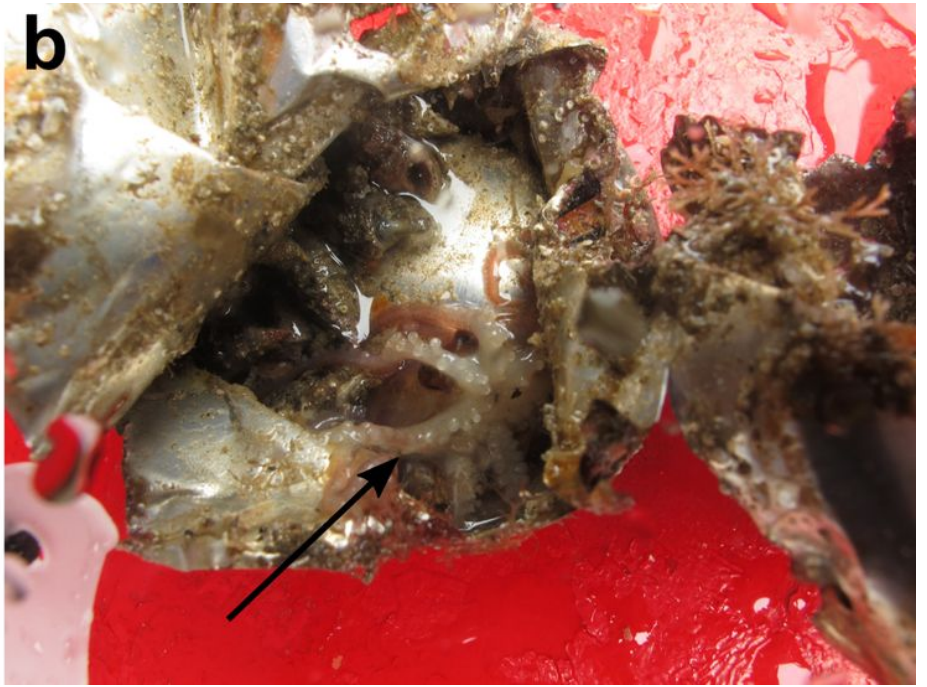


Figure 10

Type of 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 incrustation or fragmentation.