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Spawning and larval development of *Colossendeis megalonyx*, a giant Antarctic sea spider

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In the austral summer of 2021-2022, we observed spawning, post-spawning behavior, and embryonic development of the common giant Antarctic sea spider *Colossendeis megalonyx* Hoek, 1881. Sea spiders (Class Pycnogonida) are a bizarre and ancient group of marine arthropods that are distributed throughout the world's oceans (Arnaud and Bamber 1988). One characteristic of sea spiders, shared by all taxa whose reproduction was previously known, is that offspring are cared for exclusively by the male parents who brood embryos on specialized "ovigerous legs" from fertilization to hatching and often beyond (Cavanna 1877, Arnaud and Bamber 1988, Arango 2002). Exclusive male care of offspring is the rarest type of parental care, and its evolutionary origins in sea spiders and other taxa pose an intriguing puzzle for evolutionary biologists (Tallamy 2001, Goldberg et al. 2020).

While exclusive male care is often considered a feature of the sea spiders as a whole, brooding has never been observed in three of the eleven recognized extant families of pycnogonids, including the family Colossendeidae. Colossendeids include the largest and most conspicuous of sea spiders, with some species reaching leg spans of 40-50 cm (Arnaud and Bamber 1988, Child 1995, Moran and Woods 2012, Shishido et al. 2019), and the family has been collected and studied since the mid-19th century. Despite this long history of and research on colossendeids, up until very recently nothing at all was known about the reproductive ecology, embryology, or larval development of this group (Bain 2003, Arnaud and Bamber 1988, Dietz et al. 2015, Brenneis et al. 2017). To date we know of only one published observation of mating, which was based on fascinating and fortuitous visual evidence of egg production by two individuals of an unknown species of colossendeid from the deep sea (Brenneis and Wagner 2023).

Over decades of diving in McMurdo Sound, Antarctica, where colossendeids are abundant at SCUBA depths, divers had frequently observed groups of usually two, but sometimes three or four individuals stacked on top of each other (e.g. photos in Brueggeman (1998)) in what is characteristically a mating posture in other sea spiders (Bain and Govedich 2004). In the austral summer of 2021-2022, in the hopes that we could observe reproduction of colossendeids in the lab, we hand-collected several mating groups of *C. megalonyx*, transported them to McMurdo Station, kept them in a flow-through seawater system at temperatures between -1.5 and -0.5 °C, and checked them several times a day for egg production. Two of these mating groups produced eggs in the laboratory in 2021, the first on October 25 and the second on October 29. Eggs were first seen as a gelatinous cloud surrounding a single spider that had previously been part of a mating group (Figure 1a). We documented post-spawning care by one parent, which appeared to consolidate the embryos and glue the brood to the substrate (Figure 1b). We subsequently also found eggs and adults together in the field on several occasions in 2021 and 2022 (Figure 1c). A diver collected material from one of these field masses and brought them back to the lab, where we confirmed they were eggs of the same size and appearance as lab-laid eggs.

We collected the eggs from the first laboratory spawn for microscopic observation of egg morphology and embryological and larval development, and we left the second mass in place to observe post-spawning parental behavior and developmental ecology. The first mass contained thousands (though we did not count them) of light-colored, small eggs (average diameter 106.9 µm ± 3.4 (s.d.) (n = 17)) that were embedded in a loose gel. The gel was somewhat sticky but this mass never became compacted or firmly stuck to the substrate. We maintained the embryos in filtered seawater in incubators at -1.8 °C for 11 months and photographed them every two to three weeks under a compound microscope. Development was slow, as is characteristic of Antarctic ectotherms (Moran et al. 2019). Embryos reached the 2-4 cell stage by d 8 (Figure 2a) and the 8- to 16-cell stage by d 10 (Figure 2b). By d 45 a blastocoel was visible (Figure 2c). Buds of limbs were visible on d 83 (Figure 2d) and became more and more defined throughout the rest of development (Figures 2e and 2f). The first hatched larva was observed on June 28, 2022, ~ eight months post-spawn; a major hatching event occurred on July 25; and hatching continued through October 2022 when the observations were discontinued.

Hatched larvae were negatively buoyant and crawled slowly in the dish. Hatchlings had chelicerae and two other pairs of appendages with a spine extending from the scape of each one (Figure 2g, 2h). The morphology of hatched larvae of *C. megalonyx* aligned with previous descriptions of larvae of other species that were categorized as “typical protonymphons” (Bain 2003), and the small egg size and larval morphology were both consistent with the “Type I” pycnogonid larva of Brenneis et al. (Brenneis et al. 2017). This particular combination of egg size and larval morphology pattern is commonly associated with benthic and ectoparasitic development and is prevalent among various sea spider families; notably, Type I larvae are widely distributed within the Pycnogonida and are regarded as the probable ancestral mode for modern pycnogonids (Brenneis et al. 2017).

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The second egg mass remained firmly attached to the substrate through February 2022 when the seawater system was shut down. At this time, it was overgrown with diatoms and extremely cryptic, though developing embryos could be seen with a stereo microscope. In contrast, the first mass, which was removed from the sea table soon after spawning, did not become compacted or attached. Males of other pycnogonids use secretions from cement glands on their femurs to glue eggs to their ovigerous legs (Arnaud and Bamber 1988, Bain and Govedich 2004), and our observations suggest that adult colossendeids may use chemical or mechanical manipulation to glue their egg masses to the substrate. Brenneis & Wagner (2023) also suggested a role for the ovigerous legs in egg manipulation of a colossendeid. Cement glands are generally considered to be absent in the Colossendeidae (Child 1995, Arango and Wheeler 2007); however, almost 150 years ago Hoek (1881), reporting on the specimens collected by the Challenger expedition, described what he suspected were cement glands in *C. megalonyx*. Hoek's (1881) description, along with our observations, mean that the assumption that colossendeids as a family lack cement glands bears reexamination. Together, the observations that (1) development is benthic, (2) hatchlings are negatively buoyant and do not swim, and (3) hatchling morphology is consistent with an ectoparasitic lifestyle, suggest that larvae of *C. megalonyx* have limited potential for long-distance planktonic dispersal.

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FIGURE CAPTIONS

Figure 1. a. First egg mass of *C. megalonyx* observed in the laboratory, with adult standing amid the gel cloud. b. Second egg mass observed in the lab, partially consolidated onto the rock, with adult standing over it. The mass extends from the proboscis of the adult to approx. half-way across the bryozoan colony in the upper right of the figure. c. Two adults of *C. megalonyx* in the field in mating posture, with eggs underneath. em = egg mass. Scale bars = 3 cm (a), 2 cm (b). a, b, taken in the Crary Laboratory at McMurdo Station, Antarctica, on 10/25/21 and 10/29/21, respectively. c, taken by R. Robbins at the Cziko Seamount dive site, McMurdo Sound, Antarctica, on November 15, 2022 and used with permission.

Figure 2. Embryos and hatched larvae of *C. megalonyx*. a. Eggs. b. 2-4 cell embryos, 8 d after spawning. c. Blastulae, 45 d post-spawning. d. Five months after spawning, showing limb buds. e. Six months post-spawning. f. Unhatched individuals eight months after spawning. g & h, newly hatched larvae. pr = proboscis, ch = chelicera, pa = palp, o = oviger, s = spine. Scale bar in a-f = 100 µm; scale bar in g,h = 50 µm. Photos in e-g taken by J. Webber at McMurdo Station, Antarctica and used with permission.

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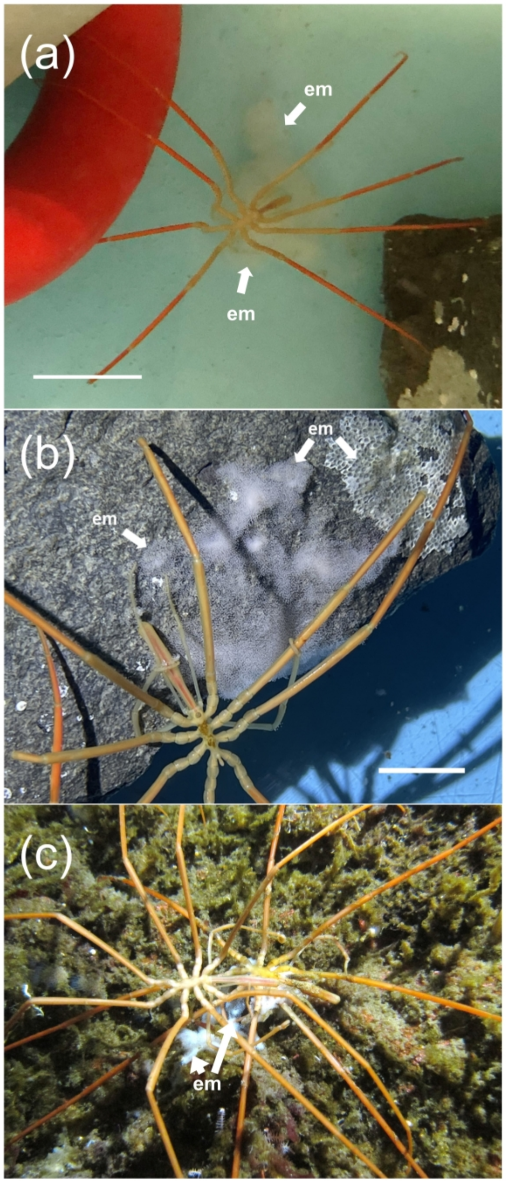
FIGURE CAPTIONS

Figure 1. a. First egg mass of *C. megalonyx* observed in the laboratory, with adult standing amid the gel cloud. b. Second egg mass observed in the lab, partially consolidated onto the rock, with adult standing over it. The mass extends from the proboscis of the adult to approx. half-way across the bryozoan colony in the upper right of the figure. c. Two adults of *C. megalonyx* in the field in mating posture, with eggs underneath. em = egg mass. Scale bars = 3 cm (a), 2 cm (b). a, b, taken in the Crary Laboratory at McMurdo Station, Antarctica, on 10/25/21 and 10/29/21, respectively. c, taken by R. Robbins at the Cziko Seamount dive site, McMurdo Sound, Antarctica, on November 15, 2022 and used with permission.

Figure 2. Embryos and hatched larvae of *C. megalonyx*. a. Eggs. b. 2-4 cell embryos, 8 d after spawning. c. Blastulae, 45 d post-spawning. d. Five months after spawning, showing limb buds. e. Six months post-spawning. f. Unhatched individuals eight months after spawning. g & h, newly hatched larvae. pr = proboscis, ch = chelicera, pa = palp, o = oviger, s = spine. Scale bar in a-f = 100 µm; scale bar in g,h = 50 µm. Photos in e-g taken by J. Webber at McMurdo Station, Antarctica and used with permission.



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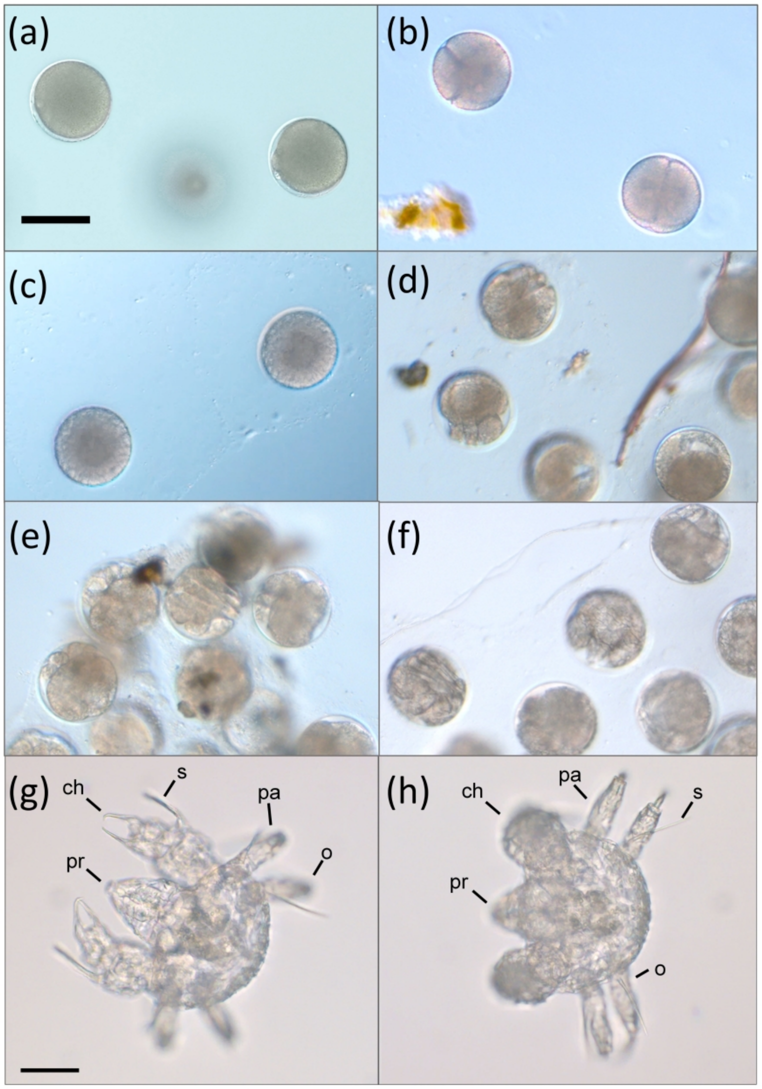

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