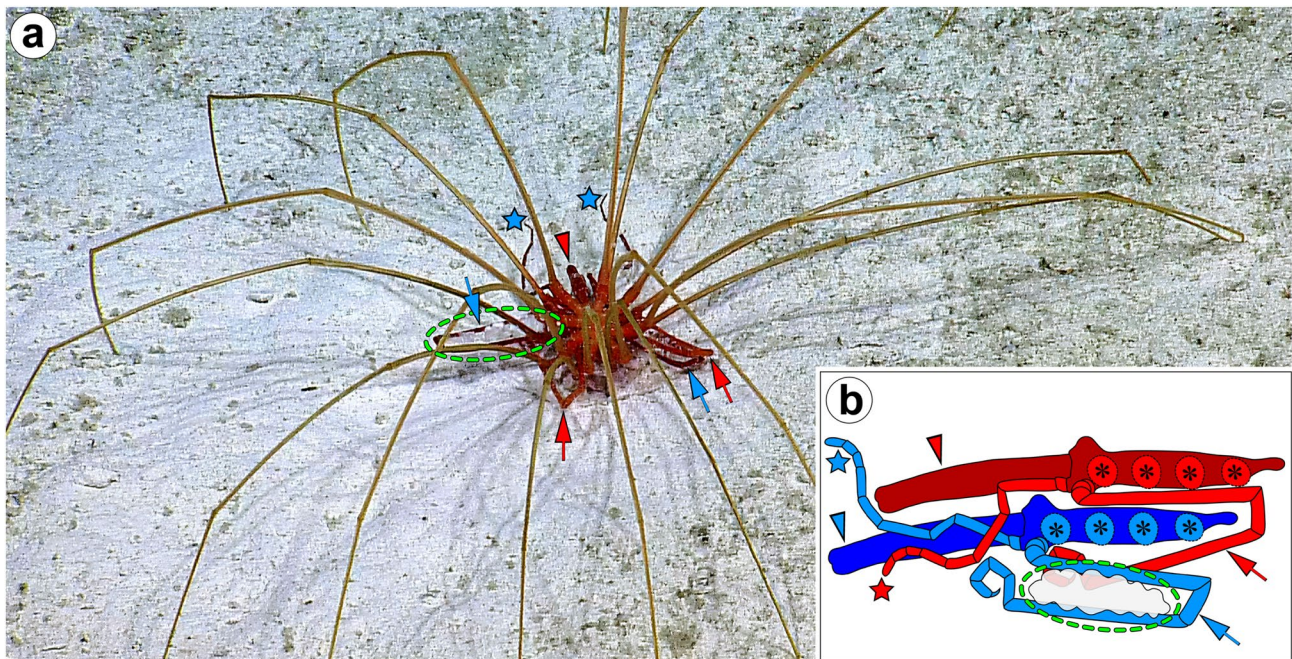




## Mating observation of giant sea spiders (Pycnogonida: Colossendeidae)

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**Fig. 1** Mating of *Colossendeis* sp. The proboscis (arrowheads), palps (stars), and ovigers (arrows) are marked (red: male, blue: female). Between the female's left oviger, the egg mass is highlighted (stippled green oval); **a** Mating pair (postero-dorsal view), recorded at a

depth of 1684 m approximately 70 km north of Kingman Reef. Photograph courtesy of Ocean Exploration Trust; **b** Schematic illustration of the mating position (lateral view). For clarity, only the leg insertion sites (asterisks) are indicated along the trunk

Sea spiders (Pycnogonida) are a common faunal component of marine benthic communities around the world. They typically possess a slender body with an anterior proboscis, four pairs of long legs, and a posterior anal tubercle (Arnaud and Bamber 1987). To this day, their reproductive

biology remains poorly studied, and in several taxa completely unknown, as is the case for Colossendeidae (Bain and Govedich 2004; Brenneis et al. 2017). This family includes the largest pycnogonids, with some species reaching up to 75-cm leg span (Bamber 2007). Pycnogonids exhibit the rare phenomenon of paternal brood care. During mating, the eggs are transferred to the male, which carries them until hatching with its ovigers, a specialized appendage pair located anterior to leg pair 1. However, females of many taxa also possess ovigers, which they may use to transiently collect freshly laid eggs during mating (Bain and Govedich 2004; Burris 2011).

During an expedition exploring deep-sea habitats around Palmyra Atoll aboard E/V *Nautilus* (NA137), we for the

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first time documented two mating colossendeids (Fig. 1a; Movie S1). The behavior was captured on high-definition video from the two-body remotely operated vehicle system (*Hercules* and *Argus*) at a depth of 1684 m on an unnamed seamount located ~70 km north of Kingman Reef (Dive H1911, 7.06750°N, 162.53226°W, 27 March 2022), within the Pacific Remote Islands Marine National Monument. Based on leg span (~35 cm) and morphology (long proboscis, multi-segmented palps, structure of prominent ovigers) (Fig. 1a, b), the animals can be assigned to the genus *Colossendeis* Jarzysky, 1870. They are aligned head to head, with one of them resting its ventral side on the dorsal surface of the other (Fig. 1a, b). This corresponds to early mating phases in other families, where the male is invariably on top of the female (Bain and Govedich 2004; Burris 2011). Accordingly, the colossendeid on top is with great likelihood the male. The male's ovigers are folded underneath the female and the two animals' interlocked bodies move occasionally up and down. The female's ovigers are pressed close to the ground and their articles 4–6 form a rectangular area with one open side. Enclosed in this area is a whitish-opaque mass that moves along with the ovigers which shift passively with the animals' bodies (Fig. 1a, b; Movie S1). Although the light sandy sediment in the background obscures some features, this mass can be confidently inferred to represent eggs, as its location corresponds to other families in which the female transiently collects eggs after laying.

Despite high sampling efforts, no egg-bearing colossendeid has been ever recorded (Child 1995; Sabroux et al. 2023). This has puzzled scientists for decades, leading some authors to suspect a deviating reproductive biology in this family (Arnaud and Bamber 1987) and an exclusive grooming function of its ovigers (Bamber 2007). Contrary to this, our observation indicates that colossendeid ovigers are at least initially involved in the handling of freshly laid eggs. What happens afterwards, however, is still unclear, as later mating stages were not recorded. It remains possible that the eggs are soon after transferred to the male, or deposited either in the sediment or on an unknown invertebrate host. Notwithstanding, more than 150 years after Colossendeidae has been described, our *in situ* observation provides the first glimpse into the reproductive biology of this iconic pycnogonid group and highlights the importance of deep-diving technologies in surveying the least explored parts of our oceans.

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## Declarations

**Conflict of interest** The authors declare no competing interests.

**Ethical approval** No animal testing was performed during this study.

**Sampling and field studies** All necessary permits for the observational field study have been obtained from the competent authorities.

**Data availability** All data analyzed in this study are included in this published article and its supplementary material.

**Author contribution** DW acquired the data. GB analyzed the data. GB and DW wrote and approved the manuscript.

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