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Research Paper

Priapulid worms from the Cambrian of China shed light on reproduction in early animals



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ABSTRACT

In the recent years, exceptional fossil sites have revealed astonishing details on the anatomy, lifestyles and behaviour of Cambrian animals but surprisingly, very little is known about one of their most vital features, reproduction. We describe here *in situ* eggs (clusters of 3 to 30 oocytes) in the tube-dwelling priapulid worm *Paraselkirkia sinica* from the Cambrian Stage 3 Xiaoshiba Lagerstätte (ca. 514 Ma, South China). These oocytes were accommodated within paired tubular ovaries located in the posterior half of the primary body cavity as in modern meiobenthic priapulid worms, thus indicating that the general organization of female tubular gonads in priapulid worms has remained virtually unchanged for half a billion years. Our findings provide for the first time, key information on the reproductive organs and strategies of early ecdysozoans, a huge animal clade that dominated Cambrian marine ecosystems and accounts for a large part of today's biodiversity (e.g. arthropods). Moreover, we also emphasize the critical role of ecology on the reproductive strategies and lifestyles of both modern and Cambrian worms.

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1. Introduction

Despite significant advances on vital aspects of the biology of early animals, many issues concerning their reproduction and developmental modes are still unresolved. Exceptionally preserved fossils from Lagerstätten such as that of the Burgess Shale and Chengjiang reveal exquisite details about the functional anatomy, development, lifestyles and behavior of early animals but amazingly do not shed light on their reproductive organs. Fossilized eggs from the Paleozoic Era are rare and limited to a few cases of isolated strands (Middle Pennsylvanian from Illinois) (Godfrey, 1992) or clusters laid by undetermined animals (Middle Cambrian Kaili Lagerstätte from China; e.g. Lin et al., 2006) and to clutches carried or brooded by Cambrian arthropods (Duan et al., 2014; Caron and Vannier, 2016; Ou et al., 2020). Secondarily phosphatized embryos found in Cambrian Orsten-type deposits from China and Siberia (Zhang and Pratt, 1994; Bengtson and Yue, 1997; Conway Morris, 1998; Dong et al., 2004; Donoghue et al., 2006) provide detailed information about the embryonic development of early animals but tell us nothing about the younger steps of their reproduction cycle (gonads, eggs, fertilization).

Ecdysozoans represent a huge proportion of the present-day biodiversity and share a unique feature, that of growing via successive moulting stages. They include arthropods (insects, chelicerates, crustaceans, and myriapods) and diverse worm groups such as nematoids and scalidophorans (including priapulids). As shown by their rich fossil record, ecdysozoan worms were abundant and diverse throughout the Cambrian and are likely to have played a significant role in early benthic ecosystems, as bioturbators (Vannier et al., 2010; Kesidis et al., 2019), predators and recyclers (Vannier, 2012). Scalidophorans in particular are well represented in several Cambrian Lagerstätten such as the Burgess Shale (Briggs et al., 1994), Chengjiang (Hou et al., 2017) and Qingjiang (Fu et al., 2019).

Recent studies of *in situ* brooded eggs found in bivalved arthropods from the Chengjiang and Burgess Shale Lagerstätten (ca. 518 Ma and 508 Ma, respectively) showed that extended investment in offspring survivorship and possible evolutionary trade-offs related to brood care (Caron and Vannier, 2016; Ou et al.,

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2020), developed soon after the Cambrian emergence of animals. Exceptionally well-preserved embryos from the early Cambrian to the Early Ordovician have provided key information on the embryonic development of ecdysozoan worms. More precisely, synchrotron-radiation X-Ray-tomographic microscopy (SRXTM) has revealed three-dimensional aspects of the development of *Markuelia* (Donoghue et al., 2006), a worm comparable to living scalidophorans, but the reproductive organs and oogenesis of this animal still remain unknown.

Selkirkiid worms share important morphological features with extant (e.g. priapulids; Schmidt-Rhaesa, 2013) and fossil scalidophoran worms, such as an eversible introvert lined with scalid rows and a circum-oral pharyngeal structure bearing teeth. Its most distinctive, possibly derived character, is the presence of a conical tube, which with numerous, evenly spaced annuli, is open at both ends, encases the whole trunk, and presumably represents a cuticular structure secreted and renewed by underlying epidermal tissues (see Wang et al. 2019 for ecdysis in early Cambrian scalidophoran worms). Selkirkiids are represented by three Cambrian genera, namely *Selkirkia* from North America (Conway Morris, 1977), *Paraselkirkia* from South China (Hou et al., 2017) and *Sullulika* from North Greenland (Peel and Willman, 2018). However, *Paraselkirkia* is likely a junior synonym of *Selkirkia* (Maas et al., 2007). Selkirkiids are often regarded as having close affinities with Priapulida and may belong to its stem group (Budd and Jensen, 2000; Smith et al., 2015).

We describe *in situ*-preserved oocytes in *Paraselkirkia sinica* (Luo et al., 1999), a stem-group priapulid from the Cambrian Stage 3 Xiaoshiba Lagerstätte (ca. 514 Ma). This finding fills an important gap of knowledge concerning the reproduction of early ecdysozoan worms. Detailed comparison with the reproductive system of modern meiobenthic priapulids leads to considering possible interactions between ecology and reproduction in the early evolution of scalidophorans.

2. Materials and methods

All studied fossils are from the early Cambrian Stage 3 (local Canglangpuan) Hongjingshao Formation at the Xiaoshiba section (ca. 3.7 km SE of Ala village, Kunming, eastern Yunnan) (Yang et al., 2018; Hou et al., 2019). Fossil specimens were prepared manually with fine needles under a Nikon SMZ800 or 1500 stereomicroscope, and then photographed using a Leica DFC 500 digital camera mounted to a Leica M205-C stereomicroscope under bright-field illumination, and a Leica DFC7000 T monochrome digital camera attached to a Leica M205 FA fluorescence stereomicroscope. Back Scattered Electron (BSE) image capture and EDX analysis were performed by using a FEI Quanta 650 scanning electron microscope (SEM) under low vacuum and high accelerating voltage (30 kV). *Priapulus caudatus* was collected from the Gullmarsfjord near the Kristineberg Marine Station, SE Sweden (low-oxygenated muds; ca. 40 m deep), and along the White Sea of Russia near the Nikolai Pertsov White Sea Biological Station (intertidal environments), then fixed with glutaraldehyde or formaldehyde, dried in a Leica critical point dryer and observed under a Zeiss Merlin Compact SEM after being coated with Au-Pd. Meiobenthic specimens (*Maccabeus tentaculatus*) collected from Cyprus (near Limassol and Paphos) and preserved in 70% ethanol were obtained from the zoological collections of the Hebrew University of Jerusalem, imaged under a Leica MZ125 binocular microscope and processed for SEM. Digital photographs were processed with Adobe Photoshop CS6 and CorelDRAW X8, diagrams with Adobe Illustrator CS6. Selected specimens were scanned using a micro-CT (Zeiss Xradia 520) to show potential internal structures, and the data were processed with Drishti (Version 2.6.4) software, resulting in a suite of 3D-projection images representing virtual sections through examined specimens. All the fossil specimens in this paper are deposited in the Key Laboratory for Palaeobiology, Yunnan University (YKLP). Correspondence and requests for materials should be addressed to X.G.Z. (xgzhang@ynu.edu.cn).

3. Results

Paraselkirkia sinica is a typical element of the early Cambrian Chengjiang and Xiaoshiba biotas from China, occurring in many localities and frequently found in dense aggregations (Lan et al., 2015).

Our study is based on about 200 specimens of *P. sinica*, all collected from the early Cambrian Stage 3 Xiaoshiba Lagerstätte (Kunming, eastern Yunnan Province, China). Like most fossils from this locality (Yang et al., 2013, 2018) and those from the Chengjiang and Qingjiang Lagerstätten (Hou et al., 2017; Fu et al., 2019), *P. sinica* occurs as two-dimensional compressions, except its pellet-like gut contents (Fig. 1a, 2c, j; Supplementary Data, Fig. S1g) which retain some relief (see Micro-CT analysis; Supplementary Data, Movie M1). Both internal (e.g. gut tract) and cuticular (e.g. introvert, pharynx, tube) features are underlined by brownish, reddish or yellowish iron oxides and locally by remains of organic matter. These oxides resulted from the weathering of both primary and secondary pyrite deposited on organic tissues through the action of sulfate-reducing bacteria under anaerobic conditions. This taphonomic scenario is common to other Lagerstätten such as that of Chengjiang (Cambrian Stage 3; Gabbott et al., 2004; Zhu et al., 2005), and Fezouata (Ordovician, Tremadocian) from Morocco (e.g. Kouraiss et al., 2018).

Among the ca. 200 specimens studied here, many are carcasses showing remains of soft parts (e.g. introvert, digestive tract); the rest are empty tubes. In particular, eleven bore clusters of up to 30 ovoid elements that invariably occur below the transverse midline of the trunk, have a relatively sharp rounded outline and often appear as conspicuous dark spots (Figs. 1, Fig. 2g, h; Supplementary Data, Fig. S1). Their diameter ranges between 300 μm and 450 μm. Some of them clearly lie on top of the intestine and are overprinted by the annulated pattern of the tube (Fig. 1g), indicating that they were located within the interspace between the digestive tract and the inner body wall (Fig. 1a, b, f, g; Supplementary Data, Fig. S1a, b) (primary body cavity as in extant priapulids; see Figs. 3 and 4, and Schmidt-Rhaesa 2007). They do not seem to be scattered randomly within the primary body cavity but instead, form relatively coherent clusters (Fig. 1c–g). In a few specimens, they seem to spread as elongated, possibly paired clusters on either side of the gut (Fig. 1h, i; Supplementary Data, Fig. S1f, g). In others, clusters occur on a single side (Supplementary Data, Fig. S1a, b, e, f, i, j). Their consistent location, shape and size all suggest that these rounded objects are eggs *sensu lato* (i.e. fertilized or not) carried by female individuals. As revealed by elemental mapping (EDS), these eggs mainly contain C, Al and Si, with small amounts of Fe and P (Supplementary Data, Fig. S2). Micro-CT analysis revealed exquisite details of scalids and digestive tract (Fig. 2j; Supplementary Data, Movie M1) but failed to provide insight into the detailed morphology of eggs (Fig. 2g–i). Fluorescence imaging of one egg cluster (Fig. 1e) allowed to distinguish a possible external envelope (weak fluorescence) with sharp external margins from a brighter inner core which appears to be detached from the peripheral structure.

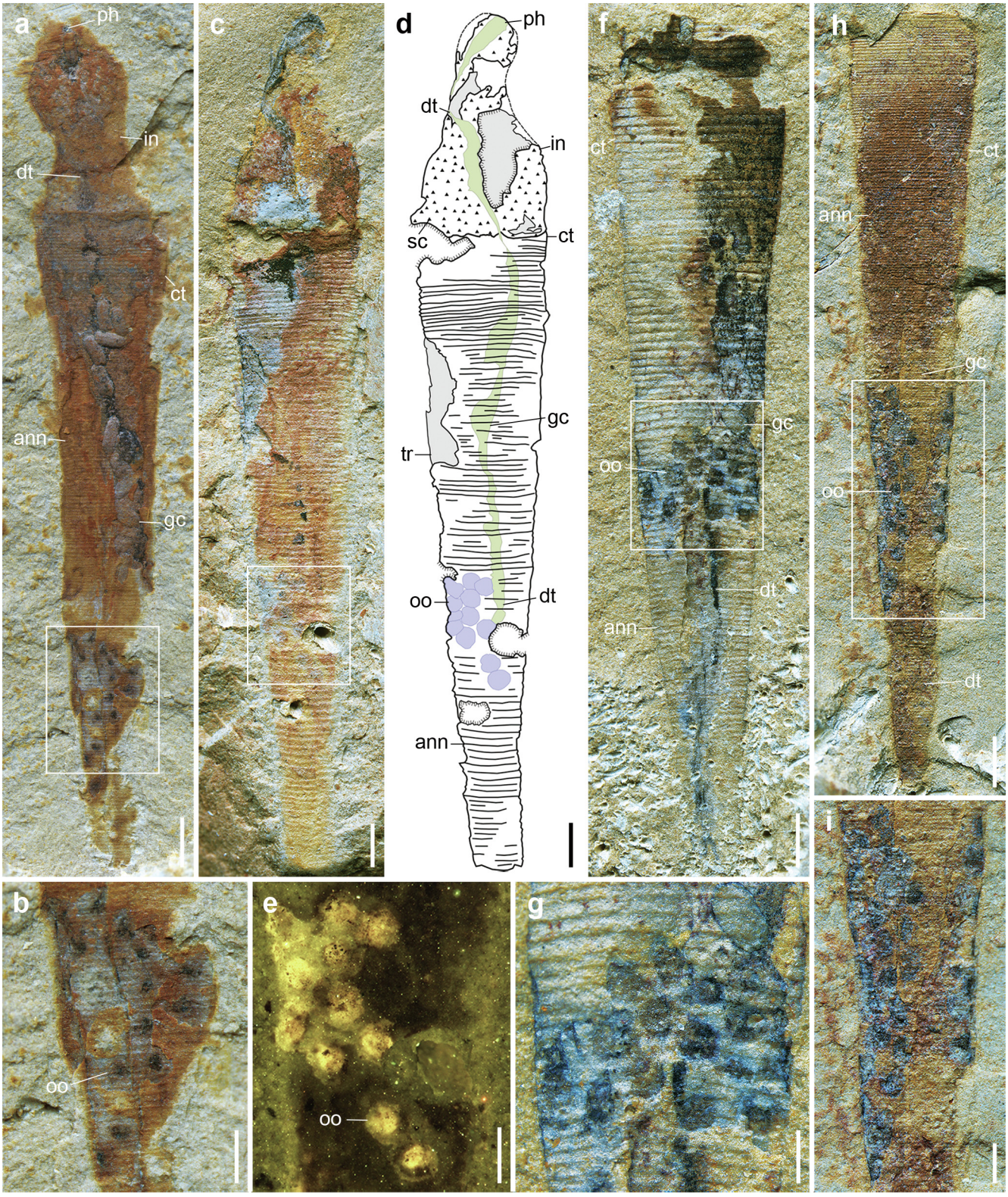


Fig. 1. Oocyte-bearing *Paraselkirkia sinica* from the Cambrian Stage 3 Xiaoshiba Lagerstätte. (a, b) YKLP 12089: (a) incomplete specimen showing partly preserved introvert, digestive tract and oocytes; (b) close-up (see location in a) showing oocytes within possible tubular ovaries. (c–e) YKLP 12350: (c) nearly complete specimen showing partly preserved introvert; (d) interpretative drawing; (e) fluorescence image (close-up, see location in c) showing oocytes. (f, g) YKLP 12351: (f) incomplete specimen (introvert missing) with oocytes within the tube; (g) close-up (see location in f) showing the egg cluster. (h, i) YKLP 12352: (h) complete tube showing oocytes; (i) close-up (see location in h) showing up to 30 eggs seemingly organized in longitudinal rows. Abbreviations: ann, annulation; ct, cuticular conical tube; dt, digestive tract; gc, gut contents; in, introvert; oo, oocytes; ph, pharynx; sc, scalid; tr, trunk. Scale bar: 1 mm (a, c, d, f, h) and 500 μm (b, e, g, i).

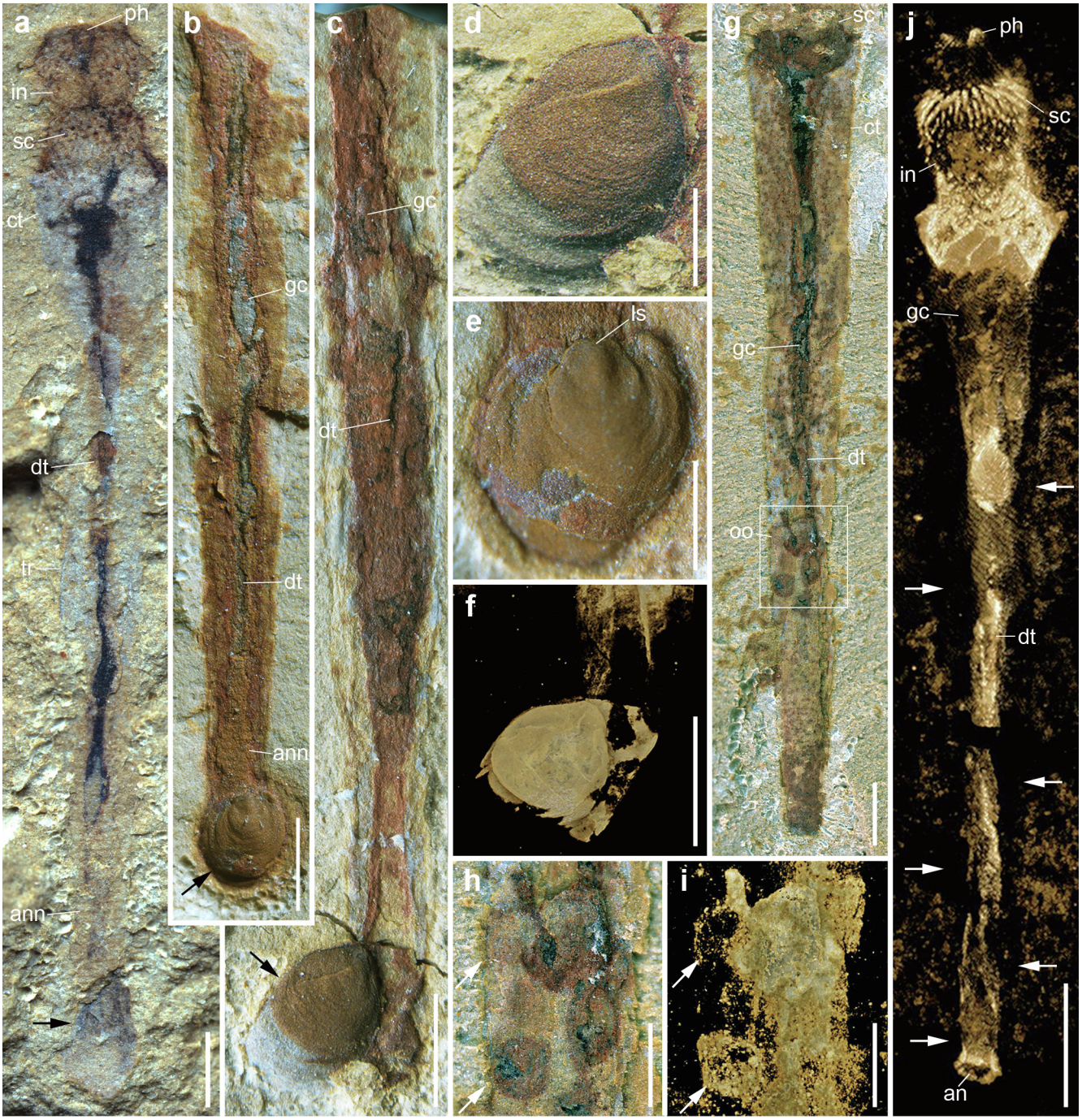


Fig. 2. *Paraselkirkia sinica* from the Cambrian Stage 3 Xiaoshiba Lagerstätte: general morphology and brachiopod epibiont. (a) YKLP 12356, complete specimen showing body features and a poorly preserved lingulate brachiopod (arrowed) attached to the tube. (b, e) YKLP 12354: (b) a kutorginate brachiopod (arrowed) attached close to the posterior opening of the tube; (e) close-up of the brachiopod. (c, d, f) YKLP 12353: (c) similar attachment by a lingulate brachiopod (arrowed); (d) close-up of the brachiopod; (f) micro-CT image showing the attached brachiopod. (g–i) YKLP 12355, specimen with complete tube and egg cluster: (g) general view; (h) close-up (see location in g) with the left three oocytes (arrowed); (i) micro-CT image showing poorly preserved oocytes (arrowed). (j) YKLP 12085, micro-CT image showing a complete, well-pyritized specimen showing detailed structures (e.g., introvert scalids, digestive tract, and the boundary between the tube wall and matrix (arrowed). Micro-CT scanning resolutions for (e), (h) and (i) are 3.56 μm, 3.96 μm and 4.92 μm, respectively. Abbreviations: an, anus; ls, larval shell, others as in Fig. 1. Scale bars: 1 mm (a–c, g), 500 μm (f, h, i), and 2 mm (d, e, j).

Twenty-two of our specimens show a remarkable association: in each case a single juvenile brachiopod of lingulate or kutorginate (Holmer et al., 2018) is attached to one side of the posterior end of the tube of *Paraselkirkia* (Fig. 2a–f). Although brachiopod epibionts occur in other Cambrian localities (Zhang et al., 2010; Topper et al., 2015) they were not seen in any other species from the Xiaoshiba Lagerstätte. The unusual co-occurrence of brachiopods and selkirkiids with preserved internal organs suggest that both animals possibly formed a symbiotic association.

4. Discussion and conclusions

4.1. Eggs in Paraselkirkia

The clusters of rounded objects observed in *Paraselkirkia* are interpreted as eggs carried by presumably female individuals, based on their consistent location, shape and size, and comparisons with extant priapulid eggs. No visible features suggest that these rounded bodies were attached to soft tissues as internal parasitic

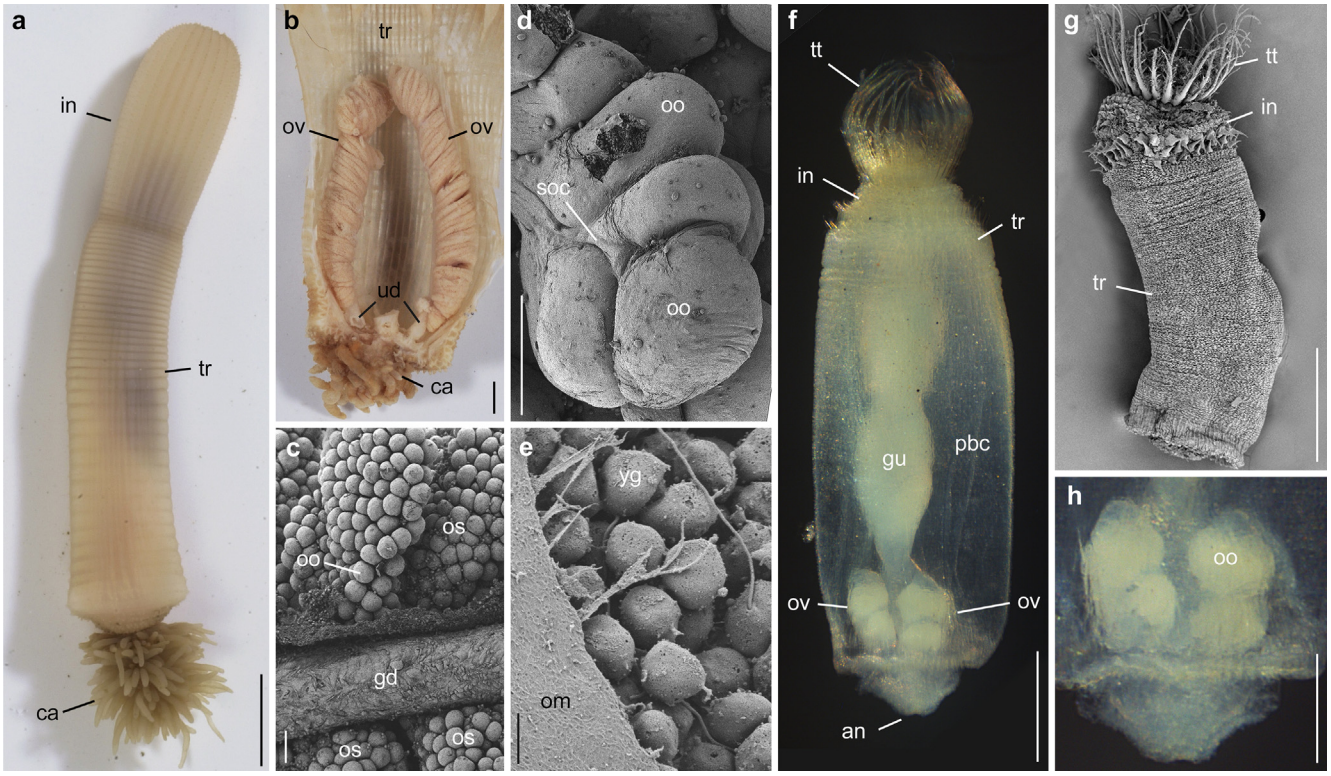


Fig. 3. Female reproductive organs and oocytes in extant macrobenthic and meiobenthic priapulid worms. (a–e) *Priapulus caudatus*. (a) Live specimen (in sea water). (b) Dissected specimen showing paired ovaries in the posterior part of the trunk. (c, d) Ovarian sacs bearing numerous oocytes and supporting structure. (e) Yolk spherules inside the oocyte (oocyte membrane removed). (f–h) *Maccabeus tentaculatus*. (f, h) Specimen bearing paired oocyte clusters (seen in transmitted light, under alcohol). (g) General view showing introvert crowned with tentacles (specialized scalids). (c–e, g) are SEM images. Abbreviations: bc, primary body cavity; ca, caudal appendage; gd, gonoduct; om, oocyte membrane; os, ovarian sac; ov, ovary; soc, supporting ovarial cells; tt, tentacle; ud, urogenital duct; ys, yolk spherule; others as in Figs. 1 and 2. (a, b) from the Gullmarsfjord, Sweden; (c–e) from the White Sea, Russia) and (f–h) from Cyprus. Scale bars: 1 cm (a), 2 mm (b); 100 μm (c, d, h) and 2 μm (e).

bodies normally do. Marked differences with the elongated pellet-like elements found within the gut (Fig. 1a; Supplementary Data, Fig. S1g) also invalidate the hypothesis that these ovoid structures might have been extruded gut contents. Comparable elemental composition was found in the brooded eggs of Cambrian bivalved arthropods (e.g. *Waptia* from the Burgess Shale and *Chuandianella* from the Chengjiang Lagerstätte (Caron and Vannier, 2016; Ou et al., 2020). C is likely to represent underlying thin carbon patches or particles of organic origin. Failure of Micro-CT analysis to provide detailed information on eggs might be due to their chemical composition such as a low concentration in iron oxides compared with other anatomical features. Fluorescence imaging of one egg cluster (Fig. 1e) allowed to distinguish a possible external envelope (weak fluorescence) from a brighter inner core that may represent the remains of other biological material (e.g. yolk or nucleus).

4.2. Reproduction in priapulid worms

Extant priapulid worms provide key information essential to the understanding of the reproductive system of *Paraselkirkia*. The gonads of extant priapulids (females and males bear ovaries and testes, respectively (Schmidt-Rhaesa, 2013) are paired structures located in the posterior part of the trunk as seen in *Priapulus caudatus* (dissected specimens, Fig. 3a, b) and *Maccabeus tentaculatus* (Fig. 3f–h), *Meiopriapulus fijiensis* with a single ovary being an exception (Storch et al., 1989). Priapulids are dioecious animals and reported cases of hermaphrodism are uncertain (Wennberg, 2008). Gonads can reach a relatively large size especially in macrobenthic species such as *Priapulus caudatus* (Fig. 3a, b) and often occupy a major part of the primary body cavity in mature females. Observations and experiments (Wennberg, 2008; Wennberg et al., 2009) have shown that fertilization is external in macrobenthic species (*P. caudatus*) with females and males spawning their gametes slightly asynchronously into the water, most probably at or near the water/sediment interface although this phenomenon requires confirmation. Usually, thousands of mature oocytes are released outside the body through paired urogenital ducts opening on either side of the anus (van der Land, 1975; Storch, 1991; Schmidt-Rhaesa, 2013). External sexual dimorphism is absent in extant priapulids, except for *Tubiluchus* in which males differ from females in the morphology of their genital area and the presence of distinctive and more abundant setae along their ventral side (van der Land, 1975; Schmidt-Rhaesa et al., 2013). The possible existence of internal fertilization in *Tubiluchus* is based on indirect evidence such as dimorphic features (van der Land, 1970), the atypical morphology of spermatozoa (Schmidt-Rhaesa, 2013), and occasional findings of spermatozoa seemingly embedded into the epithelium of the urogenital duct of female individuals of *T. phillipinensis* (Alberti and Storch, 1988) but surprisingly absent from its lumen. The assumed internal fertilization of *Meiopriapulus fijiensis* is based on the observation of a single embryo that seems to have been released from the female urogenital pore (Higgins and Storch, 1991).

In macrobenthic species such as *Priapulus caudatus* and *Halicryptus spinulosus*, ovaries consist of a large number of ovarial sacs suspended between the gonoduct and a muscular strand (van der Land, 1975; Nørrevang and van der Land, 1983), and usually contain thousands of oocytes (Figs. 3c–e and 4a) with a maximum diameter (Do) of 80 μm and 60 μm, respectively (Schmidt-Rhaesa, 2013). In contrast, the vast majority of meiobenthic species

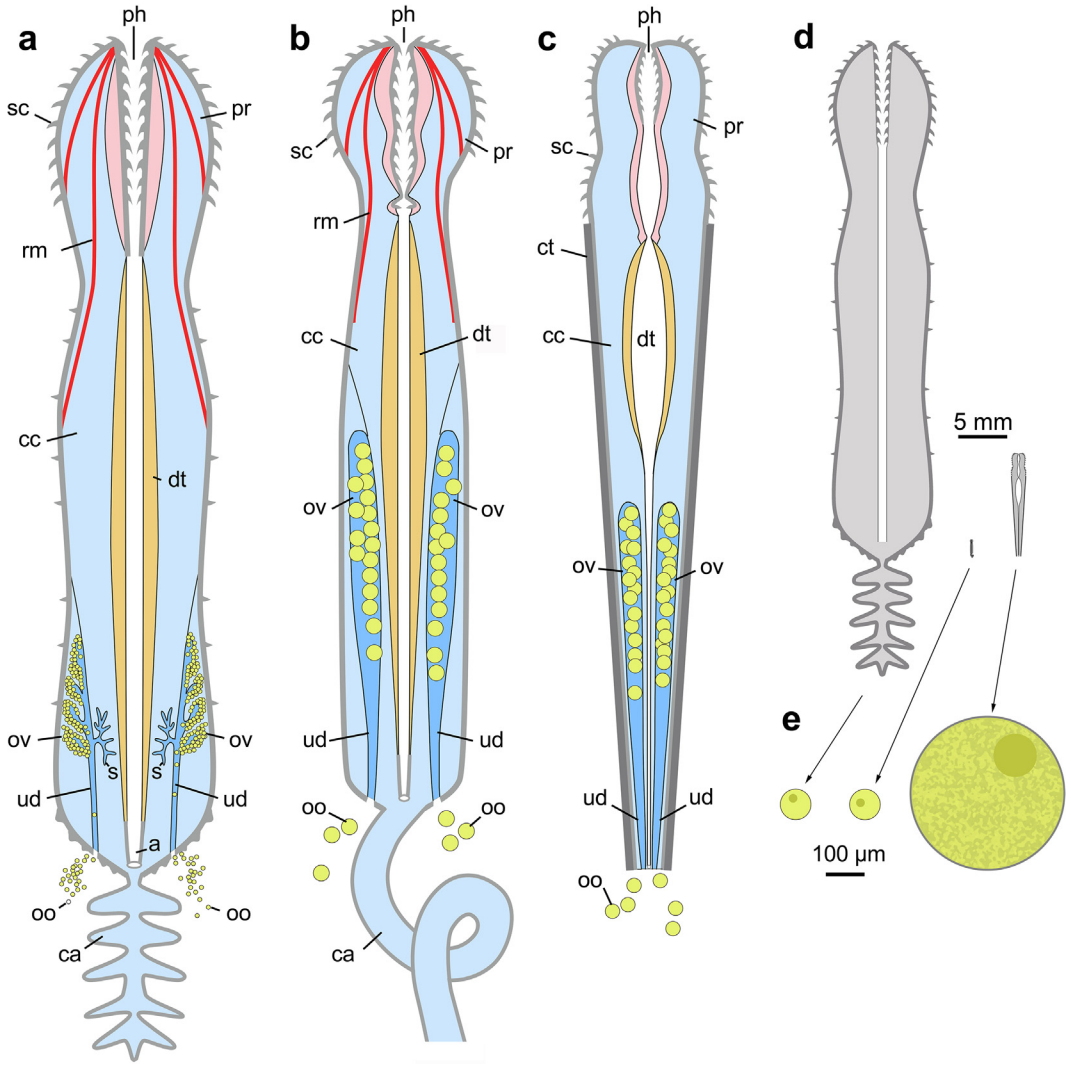


Fig. 4. Comparative diagrams showing female reproductive organs in extant priapulid worms and early Cambrian *Paraselkirkia*. (a) Macrobenthic priapulid *Priapulus caudatus* (simplified after Schmidt-Rhaesa, 2013). (b) Meiobenthic priapulid *Tubiluchus corallicola* (simplified after Kirsteuer and van der Land, 1970). (c) Early Cambrian *Paraselkirkia sinica* (simplified reconstruction). (d) Outline of the three forms represented at the same scale (from left to right: *Priapulus*, *Tubiluchus* and *Paraselkirkia*). (e) Mature oocytes of the three forms at the same scale. Primary body cavity in light blue, ovaries in dark blue, oocytes in yellow, muscular tissues in light red (around pharynx), digestive tissues in light orange, cuticle in gray. Retractor muscles may be present in *Paraselkirkia* but are not represented. Abbreviations: rm, retractor muscle; s, solenocytes; others as in Figs. 1, 2 and 3.

have simple tubular ovaries that can only produce a small number of oocytes (Fig. 4b). For example, *Meiopriapulus fijiensis* has fewer (No = 8) but much larger oocytes (Do = 250 μm) (Higgins and Storch, 1991) than in macrobenthic species. Similar reproduction modes are known in other meiobenthic species such as *Tubiluchus corallicola* (Do = 80 μm; No = 20) (Kirsteuer and van der Land, 1970) and *Maccabeus tentaculatus* (Do = 100 μm; No = 8; Por and Bromley, 1974; and see Fig. 3f–h).

Paraselkirkia sinica from the Cambrian does not show any external sexual dimorphism and no realistic mechanism can explain internal fertilization. The fertilization and embryonic development (either direct or indirect) most probably took place outside the animal. In such a case, the relatively large eggs (Do = 300–450 μm) of *P. sinica* should be interpreted as oocytes (non-fertilized eggs) rather than early embryos. The relatively low number (No < 30) and large size of these oocytes suggest marked differences with extant macrobenthic priapulids (Supplementary Data, Table S1) and instead strong similarities with the meiobenthic representatives of the group such as *Meiopriapulus fijiensis* (Do = 250 μm). However, it should be noted that the number of oocytes seen in *P. sinica* (e.g., 3 or 6 in some individuals; Supplementary Data, Fig. S1) may not faithfully correspond to the actual clutch size of the animal, since a certain percentage of them may have been lost due to decay. Several specimens of *P. sinica* (Fig. 1h, I; Supplementary Data, Fig. S1g, h) indicate that oocytes may have formed paired clusters in the posterior half of the trunk and were possibly aligned within a tubular structure (Fig. 1a, b) as seen in *Tubiluchus corallicola* (Kirsteuer and van der Land, 1970) and *Maccabeus tentaculatus* (Por and Bromley, 1974) (Fig. 3f–h).

The cohesive appearance of some of the oocyte clusters (Fig. 1c–g) suggests that they were maintained within a single (e.g., tubular; *Maccabeus tentaculatus*, Fig. 3g–h) or more complex (e.g. *Priapulus*, Fig. 3b–d) ovarian sac before being released outside through the urogenital ducts, as observed in modern priapulids. In extant macrobenthic priapulids mature oocytes bulge into the primary body cavity although tightly maintained by the ovarian basal lamina (van der Land, 1975; Schmidt-Rhaesa, 2013). Such very thin supporting structures are prone to rapid decay after death (autolysis). If present in *Paraselkirkia*, these structures may have limited the dispersal of oocytes within the primary body

cavity as observed in our fossil specimens (Fig. 1c–g). The relatively large internal structures observed within the oocytes of *Paraselkirkia* (fluorescence images, Fig. 1e) are comparable in size with the nucleus of oocytes in *Priapulus caudatus* (ca. 40% of oocyte diameter; Nørrevang and van der Land, 1983) and may therefore be interpreted as such.

4.3. Conservatism in priapulids

Our study suggests that the general organization of the female reproductive system of priapulid worms with paired tubular ovaries has remained virtually unchanged since the early Cambrian, i.e., over a period of more than 500 million years. The intriguing absence of overlap between Cambrian and post-Cambrian priapulids as seen in the character morphospace obtained by Wills et al. (2012) should not disguise the fact that numerous morphofunctional features of the priapulid body plan do appear to be highly conserved (introvert structure and symmetry, pharynx, muscle system, etc.). Although very little is known about evolutionary mechanisms that led to such extremely conservative body plans, some authors (e.g. Estes and Arnold 2007) stressed the importance of the lack of adaptation into various significantly different ecological niches. The persistence of endobenthic and burrowing lifestyles might have favoured evolutionary conservatism in priapulids through time.

4.4. Selkirkiid lifestyle and brachiopod epibionts

Selkirkiids have been interpreted as burrowers that possibly lived vertically embedded in sediment (Conway Morris, 1977; Hou et al., 2017), or alternatively as epibenthic tubicolous worms (Maas et al., 2007). They have no exact counterpart among extant priapulid worms that are overwhelmingly active infaunal burrowers (e.g., macrobenthic species such as *Priapulus*). *Maccabeus tentaculatus* (maximum length of ca. 3 mm) is the only one example of an extant tubicolous priapulid worm (Por and Bromley, 1974). Its cylindrical tube described as a flimsy structure formed by agglutinated plant fragments (e.g., *Posidonia*) is fundamentally different from the rigid, annulated, cuticular tube of *Paraselkirkia*. *Maccabeus* is seen as a sedentary meiobenthic animal that uses its crown of tentacles (modified scalids) to catch vagile microbenthic prey such as copepods (Por and Bromley, 1974). Although *Paraselkirkia* is likely to have been less mobile than non-tubicolous forms, we find no reason to consider it as a sessile animal. Its well-developed introvert suggests that this animal could move and possibly penetrate soft sediment, at least superficially. Its scalids (introvert) and tube annulations are potential anchoring features essential to locomotion and occasional excursions into the sediment. Assuming that *Paraselkirkia* is dominantly epibenthic, its tube appears as a potential protective structure against predation and physical damages.

Brachiopod epibionts also support a dominantly epibenthic lifestyle for *Paraselkirkia*. The brachiopod feeding mode (lophophoral cilia) requires constant contact with circulating water. This constraint, if applicable to the Cambrian representatives of the group, makes the host relationships with an infaunal animal unsustainable. It is therefore more likely to consider *Paraselkirkia* as a semi-sedentary epibenthic animal that had capabilities to explore the most superficial layers of the sediment for feeding and self-protecting. We hypothesize that external fertilization took place within the sediment as well (Fig. 5).

The free-swimming larvae of modern brachiopods settle on a great variety of hard substrates (via a pedicle) such as the shells of conspecific brachiopods and other invertebrates (e.g., Topper et al., 2015), and then metamorphose and remain attached. Topper et al. (2015) clearly demonstrated that mid-Cambrian Burgess Shale brachiopods such as *Nisusia burgessensis* used identical life strategies to survive in marine environments dominated by soft muds, and occurred on various invertebrates such as sponge tufts (*Pirania muricata*) and occasionally on the empty tube of *Selkirkia columbia* (single specimen attached near the posterior end; see also Conway Morris 1977, Plate 17, Figure 2). This attachment strategy seems to have developed even earlier as exemplified here by *Paraselkirkia* and other early Cambrian organisms from the Chengjiang biota (e.g., scalidophoran worm *Cricocosmia*, brachiopod *Diandongia*, alga-like *Malongitubus*; Han et al., 2004; Zhang et al., 2010; Wang et al., 2012) that served as a host to small brachiopods.

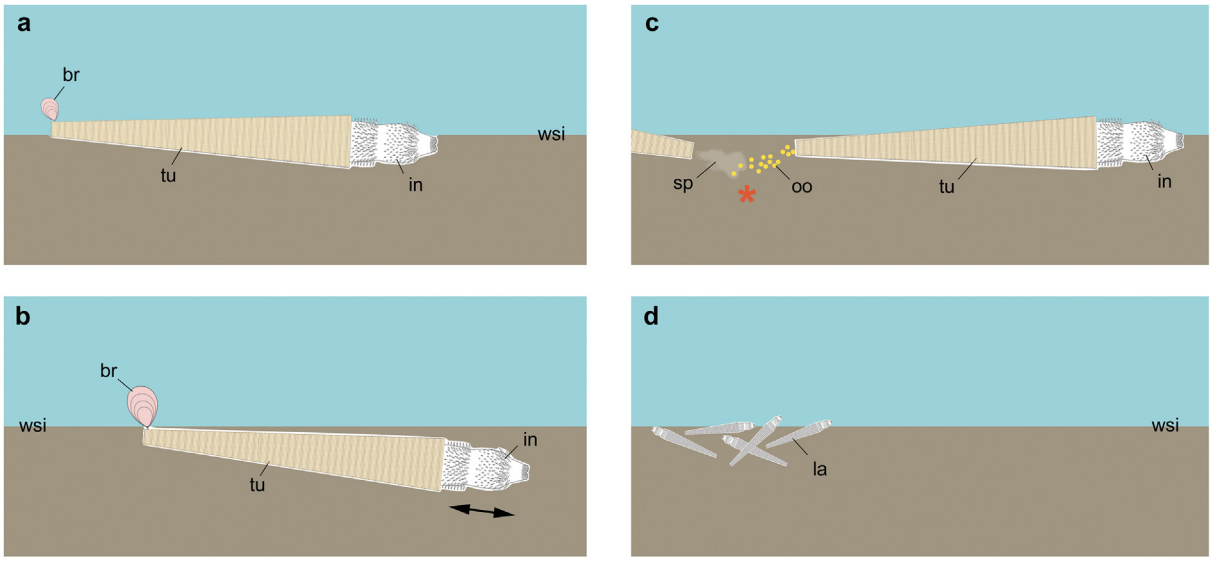


Fig. 5. Assumed lifestyle and reproductive mode of *Paraselkirkia*. (a) Living at the water/sediment interface with a juvenile brachiopod attached near the posterior end of the tube via a short pedicle. (b) Moving slightly below the water/sediment interface for feeding or protection (e.g. possibly during moulting). (c) External fertilization; oocytes and spermatozoids presumably emitted within sediment. (d) *In-situ* development of larvae. Abbreviations: br, brachiopod; la, larvae; sp, spermatozoids; tu, tube; wsi, water/sediment interface; others as in Fig. 1.

Two possible options are proposed to explain the consistent small size (ca. 1 mm) of the twenty-two brachiopod epibionts studied here: they represent either subadult stages of an unusually small-sized species or juveniles of a larger-sized species. We tentatively favour the second option on the basis of the very small size of the brachiopods, being aware that it raises the question of the intriguing absence of more mature stages. One might hypothesize that brachiopod larvae could easily settle on the tube of *Paraselkirkia* but had a relatively low probability to reach adulthood for the simple reason that their host moved and interacted with sediment (e.g., in contrast with more stable shelly substrates), possible causing a significant proportion of post-juvenile brachiopods to detach.

Equally uncertain, is the nature of the *Paraselkirkia*-brachiopod association that may have been beneficial to both parties (mutualism) or not (e.g., commensalism). Particularly enigmatic is the consistent settlement of the brachiopod close to posterior opening of the tube. We tentatively suggest that this preferential location may have favored access to possible food particles emitted through the anus of the worm.

4.5. Reproductive strategies and ecology

The reproduction mode of *Paraselkirkia* differs from that of extant macrobenthic priapulids (e.g., *Priapulus*) and instead recalls that of meiobenthic forms such as *Meiopriapulus fijiensis* (Higgins and Storch, 1991), *Tubiluchus corallicola* (Kirsteuer and van der Land, 1970) and *Maccabeus tentaculatus* (Por and Bromley, 1974) that inhabit interstitial benthic environments and typically produce a small number of large oocytes (Fig. 1f, g and 3f–h). It is worth noting that these resemblances strictly concern the reproductive mode. *Paraselkirkia* does not belong to the category of meiobenthic animals that, by definition, rarely exceeds 1 mm. This raises the question of the possible relation between ecology and reproductive mode in extant and early priapulids. In meiobenthic priapulids, optimal offspring survivorship seems to be achieved through a small number of yolk-rich embryos that presumably develop within a protective interstitial environment, suggesting that energy investment is oriented towards quality rather than quantity. Each fertilized embryo has a relatively high probability of surviving to adulthood (e.g., as in K-selected species). By releasing thousands of gametes presumably outside the substrate, macrobenthic species (e.g., *Priapulus*) seem to rely on a different reproductive strategy. Their embryos are likely to develop in a less protective environment, comparatively more exposed to predation and physical damage than in interstitial species, in which the important loss of offspring is offset by high fecundity (e.g., as in r-selected species). The reason why the reproductive strategy of selkirkiids resembles that of modern meiobenthic priapulids remains an open question but might result from shared ecological features such as a relatively small size, limited mobility and egg deposition within the sediments (Fig. 5).

In summary, we suggest that the basic organization of the reproductive system (e.g., paired ovaries and oviducts) of priapulids was established very early in the evolution of the group. This organ system created conditions for various reproductive modes and strategies to develop, in possible response to environmental factors, biological pressure within ecosystem and specific ecologies (e.g., tubicolous lifestyle, low mobility).

Interestingly, embryos and post-embryonic stages of scalidophoran worms from the basal Cambrian (Fortunian; see Donoghue et al., 2006; Liu et al., 2014) have a relatively large size that seems to match that of oocytes found in *P. sinica*. This would suggest that reproductive strategies involving a small number of large eggs may have prevailed in the early evolution of the group. However, we cannot establish with certainty that this particular reproductive mode represents the ancestral condition for scalidophorans or priapulids.

4.6. Key steps in the early evolution of reproductive systems

Present-day metazoans display a huge variety of asexual and sexual reproduction modes that include aspects related to the formation of gametes and gonads, fertilization processes and embryonic development. Gonads are either tubular organs or more complex sac-like structures often arranged in symmetrical pairs. The development mode of gonads is diverse and suggests that they probably evolved independently within various metazoan groups (Schmidt-Rhaesa, 2007). Fertilization occurs externally or internally via copulation or other methods such as the deposition of spermatophores. Diverse brood care behaviors accompany the embryonic development of numerous extant species (e.g., crustaceans). The reproductive behaviors of early animals have remained largely enigmatic because of the lack or scarcity of information on their reproductive organs and strategies. However, exceptional fossils from lower Palaeozoic Lagerstätten provide key elements that shed light on the evolution of reproductive systems of a major group of early animals, the ecdysozoans which stand as a dominant element of early animal life (Supplementary Data, Fig. S3). (i) Paired ovaries and external fertilization seem to characterize basal scalidophorans exemplified by *Paraselkirkia* (present study); (ii) brood care is known from two groups of lower Palaeozoic euarthropods, the Cambrian waptiids (Caron and Vannier, 2016; Ou et al., 2020) and Ordovician ostracods (Siveter et al., 2014). In waptiids, egg clusters are carried symmetrically on either side the female's body suggesting that they were released from paired gonads as in *Paraselkirkia*; (iii) Copulatory organs were present in early Silurian myodocope ostracods from the Herefordshire Lagerstätte (Siveter et al., 2003) (ca. 425 Ma) indicating that copulation and internal fertilization have a very ancient origin among euarthropods. Although fragmentary these few examples suggest that at least four of the most essential features of bilaterian reproduction (paired gonads, external and internal fertilization, copulation) had already evolved in the early Palaeozoic through Ecdysozoa.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author contributions

J.Y. collected the material and provided the geological information; X.Y.Y., X.G.Z. and J.V. designed the study; X.Y.Y., J.V., J.Y. and X.G.Z. performed the investigation; X.Y.Y., J.V. and X.G.Z. wrote the manuscript with input from other authors; J.Y. prepared all fossils for photography; X.Y.Y. and X.G.Z. produced the fossil figures, D.W. and J.V. studied extant priapulids and produced the relevant figures and drawings, and X.Y.Y. designed and operated the elemental map analysis.

Supplementary data

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