Early post-embryonic trilobite stages and possible eggs from the 'Túnel Ordovícico del Fabar' (Middle Ordovician, northwestern Spain)

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The sub-surface section of the Middle Ordovician Sueve Formation of the Cantabrian Zone, studied during the construction of a tunnel in the A-8 free highway of northern Spain, provided abundant fossiliferous horizons rich in trilobites of Darriwilian age. Fine-grained mudstones of the Cofiño Member delicately preserves a number of early stages of the calymenacean trilobite *Prionocheilus mendax* (Vaněk), from protaspides to meraspid degree 2, including *in situ* exuviae with disarticulated librigenae. From the upper part of the Bayo Member small clusters of pyrite spheres of ca. 0.5 mm in diameter are recorded, and may represent eggs of trilobites, being associated to the pliomerid *Placoparia* (*Coplacoparia*) *tournemini* (Rouault) in the same beds. The sphaeroids may represent the pyrite infilling of the internal cavity of possible eggs, and the covering is replaced by phyllosilicates. Differences in shape and size with presumed olenid eggs may be caused by the particular environmental adaptations of the latter group. The relatively large size of both the calymenid larvae and the pyritised ?eggs suggest a lecithotrophy development related with higher latitudes and/or with scarce or stationed organic matter.

Keywords: Development, fossil eggs, Gondwana, lecithotrophy, Spain, trilobites

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1 Introduction

2 Ordovician trilobites from the Mediterranean region, located during this period in high
 3 Gondwanan palaeolatitudes close to the South Pole (Torsvik & Cocks 2017), display a
 4 characteristic palaeobiogeographic signature related to their occurrence in shallow shelf facies,
 5 mostly dominated by siliciclastic sediments. The typical assemblages were originally named as
 6 the ‘*Selenopeltis* province’ (Whittington & Hughes 1972) and later designated as the
 7 ‘calymenacean-dalmanitacean’ shelf fauna (Cocks & Fortey 1988, 1990; Fortey & Cocks 2003).
 8 Within this South Gondwana palaeobiogeographical region, a great number of trilobite taxa
 9 have been described from the Ordovician of southwestern Europe (e.g. Dean 1966; Hammann
 10 1974, 1976, 1983, 1992; Henry 1980; Romano 1980; Rábano 1990; Hammann & Leone 1997,
 11 2007; Pereira *et al.* 2016). Nevertheless among the extensive palaeontological literature
 12 available, details of early post-embryonic development of trilobites were scarcely documented.
 13 So far, the late meraspid stages have been described for the pliomerids *Placoparia*
 14 (*Coplacoparia*) *borni* Hammann (Hammann 1974, pl. 10, figs. 167–170), *P. (Coplacoparia)*
 15 *ournemini* (Rouault) (Rábano 1989, pl. 26, fig. 10), *Pateraspis mediterranea* Hammann
 16 (Hammann 1974, pl. 11, fig. 180) and *Eccoptochile almadenensis* Romano (Romano 1980, pl.
 17 79, fig. 5; Rábano 1989, pl. 28, fig. 7); the harpetid *Eoharpes guichenensis* Henry & Philippot
 18 (Henry 1980, pl. 2, fig. 4), and the calymenines *Kerfornella miloni* Henry (Henry 1980, pl. 22,
 19 fig. 9) and *Colpocoryphe thoralis conjugens* Hammann. From the latter species a single early
 20 meraspid specimen (degree 1) was illustrated by Rábano (1989, pl. 18, fig. 7).

21 An explanation for the poor record of these early post-embryonic stages of Middle
 22 Ordovician trilobites in Ibero-Armorica may be the strong hydrodynamics of the sedimentary
 23 environments on the sea-bottom, usually placed above the storm-wave base, as well as the
 24 widespread record of relatively coarse sediments with a generalized absence of fine mudstones
 25 derived from quiet-water environments. Here, we describe a new find of protaspid and early
 26 meraspid stages from the widely-distributed calymenine trilobite *Prionocheilus mendax* (Vaněk,
 27 1965), as well as some enigmatic sphaeroidal objects, now pyritised, associated with the
 28 pliomerid trilobite *Placoparia (Coplacoparia) tournemini* (Rouault, 1847) and whose
 29 interpretation as possible eggs is considered.

31 Material and Methods

32 The trilobite specimens examined in the present study were collected from two different
 33 horizons within the tunnel, being preserved as internal and external moulds in dark brown to
 34 greenish mudstones. Study of the surficial characters of their carapaces was made through latex
 35 casts taken from external moulds. A part of the illustrated specimens were whitened with MgO
 36 and photographed using a Canon EOS 5D digital camera with a Canon Compact-Macro 100
 37 mm EF lens. Early developmental stages that needed greater magnification were mounted on a

SEM stub, carbon-coated and imaged in a Jeol JSM 6010LA Plus scanning electron microscope, in the SEM laboratory of the Geological Survey of Spain (IGME, Tres Cantos, province of Madrid). Pyritised sphaeroids were studied and analysed using an ESEM Quanta 200 environmental scanning electron microscope with EDS and WDS, low vacuum operating at a voltage of HV 20 kV using secondary and retrodispersed electrons. This equipment belongs to the non-destructive technical analytical services of the National Museum of Natural Sciences (MNCN-CSIC, Madrid).

The specimens are housed in the Museo Geominero (MGM) in Madrid. Figures were made by means of Adobe Photoshop CS6 Extended.

Geological setting

The studied material comes from the Sueve Formation, a dark shale unit (50–100 m thick) with some intercalated siltstones and ironstones, which overlies the Barrios Formation in the Laviana–Sueve thrust-sheet of the eastern Cantabrian Zone of the Iberian Massif (Fig. 1). The latter unit represents one of the most extensive and characteristic units of the Lower Palaeozoic regional succession (Aramburu & García-Ramos 1993), being in part equivalent to the Armorican Quartzite of other Ibero-Armorican areas (Gutiérrez-Alonso *et al.* 2007), although in the Cantabrian Zone the Floian beds are restricted to the uppermost part of the Formation (Gutiérrez-Alonso *et al.* 2016). Modern lithostratigraphical and palaeontological studies of the Sueve Formation were presented by Gutiérrez-Marco *et al.* (1996, 1999) based on the only available surface outcrops, which are very discontinuous. However, the excavation of a tunnel for the Cantabrian free Highway (A-8) in northern Spain, allowed a complete bed-by-bed study of the sub-surface section of the entire Sueve Formation (Gutiérrez-Marco *et al.* 2003), that is still awaiting publication. However, preliminary data found in the tunnel were presented in a catalogue for an exhibition of rocks and fossils coming from the tunnel (Gutiérrez-Marco & Bernárdez 2003). The tunnel, later renamed as ‘Túnel Ordovícico del Fabar’ due to the relevance of the scientific discoveries held in the Ordovician sequence, lies about 7 km west of the Ribadesella town (Asturias region). From a geological point of view, the Laviana–Sueve thrust-sheet penetrated by the tunnel represents an external zone of the Variscan Chain, located in the inner part of the Cantabrian-Asturian Arc (Pastor-Galán *et al.* 2011).

The Sueve Formation in the tunnel section is about 85 m thick and comprises a lower member of very fossiliferous massive dark mudstones (Cerracín Member, 12.8 m), followed by sandy siltstones with some thin intercalations of fine-grained sandstones and some shaly beds (Bayo Member, 33.4 m) and ending with dark siltstones and mudstones (Cofiño Member, 38.5 m). The base of the Sueve Formation bears an ooidal ironstone bed that in the past was an object of mining activity; a second ironstone is known in the upper part of the Bayo Member, but in this case it is a highly bioturbated sideritic layer, not ooidal in character.

Among the abundant specimens of Middle Ordovician trilobites obtained from the tunnel section, the palaeontological sample TUN-055 was the only one — from a total of 116 fossiliferous beds quoted in the Sueve Formation in which larvae and early stages of development have been recorded. The extreme rarity of these is more surprising when considering the huge volume —up to dozens of cubic meters— and the un-weathered character of the palaeontological samples yielded by the tunnel. TUN-055 is a special bed located near the base of the Cofiño Member, which offers several bedding plane concentrations of minute fossils such as ostracods (*Marquezina*, *Quadritia*), conodonts (*Drepanoistodus*, *Panderodus* and *Semiacontiodus*), machaeridians (*Plumulites*), cnidarians (*Sphenothallus*) and trilobites, including representatives of rare genera like *Dionide*, *Primaspis*, *Parabarrandia* and *Selenopeltis*, as well as diverse trilobite post-embryonic stages (i.e. protaspid and meraspid stages of *Prionocheilus*) besides diverse holaspid forms of *Isabelinia* and ?*Dionide* not studied yet. The associated record of the graptolite *Gymnograptus linnarssoni* (Moberg) makes it possible to date this bed to the middle upper Darriwilian (= lower Dobrotivian in Gutiérrez-Marco & Bernárdez 2003).

The other studied material comes from a bed 2.7 m below the former and approximately the same chronostratigraphical age, but in this case situated near the top of the Bayo Member instead of the basal part of the Cofiño Member. This fossiliferous bed (TUN-057), made of dark noduliferous mudstones, yielded eight different trilobite genera, with some abundance of complete specimens of *Placoparia*, *Neseuretus* and *Isabelinia*, besides diverse bivalves, gastropods, cephalopods, brachiopods, echinoderms, cnidarians, hyoliths, ostracods and graptolites. From this assemblage, minute clusters of sphaeroidal pyritised objects have been found, and its possible interpretation as ?trilobite eggs is discussed below.

Trilobite early stages

The record of early post-embryonic stages of calymenacean trilobites from the ‘Túnel Ordovícico del Fabar’ was briefly mentioned by Gutiérrez-Marco & Bernárdez (2003), but the material was never studied in detail. For the present work, the examined material is confidently assigned to *Prionocheilus mendax* (Vaněk, 1965), a species widely distributed in the Middle Ordovician (Darriwilian 2–3, see Bergström *et al.* 2009) of Bohemia (Vaněk 1965), southwestern Europe (Hammann 1983; Henry 1980; Romano 1980; Rábano 1989) and Great Britain (Kennedy 1989). Adult specimens of *P. mendax* (Fig. 2A) range in the section between 10.5–47.4 m above the base of the Sueve Formation, being more common near the top of the Cerracín Member and in the transitional beds between the Bayo and Cofiño members.

The assemblage of early stages of *P. mendax* recorded at the horizon TUN-055 (1 m above the base of the Cofiño Member) belongs to protaspid and meraspid periods (Fig. 3).

The earliest recorded protaspid stage (n=2) of the species is elliptical to elongate subhexagonal in outline, 1.08 to 1.04 mm long and 0.87 to 0.86 mm wide. Glabella is subrectangular in outline, comparatively large. Occipital ring bears small node. The preocular field is strongly inflated and relatively wide, ca 22% of the glabellar length. Fixigenae are comparatively narrow, 28% of the glabellar length. The specimen illustrated on Figure 3A and 3E seems to have preserved palpebral lobe located laterally. The trunk composed by three segments plus terminal piece that are tapering posteriorly. The division between protocranidium and trunk is evident only in axial part, and no distinct transverse line separating these parts is visible in the pleural part of the exoskeleton.

Calymenine trilobites usually have one or two planktonic stages (designated by Chatterton *et al.* 1990 as P1 and P2), that are followed by two benthic stages (B1 and B2 *sensu* Chatterton *et al.* 1990). However, some calymenine lineages such as Homalonotidae seem to lack planktonic stages (cf. Lu and Wu 1982; Chatterton *et al.* 1990). Based on the comparison with other Calymenina we suggest that protaspides of *P. mendax* might be homologous to the first benthic stage (B1). This assumption is based mainly on following characters: (1) absence of medial furrow crossing L1 and L2 that is characteristic for all planktonic calymenine protaspides (cf. Chatterton *et al.* 1990; Edgecombe *et al.* 1998; Lerosey-Aubril 2007); (2) absence of distinct transverse furrow separating protocranidium and trunk that is often present in B2 but less distinct in B1 (cf. Chatterton *et al.* 1990; Edgecombe *et al.* 1998); and (3) number of post-cephalic segments – B1 it seems to have about three segments while B2 usually have more (cf. Chatterton *et al.* 1990).

The known meraspid larvae (n=3) of *Prionocheilus mendax* are slightly elliptical in outline, 1.8 to 2.2 mm long and 1.6 to 1.7 mm wide. The cranidium is semicircular to subtrapezoidal in outline. The glabella tapers gently anteriorly. The facial suture seems to be proparian. The palpebral lobes are located antero-laterally. The librigena is triangular in shape bearing tiny spines along the margin (Fig. 3C). The thorax is composed of two tergites (making this meraspid degree 2), and has an axis of high convexity, well defined by deep axial furrows; the axial ring of T2 is narrower (tr.) than of T1. The pleurae are moderately flat, becoming slightly shorter (exsag.) and narrower (tr.) from T1 to T2. The meraspid pygidium is relatively large, ca 35% of the exoskeleton, semi-elliptical in outline, with anterior margin extending posterolaterally and posterior margin concave medially, W-shaped in outline. Axis convex, divided into six segments and a terminal piece (Fig. 3C, G, H). Pleural field convex with distinct pleural and interpleural furrows.

Possible trilobite eggs

Placoparia (Coplacoparia) tournemini (Rouault, 1847) is one of the most abundant pliomerid trilobites in the Sueve Formation, being represented by numerous complete exoskeletons (Fig.

2B) and also by clusters of juvenile specimens (either prone or enrolled) of up to 20 individuals in the sample TUN-057. Associated with these trilobites in the same bed, clusters of small sphaeroidal objects, preserved in un-weathered pyrite and with its characteristic shine and colour under natural light, are recorded (Fig. 4). They do not show any evidence of current alignment or re-sedimentation on bedding plane. Pyritization also occurs in some graptolites, from the Bayo Member, with preservation of the internal moulds of biserial rhabdosomes with their original relief, and even preserving details of the fusellar rings of the thecae (Gutiérrez-Marco & Bernárdez 2003, figs. p. 290–291). Significantly, pyritization did not affect other fossil groups in the sample TUN-057, where pyritic nodules and aggregates of framboidal or disseminated pyrite are absent. According to the morphology and distribution pattern on bedding plane, the aspect of these sphaeroids seems to be pre-diagenetic, corresponding to organic structures quickly pyritized after the initial burial.

The sphaeroids, with a mean diameter of 0.5 mm, are surrounded by a thin layer made of phyllosilicates (sericite: arrowed in Fig. 4D) with their main planes subperpendicular to the sphaeroid surface. This phyllosilicate layer may be interpreted as the infill of a void originally occupied by an organic envelope. The surface of the pyritic sphaeroids shows a polygonal (mainly sub-hexagonal) pattern which in some cases is densely packed whereas in another the crystalline growth is not complete, showing polygonal bodies (*ca.* 25 μ m in diameter) separated by phyllosilicates (Fig. 4E). In a slightly broken specimen (Fig. 4G–H), very small pyritic sphaeroids can be observed in a section perpendicular to the main surface, along with an octahedral pyrite crystal of a later generation. Fracturing of this specimen seems to have been caused by compaction in an early diagenetic stage, when the sphaeroid was incipiently pyritized. The pyritization, restricted to the interior of the sphaeroidal body, seems to have been initiated by the formation of very small (up to 10 μ m) framboidal aggregates, which by growing and coalescence gave rise to the greater bodies that grew until the internal cavity of each of the supposedly organic sphaeroids was completely filled. The pyritized sphaeroids from the examined sample are imperfectly spherical and show slight variation in diameter, ranging from 0.4 to 0.6 mm, 0.5 mm on average. Two of the sphaeroids show a fracture, one perpendicular to the long axis and the second in a tip of the egg. An isolated sphaeroid also shows a fracture in the tip. We hypothesised that these pyritized sphaeroids represent the mineralised internal mould of the invertebrate eggs, possibly even those of trilobites, especially concerning the fact that marine arthropod eggs seems to be quite resistant to decay (Martin *et al.* 2005) and can be replaced with framboidal pyrite (Hegna *et al.* 2017). Similar framboidal pyrite aggregates are described from hydro-carbon-bearing ecosystems where microbial organisms played an important role in its formation (Cavalazzi *et al.* 2012). This is common in modern seep environments (Merinero *et al.* 2008; Cavalazzi *et al.* 2012); however, the Bayo Member was deposited in a more normal geological setting in a marine platform. The lack of euhedral pyrite

in the spheres points to a limited diffusion of ions between the inside and outside of the spheres. The formation of framboidal aggregates is favourable under more oxidizing conditions while typical pyrite crystals are favourable under more reducing conditions (Grimes *et al.* 2002; Gabbott *et al.* 2004). This suggests that the conductions within the spheres were not too reducing or a very limited exchange with the external environment occurred. This fact agrees with the delicate pyritization process observed in the graptolites that also occur in horizon TUN-057. Their broad similarity to structures described by Hegna *et al.* (2017) and their direct association with complete carcasses of juvenile and adult specimens of *P. (C.) tournemini* (Rouault) in the same beds, where other trilobites are mostly represented by exuviae in several degrees of disarticulation, may also suggest that this pliomerid species was the producer of the purported sphaeroidal eggs. However, the lack of a direct link with this pliomerid trilobite drives us to state that the pyritized eggs could also belong to another trilobite or another unknown arthropod. Although we agree that such an interpretation is purely hypothetical, it seems the most parsimonious one so far.

The present evidence of Lower Palaeozoic arthropod eggs is very limited, being restricted to the specimens presented by Shu *et al.* (1999), Lin *et al.* (2006), Siveter *et al.* (2007, 2014), Duan *et al.* (2014), Caron & Vannier (2016) and Hegna *et al.* (2017). Shu *et al.* (1999) and Duan *et al.* (2014) described eggs and/or embryos associated with bradoriid arthropod *Kunmingella* (Cambrian, Chengjiang Lagerstätte). The structures are 150–180 μm in diameter and are preserved by pyrite and phosphates. In both of these cases, they are directly associated with adult specimens. Lin *et al.* (2006) analysed clusters of silicified sphaeroids, 767 μm of mean diameter and randomly piled in layers, which are preserved in a fine-grained siliciclastic matrix from the middle Cambrian Kaili Formation Lagerstätte, south China. According to Lin *et al.* (2006), the eggs are primarily preserved as solid silica replacement, with the original organic layer replaced by a calcite layer covering the eggs. Siveter *et al.* (2007, 2014) described brood care in myodocopid ostracods from the Silurian Herefordshire Konservat-Lagerstätte and the Ordovician Beecher's Trilobite Bed. In the first case, the eggs and possible juveniles are about 550 μm long and are preserved in calcite that co-precipitated with pyrite and infilled the voids (cf. Orr *et al.* 2000). In the second case, the eggs and possible embryos are pyritized, ranging between 240–350 μm and are associated with adult individuals. Caron & Vannier (2016) described comparatively large eggs (0.7–2.4 mm) in *Waptia fieldensis* from the Burgess Shale. Supposed trilobite eggs have been reported by Hegna *et al.* (2017). These are preserved as minute pyritised bodies, elliptical in shape, and clustered below the fixigenal area of the olenid trilobite *Triarthrus eatoni* (Hall, 1838), from the Lorraine Group of northeastern United States. Hegna *et al.* (2017) interpret these bodies as trilobite eggs by their spatial association with this particular trilobite and suggest that trilobites may have had an unmineralized ontogenetic stage

before the protaspis to explain the small size of the eggs (some 200 μm in size) relative to the first protaspis larvae.

In contrast with the scarcity of data relative to ancient eggs, the record of unassigned fossil embryos of problematic metazoans has been more extensively treated in the literature (Zhang & Pratt 1994; Bengtson & Zhao 1997; Steiner *et al.* 2004; Donoghue *et al.* 2006).

Barrande (1852, pl. 27, figs. 1–3) was the first author who reported the discovery of presumed trilobite eggs. They occur as sphaeroidal bodies of about 0.7 mm in diameter and clustered on bedding planes in number of 12 per cm^2 , or alternatively as isolated calcareous bodies of greater diameter (2–5 mm) preserved in trilobite-rich limestones. The first type was mentioned by Lin *et al.* (2006) as possible eggs, but the original sample (Barrande 1852, pl. 27, fig. 1), from the Middle Devonian Choteč Limestone of Bohemia, was reviewed by one of us (LL) and remains as unconvincing because of the preservation. The second type of sphaeroidal fossils attributed to ‘trilobite eggs’ by Barrande (1872, pl. 27, figs. 2–3), derived from Ordovician rocks, are probably too large and are possibly of faecal origin. The same probably applies to the subsequently described as ‘eggs of uncertain origin’ (Barrande, 1872, pl. 18, figs. 30–31; pl. 35, figs. 25–32, 35–36), which mostly correspond to minute ovoid pellets of *Alcyonidiopsis* [= *Tomaculum*] *problematicum* Groom, 1902 (see Eiserhardt *et al.* 2001; Bruthansová & Kraft 2003). Egg-like structures were described in thin section by Walcott (1879) but criticized by Raymond (1920). Additional records of ‘eggs of uncertain origin’ (Hermite, 1878; Bureau, 1900) and even of ‘?trilobite eggs’ (Marsille, 1910) in the Ordovician of the Armorican Massif of western France also come from classical localities where *T. problematicum* is common (Péneau, 1944).

Discussion

Speyer and Chatterton (1989) suggested that early post-embryonic stages of trilobites were, based on their functional morphology, either benthonic or planktonic. Chatterton & Speyer (1989) also proposed that some of the early developmental stages might have been either feeding on organic detritus (detritotrophic) or nourished by large yolk (lecithotrophic) and such adaptation would increase their survivorship during late Ordovician extinction. Recently, Laibl *et al.* (2017) built a database with all well-known protaspid stages from Cambrian and demonstrated that the largest, possibly lecithotrophic, post-embryonic stages of Cambrian trilobites (ca. 0.7–1.9 mm wide) were restricted to high palaeolatitudes along the West Gondwanan margin. Some of these trilobites with large stages even have accelerated development, which is another character of recent lecithotrophic crustaceans (see Anger 2001 for detailed discussion). Interestingly, the comparatively large stages of *P. mendax* are morphologically and also by size comparable to the B1 stage of *Brongniartella* sp. described by Chatterton *et al.* (1990). Supposing that there were no preceeding planktonic stages, these

similarities might suggest lecithotrophic feeding strategy in *P. mendax* as it was proposed for homalonotid trilobites (Chatterton *et al.* 1990).

Unfortunately, data about the size of the trilobite eggs are scarce. The previous supposed trilobite eggs reported from the Cambrian of South China (Lin *et al.* 2006) and from the Ordovician of New York (Hegna *et al.* 2017) are rather different from those reported herein. The Chinese specimens fall within the size range of recent arthropod eggs, and although those reported by Hegna *et al.* (2017) are quite small, some eggs are known of this size (Thiéry & Gasc 1991; Shen & Huang 2008). Conversely the possible ‘eggs’ from the ‘Túnel Ordovícico del Fabar’ are very large and could possibly have been produced by lecithotrophic taxa (cf. egg size mentioned in McEdward 1997). This is in correspondence with the large growth stages of *P. mendax* from the same locality and with distribution of large, presumably lecithotrophic, trilobite stages in high latitudes (Laibl *et al.* 2017), as all the material described herein comes from northwestern Spain, located during the Middle Ordovician in high latitudes (ca. 70°S) along the margin of Gondwana. Also, the meraspid degree 2 of *P. mendax* is larger compared with some other meraspid degree 2 specimens (at ca. 2.2 mm in length). For instance, the total length of M2 of *Hunanocephalus ovalis* and *Duyunaspis duyunensis* from South China ranges between 0.94 and 1.39 mm and between 1.05 and 1.44 mm in length respectively (Dai *et al.* 2014, 2017).

Concluding remarks

Early post-embryonic stages of trilobites are very rare fossils in the Ordovician of southwestern Europe. This is in part due to the absence of suitable marine facies for the preservation of this kind of fossils, that in southern Gondwana generally are too shallow and energetic, but mainly by the general lack of fine sediments such as black shales and limestones.

Herein, we describe the early post-embryonic growth stages of *Prionocheilus mendax*, a calymenacean trilobite that was widespread in the southern margin of the Gondwana continental shelf and its Avalonian counterpart, which were both located in high palaeolatitudes during the Middle Ordovician. The smallest protaspides of *P. mendax*, when compared with early growth stages of other calymenid trilobites, correspond with the first benthonic stage (B1) of the protaspid period. This, when combined with the relatively large size of these protaspides and early meraspides of that species, suggest that *P. mendax* may have had lecithotrophic early development. Additionally, pyritized sphaeroids found in a bed rich in complete carapaces of the trilobite *Placoparia* (*Coplacoparia*) *tournemini* are hypothesized to represent the remains of arthropod eggs, quite possibly those of *P. tournemini* because this is the only arthropod that is known to be abundant in the same bed. The size of these sphaeroids larger than previously reported for other structures that have been hypothesized to be the preserved remains of trilobite eggs (mean diameter of 0.5 mm for the Spanish material versus 0.2 mm for *T. eatoni*), when

combined with its high latitude palaeobiogeographic location, could also be explained by lecithotrophic early development for that species. However, an alternative hypothesis suggested by Hegna *et al.* (2017) is that olenid trilobites such as *T. eatoni* may have had an unmineralised larval stage or stages between the egg and the first mineralized protaspid (at present undiscovered, other than a large gap in size between the structures believed to be eggs of that species and the smallest known protaspid). Thus, the size of the smallest known protaspid of a trilobite may not necessarily be used to determine the size of the eggs of that species. It is also worthy of note that some workers have suggested that olenids lived in rather unusual, for trilobites, oxygen-depleted environments at the outer edge of the continental shelf (Clarkson & Taylor, 1995; Fortey, 2000).

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References

- Anger, K. 2001: The biology of decapod crustacean larvae. *Crustacean Issues* 14, 1–420.
- Aramburu, C. & García-Ramos, J.C. 1993: La sedimentación cambro-ordovícica en la Zona Cantábrica (NO de España). *Trabajos de Geología* 19, 45–73.
- Barrande, J. 1852: *Système Silurien du centre de la Bohême. 1ère Partie: Recherches Paléontologiques. Vol. I. Texte et Planches. Crustacés: Trilobites*. Chez l’auteur et éditeur, Prague, xxx + 935 pp.

- Barrande, J. 1872: *Système Silurien du Centre de la Bohême. 1ère Partie: Recherches Paléontologiques. Supplément au Vol. I. Trilobites, Crustacés divers et Poissons. Text et Planches*. Imprimerie de Charles Bellmann, Prague, 647 pp.
- Bengtson, S. & Zhao, Y. 1997: Fossilized metazoan embryos from the earliest Cambrian. *Science* 277, 1645–1648.
- Bergström, S.M., Chen, X., Gutiérrez-Marco, J.C. & Dronov, A.V. 2009: The new chronostratigraphic classification of the Ordovician System and its relations to major regional series and stages and $\delta^{13}\text{C}$ chemostratigraphy. *Lethaia* 42, 97–107.
- Bruthansová, J. & Kraft, P. 2003: Pellets independent of or associated with Bohemian Ordovician body fossils. *Acta Palaeontologica Polonica* 48, 437–445.
- Bureau, E. 1900: Notice sur la géologie de la Loire-Inférieure avec listes des végétaux fossiles. In: *La ville de Nantes et la Loire-Inférieure*, 3, 99–522. Nantes, Imprimerie Émile Grimaud et Fils.
- Caron, J.B. & Vannier, J. 2016: *Waptia* and the diversification of brood care in early arthropods. *Current Biology* 26, 69–74.
- Cavalazzi, B., Barbieri, R., Cady, S.L., George, A.D., Gennaro, S., Westall, F., Lui, A., Canteri, R., Rossi, A.P., Ori, G.G. & Taj-Eddine, K. 2012: Iron-framboids in the hydrocarbon-related Middle Devonian Hollard Mound of the Anti-Atlas mountain range in Morocco: Evidence of potencial microbial biosignatures. *Sedimentary Geology* 263–264, 183–193.
- Chatterton, B.D.E. & Speyer, S.E. 1989: Larval ecology, life history strategies, and patterns of extinction and survivorship among Ordovician trilobites. *Paleobiology* 15, 118–132.
- Chatterton, B.D.E., Siveter, D.J., Edgecombe, G.D. & Hunt, A.S. 1990: Larvae and relationships of the Calymenina (Trilobita). *Journal of Paleontology* 64, 255–277.
- Clarkson, E.N.K. & Taylor, C.M. 1995: The lost world of the olenid trilobites. *Geology Today*, July-August 1995, 147–151.
- Cocks, L.R.M. & Fortey, R.A. 1988: Lower Palaeozoic facies and faunas around Gondwana. In: Audley-Charles, M.G. & Hallam, A. (eds): *Gondwana and Tethys*. Geological Society London, Special Publication 37, 183–200.
- Cocks, L.R.M. & Fortey, R.A. 1990: Biogeography of Ordovician and Silurian faunas. In: McKerrow, W.S. & Scotese, C.R. (eds): *Palaeozoic Palaeogeography and Biogeography*. Geological Society London, Memoir 12, 97–104.
- Dai, T., Zhang, X.L. & Peng, S.C. 2014: Morphology and ontogeny of *Hunanocephalus ovalis* (trilobite) from the Cambrian of South China. *Gondwana Research* 25, 991–998.
- Dai, T., Zhang, X.L., Peng, S.C. & Yao, X.Y. 2017: Intraspecific variation of trunk segmentation in the oryctocephalid trilobite *Duyunaspis duyunensis* from the Cambrian (Stage 4, Series 2) of South China. *Lethaia* 50, 527–539.

- Dean, W.T. 1966: The Lower Ordovician stratigraphy and trilobites of the Landeyran Valley and the neighbouring districts of the Montagne Noire, South-Western France. *Bulletin of the British Museum (Natural History) Geology* 12/6, 245–353.
- Donoghue, P.C.J., Kouchinsky, A., Waloszek, D., Bengtson, S., Dong, X.P., Val'kov, A.K., Cunningham, J.A., & Repetski, J.E. 2006: Fossilized embryos are widespread but the record is temporally and taxonomically biased. *Evolution & Development* 8, 232–238.
- Duan, D., Han, J., Fu, D., Zhang, X., Yang, X., Komiya, T. & Shu, D. 2014: Reproductive strategy of the bradoriid arthropod *Kunmingella douvillei* from the Lower Cambrian Chengjiang Lagerstätte, South China. *Gondwana Research* 25, 983–990.
- Edgecombe, G.D., Chatterton, B.D.E., Waisfeld, B.G. & Vaccari, N.E. 1998: Ordovician (Whiterock) calymenid and encrinurid trilobites from the Precordillera of Argentina. *Journal of Paleontology* 72, 678–697.
- Eiserhardt, K.-H., Koch, L. & Eiserhardt, W.L. 2001: Revision des Ichnotaxon *Tomaculum* Groom, 1902. *Neues Jahrbuch für Geologie und Paläontologie Abhandlungen* 221, 325–358.
- Fortey, R.A. 2000: Olenid trilobites: The oldest known chemoautotrophic symbionts?. *PNAS* 97, 6574–6578.
- Fortey, R.A. & Cocks, R.L.M. 2003: Palaeontological evidence bearing on global Ordovician-Silurian continental reconstructions. *Earth-Science Reviews* 61, 245–307.
- Gabbott, S.E., Hou, X.G. & Siveter, D.J. 2004: Preservation of early Cambrian animals of the Chengjiang biota. *Geology* 32, 901–904.
- Grimes, S.T., Davies, K.L., Butler, I.B., Brock, F., Edwards, D., Rickard, D., Briggs, D.E.G. & Parkes, R.J. 2002: Fossil plants from the Eocene London Clay: the use of pyrite textures to determine the mechanism of pyritisation. *Journal of the Geological Society* 159, 493–501.
- Groom, T. 1902: The sequence of the Cambrian and associated beds of the Malvern Hills. *Quarterly Journal of the Geological Society* 58, 89–135.
- Gutiérrez-Alonso, G., Fernández-Suárez, J., Gutiérrez-Marco, J.C., Corfu, F., Murphy, J.B. & Suárez, M. 2007: U-Pb depositional age for the upper Barrios Formation (Armorican Quartzite facies) in the Cantabrian zone of Iberia: Implications for stratigraphic correlation and paleogeography. In: Linnemann, U., Nance, R.D., Kraft, P. & Zulauf, G. (Eds.), *The evolution of the Rheic Ocean: From Avalonian-Cadomian active margin to Alleghenian-Variscan collision. Geological Society of America Special Paper* 423, 287–296.
- Gutiérrez-Alonso, G., Gutiérrez-Marco, J.C., Fernández-Suárez, J., Bernárdez, E. & Corfu, F. 2016: Was there a super-eruption on the Gondwanan coast 477 My ago?. *Tectonophysics* 681, 85–94.

- 1 Gutiérrez-Marco, J.C. & Bernárdez, E. 2003: *Un tesoro geológico en la Autovía del Cantábrico.*
2 *El Túnel Ordovícico del Fabar, Ribadesella (Asturias)*. Libro-catálogo de la Exposición
3 homónima. Madrid, Ministerio de Fomento, 398 pp.
- 4 Gutiérrez-Marco, J.C., Albani, R., Aramburu, C., Arbizu, M., Babin, C., García-Ramos, J.C.,
5 Méndez-Bedia, I., Rábano, I., Truyols, J., Vannier, J. & Villas, E. 1996: Bioestratigrafía de la
6 Formación Pizarras del Suevo (Ordovícico Medio) en el sector septentrional de la Escama de
7 Laviana-Suevo (Zona Cantábrica, N de España). *Revista Española de Paleontología* 11, 48–
8 74.
- 9 Gutiérrez-Marco, J.C., Aramburu, C., Arbizu, M., Bernárdez, E., Hacar Rodríguez, M.P., Méndez-
10 Bedia, I., Montesinos López, R., Rábano, I., Truyols, J. & Villas, E. 1999: Revisión
11 bioestratigráfica de las pizarras del Ordovícico Medio en el noroeste de España (Zonas
12 Cantábrica, Asturoccidental-leonesa y Centroibérica septentrional). *Acta Geologica Hispanica*
13 34, 3–87.
- 14 Gutiérrez-Marco, J.C., Bernárdez, E., Rábano, I., Sarmiento, G.N., Sendino, M.C., Albani, R. &
15 Bagnoli, G. 2003: Ordovician on the move: geology and paleontology of the “Túnel
16 Ordovícico del Fabar” (Cantabrian free highway A-8, N Spain). *INSUGEO, Serie*
17 *Correlación Geológica* 17, 71–77.
- 18 Hall, J. 1838: Descriptions of two species of trilobites belonging to the genus *Paradoxides*.
19 *American Journal of Science* 33, 139–142.
- 20 Hammann, W. 1974. Phacopina und Cheirurina (Trilobita) aus dem Ordovizium von Spanien.
21 *Senckenbergiana lethaea* 53, 1–151.
- 22 Hammann, W. 1976. Trilobiten aus dem oberen Caradoc der östlichen Sierra Morena (Spanien).
23 *Senckenbergiana lethaea* 57, 35–85.
- 24 Hammann, W. 1983. Calymenacea (Trilobita) aus dem Ordovizium von Spanien; ihre
25 Biostratigraphie, Ökologie und Systematik. *Abhandlungen der senckenbergischen*
26 *naturforschenden Gesellschaft* 542, 1–177.
- 27 Hammann, W. 1992: The Ordovician trilobites from the Iberian Chains in the province of Aragón,
28 NE-Spain. I. The trilobites of the Cystoid Limestone (Ashgill Series). *Beringeria* 6, 1–219.
- 29 Hammann, W. & Leone, F. 1997: Trilobites of the post-Sardic (Upper Ordovician) sequence of
30 southern Sardinia. Part I. *Beringeria* 20, 3–217.
- 31 Hammann, W. & Leone, F. 2007: Trilobites of the post-Sardic (Upper Ordovician) sequence of
32 southern Sardinia. Part 2. *Beringeria* 38, 3–138.
- 33 Hegna, T.A., Martin, M.J. & Darroch, S.A.F. 2017: Pyritised *in situ* trilobite eggs from the
34 Ordovician of New York (Lorraine Group): Implications for trilobite reproductive biology.
35 *Geology* 45, 199–202.
- 36 Henry, J.-L. 1980: Trilobites ordoviciens du Massif Armoricaín. *Mémoires de la Société*
37 *Géologique et Minéralogique de Bretagne* 22, 1–254.

- 1 Hermite, H. 1878 : Étude préliminaire du terrain silurien des environs d'Angers. *Bulletin de la*
2 *Société Géologique de France* [3] 6, 531–543.
- 3 Kennedy, R.J. 1989: Ordovician (Llanvirn) trilobites from SW Wales. *Palaeontographical*
4 *Society Monograph* 141/576, 1–55.
- 5 Kimmig, J. & Pratt, B.R. 2018: Coprolites in the Ravens Throat River Lagerstätte of
6 Northwestern Canada: implications for the middle Cambrian food web. *Palaios* 33, 125–
7 140.
- 8 Laibl, L., Esteve, J. & Fatka, O. 2017: Giant postembryonic stages of *Hydrocephalus* and
9 *Eccaparadoxides* and the origin of lecithotrophy in Cambrian trilobites. *Palaeogeography,*
10 *Palaeoclimatology, Palaeoecology* 470, 109–115.
- 11 Lerosey-Aubril, R. 2007: Early Devonian calymenid larvae (Trilobita) from the Bissounel
12 Formation (Montagne Noire, France). *Bulletin de la Société Géologique de France* 178,
13 197–200.
- 14 Lin, J.-P., Scott, A.C., Li, C.-W., Wu, H.-J., Ausich, W.I., Zhao, Y.-L. & Hwu, Y.-K. 2006:
15 Silicified egg clusters from a Middle Cambrian Burgess Shale-type deposit, Guizhou,
16 South China. *Geology* 34, 1037–1040.
- 17 Lu, Y.-H. & Wu, H.-H. 1982: The ontogeny of *Platycoryphe sinensis* and its bearing on the
18 phylogeny of the Homalonotidae. *Acta Palaeontological Sinica* 21, 37–56.
- 19 Marsille, L. 1910: Note sur la faune silurienne des environs de Malestroit (Morbihan). *Bulletin de*
20 *la Société Polymathique du Morbihan* 1910, 20–36.
- 21 Martin, D., Briggs, D.E.G. & Parkes, R.J. 2005: Decay and mineralization of invertebrate eggs.
22 *Palaios* 20, 562–572.
- 23 McEdward, L.R. 1997: Reproductive strategies of marine benthic invertebrates revisited:
24 Facultative feeding by planktotrophic larvae. *The American Naturalist* 150, 48–72.
- 25 Merinero, R., Lunar, R., Martínez-Frías, J., Somoza, L. & Díaz-del-Río, V. 2008: Iron
26 oxyhydroxide and sulphide mineralization in hydrocarbon seep-related carbonate submarine
27 chimneys, Gulf of Cadiz (SW Iberian Peninsula). *Marine Petroleum Geology* 25, 706–713.
- 28 Orr, P.J., Briggs, D.E.G., Siveter, D.J. & Siveter, D.J. 2000: Three-dimensional preservation of
29 a non-biomineralized arthropod in concretions in Silurian volcanoclastic rocks from
30 Herefordshire, England. *Journal of the Geological Society* 157, 173–186.
- 31 Pastor-Galán, D., Gutiérrez-Alonso, G. & Weil, A.B. 2011: Orocline timing through joint
32 analysis: Insights from the Ibero-Armorican Arc. *Tectonophysics* 507, 31–46.
- 33 Péneau, J. 1944: Présentation des corps fossiles désignés dans les collections comme “Oeufs de
34 Trilobites” dans l’Ordovicien moyen du Synclinal d’Angers (séance du 26 juin 1941). *Bulletin*
35 *de la Société d’Études Scientifiques d’Angers, Nouvelle Série* 68–73, 13.
- 36 Pereira, S., Marques da Silva, C., Pires, M. & Sá, A.A. 2015: The oldest brachymetopid trilobite
37 record from the European peri-Gondwana. *Bulletin of Geosciences* 90, 546–553.

- 1 Rábano, I. 1989: Trilobites del Ordovícico Medio del sector meridional de la zona Centroibérica
- 2 española. Parte III. Calymenina y Cheirurina. *Boletín Geológico y Minero* 100, 767–841.
- 3 Rábano, I. 1990: Trilobites del Ordovícico Medio del sector meridional de la zona Centroibérica
- 4 española. *Publicaciones Especiales del Boletín Geológico y Minero*, 233 pp.
- 5 Raymond, P.E. 1920: The appendages, anatomy, and relationships of trilobites. *Connecticut*
- 6 *Academy of Arts and Science Memoirs* 7, 1–169.
- 7 Romano, M. 1980: The trilobite genus *Eccoptochile* from the Ordovician of northern Portugal.
- 8 *Palaeontology* 23, 605–616.
- 9 Rouault, M. 1847: Extrait du mémoire sur les Trilobites du Département d'Île-et-Vilaine.
- 10 *Bulletin de la Société Géologique de France* [2] 4, 309–328.
- 11 Shen, Y.B. & Huang, D.Y. 2008: Extant clam shrimp egg morphology: Taxonomy and
- 12 comparison with other fossil branchiopod eggs. *Journal of Crustacean Biology* 28, 352–360.
- 13 Shu, D., Vannier, J., Luo, H., Chen, L., Zhang, X. & Hu, S. 1999: Anatomy and lifestyle of
- 14 *Kunrningella* (Arthropoda, Bradoriida) from the Chengjiang fossil Lagerstätte (lower
- 15 Cambrian; Southwest China). *Lethaia* 32, 279–298.
- 16 Siveter, D.J., Siveter, D.J. Sutton, M.D. & Briggs, D.E.G. 2007: Brood care in a Silurian
- 17 ostracod. *Proceedings of the Royal Society B* 274, 465–469.
- 18 Siveter, D.J., Tanaka, G., Farrell, Ú.C., Martin, M.J., Siveter, D.J. & Briggs, D.E.G. 2014:
- 19 Exceptionally preserved 450-Million-year-old Ordovician ostracods with brood care.
- 20 *Current Biology* 24, 801–806.
- 21 Speyer, S.E. & Chatterton, B.D.E. 1989: Trilobite larvae and larval ecology. *Historical Biology*
- 22 3, 27–60.
- 23 Steiner, M., Zhu, Mao-yan, Li, Guo-xiang, Qian, Yi, & Erdtmann, B.-D. 2004: New Early
- 24 Cambrian bilaterian embryos and larvae from China. *Geology* 32, 833–836.
- 25 Thiéry, A. & Gasc, C. 1991: Resting eggs of Anostraca, Notostraca and Spinicaudata (Crustacea,
- 26 Branchiopoda) occurring in France: Identification and taxonomical value. *Hydrobiologia*
- 27 212, 245–259.
- 28 Torsvik, T.H. & Cocks, L.R.M. 2017: *Earth History and Palaeogeography*. Cambridge
- 29 University Press, Cambridge, 332 pp.
- 30 Vaněk, J. 1965: New species of the suborder Calymenina Swinnerton, 1915 (Trilobita) from the
- 31 Barrandian area. *Sborník Geologických Věd, Paleontologie* 6, 21–37.
- 32 Walcott, C.D. 1879: Note upon the eggs of the trilobite. *New York State Museum of Natural*
- 33 *History* 31st Regent's Report, 66–67.
- 34 Whittington, H.B. & Hughes, C.P. 1972: Ordovician geography and faunal provinces deduced
- 35 from trilobite distribution. *Philosophical Transactions of the Royal Society of London B* 263,
- 36 235–278.

Zhang, X.G. & Pratt, B.R. 1994: Middle Cambrian arthropod embryos with blastomeres.
Science 266, 637–639.

Fig. 1. A, Geological sketch map of the area between Caravia and Ribadesella (Asturias, northern Spain) showing vertically dipping outcrops of the Barrios and Sueve formations (Ordovician) in the northeastern ends of the Laviana (W) and Rioseco nappes, and the path of the A8 free highway, including the El Fabar tunnel (thick black line). The star indicates the tunnel section through the Sueve Formation. B, Stratigraphical column of the main units traversed by the tunnel, showing the position of the studied horizons in the Sueve Formation (TUN-055 and -057) as well as significant beds and fossils occurring in the Barrios Formation: 1, trilobites; 2, *Cruziana* (trace fossil); 3, *Skolithos* pipe rock; 4, phyllocarids; 5, organic-walled microfossils; 6, graptolites; and 7, lingulid shell bed. Members within the Sueve Formation are as follows: a, Cerracín; b, Bayo; c, Cofiño; Or, Oretanian and Db, Dobrotivian.

Fig. 2. **A**, Internal mould of *Prionocheilus mendax* (Vaněk, 1965) from TUN-097 Cofiño Member, upper Darriwilian (MGM-2554O). **B**, Internal mould of *Placoparia* (*Coplacoparia*) *tournemini* (Rouault, 1847) with abraded pygidium from the sample TUN-057 (MGM-2560O). Scale bars, 5 mm (A) and 10 mm (B).

Fig. 3. Post-embryonic stages of *Prionocheilus mendax* (Vaněk, 1965) from the sample TUN-55, Cofiño Member, upper Darriwilian. **A**, **E**, Internal mould and latex cast of the first benthonic stage, B1 *sensu* Chatterton *et al.* 1990 (MGM-2795O-1). **B**, **F**, Internal mould and latex cast of the first benthonic stage, B1 *sensu* Chatterton *et al.* 1990 (MGM-2795O-2). **C**, Internal mould of a meraspid degree 2, see disarticulated free cheek bearing tiny spines along the margin (MGM-2800Oa-1). **D**, Internal mould of a meraspid degree 2, with disarticulated pygidium (MGM-2800Oa-2). **G**, **H**, Latex cast and internal mould of a meraspid degree 2 (MGM-2800Ob-3). Scale bars, 1 mm (C, D, H), 0.5 mm (A, B, G) and 0.2 mm (E, F).

Fig. 4. Possible trilobite eggs from the sample TUN-057, Darriwilian of northern Spain (MGM-8111O). **A–C**, cluster photographed under natural light showing pyrite shine (**A**), coated with MgO (**B**) and in ESEM image using retrodispersed electrons (**C**). **D**, Upper-left sphaeroid of cluster shown in **A–C**, showing the outer phyllosilicated envelope (arrowed). **E**, Detail of polygonal pattern on the surface of a sphaeroid indicating incomplete crystalline growth. **F**, Two sphaeroids, the left-one showing a fracture. **G**, **H**, Detail of sphaeroid on left hand side of **F** with aggregates of pyrite framboids and a octahedral pyrite crystal (at the bottom of **H**) seen in the fracture. **I**, Cluster of enrolled specimens of *Placoparia* (*Coplacoparia*) *tournemini* (Rouault) from bed TUN-057 (MGM-2564O). Figures **E–H** were taken using an Environmental SEM in the National Museum of Natural Sciences, Madrid. Scale bars = 10 mm (**A–C**), 5 mm (**F**, **I**), 100 μ m (**D**, **G**) and 50 μ m (**E**, **H**).