Pyritized *in situ* trilobite eggs from the Ordovician of New York (Lorraine Group): Implications for trilobite reproductive biology

Thomas A. Hegna[1]*, Markus J. Martin[2], and Simon A.F. Darroch[3]

[1]Department of Geology, Western Illinois University, 113 Tillman Hall, 1 University Circle, Macomb, Illinois 61455, USA
[2]371 Pawling Street, Watertown, New York 13601, USA
[3]Department of Earth and Environmental Sciences, Vanderbilt University, Nashville, Tennessee 37235, USA

ABSTRACT

Despite a plethora of exceptionally preserved trilobites, trilobite reproduction has remained a mystery. No previously described trilobite has unambiguous eggs or genitalia preserved. This study reports the first occurrence of *in situ* preserved eggs belonging to *Triarthrus eatoni* (Hall, 1838) trilobites from the Lorraine Group in upstate New York, USA. Like other exceptionally preserved trilobites from the Lorraine Group, the complete exoskeletons are replaced with pyrite. The eggs are spherical to elliptical in shape, nearly 200 µm in size, and are clustered in the genal area of the cephalon. The fact that the eggs are smaller than the earliest-known trilobite ontogenetic (protaspis) stage suggests that trilobites may have had an unmineralized preliminary stage in their ontogeny, and that the protaspis shield formed only after hatching. The eggs are only visible ventrally with no dorsal brood pouch or recognized sexual dimorphism. The location of the eggs is consistent with where modern female horseshoe crabs release their unfertilized eggs from the ovarian network within their head. Trilobites likely released their gametes (eggs and sperm) through a genital pore of as-yet unknown location (likely near the posterior boundary of the head). If the *T. eatoni* reproductive biology is representative of other trilobites, they spawned with external fertilization, possibly the ancestral mode of reproduction for early arthropods. Because pyritization preferentially preserves the external rather than internal features of fossils, it is suggested that there is likely a bias in the fossil record toward the preservation of arthropods that brood eggs externally: arthropods that brood their eggs internally are unlikely to preserve any evidence of their mode of reproduction.
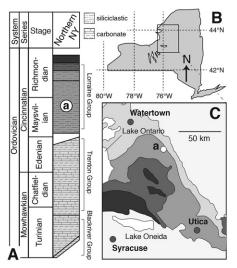
Figure 1. Origin of the pyritized, egg-bearing specimens. A: Geologic and stratigraphic context (after Farrell et al., 2011). B: Map of New York State (northeastern USA); box indicates location of the inset map. C: Inset map showing the context Ordovician geology near the site of the Martin Quarry (a).

INTRODUCTION

Trilobites are one of the most recognizable and fascinating Paleozoic fossils. Despite the fact that we know much about their ontogeny, ecology, and evolution, almost nothing is known about how they reproduced. Much of what has been published about trilobite reproduction has been based on ad hoc interpretations of supposed associations of trilobites preserved while copulating (Endo and Resser, 1937; Hu, 1971) and dimorphic exoskeletal features that suggest brooding pouches (Fortey and Hughes, 1998). By knowing more about their reproductive biology, we expand our knowledge about trilobite autecology and can begin to address longstanding research questions about trilobite mating behavior and reproductive strategies. All of these questions will ultimately help illuminate a much bigger question, i.e., what was the primitive mode of reproduction for arthropods?

Until recently, little was known about how any arthropod reproduced in the Paleozoic; several papers have helped address this issue (Briggs et al., 2016a, 2016b; Caron and Vannier, 2016;

Duan et al., 2014; Siveter et al., 2007, 2014). These contributions implicitly suggest that external brooding was common among early arthropods, i.e., the eggs or developing embryos were carried externally under the carapace (Caron and Vannier, 2016; Siveter et al., 2007, 2014), attached to limbs (Duan et a., 2014), or attached to enigmatic trailing filaments (Briggs et al., 2016a, 2016b; see Piper, 2016, for an alternate interpretation).

MATERIALS AND METHODS

The descriptions below are based on two specimens of *Triarthrus eatoni* (Hall, 1838) from the Martin Quarry in the Whetstone Gulf Formation, Lorraine Group (Fig. 1; see Farrell et al., 2009, for stratigraphic description). The Whetstone Gulf Formation is a set of dark mudstones and siltstones that represent distal turbiditic sedimentation in a deep-water, low-oxygen environment (Farrell et al., 2011). The paleoenvironmental conditions at the Martin Quarry are relatively similar to those at Beecher's Trilobite Bed (Farrell and Briggs, 2008, Farrell et al., 2009).

The specimens were collected and prepared by one of us (Martin) using an air abrasion

system. Computed tomography (CT) data sets of one of the fossils were obtained using the North Star Imaging micro-CT scanner housed at Vanderbilt University (Tennessee, USA), at a resolution (i.e., voxel size) of 12.3 µm (see the GSA Data Repository[1] for details of scan parameters). CT data sets were turned into .bmp stacks, and then segmented using Image-J and the SPIERSedit and SPIERSview software packages (see Sutton et al., 2012) to produce a three-dimensional (3-D) digital model of the specimen. They are housed at the Yale Peabody Museum (Connecticut, USA; specimen prefix YPM).

DESCRIPTION

When viewed from above, the egg-like structures (Figs. 2C, 2E, 2F, 2K, and 2L) are ovoid bodies averaging 167 µm (n = 6, range = 159–177
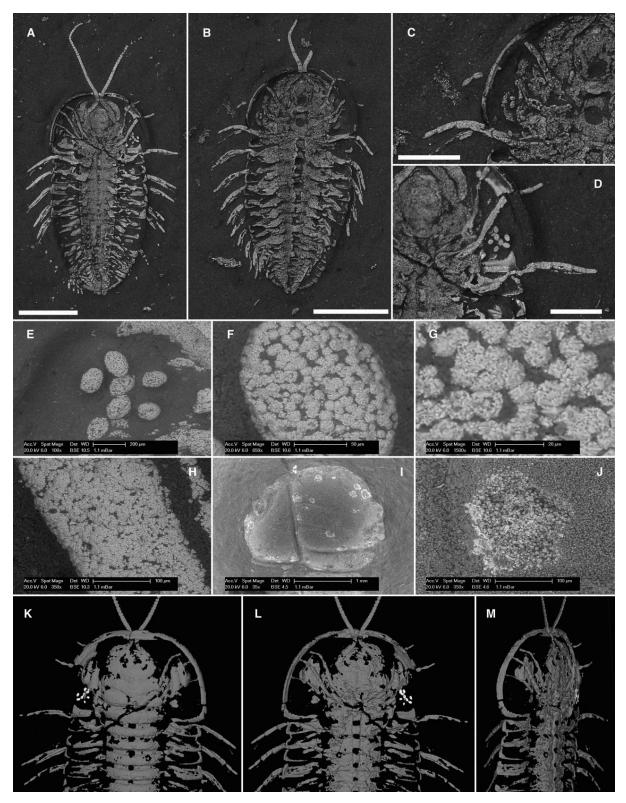
*E-mail: ta-hegna@wiu.edu

Figure 2. Pyritized specimens of *Triarthrus eatoni* from the Ordovician Whetstone Gulf Formation (Lorraine Group), upstate New York (USA). A: Ventrally preserved specimen (Yale Peabody Museum [YPM] 535703) showing nine eggs in the specimen's left genal angle. B: Ventrally preserved specimen (YPM 535704) showing four eggs in the specimen's right genal angle. C: Close-up of the egg-bearing region from the specimen in B. D: Close up of the egg-bearing region from the specimen in A. E: Scanning electron microscopy (SEM) image of the eggs from the specimen in A and D. Note that the perspective is twisted ~180° from that in D. F: SEM image of an egg from E. G: Close-up SEM image of the egg surface from F. Note the dominant framboids and rare euhedral crystals of pyrite. H: Close-up of a limb from A. Note the dominant framboids and rare euhedral crystals of pyrite. I: SEM image of a disarticulated cranidium (YPM 238366) that was replaced with pyrite. J: SEM image showing the replacement fabric from I. Note the dominant euhedral crystals of pyrite. K–M: Dorsal digital reconstruction of the specimen in A derived from microcomputed tomography scan data. L: Ventral reconstruction. M: Left-ventral reconstruction. Scale bars in A and B are 5 mm long; scale bars in C and D are 2 mm long.

µm) in length along long axes. When viewed from the side, they are compressed much like the trilobite itself (which is compressed along the dorsal-ventral axis). The structures appear solid in the micro-CT scan data with no internal detail preserved. They are replaced dominantly with framboidal pyrite and rare euhedral pyrite crystals (Figs. 2F and 2G). This is similar to the pattern of pyritization observed for the trilobite legs (Fig. 2H), but is different than that observed for the exoskeleton (dominated by euhedral crystals with rare framboids; Figs. 2I and 2J). The eggs have thus far only been identified on the underside of cephala belonging to *T. eatoni*. They are located in the genal angle set away from the doublure of one side of the cephalon (the egg-bearing side varies from trilobite to trilobite). The eggs have been observed in clusters of three to nine eggs. This is likely a low estimate; eggs may have been accidentally destroyed during the air abrasion preparation process, or additional eggs may remain hidden in the sediment (although micro-CT scans have not yet revealed any hidden masses of eggs). There is no evidence for any sort of connecting filaments or egg pouch that would indicate how the eggs were attached to the cephalic region.

Due to the small number of specimens available for study, there is no observed relationship between the size of the trilobite and presence of eggs, or proportion of egg-bearing specimens.

DISCUSSION

The structures described here do not fit alternate interpretations. They are too large to be microbial fossils. Their distribution on the exoskeleton would be puzzling for a type of small epibiont, a fecal pellet, or localized pyrite growth. Thus, the structures more readily fit an egg interpretation. Their size, though small, is within the size range known from other modern arthropods (e.g., Shen and Huang, 2008; Thiéry and Gasc, 1991) and fossil arthropods (Duan et al., 2014; Siveter et al., 2014). Notably, the eggs are somewhat smaller than the earliest growth stage of trilobites, the protaspis. Edgecombe et al. (2005) illustrated triarthrinid trilobite protaspids that were ~600 µm long, and Månsson and Clarkson (2012) illustrated olenid protaspids that were as small as ~270 µm. This difference in size suggests that trilobites may have an early, unmineralized stage in their life cycle that preceded the protaspis phase. They are nearly the same size as the enigmatic phaselus larva attributed to trilobites by Fortey and Morris (1978). The eggs are quite small compared to the body size of *T. eatoni*: although the eggs of the Cambrian bradoriid arthropod *Kunmingella douvillei* are approximately the same size as *T. eatoni* eggs, its body is much smaller (giving *K. douvillei* proportionally larger eggs; Duan et al., 2014). The number of eggs carried by *T. eatoni* is rather small by arthropod standards

(e.g., *K. douvillei*), but overlaps with the range of variation observed for the Cambrian arthropod *Waptia fieldensis* (Caron and Vannier, 2016).

While carrying eggs on the head may seem unusual, it is far from unknown in arthropods. Fortey and Hughes (1998) proposed that trilobites brooded their eggs in anterior cephalic pouches. One of the trilobites' closest living relatives, the horseshoe crab, carries its unfertilized eggs internally within a prosomal ovarian network within the head (Hong, 2011), though they do not brood them externally.

This is not the first time that supposed trilobite eggs have been putatively identified; however, it is the first time that eggs have been positively identified in association with a body fossil. This firmly establishes the identity of the eggs. Previous putative trilobite eggs have lacked such a close association with a body fossil (Barrande, 1872; Lin et al., 2006; Wetzel, 1968). Barrande (1872) reported some egg-like structures in the glabela of *Parabarrandia crassa* (Barrande, 1872), but they were housed in an area now known to have held the gut tract (Lerosey-Aubril et al., 2012) and have since been reinterpreted as fecal pellets (Brunthansová and Kraft, 2003). The egg-like structures reported by Billings (1870) and Walcott (1879, 1881) are from thin sections and have been treated with skepticism (Raymond, 1920).

If these structures are indeed eggs, what can we infer about trilobite biology from them? First among the inferences we can make is the position of the eggs: are we seeing eggs still inside the ovaries, or are they being held externally? Due to the fact that internal organs are not frequently preserved during the pyritization of Beecher's Bed trilobites (with the rare exception of putative gut tracts; see Cisne, 1981), the eggs are likely to be external to the body and within the same geochemical microenvironment that led to the pyritization of the exoskeleton. Trilobites may have had innocuous paired genital pores hidden at the base of one of the anterior appendages, much like modern horseshoe crabs, rather than external genitalia. If this suggestion is true, the location of the eggs in the genal angle and the comparison with modern horseshoe crabs suggest that the location of the paired genital pores was near the back of the cephalon (as suggested by Raymond, 1920). No exceptionally preserved trilobite has ever preserved evidence of external genitalia or specialized appendages for sperm transfer or carrying eggs, supporting the idea of innocuous genitalia and thus external fertilization. Almost no exceptionally preserved arthropods from the early Paleozoic preserve any evidence of external genitalia (eurypterids are the exception to this; they have well-known genital appendages; see Kamenz et al., 2011). Having genital pores makes it unlikely that trilobites engaged in any sort of copulation (i.e., intercourse with

a penetrative organ; for examples of supposed copulating trilobites, see Endo and Resser, 1937; Hu, 1971) and even less likely that they had internal fertilization (Fortey and Hughes, 1998). Thus, it is ambiguous whether the eggs present in the specimens are fertilized. It is possible that trilobites may have had internal fertilization via exchange of spermatophores, but they certainly had no specialized morphology to pass along the spermatophores. Thus, external fertilization was likely for trilobites, and was probably the primitive mode for fertilization in Arthropoda.

In the past, authors seem to have imprinted the idea of mammalian-style sex onto their interpretations of trilobite reproduction, going so far as to interpret some specimens as being fossilized in the act of copulation (Endo and Resser, 1937; Hu, 1971). These trilobites with fossilized eggs preserved make it exceedingly unlikely that any trilobites will be discovered in copula, because animals without external genitalia generally do not copulate. Instead, trilobites may have spawned like horseshoe crabs, with males clustering by females to ensure fertilization. This is supported by accumulations of trilobite exoskeletons that have been previously interpreted as possible evidence of spawning behaviors (e.g., Karim and Westrop, 2002).

A number of fossil arthropods known from the early Paleozoic preserve evidence for carrying their eggs (or developing young) externally (Briggs et al., 2016a; Caron and Vannier, 2016; Duan et al., 2014; Siveter et al., 2007, 2014). One might be tempted to infer from these that external brooding was the norm for early Paleozoic arthropods. However, there is a possible taphonomic bias at play. External structures, as opposed to internal organs, are much easier to preserve and interpret. Arthropods that did not brood their young would not preserve any evidence of reproductive behaviors; this would be indistinguishable from males or nongravid females from species that practiced brooding of young. Arthropods that laid eggs and left them are unlikely to be preserved in association with their eggs.

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