\prod The University of Georgia

Symbiotic Trilobite Morphology in the Cambrian: Phylogeny versus Environment **Douglas L. John and Sally E. Walker** Dept. of Geology, University of Georgia, 210 Field St., Athens, Georgia, USA d.john@uga.edu

Abstract

The early to middle Cambrian represents a period of mostly warm, stratified oceans conducive to the formation of dysoxic (low-oxygen, <.01 ml/l O2) conditions. These conditions may have driven evolutionary changes in benthic organisms such as trilobites. Previous work established a set of qualitative morphological characters associated with olenimorph trilobites (i.e., *Hypermecaspis*) that may infer symbiosis with bacteria as an adaptation for life in dysoxic habitats. The ubiquity of this symbiotic morphology in the Cambrian is not well known, with the exception of *Elrathia kingii* from the Wheeler Shale. Our study determined if symbiotic morphology was common, and whether this morphology occurred in oxic and dysoxic habitats within eight Cambrian trilobite-rich Lagerstätten. We also examined how symbiotic morphology varied between genera from three major trilobite orders.

Symbiotic morphology was converted into a ranked character matrix from which non-metric multidimensional scaling (NMDS) scores were generated for 67 Cambrian trilobite genera. The NMDS scores described overall fitness to dysoxic conditions with higher scores indicating high fitness for symbiosis, while lower scores indicated poor fitness. Trilobites scored most commonly in the moderately well-fit range, driven to more positive values by ptychopariids. Five ptychopariids scored > 0.700, similar to the iconic Ordovician Hypermecaspis (0.777), while Elrathia scored lower (0.535); highest values for corynexochids and redlichiids were 0.340 and 0.193, respectively. There was no significant morphological distinction between oxic and dysoxic habitats. Ptychopariids scored high, regardless of environment, while corynexochids and redlichiids, from predominately dysoxic and oxic environments, respectively, both scored low in fitness for symbiotic morphology. Therefore, phylogenetic bias may be the primary factor that explains symbiotic morphology: olenimorph morphology appears to describe general ptychopariid morphology, rather than a symbiotic adaptation for dysoxia. Yet, some ptychopariids scored similarly to Hypermecaspis, suggesting that symbiosis, if it exists, may be more pervasive among ptychopariids, but is generally rare within Cambrian assemblages.

Introduction

The Cambrian period represented a global climate conducive to the formation of benthic dysoxia, which could also have led to a buildup of free H₂S (euxinia). These dysoxic conditions, when they appeared, would have effected benthic fauna including the common Paleozoic arthropods, the trilobites. Fortey (2000) described a suite of morphological characteristics associated with "olenimorph" trilobites, which he hypothesized were related to adaptation for dysoxic environments through symbiosis with chemoautotrophic sulfur-oxidizing bacteria. Fortey's symbiotic characters included a broad, flat morphology, with abundant thoracic segmentation, which would increase the surface area for harboring symbionts. Symbiotic trilobites would also exhibit atrophied feeding structures and reduced musculature, as symbionts would provide nutrition and the trilobite could maintain a sedentary lifestyle. Lastly, they would have a thin exoskeleton which would have reduced metabolic costs in extremely low-oxygen environments, where predation pressures were reduced.

Fortey's characters were also used by Gaines and Droser (2003) to suggest that the iconic middle Cambrian trilobite Elrathia kingii, from the Wheeler Shale of Utah, may have also been symbiotic, and is the earliest such trilobite yet recognized. However, the extent of symbiotic morphology within trilobites in general has not been systematically examined.

We assessed the ubiquity, antiquity, and potential phylogenetic bias of symbiotic morphology among a suite of widely-distributed Cambrian trilobites using a multidimensional scaling model to quantify their morphologies, and also sought additional candidates for symbiosis. We hypothesize that, because dysoxia was more common in the Cambrian (Frakes et al., 1992; Gill et al., 2011; Xu et al., 2012), symbiotic morphologies should be widespread. Similarly, symbiotic morphology should arise earlier than the middle Cambrian, with the prevalence of dysoxia. Lastly, symbiotic morphology should occur more often in dysoxic conditions, irrespective of taxonomy.

Methods

A model was constructed to quantify and compare trilobite morphologies in four steps: First, eight early-to-middle Cambrian Lagerstätten with well-documented trilobite assemblages were selected (Fig. 1). These Lagerstätten were categorized based sedimentology or geochemistry as "mixed" or "mostly oxic": mixed sites had regular recurring dysoxia; mostly oxic sites had only irregular dysoxia, or dysoxia only below the sediment-water interface. Both types of localities contained individually oxic and dysoxic horizons, though the available data did not allow for identification of trilobites from individual horizons.



Figure 1. Locations of eight early to middle **Cambrian Lagerstätten.** From west to east: 1) Chengjiang, 2) Latham and Spence Shales, 3) Wheeler and Marjum shales, 4) Burgess Shale, 5) Jince, and 6) Emu Bay Shale.

Second, 66 trilobite genera from the eight localities were selected for which morphologic descriptions were complete. The middle Cambrian-Ordovician Hypermecaspis, one of Fortey's (2000) original examples of symbiotic morphology, was also included as a point of orientation for the model. Hypermecaspis, along with *Elrathia*, represent putatively symbiotic morphologies. *Olenellus*, a redlichiid with spiny pleurae, a tapered and complex thorax, and a highly arched carapace, represents a morphology qualitatively unfit for symbiosis (Fig. 2). If the model adequately describes symbiotic morphology, Hypermecaspis and Elrathia should fall near each other at one end, while *Olenellus* falls at the opposite end.

Third, Fortey's qualitative morphological characters were converted to a quantitative ranked character matrix, and the 66 genera plus Hypermecaspis were scored for each of five ranked characters: number of segments, carapace morphology, pleural morphology, hypostome attachment, and exoskeletal ornamentation. Lastly, the matrix was reduced via non-metric multidimensional scaling (NMDS) to a two-dimensiona model. Genera scoring higher on the primary axis of this model (NMDS1) had an overall morphology closer to Fortey's description of symbiotic morphology; those scoring lower had morphologies unsuited for symbiosis. Trilobites scoring very high on NMDS1 were assessed as potential candidates for symbiosis.

Patterns along NMDS1 were broken down and analyzed by taxonomic order (ptychopariid, corynexochid, or redlichiid), by oxic versus mixed environments, and through time. Additional trilobites were regressed against the NMDS model to assess the independent effects of phylogeny and environment. The effect of environment was tested by comparing the Devonian phacopids Phacops (from oxic horizons) and Greenops (from dysoxic horizons); the difference in their NMDS1 scores reflects a purely environmental gradient. The effect of phylogeny was tested by comparing the Ordovician olenid *Triarthrus* to the closely related olenid Hypermecaspis, and then to the more distally related non-olenid olenimorph Elrathia; the difference in their NMDS1 scores reflects phylogenetic distance, and Triarthrus and Hypermecaspis are expected to score more closely to each other than either is to *Elrathia*.



Figure 2. Trilobites with high or low qualitative fitness for symbiosis. Images not to scale. High Fitness: A, Hypermecaspis from the Ordovician of Bolivia (image from www.litmir.net); B, Elrathia from the Cambrian of Utah (image from www.trilobites.info). Low fitness: C, Olenellus from the Cambrian of British Columbia (image from www.westerntrilobites.com)

To assess the robustness of the NMDS model in describing symbiotic morphology, several statistical analyses were performed. A chi-squared test was performed to address sample bias with respect to taxonomy (ptychopariids versus non-ptychopariids) and environment (mixed versus mostly oxic), and showed no significant bias ($\chi^2 = 0.926$, df = 1, and p = 0.336).

The correlation of each pairwise combination of the five character states was determined by a Spearman's rank correlation (Table 1). Each pairwise combination of characters showed moderate to weak correlations. This indicated that each of the five characters were independent of one another, and described unique variation in the dataset, as opposed to two or more codependent characters describing the same variation. It also suggested that there was only one source of variation for the five characters, as opposed to having a second mechanism, potentially not related to symbiosis, that might be controlling two or more characters but not all.

The correlation of each character to NMDS1 was also determined (Table 1). In contrast to the correlations of pairwise characters, correlations between characters and NMDS1 were moderate to high, and all were statistically significant. All characters were better correlated to NMDS1 than to any other character. This suggested that NMDS1 was an adequate proxy for each individual character state, and thus a robust proxy for overall fitness to symbiotic morphology.

Character Segments Carapace morphology (CM) Pleural morpholog (PM) Hypostome attachment (HA) Exoskeletal ornamentation (statistical significance (p > 0.10).

> Table 1. Pairwise correlations and statistical significance between each character state, and between characters and NMDS1.

Methods (cont.)

Testing the Model

Cha	racter Stat (Spe	e Pairwise (arman's rh				
er	СМ	PM	НА	EO	Corr. with NMDS1 (rho)	<i>p</i> -value for corr. with NMDS1
nts	0.03	0.05	0.41	0.23	0.42	0.001
ce blogy (CM)		0.17	0.16	0.12	0.36	0.003
morphology			0.21	0.38	0.57	<0.0001
ome nent (HA)				0.39	0.84	<0.0001
etal entation (EO)					0.61	<0.0001

Note: pairwise correlations for the five character states determined via Spearman's rank correlation; correlation (corr.) of each character to the primary NMDS axis, with a *p*-value for the statistical significance of the correlation; n=66 unique genera plus *Hypermecaspis*. All *p*-values for character state pairwise correlations show no

NMDS1 was an adequate approximation of overall fitness to symbiotic morphology: each of the five ranked characters agreed well with fitness along NMDS1. For all five characters, NMDS1 scores increased monotonically with genera exhibiting character states better-fit to symbiosis (Fig. 3). Ornamentation and hypostome attachment provided the most distinct groupings, with the lowermost group on NMDS1 characterized by both attached hypstomes and ornamentation. Scores on NMDS2 were more ambiguous but did not appear to be related to symbiosis. Hypermecaspis, an example of Fortey's orgininal morphological description, scored high on NMDS1, while Olenellus, a redlichiid with a qualitatively poorly-fit morphology for symbiosis, scored low on NMDS1. This demonstrates the NMDS model matched broad expectations of fitness (Fig. 4).

We found that symbiotic morphology as described by NMDS1 was related to general ptychopariid morphology. Ptychopariids as a taxonomic order scored significantly higher on NMDS1 than corynexochids or redlichiids (t = -6.416, df = 16.85, p < 0.0001), while the latter two were not significantly different from each other (t = 0.412, df = 26.98, p = 0.684) (Fig. 5). Additionally, Lagerstätten with more ptychopariids had higher mean NMDS1 scores. Phylogeny had a stronger influence on NMDS1 scores than environment, and NMDS1 scores, in general, were not significantly different between trilobites from mostly oxic or mixed localities (t = 0.736, df = 63.90, p = 0.465) (Fig. 6). The weak environmental signal suggested that overall morphology was not related to the presence of dysoxia, as would be expected if morphology was related to symbiosis. Likewise, there was no difference within the ptychopariids or redlichiids with respect to mostly oxic versus mixed assemblages. There was a difference within the corynexochids, but the higher-scoring corynexochids (i.e., those with morphologies more fit for symbiosis) occurred in mostly oxic environments, counter to expectations for symbiosis (Fig. 7). Ptychopariids were represented evenly in both mixed and mostly oxic conditions, while corynexochids were predominantly in mixed, and redlichiids were predominantly in mostly oxic; this indicated that corynexochids and redlichiids may have maintained a preference for environment, while the ptychopariids (which contained all genera scoring high on NMDS1) tolerated a wider range of fluctuation in oxygen.

Phacops and Greenops, both Devonian phacopid trilobites but from disparate habitats, scored closely to each other, suggesting that environment does not strongly influence morphology. Hypermecaspis and Triarthrus, both olenids, scored more closely along NMDS1 than either did to Elrathia, a non-olenid olenimorph. All three came from mixed environments. This also suggested that phylogenetic distance impacted NMDS scores more than environment (refer to Fig. 4).

Five out of 66 genera of trilobites (i.e., the ptychopariids Alokistocarella, Altiocculus, Asaphiscus, Germaropyge, and Palaeolenus) were identified as potential candidates for symbiosis. Palaeolenus, from the lower Cambrian Chengjiang, was the earliest potential symbiotic trilobite. These five, plus the previously identified candidate Hypermecaspis, scored higher on NMDS1 than other genera (NMDS1 > 0.700), and occurred primarily in mixed localities. Only Germaropyge from the Jince was interpreted as coming from a mostly oxic environment. These five were represented in six of the eight Lagerstätten; only the oldest Lagerstätte, the Emu Bay, and one with no ptychopariids, the Latham, were without a symbiotic candidate. Symbiotic trilobites remained rare, though, and no site had more than two potentially symbiotic genera (**Table 2**).



biotic trilobites would have less developed musculature, leading to a flatter carapace. C, Pleural morphology, separated into flat, arched, and arched-and-spiny groups. Flat pleurae without spines would provide the most area for symbionts. D, Hypostome (mouthpart) attached or non-attached. Symbiotic trilobites would have simple feeding behavior and a less developed (non-attached) hypostome. E, Exoskeletal ornamentation, either ornamented or non-ornamented. Exoskeletal ornamentation would be an unnecessary metabolic cost for symbiotic trilobites living in dysoxic conditions where predatory pressures are reduced.



Result

Figure 4. NMDS scores for six trilobite genera used to test the robustness of the model, and independently assess the effects of phylogeny and environment.

Locality	Age	Total mean NMDS1	Number of ptychopariids	Ptychopariid mean NMDS1	Moderately fit trilobites (NMDS1 0.500- 0.700)	Well fit trilobites (NMDS1 > 0.700)
Emu Bay	e. Camb. (525 Ma)	-0.459	1	-0.227	0	0
Chengjiang	e. Camb. (525 Ma)	-0.067	2	0.475	0	1
Latham	e. Camb. (515 Ma)	-1.150	0	-	-	-
Spence	m. Camb.	-0.267	4	0.629	1	2
Jince	m. Camb. (510 Ma)	0.294	16	0.399	8	1
Wheeler	m. Camb. (507 Ma)	0.119	9	0.395	2	2
Burgess	m. Camb. (505 Ma)	-0.148	6	0.532	3	1
Marjum	m. Camb. (504 Ma)	0.133	11	0.372	2	2

Table 2. NMDS1 scores by locality, with prevalence of ptychopariids and symbiotic morphology.



Figure 5. Frequency of NMDS1 scores by taxonomic order. Means indicated with dashed line, medians with dotted line.





Mixed Genera

Figure 6. Frequency of NMDS1 scores by environment



Figure 7. NMDS scores by taxonomic order and environment.



Discussion

The NMDS methodology proved useful in converting a set of qualitative morphological characters to a single quantitative approximation that could be assessed statistically. When applied to the 66 trilobite genera, the NMDS model suggested that ptychopariids were collectively more fit to Fortey's description of symbiotic morphology than corynexochids or redlichiids, and that environment wa not a major influence on NMDS1 scores. This suggests that symbiosis is not a major factor that influences broad trilobite morphology However, a subset of trilobites with the highest NMDS1 scores did occur in primarily mixed (i.e., more dysoxic) environments, meeting the expectations for symbiosis.

Five of the 66 trilobite genera were identified as potential candidates for symbiosis, present in six of the eight Lagerstätten. These trilobites, all ptychopariids, represented a discrete group of very high NMDS1 scores (> 0.700) that were associated with mixed localities, as would be expected for symbiotic trilobites. While NMDS1 scores (and thus, morphology) did not appear related to symbiosis among the wider group of trilobites, very high NMDS1 scores could be used in concert with paleoenvironmental information to identipotential symbiotic trilobites. Ptychopariids in general had morphologies innately better-suited for symbiosis, and the most closely-factorial symbiosis and the most closely-factorial symbol and th ptychopariids may have been able to make the transition to symbiosis.

Symbiotic morphology arose early in the Cambrian, with the lower Cambrian ptychopariid Palaeolenus in the Chengjiang, and remained rare, even as ptychopariids as a group flourished in the middle Cambrian. This suggests that while symbiotic morphology arose quickly following the Cambrian explosion, symbiotic trilobites did not significantly flourish or diversify through the middle Cambrian after that initial innovation. Factors driving diversification within the ptychopariids in the middle Cambrian, such as predation or competition, may not have selected as strongly for trilobites in strongly dysoxic habitats, where predatory and competitive pressures were reduced.

Previous studies (Hughes, 2005, 2014; Webster, 2007) have suggested that trilobites in the Cambrian were more morphologically flexible than later trilobites, particularly in segmentation, and particularly immediately after the initial appearance of a group. The Cambrian was a period of intense diversification among trilobites, and the advent of symbiotic morphologies (which involves increasing segmentation) may have been facilitated by an increase in morphological plasticity. Once specialized for dysoxia, however, the morphology may have stabilized and remained fairly constant despite further diversification among the ptychopariids, similar to the findings of Hunda (2008) that trilobites generally show low plasticity in response to environmental change. Changes in oxygenation in the Cambrian also may not have been severe enough to trigger evolutionary changes, whereas trilobites in the Ordovician have been suggested to respond to greater global climatic changes (Hopkins, 2014).

Implications for Modern Arthropods

Modern arthropods show a low tolerance for reduction in oxygenation levels (Rabalais et al., 2002; Vaquer-Sunyer and Duarte, 2008). Arthropods are not a major component of modern dysoxic assemblages (Levin 2003; Sellanes et al., 2010), and symbiosis with bacteria is an uncommon adaptation for arthropods, restricted mainly to sulfidic (but oxic) hydrothermal systems (Petersen et al., 2010

In modern icehouse ocean systems, oxygen is generally well-mixed and dysoxic events are limited in size and duration (Toggweiler and Russell, 2008). Modern arthropods respond to dysoxic events by avoiding dysoxia and recolonizing the benthos after reoxygenation (Lu and Wu, 2008). In the past, particularly in the warmer Cambrian, oceans were more stratified and dysoxic events were more widespread and persistent (Slomp, 2013). Some fossil arthropods that were not able to migrate to more oxic waters when faced with dysoxic conditions went extinct (Williams et al., 2011).

It appears that arthropods are poorly suited to adapting their body plan for low-oxygen conditions. Among our 66 trilobite genera, only those trilobites already closely fit to a symbiotic morphology (i.e., the ptychopariids) showed potential to make a transition to symbiosis. Corynexochids and redlichiids showed a strong preference for environment, suggesting they followed their preferred habitat (or died out) when environmental conditions changed rather than adapt to that change.

As global temperatures continue to rise through the next century, modern oceans may experience stronger dysoxic conditions (after Cannariato and Kennett, 1999), which could lead to extirpation of arthropods within suddenly dysoxic environments and a loss of overall arthropod abundance as suitable habitat is lost. Given the economic importance of many benthic arthropods as food stocks, and their ecological importance as predators and scavengers, more widespread dysoxia could have a distinct effect on both human and environmental sustainability.

References

Cannariato, K. G. and J. P. Kennett. 1999. Climatically related millennial-scale fluctuations in strength of California margin oxygen-minimum zone during the past 60 ky. Geology, 27(11):975-978

Fortey, R. 2000. Olenid trilobites: The oldest known chemoautotrophic symbionts? Proc. Nat. Acad. Sci., 97:6574-6578. Frakes, L. A., J. E. Francis, and J. I. Syktus. 1992. Climate modes of the Phanerozoic. Cambridge University Press, 288 pp.

Gill, B. C., T. W. Lyons, S. A. Young, L. R. Kump, A. H. Knoll, and M. R. Saltzman. 2011. Geochemical evidence for widespread euxinia in the Later Cambrian ocean. Nature, 469:80-83.

Hopkins, M. J. 2014. The environmental structure of trilobite morphologal disparity. Paleobio., 40(3):352-373. Hughes, N. C. 2005. Trilobite construction: building a bridge across the micro- and macroevolutionary divide. Pp. 139-158 in Evolving form and function: proceedings of a symposium honoring Adolf Seilacher for his contributions to paleontology, in honor of his 80th birthday. D.E.G. Briggs, ed., Yale Univeristy, New Haven.

Hughes, N. C. 2014. Morphological plasticity and genetic flexibility in a Cambrian trilobite. Geology, 19:913-916. Hunda, B. R., A. Thompson, A. J. Webber, and N. C. Hughes. 2008. Short-term paleoenvironmentally-related morphological variation in the Ordovician trilobite Flexicalymene granulosa.

in Advances in trilobite research, I. Rabano, R. Gozalo, and D. Garcia-Bellido, eds., Cuadernos del Museo Geominero, 9, Instituto Geologico y Minero de Espana, Madrid, pp.

Levin, L. A. 2003. Oxygen minimum zone benthos: adaptation and community response to hypoxia. Oceanic and Marine Biology: an Annual Review, 41:1-45. Lu, L., and R. S. S. Wu. 2000. An experimental study on recolonization and succession of marine macrobenthos in defaunated sediment. Marine Biology, 136:291-302.

Petersen, J. M., A. Ramette, C. Lott, M. A. Cambon-Bonavita, M. Zbinden, and N. Dubilier. 2010. Dual symbiosis of the vent shrimp Rimicaris exoculata with filamentous gamma- and epsilonproteobacteria at four Mid-Atlantic Ridge hydrothermal vent fields. Env. Microbio., 12:2204-2218.

Rabalais, N. N., R. E. Turner, and W. J. Wiseman, Jr. 2002. Gulf of Mexico Hypoxia, AKA "The Dead Zone". Ann. Rev. Ecol. System., 33:235-263.

Sellanes, J., C. Neira, E. Quiroga, and N. Teixido. 2010. Diversity patterns along and across the Chilean margin: a continental slope encompassing oxygen gradients and methane seep benthic habitats. Marine Ecology, 31(1):111-124.

Slomp, C. P. 2013. Reconstructing the history of euxinia in a coastal sea. Geology, 41:523-524.

Toggweiler, J. R., and J. Russell. 2008. Ocean circulation in a warming climate. Nature, 451(7176):286-288.

Vaguer-Sunyer, R., and C. M. Duarte. 2008. Thresholds of hypoxia for marine biodiversity. Proc. Nat. Acad. Sci., 105:15452-15457 Webster, M. 2007. A Cambrian peak in morphological variation within trilobite species. Science, 317:499-502.

Williams, M., J. Vannier, L. Corbari, and J. C. Massabau. 2011. Oxygen as a driver of early arthropod micro-benthos evolution. PLoS ONE, 6(12):e28183.

Acknowledgements

The first author gratefully acknowledges the support of the Geological Society of America, the Watts-Wheeler Fund at the University of Georgia, the Society for Sedimentary Geology (SEPM), and the UGA Office of the Vice President for Research. We also thank Steve Holland of the University of Georgia for assistance with statistical methods.