QUANTITATIVE ANALYSIS OF REPAIRED AND UNREPAIRED DAMAGE TO TRILOBITES FROM THE CAMBRIAN (STAGE 4 - DRUMIAN) IBERIAN CHAINS, NE SPAIN

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RRH: BROKEN AND REPAIRED CAMBRIAN TRILOBITES FROM NE SPAIN

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ABSTRACT

Repaired fossil skeletons provide the opportunity to study predation rates, repair mechanisms, and ecological interactions in deep time. Trilobites allow the study of repaired damage over long time periods and large geographic areas due to their longevity as a group, global distribution, and well-preserved mineralized exoskeletons. Repair frequencies on trilobites from three sites representing offshore marine environments in the Iberian Chains (Spain) show no injuries on 45 complete redlichiid thoraces from Minas Tierga (Huérmeda Formation, Cambrian Series 2, Stage 4), or 23

complete *Eccaparadoxides pradoanus* thoraces from Mesones de Isuela (Murero Formation, Cambrian Series 3, Drumian). 10 injuries on 69 *E. pradoanus* thoraces from Purujosa (Murero Formation, Cambrian Series 3, Drumian) were noted. There is no evidence for laterally asymmetric predation or size selection on the trilobites in this study. Weak evidence for selection for the rear of the thorax is documented. A series of injured trilobites illustrates four stages of the healing process. Analysis of injury locations and frequency suggests that injuries to these trilobites are predatory in origin. Semilandmark analysis of previously described exoskeletons with unrepaired damage assigned to the ichnotaxon *Bicrescomanducator serratus* alongside newly collected damaged exoskeletons from Purujosa (Mansilla and Murero Formations, Stage 5 - Drumian), Mesones de Isuela (Murero Formation, Drumian), and Minas Tierga (Huérmeda Formation, Stage 4) found that shapes of biotic and abiotic breaks could not be distinguished.

INTRODUCTION

Predator-prey interactions, including sub-lethal and lethal damage, have been studied using repaired exoskeletons, shells, drillholes, and broken sclerites, exemplifying the importance of predation as a potential evolutionary driver (e.g., Vermeij 1987; Kowalewski et al. 1998; Kowalewski 2002; Kelley et al. 2003). Repaired injuries preserved in different depositional environments and on skeletons of a wide variety of prey offer direct evidence of biotic interaction between predators and prey (e.g., Kowalewski 2002; Babcock 2003). Studies on repaired injuries have shown that the morphology of prey affects the frequency of repaired damages (e.g., Alexander 1986; Dietl et al. 2000; Alexander and Dietl 2001; Dietl 2003a, b; Dietl and Hendricks 2006; Harper et al. 2009), and morphological characters such as spiny shells and reduced aperture sizes are adaptations in response to predation pressure

(Vermeij 1977, 1987; Kelley 1989; Klompmaker and Kelley 2015). Potential physical defensive adaptations in trilobites include growth to large size (e.g., *Paradoxides davidis* – Bergström and Levi-Setti 1978), development of additional spines and lengthening existing spines (e.g., *Psychopyge elegans* – Morzadec 1988), and thickening of the exoskeleton. Behavioral defensive adaptations include enrolment (e.g., *Eccaparadoxides pradoanus* – Esteve et al. 2011, 2013), burrowing (e.g., *Symphysurus angustatus* – Fortey 1986), or infaunal habit (e.g., *Paciphacops* - Rustán et al. 2011). Finally, some trilobites occupied low oxygen environments, potentially as refugia from predation (e.g., *Elrathia kingii* – Gaines and Droser 2003). Many of these adaptations (enrolment, occupation of low oxygen environments, growth of elongated pleural spines) originated in the Cambrian, perhaps driven by predatory pressure. The sophistication of predatory behavior also increased over time, showing the importance of predator-prey escalation as an evolutionary driver (Kowalewski et al. 1998; Brett and Walker 2002; Aberhan et al. 2006).

Trilobites were preyed upon since the Cambrian, and have been reported in the gut contents of *Ottoia prolifica* Walcott 1911, *Sidneyia inexpectans* Walcott 1911, *Wisangocaris barbarahardyae* Jago et al. 2016, and a *Fuxianhuia*-like arthropod (Conway Morris 1977; Bruton 1981; Zhu et al. 2004; Vannier 2012; Zacaï et al. 2015; Jago et al. 2016) as well as in coprolites (Sprinkle 1973; Conway Morris and Robison 1988; Nedin 1999; Babcock 2003, Skinner 2005; Vannier and Chen 2005; English and Babcock 2010; Daley et al. 2013; Kimmig and Strotz 2017). Such examples are only recorded in exceptional preservation fossilization events, whereas damage and repair of mineralized trilobite exoskeletons are more easily preserved (Lochman 1941; Sinclair 1947; Šnajdr 1978; Rudkin 1979; Owen 1985; Babcock 1993). Trilobite abnormalities and repair have been attributed to predation, problematic moulting, genetic malfunction, parasites, and accidental damage. The predation or scavenging trace fossil taxon *Bicrescomanducator serratus* (Zamora et al. 2011) describes unrepaired damage on trilobite sclerites that consists of asymmetric V- or W-shaped serrated breakage of variable length, with a first-order path that is straight, or sometimes slightly arcuate (Zamora et al. 2011; Buatois et al. 2017). This trace can be seen on trilobite exoskeletons and fragments from the Cambrian Series 3 (Drumian) Purujosa 3 section of the Murero Formation, NE Spain (Zamora et al. 2011, fig. 2), Cambrian Drumian (Marjuman) section of the Rabbitkettle Formation, SW Canada (Pratt 1998, figs. 8, 9, 10), and the Ordovician Valongo Formation in Portugal (Sá and Gutiérrez-Marco 2015, fig. 12), and is attributed to *Anomalocaris* in the Cambrian and orthoceratids in the Ordovician (Zamora et al. 2011; Sá and Gutiérrez-Marco 2015). Sá and Guitiérrez-Marco (2015) synonymized *Mandibulichnus* Zamora et al. 2011 with *Bicrescomanducator* Donovan et al. in Andrews et al. 2010, as both describe irregular asymmetric breaks which occur singularly, with the difference between the type species *Bicrescomanducator rolli* Donovan et al. in Andrews et al. 2010 and *B. serratus* being the shape of the breaks: *B. rolli* is sub-crescentic and *B. serratus* is serrated. Although subsequent authors have continued to use *Mandibulichnus* (e.g., Neto de Carvalho et al. 2016; Buatois et al. 2017), here we treat the differences between these bioerosion traces at the species level, and so use *Bicrescomanducator serratus*. Not all broken trilobite sclerites are caused by the action of predators or scavengers and abiotic damage in trilobites has been recognized (e.g., Webster and Hughes 1999, Webster et al. 2008).

Injuries and abnormalities in trilobites are used to understand the repair mechanisms of exoskeletons, as reviewed in the landmark publication by Owen (1985) and subsequently by Bicknell and Paterson (2017). Trilobite abnormalities can result from injuries, teratologies and pathologies (Owen 1985). Injuries can be caused by predation, accidental damage, intraspecific competition, or damage during moulting (Owen 1985; Babcock 1993). Trilobites healed over a number of moult cycles (e.g., Šnajdr 1978; Owen 1985) that followed an initial callousing and regrowth over the injury (e.g., Schoenemann et al. 2017). When attacked during the soft post-ecdysial stage, trilobite spines could wrinkle or distort (Conway Morris and Jenkins 1985), and rarely additional pleural spines grew from injured areas (Babcock 1993). Regeneration of spines is controlled by segment polarity genes (McNamara and Tuura 2011), and begins during ecdysis after the damage was sustained (Lochman 1941). Regrown spines remain shorter than original spines for a variable number of moults, likely dependent on the severity of the injury. This process is comparable to the regeneration of tail spines in *Daphnia* and crinoid arms (Murtaugh 1981; Baumiller and Gahn 2012).

Examining repaired injuries and drillholes on exoskeletons at various locations and in different formations is important for understanding the variability of predation pressure across space and time (Harper 2016). Such data can also be used to identify stereotypy of predators targeting specific locations on prey exoskeletons or specific prey sizes (e.g., Conway Morris and Bengtson 1994; Leighton 2001, 2011; Robson and Pratt 2007). Previous quantitative studies on repaired trilobites showed non-random distribution of repaired injuries as evidence for predator site selection (Babcock and Robison 1989; Babcock 1993, 2003). When data from Cambrian trilobites was treated statistically it was shown that most scars on trilobites were incurred on the posterior right-hand side of the thorax (Babcock 1993).

Using exoskeletons and broken sclerites of trilobites from three sites (Purujosa, Mesones de Isuela, and Minas Tierga) from two formations (Murero Formation and Huérmeda Formation) in the Iberian Chain, NE Spain, evidence of sub-lethal predation and broken sclerites was recorded. Repaired injuries demonstrate unequivocally damage during the life of the animal. The proportion of injured trilobites at each site, and position of repaired injuries on the exoskeletons are analyzed to provide information about causes, selection pressures, and predation intensity. The location of repaired injuries is statistically tested for lateral asymmetry and anteroposterior selection. Breaks on isolated sclerites from these sites and additional specimens from the Mansilla Formation (Cambrian Series 3, Stage 5) and *Bicrescomanducator serratus* breaks from the literature are assessed using a semilandmark morphometric analysis to quantify the variance of abiotic and *B. serratus* breaks.

GEOGRAPHIC AND GEOLOGICAL SETTING

All trilobites were collected from the Iberian Chain (NE Spain), near Zaragoza (Fig. 1A). Specimens from the Murero Formation (Cambrian Series 3, Drumian) were collected at two localities, the first near the village of Mesones de Isuela (Fig. 1C) and the second near Purujosa (Fig. 1D), specifically the Purujosa 3 section (2 km south of Purujosa village, Zaragoza province) inside the limits of the Moncayo Natural Park (Fig. 1D). The trilobite taxon examined for predation traces at this site, *Eccaparadoxides pradoanus* (Verneuil and Barrande in Prado et al. 1860) is found as abundant broken sclerites, together with ptychopariids in the uppermost red shales of the upper Murero Formation (Fig. 2–star). The Murero Formation at both Mesones de Isuela and Purujosa represents an offshore marine environment (Álvaro and Vennin 1997; Gámez Vintaned et al. 2009). Other biomineralized groups within the faunal assemblage include protorthacean and lingulid brachiopods, sponges, echinoderms, and agnostids (Zamora 2010; Mergl and Zamora 2012). *Eccaparadoxides* is also known from lower levels in the Purujosa 3 section, and both repaired injuries and broken sclerites have been reported (Zamora et al. 2011, fig. 1C). The Mesones de Isuela locality is an outcrop 500 m east of the M3 section (Valenzuela et al. 1990) (Fig. 1C). At the Mesones de Isuela locality, the Murero Formation has been subdivided into two parts: a lower part with green shales alternating with sandy units, and an upper part with red shales. Articulated *E. pradoanus* were collected from upper section of the green shale, just below the appearance of the first sandy unit, alongside numerous isolated broken sclerites of the same taxon (Fig. 2).

The Huérmeda Formation, Cambrian Series 2, Stage 4 (Gozalo et al. 2008) was also deposited in open marine offshore conditions (Gámez Vintaned et al. 2009). It is a monotonous succession of green-grey shales with subsidiary dolostone interbeds (Figs. 1C, 2). Trilobites, mostly redlichiid taxa, are present at the base of the Huérmeda Formation (e.g., Sdzuy 1961; Schmitz 1971; Schmidt-Thomé 1973). Beds consisting of large accumulations of isolated sclerites are found between the beds containing complete trilobites.

MATERIALS AND METHODS

Collection of trilobites

Bulk samples of trilobites were collected from all sites, and broken trilobite sclerites were collected from the Mansilla Formation (Cambrian Series 3, Stage 5) near Purujosa (Figs. 1C, 2), allowing the analysis of broken sclerites to be extended through the Cambrian Stage 4, Stage 5, and Drumian. Specimens collected from the field, studied and illustrated herein, are housed at the Museo de Ciencias Naturales de la Universidad de Zaragoza (MPZ). Additional specimens, collected from the nearby Barranco del Judio and Las Cuevas/Las Coronadas localities (Fig. 1C) and other levels at the Minas Tierga locality (Fig. 2), were examined from private collections (Supp Fig. 1) and the MPZ collections. The data from private specimens were recorded separately from field specimens in case of collection bias in private collections, however as these collectors assisted in the field for this study, collection bias in the private collection is unlikely. Measurements of trilobites and abnormalities were made using digital callipers. Specimens in private collections were measured using photographs and ImageJ.

Measurement collection and observations

Specimens with an equal number of thoracic spines visible on each side were used to calculate potential asymmetry in repair location, and only specimens with complete thoraces were used to calculate repair frequencies and multiple repair frequencies. Other incomplete specimens were not used for any analysis. The pygidium and cephalon were not studied for injuries as they were not always preserved. Comparative data on repaired injuries from the Wheeler Formation was obtained from Babcock (1993). Repaired injuries were identified as shortened spines with recognizable healing, either in the form of rounded edges or partially regrown distal spine tips.

Specimens from the northern part of the Iberian Chains are preserved slightly flattened, but still show some three-dimensional features as fragile regional deformation and carbonate interbeds prevented strong deformation. This is different from specimens from the classic Murero locality, which are more flattened. Other studies on trilobites from the northern Iberian Chains (and the same locality as specimens in this study) have shown three dimensional behaviors, such as enrolment (Esteve et al. 2011, 2013).

Statistics and calculations for repaired injuries

Frequency of repairs.— Repair frequency was calculated using the following metrics to allow direct comparison between sites:

$$\mathrm{F} = \frac{\text{Number of repairs}}{\text{Number of animals}}$$

$$\mathrm{MF} = \frac{\text{Number of animals with} > 1\ \mathit{repaired\ injury}}{\text{Number of animals with 1 repaired injury}}$$

$$\mathrm{R} = \frac{\text{Number of animals with } \geq 1 \text{ repaired injury}}{\text{Number of animals}}$$

Metric F gives an inflated representation of the percentage of individuals damaged and subsequently repaired (Dietl et al. 2000), and metric R gives an underestimated frequency of individuals repaired (Alexander and Dietl 2003). Following Alexander and Dietl (2003), both are presented to mitigate the limitations of both methods. For the Huérmeda Formation, the repair frequency was calculated using all redlichiid trilobites, and for the Murero Formation the repair frequency was calculated using *Eccaparadoxides pradoanus*. Although repair frequencies for the Wheeler Formation could not be calculated from the literature, a multiple repair frequency value, MF, was derived using data from Babcock (1993, p. 222).

Collecting very large sample sizes of complete or near-complete trilobites is not always possible, and this affects the uncertainty of calculated repair frequencies. We use a Bayesian Inference method to estimate the effect of sample size on repair frequencies, calculating 5th and 95th percentile confidence values. This analysis was run in R Studio (R Core Team 2017; see Supp Info 1 for code).

Origin and location of repaired injuries.— Distinguishing between accidental damage, damage due to problems during moulting, or predatory damage is a complicated task when considering the cause of repaired injuries. We propose a statistical method to estimate the likelihood of damage having occurred during moulting. For trilobites that have thoracic spines of approximately equal length and similar morphology, the likelihood that a given spine is injured due to moulting complications is expected be the same as all other spines. This would result in randomly distributed injuries on trilobite exoskeletons, assuming that injuries occurred during the holaspis phase. As segments are added at the posterior of the thorax throughout meraspid stages, individual injuries due to moulting could be more common at the anterior than at the posterior, as these segments undergo more moult stages. For species with particularly long, thin, or intricate spines, this expectation changes as such spines are more susceptible to moulting damage than others. Accidental injuries from copulation, interspecific combat, or unsuccessful predatory attacks would be more likely to injure multiple adjacent spines, and so injured spines would not be randomly distributed across the thorax (Babcock 1993). The ‘stats’ package (R Core Team 2017) in R Studio was employed to do a binomial test, comparing the observed number of adjacent injured spines to the expected distribution of randomly arranged injured spines (see Supp Info 1 for code). A random distribution of injured spines would suggest that moulting was a major cause, whereas a significant number of short spines adjacent to each other supports a predatory or accidental origin of the injuries.

Repair frequencies at Purujosa and Mesones de Isuela were calculated, as these two sites house the same species (*Eccaparadoxides pradoanus*), and were deposited in similar environments of the same age (Cambrian Series 3, Drumian). Repair frequencies were also calculated on trilobites from Minas Tierga (Cambrian Series 2, Stage 4) as they are morphologically similar to *E. pradoanus*, from a similar environment, and geographically close to Mesones de Isuela.

We tested both lateral and anteroposterior selection of injury location. Lateral asymmetry was tested using a two-tailed binomial test, so that selection for either the left or right could be detected. Our null hypothesis was that there is no lateral asymmetry in injury location, so an equal distribution of injuries on the left and right sides is expected. A rejection of this hypothesis supports the existence of lateral asymmetry in injury location. A two-tailed binomial test facilitates the detection of laterally asymmetric selection for the left and right sides of the thorax. A one-sided binomial, as used in other studies (e.g., Babcock and Robison 1989; Babcock 1993, 2003) allows only detection for either the right side or the left side (which must be determined before the analysis is undertaken).

Anteroposterior selection for the most posterior three thoracic segments of the trilobite was also tested using a two-tailed binomial test. These three thoracic segments have posterior-pointing thoracic spines, and would have covered the anterior of the cephalon during enrolment. The null hypothesis was that there was no selectivity in injury location: the probability of injuring each spine is equal. This gives an expected percentage of 18.75% of injuries occurring on the rear three thoracic segments in a thorax of 16 segments. Rejecting this null hypothesis in favour of the alternative would illustrate selection either for the front 13 or rear 3 thoracic segments.

Size distribution and selection.— To test if the size distribution of trilobites was similar between the three sites and to assess whether size impacted the frequency of repaired injury frequency, a Mann-Whitney U test was undertaken using the ‘stats’ package in R Studio. The Mann-Whitney U test is a non-parametric test that determines whether the means of two independent samples are equal. In this case, if the mean lengths of injured trilobites are distinguished from the mean lengths of uninjured trilobites using a Mann-Whitney U test, a size preference for attacks can be demonstrated.

Statistics and calculations for broken sclerites

A morphometric analysis was used to quantitatively assess broken sclerites. A semilandmark analysis of sclerites collected from the Murero Formation (Drumian) the Mansilla Formation (Stage 5), the Huérmeda Formation (Stage 4), *B. serratus* from the Rabbitkettle Formation (Pratt 1998), and Middle Darriwilian Valongo Formation (Sá and Gutiérrez-Marco 2015). Semilandmarking was conducted using the Thin-Plate Spline (tps) suite (http://life.bio.sunysb.edu/morph/index.html). A tps file was constructed using tpsUtil64 (v.1.7). The tps file was imported into tspDig2 (v.2.26), which was used to place the 80 semi-landmarks along the breaks in a counter clockwise direction. As these outlines are not closed curves, a consistent placement of semilandmark direction was needed. These points were used to populate the tps file with the semilandmark data. The tps file was imported into an R environment. The ‘geomorph’ package (Adams and Otarola-Castillo 2013) was used to conduct the Procrustes Superposition and Principal Components Analysis (PCA) of the superimposed data. The Procrustes Superposition was standardized for size and orientation, and so the analysis was performed solely on the variation of the outline shapes. Note that as these breaks do not have a biologically homologous landmark, no landmarked points were produced.

RESULTS OF ANALYSES ON REPAIRED INJURIES

Description of repaired injuries

Injuries at a number of stages of regeneration were recognized in this study (Figs. 3, 4, Zamora et al. 2011, fig. 4). These injuries healed over multiple moult stages: after callousing and initial repair (Fig. 3A, E) the end of the spine became rounded (Fig. 3B, F). This stage was followed by a thin growth with a pointed end during the subsequent moult(s) (Fig. 3C, G, Zamora et al. 2011, fig. 4D, E). Complete, but comparably shorter spines arose in the following moults (Fig. 3D, H, Zamora et al. 2011, fig. 4A-C, F-K). In one case (Fig. 4) multiple spines grew from an injured area.

Frequency of repairs

In the Murero Formation, a record of predation is only reported from the Purujosa locality (Table 1). No evidence for sublethal predation is reported from Mesones de Isuela (Murero Formation), from any locality, or private collection of material from the Huérmeda Formation. The 5th and 95th confidence intervals suggest that even though different population sizes were considered, the repair frequencies (both R and F) are significantly different (Table 1). The multiple repair frequency in the Murero Formation (0.22) is an order of magnitude higher than the Wheeler Formation (0.04) (Table 1).

Origin and location of injuries

Injured spines are not randomly distributed on trilobite exoskeletons, instead they are found adjacent to each other (Binomial text, n=15, p-value<0.001, Table 2). Injuries on *E. pradoanus* are found at Purujosa (F = 0.14, R = 0.13) but not Mesones de Isuela (F = 0, R = 0) (Table 1).

Spines are not significantly more likely to be injured on the left or right sides (two-tailed binomial test, n=16, p-value=1) and spines on the rear three thoracic segments were more likely to be injured than other segments (two-tailed binomial test, n=10, p-value=0.0045) (Table 3).

Size distribution and selection in the Murero Formation

No significant size selection was detected between the injured trilobite sample (mean = 38.98 mm, median = 32.83 mm, sd = 21.81 mm) and the non-injured sample (mean = 32.14 mm, median = 30.91 mm, sd = 13.31 mm) at Purujosa (Mann Whitney U test, W=224, p=0.46). This suggests that predators in the Murero Formation did not target smaller or larger trilobites at Purujosa. The size distributions of trilobites collected from the field at Purujosa, Mesones, and Minas Tierga cannot be distinguished according to Mann-Whitney U tests (thoracic means: Purujosa, 33.02 mm, Mesones, 32.42 mm, Minas Tierga, 41.45 mm; Mann Whitney U tests: Purujosa and Mesones, W= 816.5, p=0.86; Purujosa and Minas Tierga, W = 786, p= 0.058; Mesones and Minas Tierga, W = 439, p = 0.094, lengths of specimens plotted in Supp Fig. 2), suggesting that size differences are not the cause of differences in calculated repair frequencies.

RESULTS OF ANALYSES ON BROKEN SCLERITES

A biotic origin for trace fossils can be inferred if they have a distinct geometric shape, a narrow size range, and/or a non-random distribution of traces across taxa, size of prey, or location on the skeletons (Kowalewski 2002). Some broken sclerites found here are putatively of biotic origin (Figs. 5A, 6 white arrows, 7), some abiotic (Figs. 5C, 6 black arrows), and some breaks are of indeterminate origin (Fig. 5B). Semilandmark analyses describe the shape variation of the broken sclerites very effectively, as the two illustrated Principal Components explain 46.3% of the shape variation (Fig. 8). PC1 shows a change from a deep to shallow break, and PC2 shows a change from a break indented on the left side to one on the right side. Fossils that are referred to *Bicrescomanducator serratus* do not have a restricted shape variation in the Principal Coordinate (PC) space: there is a large spread of *B. serratus* specimens (red squares) across both PC1 and PC2 (Fig. 8). As specimens assigned to *B. serratus* overlap in morphospace with previously described *B. serratus* specimens (Fig. 9, red shapes: compare the points with black dots to those without), the assignment of *B. serratus* is acceptable. However, the new specimens also overlap with putative abiotic shapes (Fig. 9, blue shapes) and so a biotic origin of the new specimens cannot unambiguously be assigned.

DISCUSSION

Repaired injuries

Complete repair of trilobite injuries? – Trilobite injuries at a number of stages of repair are reported in this study (Figs 3, 4). Trilobites are thought to have had indeterminate growth, and so continued to moult after reaching adult morphology (Daley and Drage 2016). Therefore it is likely given enough time a trilobite with an injury would heal completely, removing all evidence that an injury occurred. Each moult stage is a stage of healing (Fig. 10A-D) and after a number of moults, dependent on the location and severity of the injury, all evidence of the injury would be removed (Fig. 10E). This has a direct implication for the comparison of repair frequencies of trilobites (and other ecdysozoans) with other groups which do not moult. The calculated repair frequency for ecdysozoans is likely an underestimate of the true frequency of injuries in the population. Larger injuries, which would require more moult stages to heal, therefore have a greater impact on the repair frequency than small injuries which would heal more quickly.

Origin of injuries—The low statistical likelihood of adjacent spines being injured by chance illustrates that injuries on *Eccaparadoxides pradoanus* likely result from predatory attacks, rather than from accidents and/or problematic moulting (Table 2). This is corroborated by the lower frequency of injuries on *E. pradoanus* from Mesones: if injuries were the result of moulting problems or another consistent behavior of the trilobites, the injury value would not vary markedly between sites. As the 95th percentile repair frequency value at Mesones is lower than the measured repair frequency at Purujosa, the smaller sample size at Mesones does not account for the difference in injury frequency between these two sites. Consequently, when using the frequency of repaired trilobite injuries as a proxy for predation on *E. pradoanus*, the ‘noise’ from non-predatory damage is likely to be minimal. This may not be the case for all trilobite injuries. Indeed Šnajdr (1978) considered moulting damage the most significant cause of injury in Bohemian paradoxidids.

Comparison between sites.— A difference in repair frequency between two sites of the same age, environment, and species of trilobite suggests a difference in predator pressure at those two sites. This may not follow when the energy of the depositional environment is significantly different (as this may affect the likelihood of accidental injuries), or where the sizes of trilobites vary significantly (as predators may preferentially attack smaller or larger animals). At all three field sites, complete trilobites have similar body plans with similar sized pleural spines on relatively large thoraces. Furthermore both the Huérmeda and Murero

Formations are considered to represent offshore environmental conditions, sporadically affected by storms. These similarities facilitate comparison between all three sites.

The repair frequency metrics (R, F, and MF) in the Huérmeda Formation (Cambrian Stage 4) are lower than the Murero Formation (Drumian), however trilobites at neither Minas Tierga (Huérmeda Formation) or Mesones de Isuela (Murero Formation) show evidence for repaired injuries. These two sites are geographically closer to each other than to Purujosa, where there is evidence for repaired injuries in the Drumian (see Figs. 1, 2; Table 1) and from other levels of the Purujosa 3 section (Table 1; Zamora et al. 2011). This suggests a geographic rather than temporal cause for the difference in predation, although the absolute distances between these sites in the Cambrian would have been different than at present as the Iberian Chains have been subjected to substantial tectonic activity. A lack of injured individuals suggests that either predation intensity on the trilobites studied was very low, or that predators were 100% efficient (Schoener 1979; Alexander 1981). Three lines of evidence support an interpretation of low predation intensity in this case: failure by predators is common; trilobites could repair even extensive damage; and trilobites of the same species at a different site show repaired injuries (Vermeij 1982; McNamara and Tuura 2011; Schoenemann et al. 2017).

Selectivity in repaired damage location.— Eccaparadoxides pradoanus likely enrolled as a defensive measure (Esteve et al. 2013). During enrolment, the posterior part of the thorax was located over the cephalon, an area where damage would have been more likely to be fatal (Babcock 1993). The high occurrence of damage to the posterior three segments may be the result of damage incurred during defensive enrolment. Alternatively, posterior injuries may indicate that predators attacked from the rear (Babcock 1993). The lack of anterior injuries supports the observation that posterior injuries are less lethal than anterior attacks (Babcock 1993), especially as the anterior segments were created before the posterior, although the small sample size and p-value (0.0045), means this result is only tentative.

Comparison between the Wheeler Formation and the Murero Formation—There is a higher multiple repair frequency in the Murero than the Wheeler Formation (Table 1). Assuming that the data collected are directly comparable, this indicates that injured specimens were more likely to be attacked a second time in the Murero Formation than the Wheeler.

The data from the Murero Formation show no lateral asymmetry of injuries on Cambrian trilobites, differing from the results of previous studies where site selectivity was reported for the right side of the body (Babcock and Robison 1989; Babcock 1993, 2003), specifically in the Wheeler Formation (Babcock 1993). Differences between the Wheeler and Murero Formations including environment, paleolatitude, and taxa studied, are factors that may impact on the differences observed for site selectivity of injuries and multiple repair frequency. More studies of trilobites using large datasets and similar taxa are needed to understand variation in repair frequencies and injury location selectivity.

Broken sclerites

It is often not possible to distinguish between predation and scavenging (e.g., Babcock 1993). Similar complications occur when differentiating between abiotic and biotic breakages, as fragmentation of shapes can arise from abiotic and biotic factors (Kowalewski 2002; Webster et al. 2008). While taphonomic alteration of cuticle is thought to be uncommon, trilobite cephala often fractured sagitally along the axis of highest vertical relief (Pratt 1998; Webster and Hughes 1999; Fig. 5C). Both biotic and abiotic damage to sclerites are presented and described morphometrically here.

V- and W-shaped traces on broken sclerites from the Huérmeda, Mansilla and Murero Formations fit the current definition of *Bicrescomanducator serratus* and overlap in PC space, but show a wide variety of shapes, sizes, and angles. The large morphological variation in *B. serratus* is problematic because the semilandmark analyses did not clearly distinguish between abiotic and biotic damage, suggesting that the species definition requires refining and that caution must be applied when assigning biotic origin. Circumstantial evidence, such as co-occurring predators or repaired predatory damage, could be used to strengthen future assignments. If a biotic origin is confirmed, breaks from the Huérmeda Formation would be the oldest known representatives of *B. serratus*.

CONCLUSIONS

Based on the relative frequencies of damage in trilobites from multiple localities, repaired injuries on *Eccaparadoxides pradoanus* appear to be predatory in nature. Variation in repair frequencies between the Murero, Huérmeda, and Wheeler Formations show that predation intensity can vary even on the same species of trilobite at different sites, and between different trilobite species worldwide. Evidence for predation is present at numerous levels at Purujosa, but absent in localities of the same age that are geographically more distant, suggesting that geography may be one factor affecting predation pressure. This does not mean that predation is absent at Minas Tierga or Mesones de Isuela. The location of injuries on trilobites from Purujosa showed no significant lateral asymmetry, differing from previous reports on Cambrian trilobites (Babcock and Robison 1989; Babcock 1993, 2003). The posterior three thoracic segments were targeted more often than expected from random attacks, perhaps due to *E. pradoanus* enrolling as a defensive measure.

A novel approach was employed using morphometric analyses to assess and compare the shape of unrepaired damage on trilobite sclerites. While the shape variation of serrated breaks on isolated sclerites was well described using this technique, *Bicrescomanducator* *serratus* traces could not be distinguished from abiotically broken sclerites, suggesting that a revision to the definition of this trace fossil taxon is needed.

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FIGURE CAPTIONS

FIG 1.—Map of study area. **A**) IC: Iberian Chains; box shows area in B; other abbreviations: BC: Betic Cordillera; CCR: Catalan Coastal Ranges; CIZ: Central Iberian Zone; CZ: Cantabrian Zone; OMZ: Ossa-Morena Zone; PY: Pyrenees; SPZ: South Portuguese Zone;

WALZ: West Asturian-Leonese Zone. **B**) Box from A with Purujosa, Tierga, and Mesones de Isuela marked. **C**) MI: Mesones de Iseula; MT: Minas Tierga; BJ: Barranco del Judio; CC: Las Cuevas/ Las Coronadas. **D**) Town of Purujosa with Purujosa 3 series marked; stars indicate field sites in Murero and Mansilla Formations. Key: Units increase in age from left to right, top older than bottom (see Figure 2). (Jalón Formation is older than Ribota Formation, and Embid Formation is older than Jalón Formation.)

FIG 2.—Stratigraphy of the studied sections. Unit thicknesses and local lithologies at each of the three field sites are depicted. Stars mark levels where articulated trilobites were collected. Adapted from Gozalo et al. (2008), Gámez Vintaned et al. (2009), and Zamora et al. (2011).

FIG 3.—*Eccaparadoxides pradoanus* (Verneuil and Barrande in Prado et al. 1860) from the Purujosa Red Beds, Murero Formation (Cambrian Series 3, Drumian), Iberian Chains, Spain, at a number of stages of repair. **A**) MPZ 2011/6: nearly complete thorax with crescent shaped recent injury on a thoracic spine. **B**) MPZ 2012/844: slightly disarticulated specimen with two shortened thoracic spines. **C**) MPZ 2012/1009: rear of a thorax showing thoracic spine with slight regrowth of the tip. **D**) MPZ 2012/7808: near complete thorax with two shortened thoracic spines. **E**) Box from A, arrow indicates injured spine. **F**) Box from B, arrow indicates shortened spines. **G**) Box from C, arrow indicates spine beginning regrowth. **H**) Box from D, arrow indicates two nearly fully repaired spines. Scale bars: **A-D**) 10 mm, **E-H**) 1 mm.

FIG 4.— *Eccaparadoxides pradoanus* (Verneuil and Barrande in Prado et al. 1860) nearly complete thorax from the Purujosa Red Beds, Murero Formation (Cambrian Series 3, Drumian), Iberian Chains, Spain, with additional spines growing from a previously injured area. **A**) MPZ 2017/1088. **B**) Box from A, white arrows indicate spines fully repaired, black arrows indicate additional spines grown from previously injured area. Scale bars: **A)** = 5 mm, **B)** = 1 mm.

FIG 5.—Broken sclerites of *Eccaparadoxides pradoanus* (Verneuil and Barrande in Prado et al. 1860) from the Purujosa Red Beds and near Mesones de Isuela, Murero Formation (Cambrian Series 3, Drumian), Iberian Chains, Spain. **A**) MPZ 2017/398: hypostome with *Bicrescomanducator serratus* (Zamora et al. 2011) trace. **B**) MPZ 2017/398 curved break on posterior of cranidium. **C**) MPZ 2017/358 abiotic fracture at rear of cranidium. Arrows point to breaks. Scale bars = 5 mm.

FIG 6.—Broken sclerites of redlichiid trilobites with *Bicrescomanducator* serratus (Zamora et al. 2011) traces and abiotic breaks from the Huérmeda Formation near Minas Tierga (Cambrian Series 2, Stage 4), Iberian Chains, Spain. **A**) MPZ 2017/338: partial cranidium showing biotic (white arrow) and abiotic (black arrow) breaks. **B**) Close up of biotic break in A. **C)** MPZ 2017/349: partial cranidium showing biotic and abiotic breaks. **D**) Close up of C. *B. serratus* indicated by white arrow, abiotic break indicated by black arrow. Scale bars: A, C = 5 mm; B, D = 1 mm.

FIG 7.—Broken sclerites with *Bicrescomanducator serratus* (Zamora et al. 2011) traces from the Mansilla Formation near Purujosa (Cambrian Series 3, Stage 5), Iberian Chains, Spain. **A**) MPZ 2017/427: cranidium. **B**) MPZ 2017/431: fragmentary trilobite sclerite. **C**) MPZ 2017/428: cranidium. **D**) MPZ 2017/430: fragmentary trilobite sclerite. *B. serratus* indicated by white arrows. Scale bars = 5 mm.

FIG 8.—Principal Component Analysis of *Bicrescomanducator serratus* (Zamora et al. 2011) and abiotic breaks. Outline of the shapes of the breaks semilandmarked, as shown by dotted red line in **A**) and **B**). Black arrows indicate the direction of semilandmark placement. Scale bars = 5 mm.

FIG 9.—Principal Component Analysis plot of the semilandmarked breaks (same as Figure 8), separating specimens by formation and by origin of the breaks. The overlap of previously described examples of *Bicrescomanducator serratus* (Zamora et al. 2011) (points with black dots) with new examples (points without black dots), shows the positive assignment of new material to the ichnotaxon. Circles: Huérmeda Formation (Spain, Cambrian Stage 4); triangles with point upwards: Mansilla Formation (Spain, Cambrian Stage 5); diamonds: Murero Formation (Spain, Cambrian Drumian); triangles with point downwards: Rabbitkettle Formation (Canada, Cambrian Drumian); squares: Stephen Formation (Canada, Cambrian, Stage 5); stars: Valongo Formation (Portugal, Ordovician Darriwilian); blue: abiotic; red: biotic.

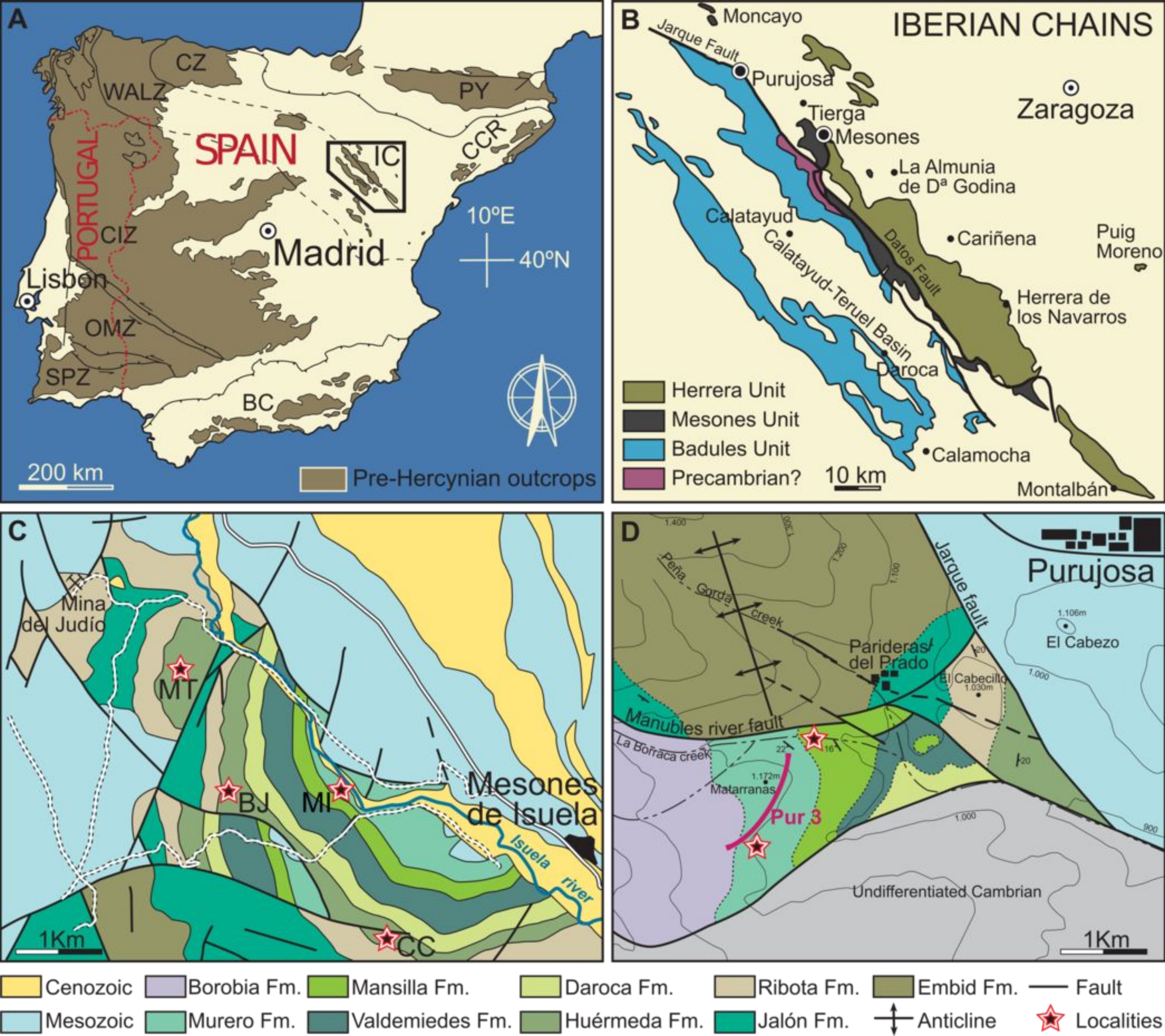
FIG 10.—Idealized repair sequence of trilobite pleural spines, based on Figure 3. Grey lines show uninjured shape of middle spine, dotted black line shows previous stage of healing. Healing stages: **A**) Immediately after injury; **B**) Rounded spine after initial healing; **C**) Regrowth begins, with thin tip of pleural spine; **D**) Short spine; **E**) Healing complete, spine same length as uninjured spines.

TABLE CAPTIONS

TABLE 1.—Frequency of repairs in Cambrian trilobites. Formation and locality information for trilobite repair frequencies and Bayesian Inference 5th and 95th percentile values. F and R are repair frequency metrics and MF is the multiple repair frequency metric (defined in methods).

TABLE 2.—Adjacent injuries in complete trilobite thoraces from the Purujosa Red Beds. P-values calculated using a binomial test with 10 successes from 14 attempts. The random probability of a success (a short spine adjacent to another short spine) is 2/2207 (2 available adjacent spines, with 2207 available spines in total).

TABLE 3.—Analysis of the location of injuries on trilobites from the Purujosa Red Beds. Expected values calculated using a two-tailed binomial analysis. Measured values are from observations. P-values calculated as described in methods.


A
CZ
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10ºE
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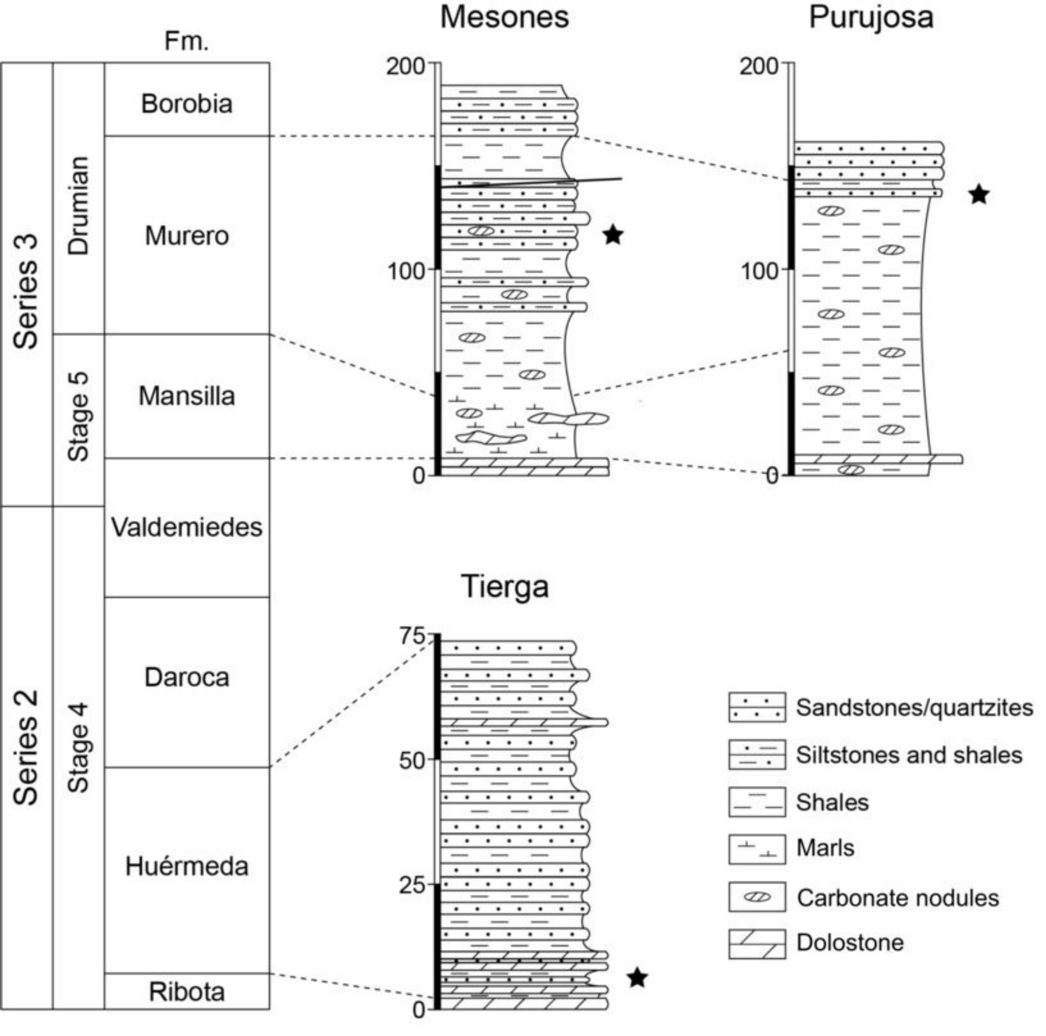
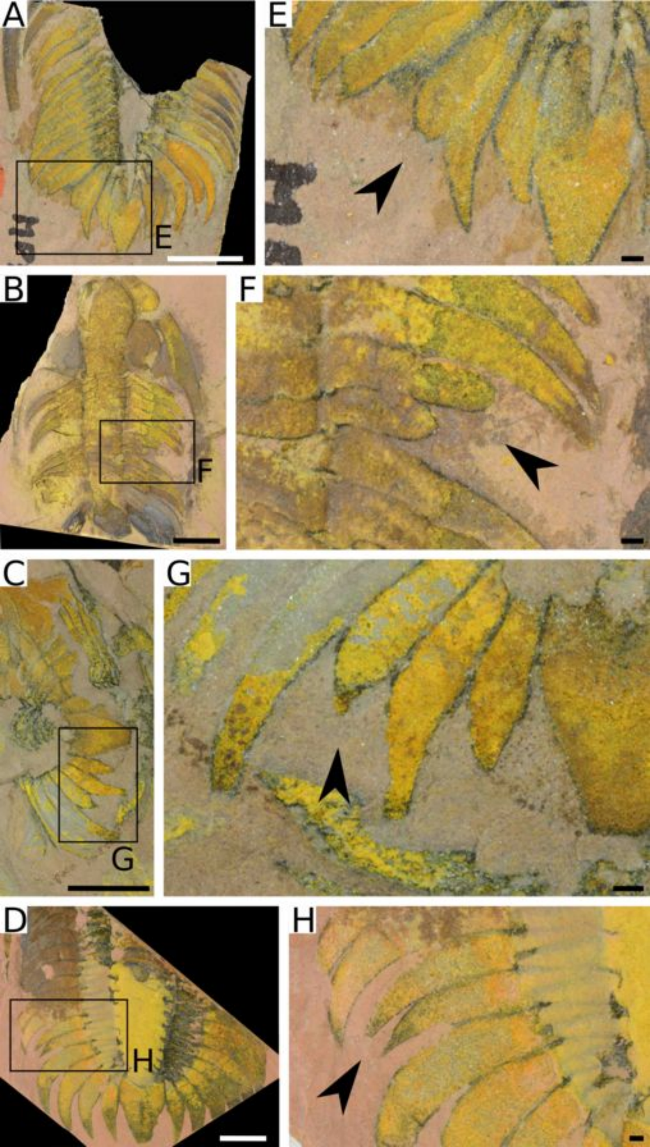
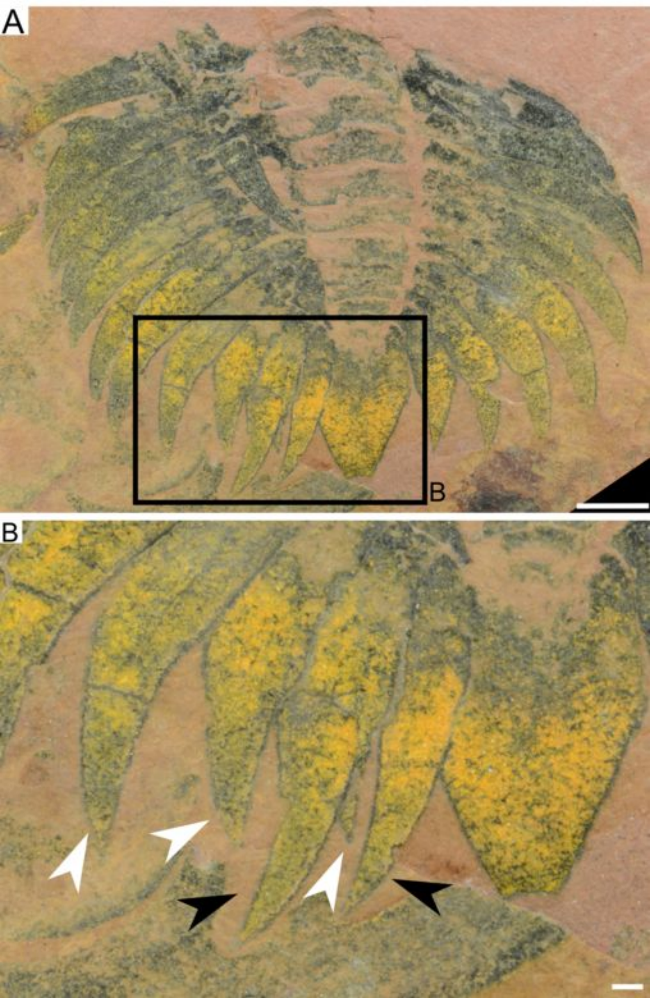
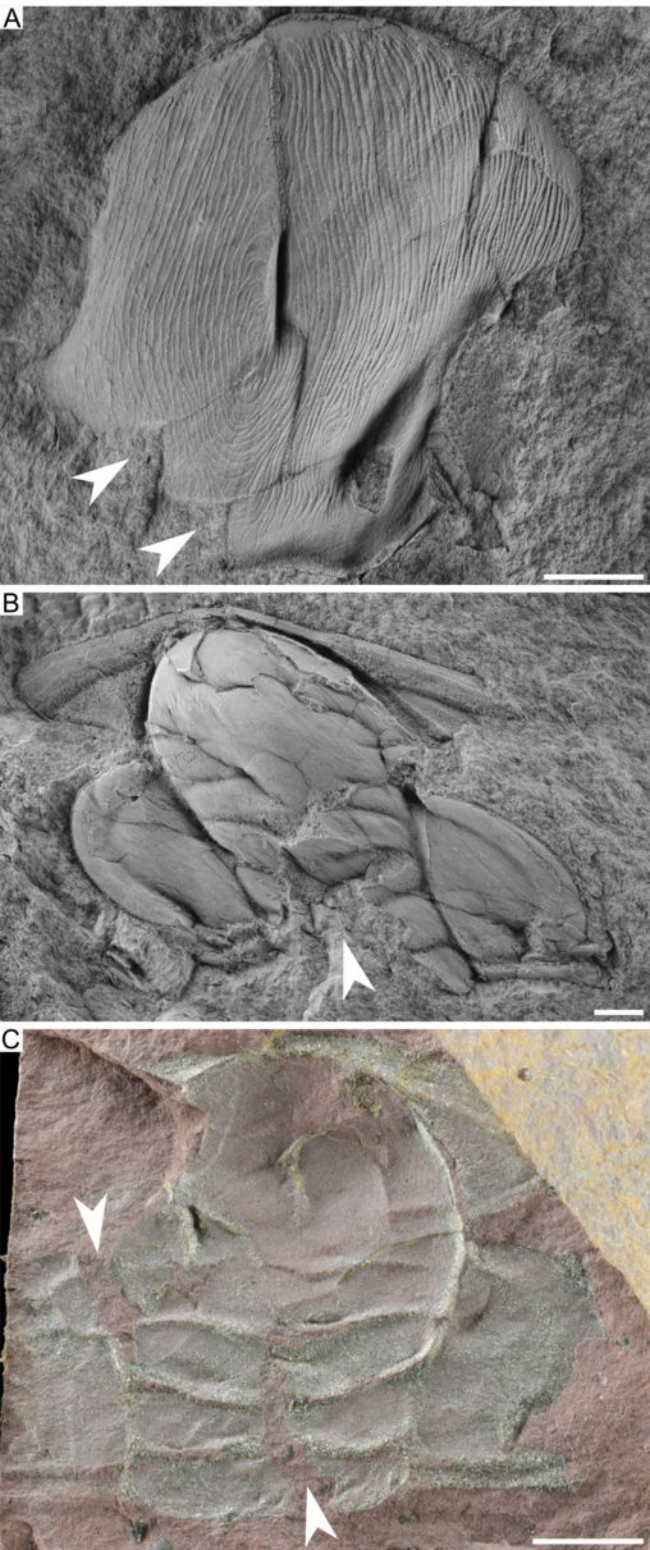
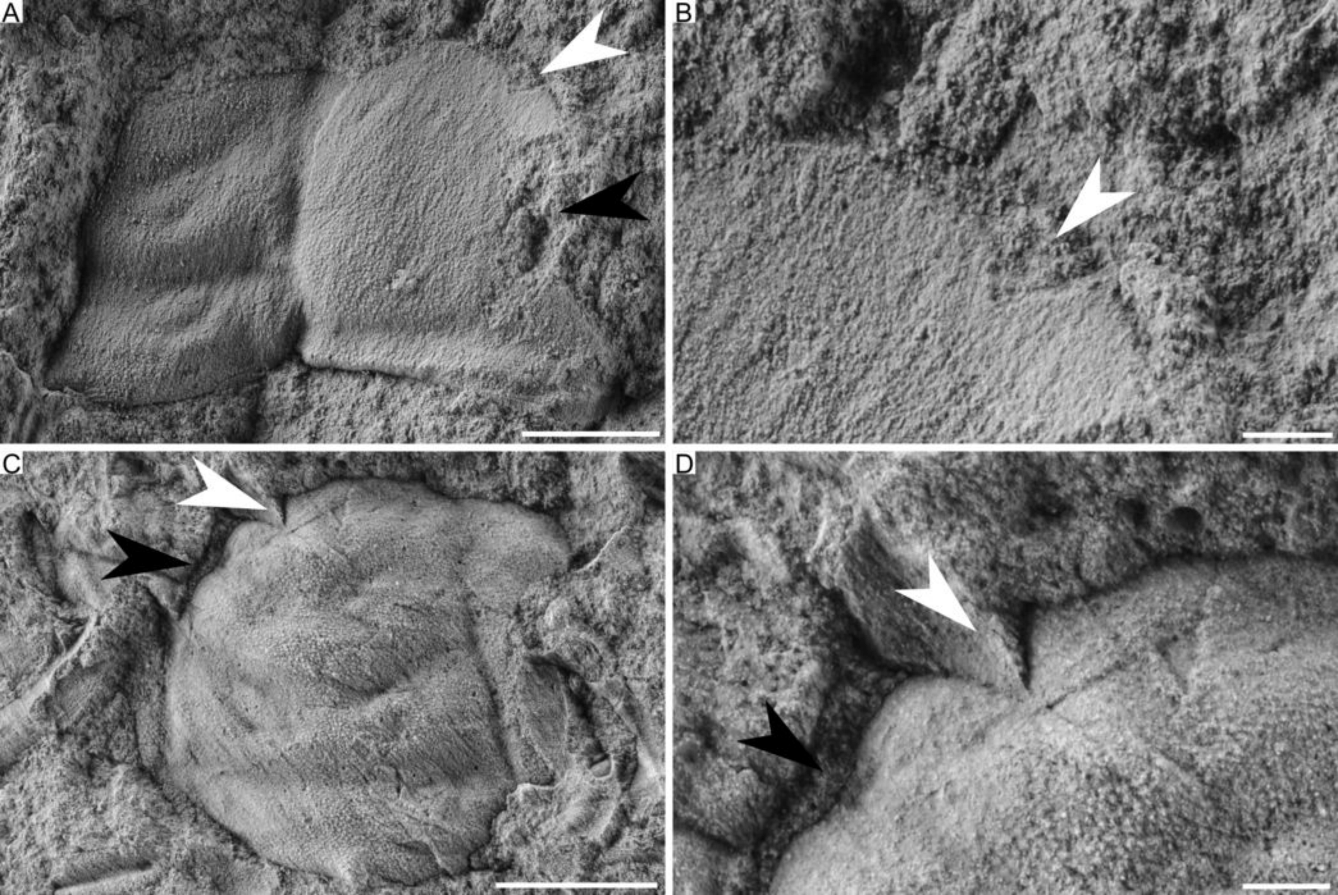
Lisbon
OMZ
SPZ
BC
200 km
Pre-Hercynian outcrops
B
Moncayo
IBERIAN CHAINS
Jarque Fault
Purujosa
Tierga
Zaragoza
Mesones
La Almunia de Dª Godina
Calatayud
Cariñena
Puig Moreno
Datos Fault
Calatayud-Teruel Basin
Herrera de los Navarros
Daroca
Calamocha
Montalbán
Herrera Unit
Mesones Unit
Badules Unit
Precambrian?
10 km
C
Mina del Judío
MT
BJ
MI
Mesones de Isuela
Isuela river
CC
1Km
D
1.400
1.300
1.200
1.100
Peña Gorda creek
Jarque fault
Purujosa
1.106m
El Cabezo
Parideras del Prado
El Cabecillo
1.030m
1.000
20
Manubles river fault
La Borraca creek
22
16
1.172m
Matarranas
Pur 3
1.000
900
Undifferentiated Cambrian
1Km
Cenozoic
Borobia Fm.
Mansilla Fm.
Daroca Fm.
Ribota Fm.
Embid Fm.
Fault
Mesozoic
Murero Fm.
Valdemiedes Fm.
Huérmeda Fm.
Jalón Fm.
Anticline
Localities


Mesones
Purujosa
Tierga
Fm.
Series 3
Series 2
Drumian
Stage 5
Stage 4
Borobia
Murero
Mansilla
Valdemiedes
Daroca
Huérmeda
Ribota
200
100
0
200
100
0
75
50
25
0
Sandstones/quartzites
Siltstones and shales
Shales
Marls
Carbonate nodules
Dolostone

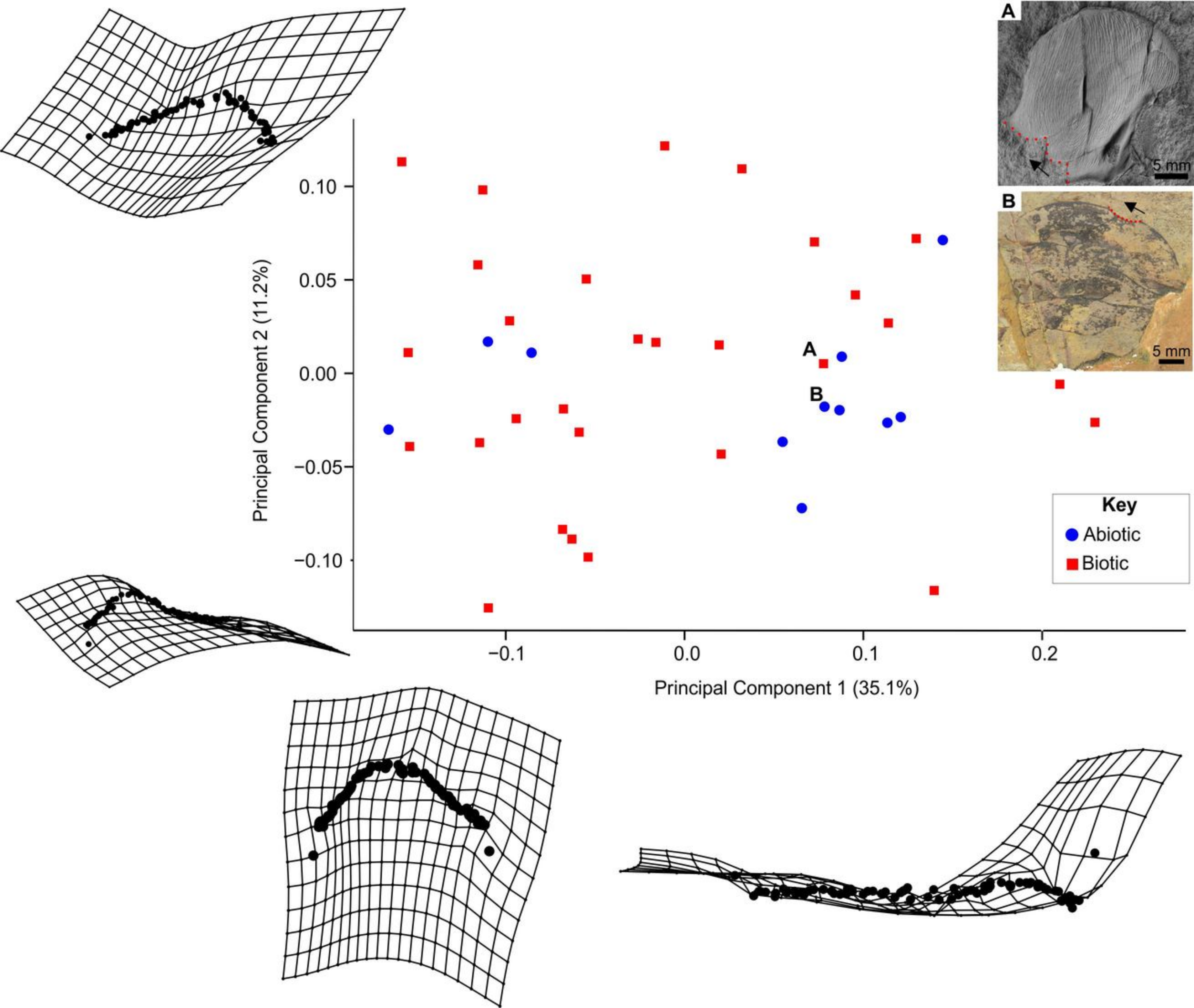

A
E
E
B
F
F
C
G
G
D
H
H


A
B
B


A
B
C


A
B
C
D


A
B
C
D


A
5 mm
B
5 mm
0.10
0.05
0.00
−0.05
−0.10
Principal Component 2 (11.2%)
−0.1
0.0
0.1
0.2
Principal Component 1 (35.1%)
A
B
Key
● Abiotic
🟥 Biotic


Key
Huérmeda Formation
Mansilla Formation
Murero Formation
Rabbitkettle Formation
Stephen Formation
Valongo Formation
Abioltic
Bioltic
Previously described
B. serratus
Principal Component 2 (11.2%)
Principal Component 1 (35.1%)
0.10
0.05
0.00
−0.05
−0.10
−0.1
0.0
0.1
0.2

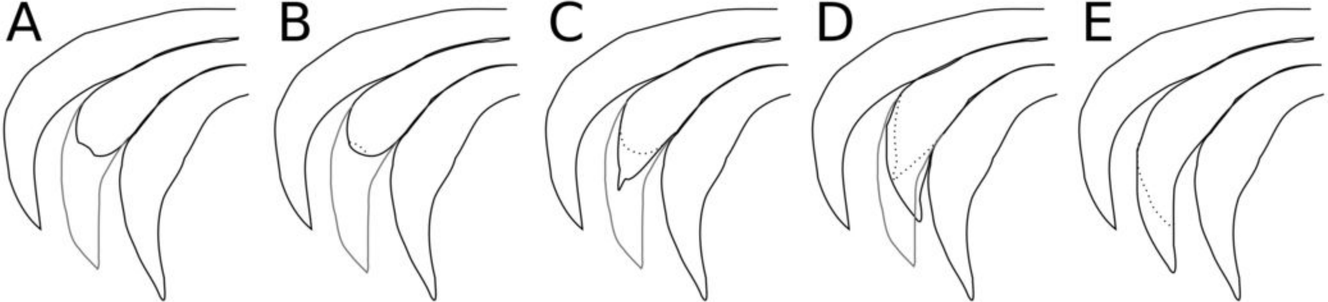

A
B
C
D
E

Table 1: Frequency of repairs in Cambrian trilobites

Formation/ Site	Age	Number of trilobites	Number of injured trilobites	Number of injuries	F	R	MF	F 5th Percentile	F 95th Percentile	R 5th Percentile	R 95th Percentile
Huérmeda Formation	Stage 4	45	0	0	0	0	0	0.00	0.06	0.00	0.06
Murero Formation	Drumian	97	10	12	0.11	0.09	0.20	0.08	0.19	0.06	0.17
Wheeler Formation (data from Babcock 1993)	Drumian	N/A	27	28	N/A	N/A	0.04	N/A	N/A	N/A	N/A
Minas Tierga (Huérmeda Fm)	Stage 4	30	0	0	0	0	0	0.00	0.09	0.00	0.09
Barranco del Judio (Huérmeda Fm)	Stage 4	1	0	0	0	0	0	0.02	0.78	0.02	0.78
Private collection, (Huérmeda Formation)	Stage 4	14	0	0	0	0	0	0.00	0.18	0.00	0.18
Mesones de Isuela (Murero Fm)	Drumian	23	0	0	0	0	0	0.00	0.12	0.00	0.12

Purujosa Red Beds (Murero Fm)	Drumian	69	9	10	0.14	0.13	0.11	0.09	0.23	0.08	0.21
Purujosa (other levels, from MPZ) (Murero Fm)	Drumian	5	1	2	0.4	0.2	1	0.15	0.73	0.06	0.58

Formation and locality information for trilobite repair frequencies and Bayesian Inference 5th and 95th percentile values. F and R are repair frequency metrics and MF is the multiple repair frequency metric (defined in methods).

Table 2. Adjacent injuries in complete trilobite thoraxes from Purujosa Red Beds

Number of short/injured spines	Total number of spines (assuming 32 per animal)	Number of expected injuries adjacent to another injury	Number of injured spines adjacent to injured spines	p-value
15	2208	0.013	10	2.2×10^{-16}

P-values calculated using a binomial test with 10 successes from 14 attempts. The random probability of a success (a short spine adjacent to another short spine) is 2/2207 (2 available adjacent spines, with 2207 available spines in total).

Table 3. Location of injuries and size analysis for trilobites from Purujosa locality

Measured location of injuries		Expected location of injuries		Measured location of injuries		Expected location of injuries		2-tailed binomial p-value	
Anterior 13 thoracic segments	Posterior 3 thoracic segments	Anterior 13 thoracic segments	Posterior 3 thoracic segments	Left side of thorax	Right side of thorax	Left side of thorax	Right side of thorax	Posterior 3 thoracic segments	Left/Right side of thorax
4	6	7.3	1.7	8	8	7.5	7.5	0.0045	1

Expected values calculated using a binomial analysis. Measured values are from observations. P-values calculated as described in methods.