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

STEPHEN PATES[1,2], RUSSELL D. C. BICKNELL[3], ALLISON C. DALEY[2] and SAMUEL ZAMORA[4, 5]

[1]*Department of Zoology, University of Oxford, Oxford, OX1 3PS, United Kingdom*

[2]*Institute of Earth Sciences, University of Lausanne, Lausanne, CH-1015, Switzerland*

[3]*Paleoscience Research Centre School of Environmental and Rural Science, University of New England, Armidale, New South Wales, 2351, Australia*

[4]*Instituto Geológico y Minero de España, C/Manuel Lasala, 44, 9ºB, 50006, Zaragoza, Spain*

[5]*Unidad Asociada en Ciencias de la Tierra, Universidad de Zaragoza-IGME, Zaragoza, Spain*

email: stephen.pates@zoo.ox.ac.uk

RRH: BROKEN AND REPAIRED CAMBRIAN TRILOBITES FROM NE SPAIN

LRH: S. PATES ET AL.

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

101 followed an initial callousing and regrowth over the injury (e.g., Schoenemann et al. 2017).
102 When attacked during the soft post-ecdysial stage, trilobite spines could wrinkle or distort
103 (Conway Morris and Jenkins 1985), and rarely additional pleural spines grew from injured
104 areas (Babcock 1993). Regeneration of spines is controlled by segment polarity genes
105 (McNamara and Tuura 2011), and begins during ecdysis after the damage was sustained
106 (Lochman 1941). Regrown spines remain shorter than original spines for a variable number
107 of moults, likely dependent on the severity of the injury. This process is comparable to the
108 regeneration of tail spines in *Daphnia* and crinoid arms (Murtaugh 1981; Baumiller and Gahn
109 2012).

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

119 Using exoskeletons and broken sclerites of trilobites from three sites (Purujosa,
120 Mesones de Isuela, and Minas Tierga) from two formations (Murero Formation and
121 Huérmeda Formation) in the Iberian Chain, NE Spain, evidence of sub-lethal predation and
122 broken sclerites was recorded. Repaired injuries demonstrate unequivocally damage during
123 the life of the animal. The proportion of injured trilobites at each site, and position of repaired
124 injuries on the exoskeletons are analyzed to provide information about causes, selection
125 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:

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

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

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

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

196 frequency of individuals repaired (Alexander and Dietl 2003). Following Alexander and Dietl
197 (2003), both are presented to mitigate the limitations of both methods. For the Huérmeda
198 Formation, the repair frequency was calculated using all redlichiid trilobites, and for the
199 Murero Formation the repair frequency was calculated using *Eccaparadoxides pradoanus*.
200 Although repair frequencies for the Wheeler Formation could not be calculated from the
201 literature, a multiple repair frequency value, MF, was derived using data from Babcock
202 (1993, p. 222).

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

208 *Origin and location of repaired injuries.*— Distinguishing between accidental
209 damage, damage due to problems during moulting, or predatory damage is a complicated task
210 when considering the cause of repaired injuries. We propose a statistical method to estimate
211 the likelihood of damage having occurred during moulting. For trilobites that have thoracic
212 spines of approximately equal length and similar morphology, the likelihood that a given
213 spine is injured due to moulting complications is expected be the same as all other spines.
214 This would result in randomly distributed injuries on trilobite exoskeletons, assuming that
215 injuries occurred during the holaspis phase. As segments are added at the posterior of the
216 thorax throughout meraspid stages, individual injuries due to moulting could be more
217 common at the anterior than at the posterior, as these segments undergo more moult stages.
218 For species with particularly long, thin, or intricate spines, this expectation changes as such
219 spines are more susceptible to moulting damage than others. Accidental injuries from
220 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 5^{th} and 95^{th} 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).

296

297 Origin and location of injuries

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

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

306

307 Size distribution and selection in the Murero Formation

308 No significant size selection was detected between the injured trilobite sample (mean = 38.98
309 mm, median = 32.83 mm, sd = 21.81 mm) and the non-injured sample (mean = 32.14 mm,
310 median = 30.91 mm, sd = 13.31 mm) at Purujosa (Mann Whitney U test, W=224, p=0.46).
311 This suggests that predators in the Murero Formation did not target smaller or larger trilobites
312 at Purujosa. The size distributions of trilobites collected from the field at Purujosa, Mesones,
313 and Minas Tierga cannot be distinguished according to Mann-Whitney U tests (thoracic
314 means: Purujosa, 33.02 mm, Mesones, 32.42 mm, Minas Tierga, 41.45 mm; Mann Whitney
315 U tests: Purujosa and Mesones, W= 816.5, p=0.86; Purujosa and Minas Tierga, W = 786, p=
316 0.058; Mesones and Minas Tierga, W = 439, p = 0.094, lengths of specimens plotted in Supp
317 Fig. 2), suggesting that size differences are not the cause of differences in calculated repair
318 frequencies.

319

320 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

346 evidence of the injury would be removed (Fig. 10E). This has a direct implication for the
347 comparison of repair frequencies of trilobites (and other ecdysozoans) with other groups
348 which do not moult. The calculated repair frequency for ecdysozoans is likely an
349 underestimate of the true frequency of injuries in the population. Larger injuries, which
350 would require more moult stages to heal, therefore have a greater impact on the repair
351 frequency than small injuries which would heal more quickly.

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

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

371 Formations are considered to represent offshore environmental conditions, sporadically
372 affected by storms. These similarities facilitate comparison between all three sites.
373 The repair frequency metrics (R, F, and MF) in the Huérmeda Formation (Cambrian
374 Stage 4) are lower than the Murero Formation (Drumian), however trilobites at neither Minas
375 Tierga (Huérmeda Formation) or Mesones de Isuela (Murero Formation) show evidence for
376 repaired injuries. These two sites are geographically closer to each other than to Purujosa,
377 where there is evidence for repaired injuries in the Drumian (see Figs. 1, 2; Table 1) and from
378 other levels of the Purujosa 3 section (Table 1; Zamora et al. 2011). This suggests a
379 geographic rather than temporal cause for the difference in predation, although the absolute
380 distances between these sites in the Cambrian would have been different than at present as
381 the Iberian Chains have been subjected to substantial tectonic activity. A lack of injured
382 individuals suggests that either predation intensity on the trilobites studied was very low, or
383 that predators were 100% efficient (Schoener 1979; Alexander 1981). Three lines of evidence
384 support an interpretation of low predation intensity in this case: failure by predators is
385 common; trilobites could repair even extensive damage; and trilobites of the same species at
386 a different site show repaired injuries (Vermeij 1982; McNamara and Tuura 2011;
387 Schoenemann et al. 2017).
388 *Selectivity in repaired damage location*.— *Eccaparadoxides pradoanus* likely
389 enrolled as a defensive measure (Esteve et al. 2013). During enrolment, the posterior part of
390 the thorax was located over the cephalon, an area where damage would have been more
391 likely to be fatal (Babcock 1993). The high occurrence of damage to the posterior three
392 segments may be the result of damage incurred during defensive enrolment. Alternatively,
393 posterior injuries may indicate that predators attacked from the rear (Babcock 1993). The
394 lack of anterior injuries supports the observation that posterior injuries are less lethal than
395 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*

445 *serratus* traces could not be distinguished from abiotically broken sclerites, suggesting that a
446 revision to the definition of this trace fossil taxon is needed.
447
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460
461 References
462
463 ABERHAN, M., KIESSLING, W. and FÜRSICH, F.T., 2006, Testing the role of biological
464 interactions in the evolution of mid-Mesozoic marine benthic ecosystems:
465 Paleobiology, v. 32, p. 259—277.
466 ADAMS, D.C., and E. OTAROLA-CASTILLO, 2013. geomorph: an R package for the
467 collection and analysis of geometric morphometric shape data: Methods in Ecology
468 and Evolution, v. 4, p. 393—399.

ALEXANDER, R.R., 1981, Predation scars preserved in Chesterian brachiopods: probable culprits and evolutionary consequences for the articulates: Journal of Paleontology, v. 55, p. 192—203.

ALEXANDER, R.R.,1986, Resistance to and repair of shell breakage induced by durophages in late Ordovician brachiopods, Journal of Paleontology, v. 60, p. 273—285.

ALEXANDER, R.R. and DIETL, G.P., 2001. Latitudinal trends in naticid predation on *Anadara ovalis* (Bruguiere, 1789) and *Divalinga quadrisulcata* (Orbigny, 1842) from New Jersey to the Florida Keys: American Malacological Bulletin, v. 16, p. 179—194.

ALEXANDER, R.R. and DIETL, G.P., 2003, The fossil record of shell-breaking predation on marine bivalves and gastropods, *in* Kelly, P., Kowalewski, M., and Hansen, T.A. (eds.), Predator—Prey Interactions in the Fossil Record, Kluwer Academic, New York, p. 141—176.

ÁLVARO, J.J. and VENNIN, E., 1997, Episodic development of Cambrian eocrinoid-sponge meadows in the Iberian Chains (NE Spain): Facies, v. 37, p. 49—63.

ANDREW, C., HOWE, P., PAUL, C.R.C., and DONOVAN, S.K., 2010, Fatally bitten ammonites from the lower Lias Group (Lower Jurassic) of Lyme Regis, Dorset : Proceedings of the Yorkshire Geological Society, v. 58, p. 81—94.

BABCOCK, L. E., 1993, Trilobite malformations and the fossil record of behavioral asymmetry: Journal of Paleontology, v. 67, p. 217—229.

BABCOCK, L. E., 2003, Trilobites in Paleozoic predator-prey systems, and their role in reorganization of early Paleozoic ecosystems, *in* Kelly, P., Kowalewski, M., and Hansen, T.A. (eds.), Predator—Prey Interactions in the Fossil Record, Kluwer Academic, New York, p. 55—92.

493 BABCOCK, L.E. and ROBISON, R.A., 1989, Preferences of Palaeozoic predators: Nature, v.
494 337, p. 695—696.
495 BAUMILLER, T.K. and GAHN, F.J., 2012, Reconstructing predation pressure on crinoids:
496 estimating arm-loss rates from regenerating arms: Paleobiology, v. 39, p. 40—51.
497 BERGSTRÖM, J. and LEVI-SETTI, R., 1978, Phenotypic variation in the Middle Cambrian
498 trilobite *Paradoxides davidis*: Geologica et Palaeontologica, v. 12, p. 1—40.
499 BICKNELL, R.D.C and PATERSON J.R., 2017, Reappraising the early evidence of
500 durophagy and drilling predation in the fossil record: Implications for escalation and
501 the Cambrian Explosion: Biological Reviews, doi: 10.1111/brv.12365.
502 BRETT, C.E. and WALKER, S.E., 2002, Predators and predation in Paleozoic marine
503 environments: Paleontological Society Papers, v. 8, p. 93—118.
504 BRUTON, D.L., 1981, The arthropod *Sidneyia inexpectans*, Middle Cambrian, Burgess
505 Shale, British Columbia: Philosophical Transactions of the Royal Society of London.
506 Series B, Biological Sciences, v. 296, p. 619—653.
507 BUATOIS, L.A., WISSHAK, M., WILSON, M.A. and MÁNGANO, M.G., 2017, Categories
508 of architectural designs in trace fossils: A measure of ichnodisparity: Earth-Science
509 Reviews, v. 164, p. 102—181.
510 CONWAY MORRIS, S., 1977, Fossil priapulid worms: Special Papers in Palaeontology, v.
511 20, p. 1—155.
512 CONWAY MORRIS, S. and BENGTSON, 1994, Cambrian predators: possible evidence
513 from boreholes: Journal of Paleontology, v. 68, p. 1—23.
514 CONWAY MORRIS, S. and JENKINS, R., 1985, Healed injuries in early Cambrian
515 trilobites from South Australia: Alcheringa, v. 9, p. 167—177.

CONWAY MORRIS, S. and ROBISON, R.A., 1988, More soft-bodied animals and algae from the Middle Cambrian of Utah and British Columbia: University of Kansas Paleontological Contributions, v. 122, p. 1—48.

DALEY, A.C., and DRAGE, H.B. 2016, The fossil record of ecdysis, and trends in the moulting behavior of trilobites: Arthropod Structure and Development, v. 45, p. 71—96.

DALEY, A.C., PATERSON, J.R., EDGECOMBE, G.D., GARCIA-BELLIDO, D. C. and JAGO, J. B., 2013, New anatomical information on *Anomalocaris* from the Cambrian Emu Bay Shale of South Australia and a reassessment of its inferred predatory habits: Palaeontology, v. 56, p. 971—990.

DIETL, G.P., ALEXANDER, R.R. and BIEN, W.F., 2000, Escalation in Late Cretaceous–early Paleocene oysters (Gryphaeidae) from the Atlantic Coastal Plain: Paleobiology, v. 26, p. 215—237.

DIETL, G.P., 2003a, Interaction strength between a predator and dangerous prey: *Sinistrofulgur* predation on *Mercenaria*: Journal of Experimental Marine Biology and Ecology, v. 289, p. 287—301.

DIETL, G.P., 2003b, Coevolution of a marine gastropod predator and its dangerous bivalve prey: Biological Journal of the Linnean Society, v. 80, p. 409—436.

DIETL, G.P. and HENDRICKS, J.R., 2006, Crab scars reveal survival advantage of left-handed snails: Biology Letters, v. 2, p. 439—442.

ENGLISH, A.M. AND BABCOCK, L.E., 2010, Census of the Indian Springs Lagerstätte, Poleta Formation (Cambrian), western Nevada, USA: Palaeogeography, Palaeoclimatology, Palaeoecology, v. 295, p. 236—244.

ESTEVE, J., HUGHES, N.C. and ZAMORA, S., 2011, Purujosa trilobite assemblage and the evolution of trilobite enrolment: Geology, v. 39, p. 575—578.

ESTEVE, J., HUGHES, N.C. and ZAMORA, S., 2013, Thoracic structure and enrolment style in Middle Cambrian *Eccaparadoxides pradoanus* presages caudalization of the derived trilobite trunk: Palaeontology, v. 56, p. 589—601.

FORTEY, R., 2000, Olenid trilobites: The oldest known chemoautotrophic symbionts?: Proceedings of the National Academy of Sciences, v. 97, p. 6574—6578.

FORTEY, R.A., 1986, The type species of the Ordovician trilobite *Symphysurus*: systematics, functional morphology and terrace ridges: Paläontologische Zeitschrift, v. 60, p.255—275.

GÁMEZ VINTANED, J.A., SCHMITZ, U. AND LIÑÁN, E., 2009, Upper Vendian-lowest Ordovician sequences of the western Gondwana margin, NE Spain: Geological Society, London, Special Publications, v. 326, p. 231—244.

GAINES, R.R. and DROSER, M.L., 2003, Paleoecology of the familiar trilobite *Elrathia kingii*: An early exaerobic zone inhabitant: Geology, v. 31, p. 941—944.

GOZALO, R., LIÑÁN, E., GÁMEZ VINTANED, J.A., DIES ÁLVAREZ, M.E., CHIRIVELLA MARTORELL, J.B., ZAMORA, S., ESTEVE, J. and MAYORAL, E., 2008, The Cambrian of the Cadenas Ibéricas (NE Spain) and its trilobites: Cuadernos del Museo Geominero, v. 9, p. 137—151.

HARPER, E.M., 2016. Uncovering the holes and cracks: from anecdote to testable hypotheses in predation studies, Palaeontology, v. 59, p. 597—609.

HARPER, E.M., PECK, L.S. and HENDRY, K.R., 2009, Patterns of shell repair in articulate brachiopods indicate size constitutes a refuge from predation: Marine Biology, v. 156, p. 1993—2000.

JAGO, J.B., GARCÍA-BELLIDO, D.C, and GEHLING, J.G., 2016, An early Cambrian chelicerate from the Emu Bay Shale, South Australia: Palaeontology v. 59, p. 549—562.

566 KELLEY, P.H., 1989. Evolutionary trends within bivalve prey of Chesapeake Group naticid
567 gastropods: Historical Biology, v. 2, p. 139—156.
568 KELLY, P.H., KOWALEWSKI, M., and HANSEN, T.A., 2003, Predator—Prey Interactions
569 in the Fossil Record: Kluwer Academic, New York, p. 455.
570 KIMMIG, J. and STROTZ, L.C., 2017, Coprolites in mid-Cambrian (Series 2-3) Burgess
571 Shale-type deposits of Nevada and Utah and their ecological implications, Bulletin of
572 Geosciences, v. 92, p. 297—309.
573 KLOMPMAKER, A.A. and KELLEY, P.H., 2015, Shell ornamentation as a likely
574 exaptation: evidence from predatory drilling on Cenozoic bivalves, Paleobiology, v.
575 41, p.187—201.
576 KOWALEWSKI, M., DULAI, A. and FÜRSICH, F.T., 1998, A fossil record full of holes:
577 the Phanerozoic history of drilling predation: Geology, v. 26, p.1091—1094.
578 KOWALEWSKI, M., 2002, The fossil record of predation: an overview of analytical
579 methods: Paleontological Society Papers, v. 8, p. 3—42.
580 LEIGHTON, L.R., 2001, New example of Devonian predatory boreholes and the influence of
581 brachiopod spines on predator success: Palaeogeography, Palaeoclimatology,
582 Palaeoecology, v. 165, p. 53—69.
583 LEIGHTON, L.R., 2011, Analyzing Predation from the Dawn of the Phanerozoic, *in*
584 Laflamme, M., Schiffbauer, J.D., and Dornbos, S.Q. (eds.), Quantifying the Evolution
585 of Early Life, Springer Netherlands, p. 73—109.
586 LOCHMAN, C., 1941, A pathologic pygidium from the Upper Cambrian of Missouri:
587 Journal of Paleontology, v. 15, p. 324—325.
588 MCNAMARA, K. J. and TUURA, M. E., 2011, Evidence for segment polarity during
589 regeneration in the Devonian asteropygine trilobite *Greenops widderensis*: Journal of
590 Paleontology, v. 85, p. 106—110.

591 MERGL, M. and ZAMORA, S., 2012, New and revised occurrences of rhynchonelliformean
592 brachiopods from the middle Cambrian of the Iberian Chains, NE Spain: Bulletin of
593 Geosciences, v. 87, p. 571—586.
594 MORZADEC, P., 1988, Le genre *Psychopyge* (Trilobita) dans le Dévonien inférieur du Nord
595 de l'Afrique et l'Ouest de l'Europe: Palaeontographica Abteilung A, v. 200, p. 153—
596 161.
597 MURTAUGH, P.A., 1981, Inferring properties of mysid predation from injuries to *Daphnia*:
598 Limnology and Oceanography, v. 26, p. 811—821.
599 NEDIN, C., 1999, *Anomalocaris* predation on nonmineralized and mineralized trilobites:
600 Geology, v. 27, p. 987—990.
601 NETO DE CARVALHO, C., COUTO, H., FIGUEIREDO, M. V. and BAUCON, A., 2016,
602 Estruturas biogénicas com relação microbiana nas ardósias Ordovícico Médio de
603 Canelas (Norte de Portugal): Comunicações Geológicas, v. 103, Especial 1, p. 23—
604 38.
605 OWEN, A.W., 1985, Trilobite abnormalities: Transactions of the Royal Society of
606 Edinburgh: Earth Sciences, v. 76, p. 255—272.
607 PRADO, M.C., VERNEUIL, E. and BARRANDE, J., 1860, Sur l'existence de la faune
608 primordial dans la Chaıˆne Cantabrique: Bulletin de la Société Géologique de France,
609 Series 2, v. 17, p. 516—542.
610 PRATT, B.R., 1998, Probable predation on upper Cambrian trilobites and its relevance for
611 the extinction of soft-bodied Burgess Shale-type animals: Lethaia, v. 31, p. 73—88.
612 R CORE TEAM, 2017, R: A language and environment for statistical computing: R
613 Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/.

ROBSON, S.P. and PRATT, B.R., 2007, Predation of late Marjuman (Cambrian) linguliformean brachiopods from the Deadwood Formation of South Dakota, USA: Lethaia, v. 40, p. 19—32.

RUDKIN, D.M., 1979, Healed injuries in *Ogygopsis klotzi* (Trilobita) from the Middle Cambrian of British Columbia: Royal Ontario Museum Life Sciences Occasional Papers, v. 32, p. 1—8.

RUSTÁN, J.J., BALSEIRO, D., WAISFELD, B., FOGLIA, R.D. and VACCARI, N.E., 2011, Infaunal molting in Trilobita and escalatory responses against predation: Geology, v. 39, p. 495—498.

SÁ, A.A. and GUTIÉRREZ-MARCO, J.C., 2015, *Aroucaichnus* igen. nov. y otros icnofósiles singulares del Ordovícico del Geoparque Arouca (Portugal): Boletín de la Sociedad Geológica del Perú, v. 110, p. 8—23.

SCHMIDT-THOMÉ, M., 1973, Beiträge zur Feinstratigraphie des Unter-Kambriums in den-Iberischen Ketten (Nordost-Spanien): Geologisches Jahrbuch Reihe, Band B7, p. 3—43.

SCHMITZ, U., 1971, Stratigraphie und sedimentologie im Kambrium und Tremadoc der Westlichen Iberischen Ketten nördlich Ateca (Zaragoza), NE-Spanien: Münstersche Forschungen zur Geologie und Paläontologie, v. 22, p. 1—123.

SCHOENEMANN, B., CLARKSON, E.N. and HØYBERGET, M., 2017, Traces of an ancient immune system–how an injured arthropod survived 465 million years ago: Scientific Reports, v. 7, p. 40330.

SCHOENER, T.W., 1979, Inferring the properties of predation and other injury-producing agents from injury frequencies: Ecology, v. 60, p. 1110—1115.

SDZUY, K., 1961, Das Kambrium Spaniens. Teil II: Trilobiten: Abhandlungen der Akademie der Wissenschaften Lit Mainz Math Nat Kl, v. 7, p. 499—594.

639 SINCLAIR, G.W., 1947, Two examples of injury in Ordovician trilobites: American Journal
640 of Science, v. 245, p. 250—257.
641 SKINNER, E.S., 2015, Taphonomy and depositional circumstances of exceptionally
642 preserved fossils from the Kinzers Formation (Cambrian), southeastern Pennsylvania:
643 Palaeogeography, Palaeoclimatology, Palaeoecology, v. 220, p. 167—192.
644 SPRINKLE, J., 1973, Morphology and evolution of blastozoan echinoderms: Museum of
645 Comparative Zoology Harvard University Special Publications, Boston, p. 283.
646 ŠNAJDR, M., 1978, Anomalous carapaces of Bohemian paradoxid trilobites: Sborník
647 Geologických Věd Paleontologie, v. 20, p. 7—31.
648 VALENZUELA, J.I., GÁMEZ, J.A., LIÑÁN, E. and SDZUY, K., 1990, Estratigrafía del
649 Cámbrico de la región de Brea. Cadena Ibérica oriental: Boletín de la Real Sociedad
650 Española de Historia Natural (Sección Geológica), v. 85, p. 45—54.
651 VANNIER, J., 2012, Gut contents as direct indicators for trophic relationships in the
652 Cambrian marine ecosystem: PLoS ONE, v. 7, p. 1—20. e52200.
653 https://doi.org/10.1371/journal.pone.0052200.
654 VANNIER, J. and CHEN, J., 2005, Early Cambrian food chain: new evidence from fossil
655 aggregates in the Maotianshan Shale biota, SW China: Palaios, v. 20, p. 3—26.
656 VERMEIJ, G.J., 1977, The Mesozoic marine revolution: evidence from snails, predators and
657 grazers: Paleobiology, v. 3, p. 245—258.
658 VERMEIJ, G.J., 1982, Unsuccessful predation and evolution: The American Naturalist, v.
659 120, p. 701—720.
660 VERMEIJ, G.J., 1987, Evolution and Escalation: an ecological history of life: Princeton
661 University Press, New Jersey, p. 544.
662 WALCOTT, C.D., 1911, Cambrian geology and paleontology. II. Middle Cambrian
663 Merostomata: Smithsonian Miscellaneous Collections, v. 57, p. 17—40.

664 WEBSTER, M. and HUGHES, N.C., 1999, Compaction-related deformation in Cambrian
665 olenelloid trilobites and its implications for fossil morphometry: Journal of
666 Paleontology, v. 73, p. 355—371.
667 WEBSTER, M., GAINES, R.R. and HUGHES, N.C., 2008, Microstratigraphy, trilobite
668 biostratinomy, and depositional environment of the "Lower Cambrian" Ruin Wash
669 Lagerstätte, Pioche Formation, Nevada: Palaeogeography, Palaeoclimatology,
670 Palaeoecology, v. 264, p. 100—122.
671 ZACAÏ, A., VANNIER, J. and LEROSEY-AUBRIL, R., 2015, Reconstructing the diet of a
672 505-million-year-old arthropod: *Sidneyia inexpectans* from the Burgess Shale fauna:
673 Arthropod structure and development, v. 45, p. 200—220.
674 ZAMORA, S., 2010, Middle Cambrian echinoderms from North Spain show echinoderms
675 diversified earlier in Gondwana: Geology, v. 38, p. 507—510.
676 ZAMORA, S., MAYORAL, E., ESTEVE, J., GÁMEZ VINTANED, J. A. and SANTOS, A.,
677 2011, Exoskeletal abnormalities in paradoxidid trilobites from the Cambrian of Spain,
678 and a new type of bite trace: Bulletin of Geosciences, v. 86, p. 665—673.
679 ZHU, M.-Y., VANNIER, J., ITEN, H. and ZHAO, Y.-L., 2004, Direct evidence for predation
680 on trilobites in the Cambrian: Proceedings of the Royal Society of London B:
681 Biological Sciences, v. 271, p. S277—S280.
682
683
684 FIGURE CAPTIONS
685
686 FIG 1.—Map of study area. **A**) IC: Iberian Chains; box shows area in B; other abbreviations:
687 BC: Betic Cordillera; CCR: Catalan Coastal Ranges; CIZ: Central Iberian Zone; CZ:
688 Cantabrian Zone; OMZ: Ossa-Morena Zone; PY: Pyrenees; SPZ: South Portuguese Zone;

689 WALZ: West Asturian-Leonese Zone. **B**) Box from A with Purujosa, Tierga, and Mesones de
690 Isuela marked. **C**) MI: Mesones de Iseula; MT: Minas Tierga; BJ: Barranco del Judio; CC:
691 Las Cuevas/ Las Coronadas. **D**) Town of Purujosa with Purujosa 3 series marked; stars
692 indicate field sites in Murero and Mansilla Formations. Key: Units increase in age from left
693 to right, top older than bottom (see Figure 2). (Jalón Formation is older than Ribota
694 Formation, and Embid Formation is older than Jalón Formation.)
695
696 FIG 2.—Stratigraphy of the studied sections. Unit thicknesses and local lithologies at each of
697 the three field sites are depicted. Stars mark levels where articulated trilobites were collected.
698 Adapted from Gozalo et al. (2008), Gámez Vintaned et al. (2009), and Zamora et al. (2011).
699
700 FIG 3.—*Eccaparadoxides pradoanus* (Verneuil and Barrande in Prado et al. 1860) from the
701 Purujosa Red Beds, Murero Formation (Cambrian Series 3, Drumian), Iberian Chains, Spain,
702 at a number of stages of repair. **A**) MPZ 2011/6: nearly complete thorax with crescent shaped
703 recent injury on a thoracic spine. **B**) MPZ 2012/844: slightly disarticulated specimen with
704 two shortened thoracic spines. **C**) MPZ 2012/1009: rear of a thorax showing thoracic spine
705 with slight regrowth of the tip. **D**) MPZ 2012/7808: near complete thorax with two shortened
706 thoracic spines. **E**) Box from A, arrow indicates injured spine. **F**) Box from B, arrow
707 indicates shortened spines. **G**) Box from C, arrow indicates spine beginning regrowth. **H**)
708 Box from D, arrow indicates two nearly fully repaired spines. Scale bars: **A**-**D**) 10 mm, **E**-**H**)
709 1 mm.
710
711 FIG 4.— *Eccaparadoxides pradoanus* (Verneuil and Barrande in Prado et al. 1860) nearly
712 complete thorax from the Purujosa Red Beds, Murero Formation (Cambrian Series 3,
713 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.

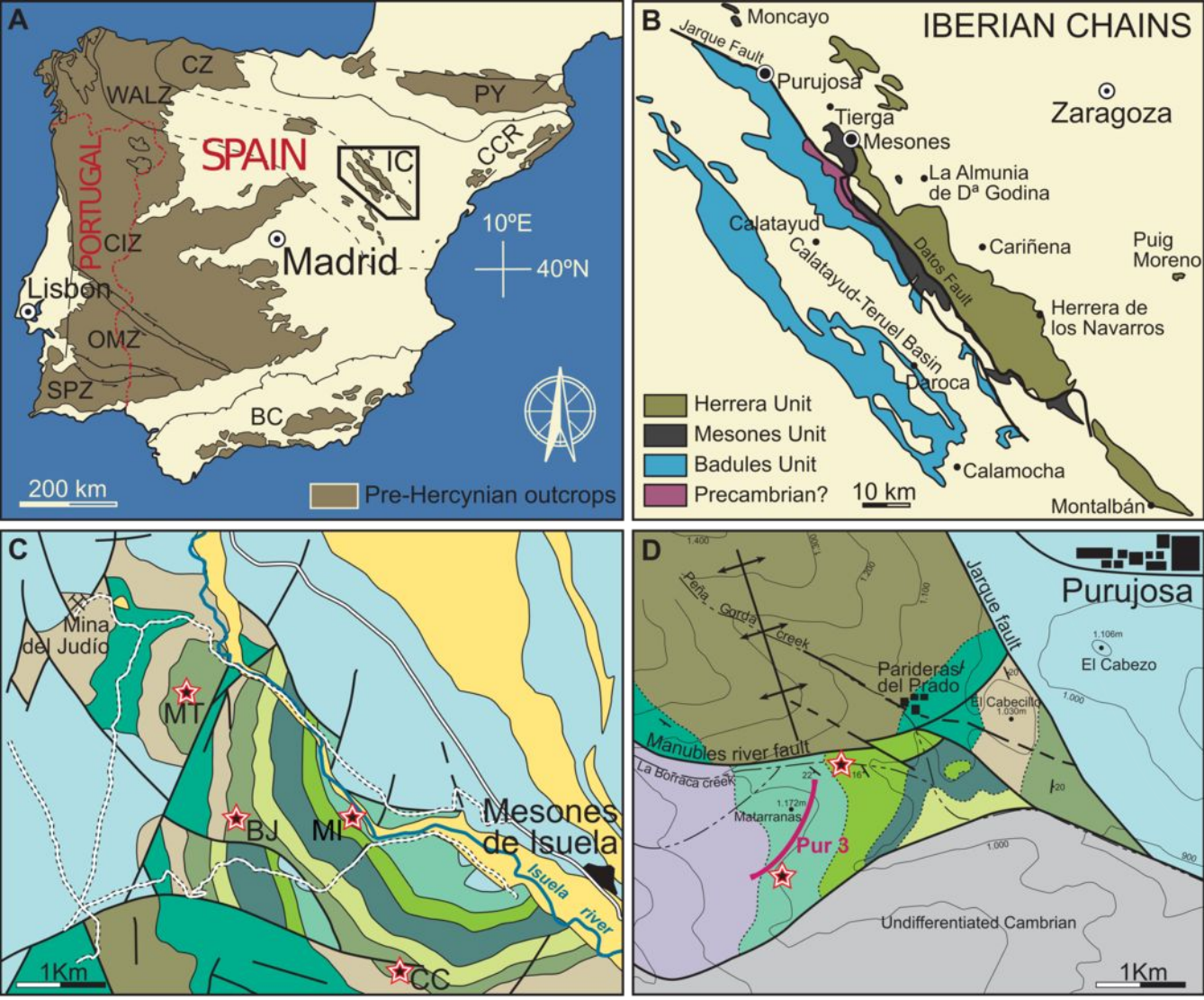
FIG 8.—Principal Component Analysis of *Bicrescomanducator serratus* (Zamora et al. 2011) and abiotic breaks. Outline of the shapes of the breaks semilandmarked, as shown by dotted red line in **A**) and **B**). Black arrows indicate the direction of semilandmark placement. Scale bars = 5 mm.

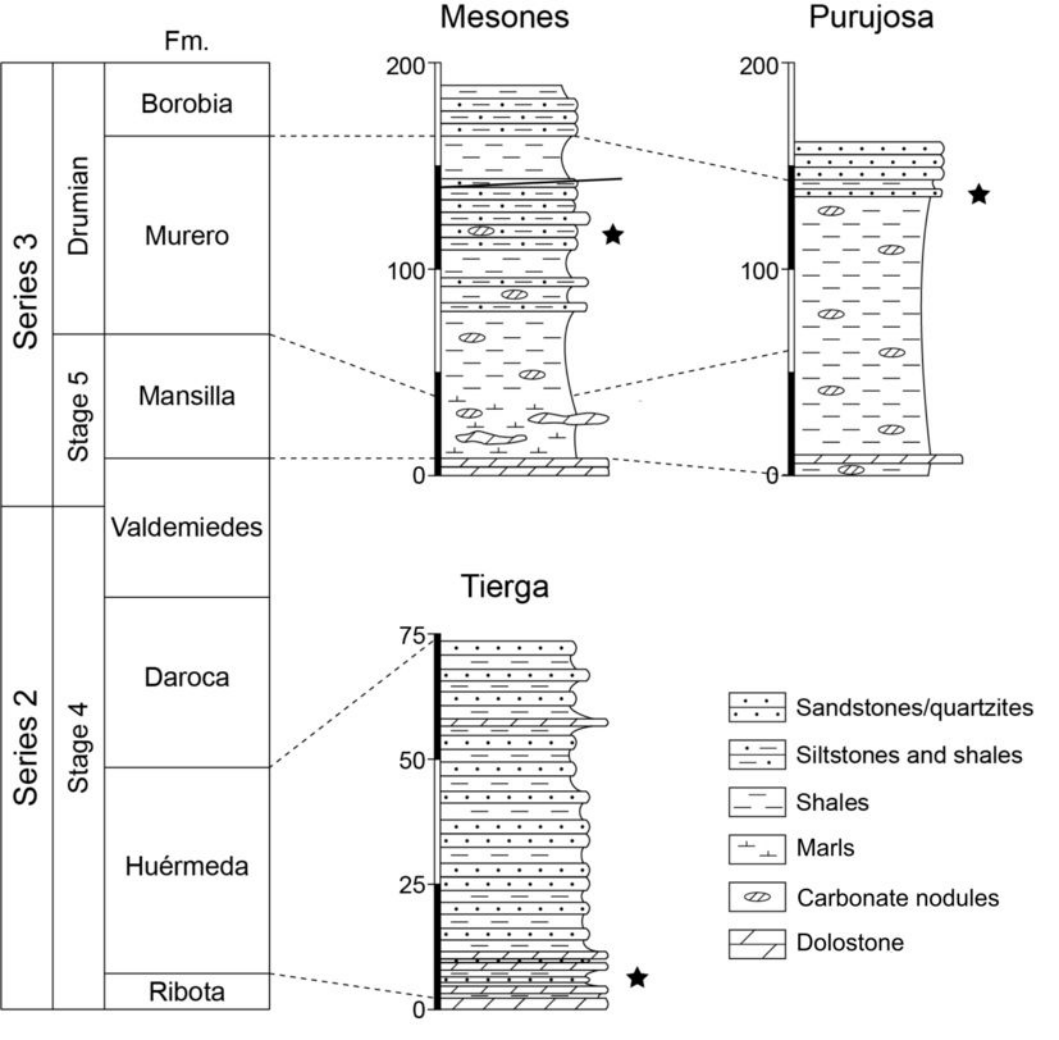
FIG 9.—Principal Component Analysis plot of the semilandmarked breaks (same as Figure 8), separating specimens by formation and by origin of the breaks. The overlap of previously described examples of *Bicrescomanducator serratus* (Zamora et al. 2011) (points with black dots) with new examples (points 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.

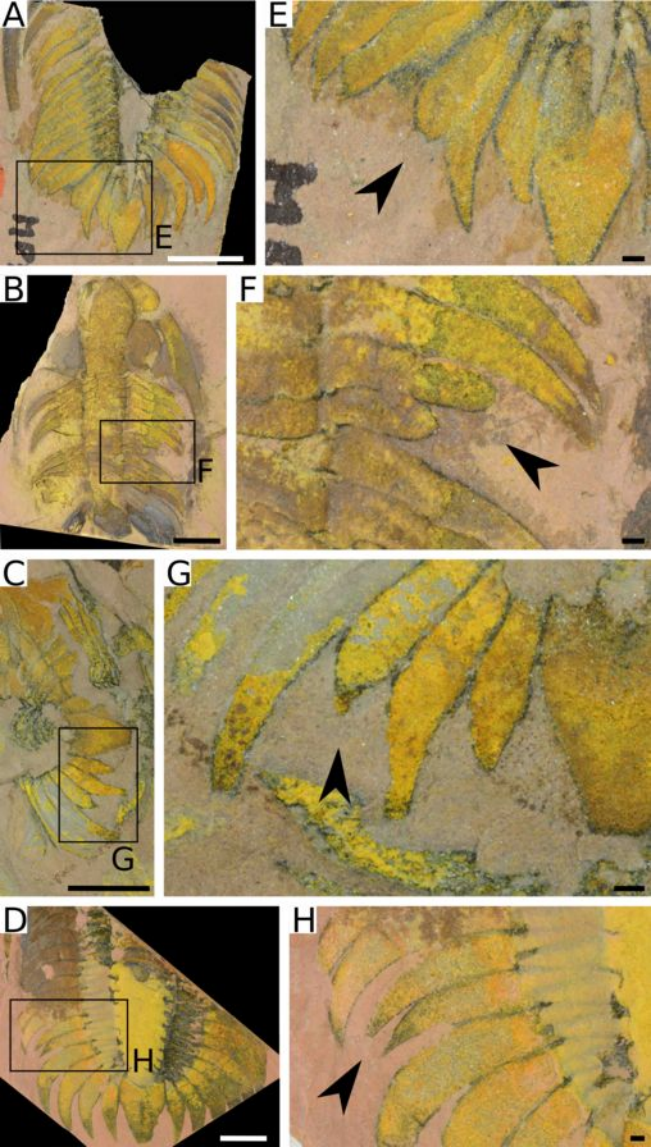
FIG 10.—Idealized repair sequence of trilobite pleural spines, based on Figure 3. Grey lines show uninjured shape of middle spine, dotted black line shows previous stage of healing. Healing stages: **A**) Immediately after injury; **B**) Rounded spine after initial healing; **C**) Regrowth begins, with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

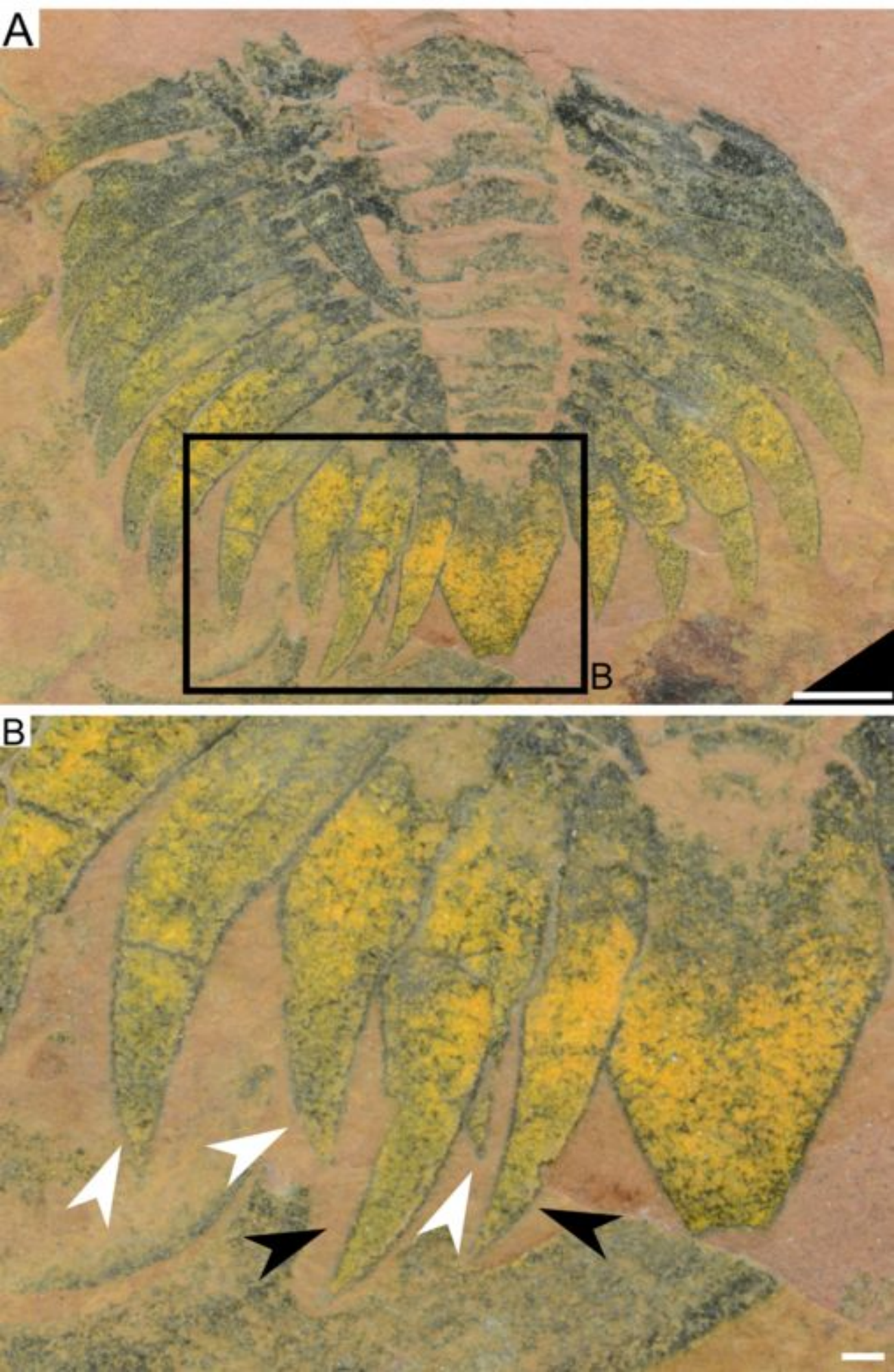
TABLE CAPTIONS

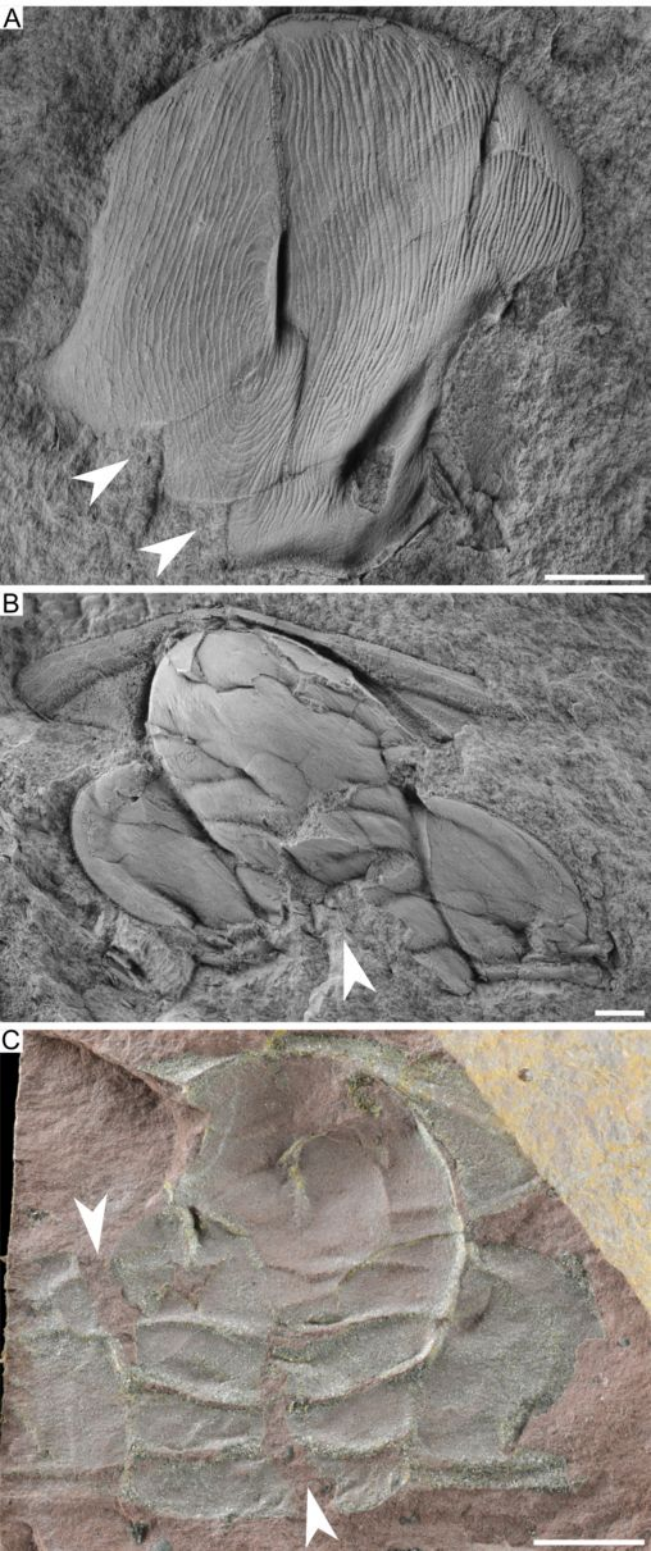
763 TABLE 1.—Frequency of repairs in Cambrian trilobites. Formation and locality information
764 for trilobite repair frequencies and Bayesian Inference 5^{th} and 95^{th} percentile values. F and R
765 are repair frequency metrics and MF is the multiple repair frequency metric (defined in
766 methods).
767 TABLE 2.—Adjacent injuries in complete trilobite thoraces from the Purujosa Red Beds. P-
768 values calculated using a binomial test with 10 successes from 14 attempts. The random
769 probability of a success (a short spine adjacent to another short spine) is 2/2207 (2 available
770 adjacent spines, with 2207 available spines in total).
771 TABLE 3.—Analysis of the location of injuries on trilobites from the Purujosa Red Beds.
772 Expected values calculated using a two-tailed binomial analysis. Measured values are from
773 observations. P-values calculated as described in methods.
774


A
CZ
WALZ
PY
SPAIN
IC
CCR
PORTUGAL
CIZ
Madrid
10ºE
40ºN
Lisbon
OMZ
SPZ
BC
200 km
Pre-Hercynian outcrops
B
IBERIAN CHAINS
Moncayo
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
Herrera Unit
Mesones Unit
Badules Unit
Precambrian?
Calamocha
10 km
Montalbán
C
Mina del Judío
MT
BJ
MI
Mesones de Isuela
Isuela river
1Km
CC
D
Jarque fault
Purujosa
Peña Gorda creek
Parideras del Prado
El Cabezo
El Cabecillo
Manubles river fault
La Borraca creek
Matarranas
Pur 3
Undifferentiated Cambrian
1Km
Cenozoic
Mesozoic
Borobia Fm.
Murero Fm.
Mansilla Fm.
Valdemiedes Fm.
Daroca Fm.
Huérmeda Fm.
Ribota Fm.
Jalón Fm.
Embid Fm.
Anticline
Fault
Localities


Fm.
Mesones
Purujosa
Tierga
Series 3
Series 2
Drumian
Stage 5
Stage 4
Borobia
Murero
Mansilla
Valdemiedes
Daroca
Huérmeda
Ribota
200
100
0
75
50
25
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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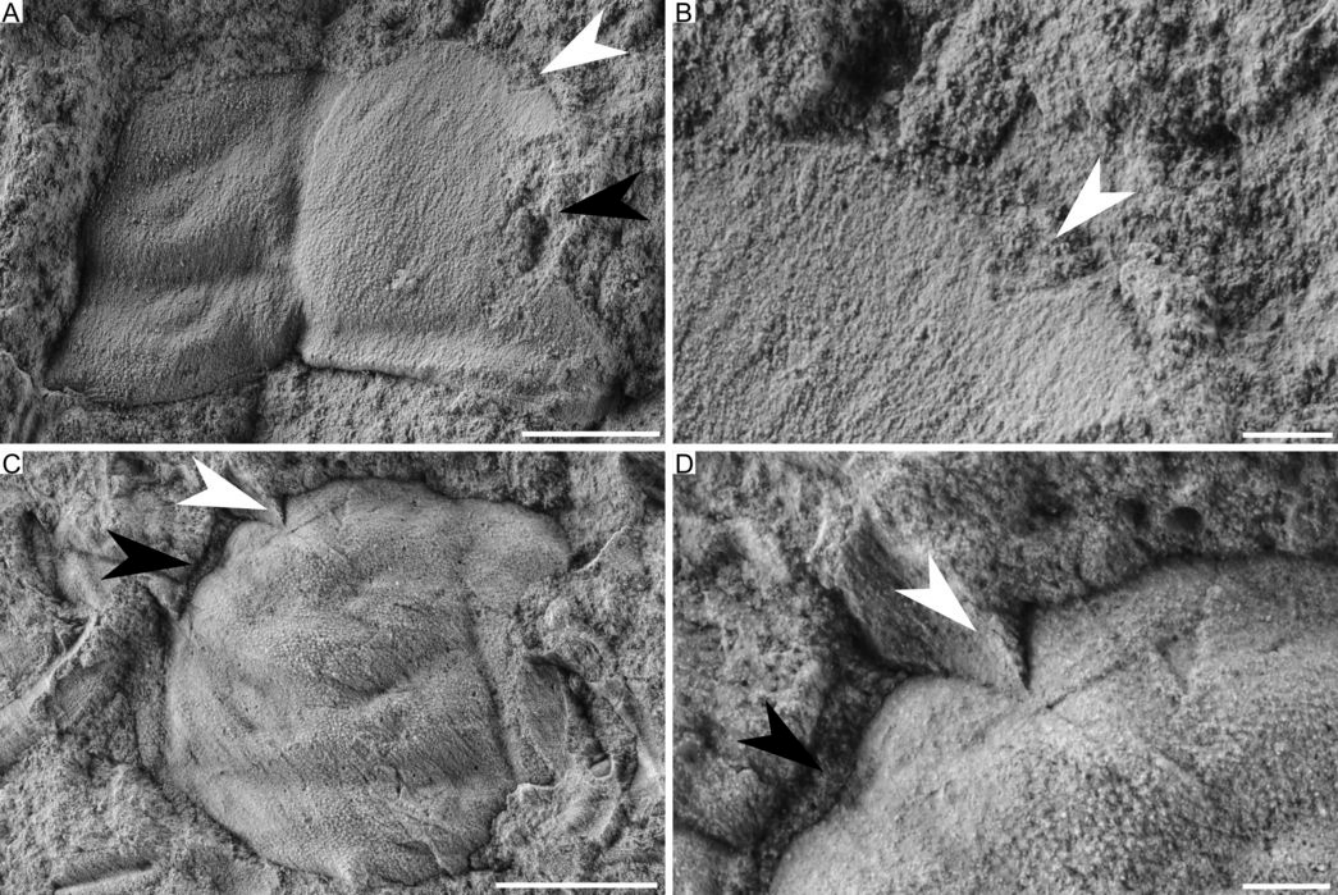
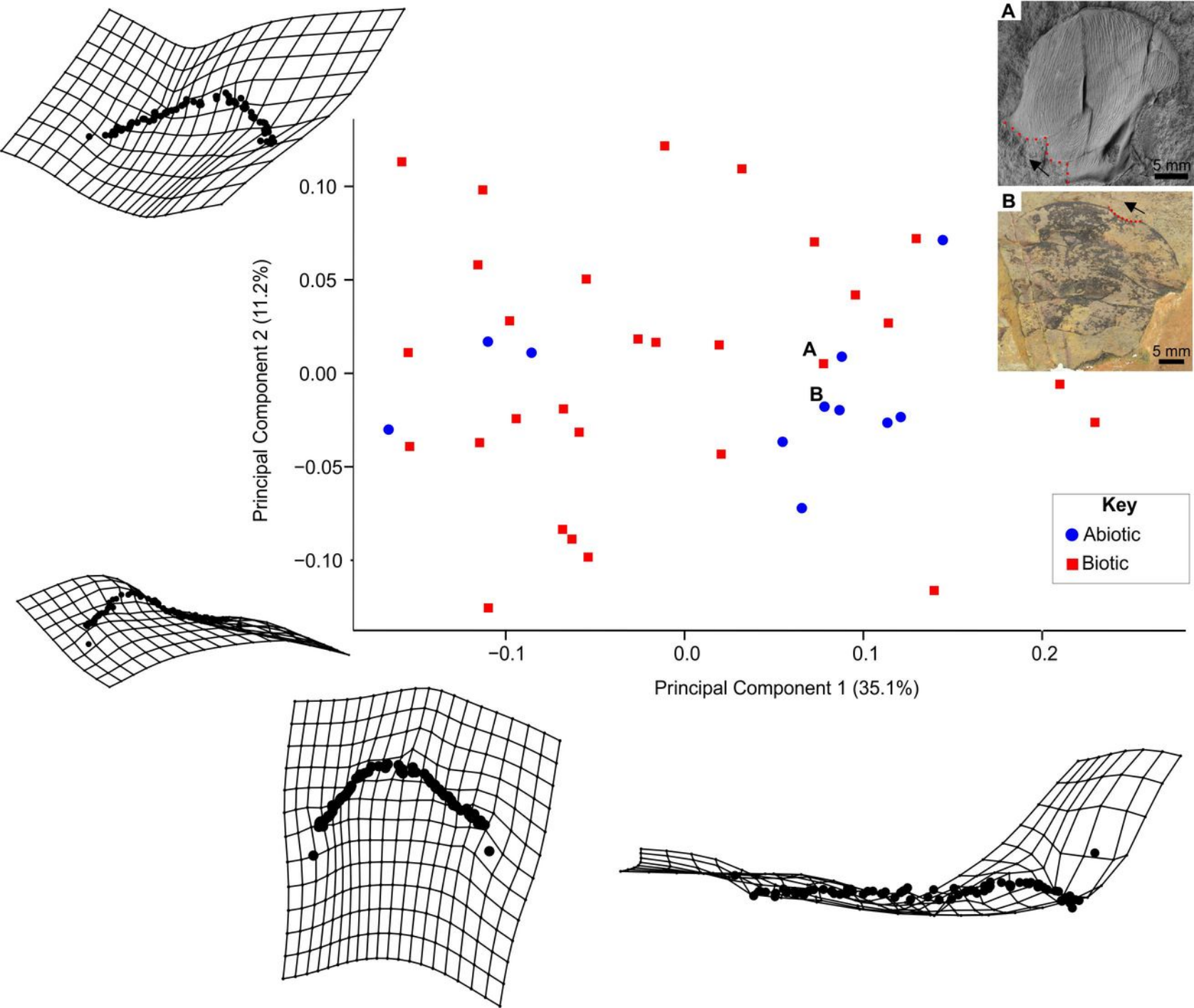
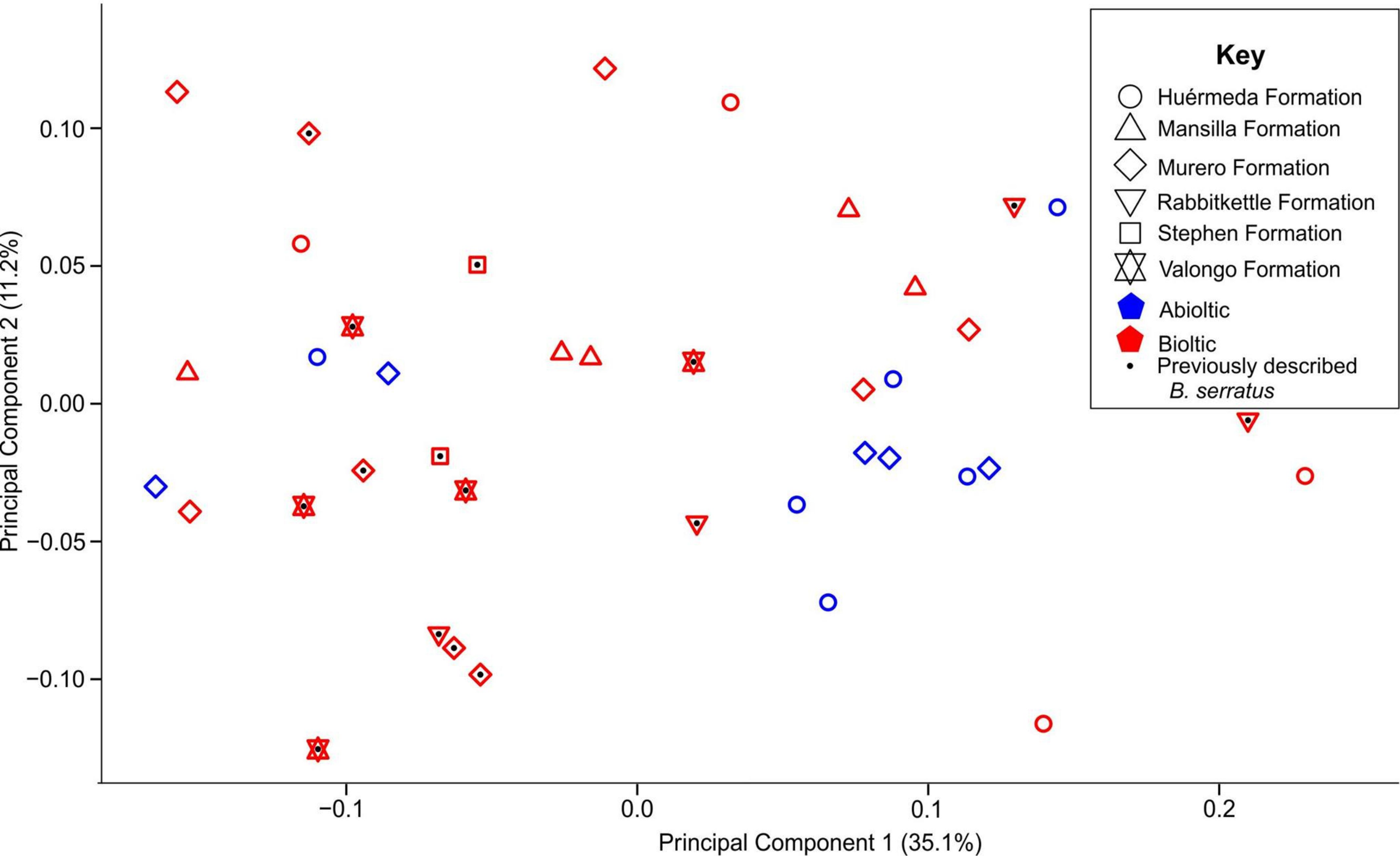
B
C


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%)
A
B
−0.1
0.0
0.1
0.2
Principal Component 1 (35.1%)
Key
Abiotic
Biotic


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

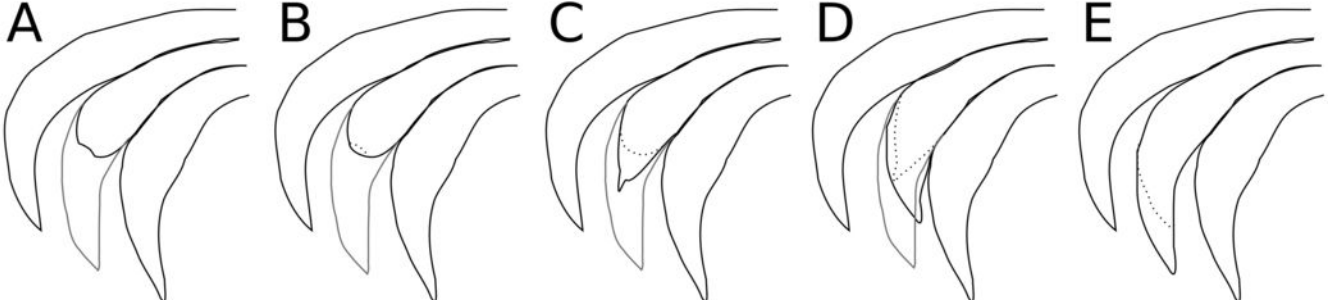

A
B
C
D
E

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.