



Associations between trilobite intraspecific moulting variability and body proportions: *Estaingia bilobata* from the Cambrian Emu Bay Shale, Australia

by HARRIET B. DRAGE^{1*} , JAMES D. HOLMES² ,
DIEGO C. GARCÍA-BELLIDO^{3,4}  and JOHN R. PATERSON⁵ 

¹Institute of Earth Sciences, University of Lausanne 1015, Lausanne, Switzerland; harriet.drage@unil.ch

²Department of Earth Sciences, Palaeobiology, Uppsala University, Uppsala 752 36, Sweden; james.holmes@geo.uu.se

³School of Biological Sciences, University of Adelaide, Adelaide, South Australia 5005, Australia; diego.garcia-bellido@adelaide.edu.au

⁴South Australian Museum, Adelaide, South Australia 5000, Australia

⁵Palaeoscience Research Centre, School of Environmental and Rural Sciences, University of New England, Armidale, New South Wales 2351, Australia; jpater20@une.edu.au

*Corresponding author

Typescript received 27 July 2022; accepted in revised form 11 April 2023

Abstract: Trilobites were notably flexible in the moulting behaviours they employed, producing a variety of moult configurations preserved in the fossil record. Investigations seeking to explain this moulting variability and its potential impacts are few, despite abundant material being available for study. We present the first quantitative study on moulting in a single trilobite species using a dataset of almost 500 moult specimens of *Estaingia bilobata* from the Cambrian (Series 2, Stage 4) Emu Bay Shale, South Australia. Specimens were categorized by moulting mode (Salter's or Sutural Gape) and their associated configurations, and their body proportions measured from both a museum collection (including a bycatch sample) and a randomly-collected field sample. This enabled analysis of the proportion of *E. bilobata* specimens displaying the Sutural Gape and Salter's modes of moulting and their different configurations, and tests for association between moulting behaviour and

body proportions. The results show a wide range of *E. bilobata* moulting configurations in all samples, suggesting that configurations represent definable instances in a largely continuous spectrum of variation. Analyses comparing body proportions of specimens showing the two modes of moulting were non-significant, suggesting there is no true association between moulting behaviour and body proportion, except for a single significant result for body length. All results were relatively consistent between the museum and field samples. However, removing accessioned specimens from the museum sample brought results even further in line with the field sample, supporting the need for consideration of museum collection bias in palaeontological analyses.

Key words: moulting, Emu Bay Shale, behaviour, trilobite, Cambrian, collection bias.

EXPLORATION of moulting behaviours preserved in the arthropod fossil record is of great importance for understanding the evolution of this most diverse and abundant animal group (Vevea & Hall 1984; Brandt 2002). All arthropods, extinct and extant, must periodically moult their exoskeletons in order to grow, develop, and repair their bodies (Ewer 2005). The restrictiveness of supporting and replacing an exoskeleton presumably impacts on other aspects of arthropod life history and evolution, such as body morphology, proportions, and their scaling with growth (Henningsmoen 1975).

Trilobites had highly biomineralized exoskeletons preserving vast numbers of moults, and have been described as showing seemingly unrivalled levels of both

interspecific and intraspecific variability amongst Arthropoda, including variance with ontogenetic stage (Brandt 2002; Daley & Drage 2016; Drage *et al.* 2018; Drage 2019a; Wang *et al.* 2021). They also demonstrate a variety of morphological adaptations to different moulting behaviours, such as the facial sutures used specifically to create an ecdysial gape for moulting in some of the earliest trilobites and across all major clades (Stubblefield 1959; Whittington *et al.* 1997), as well as the repeated loss of these sutures (Stubblefield 1959), and the acquisition of a marginal cephalic suture for moulting in several groups (Stubblefield 1959; Henningsmoen 1975). This variability, given the central importance of moulting in the lives of all arthropods, may have conveyed an

evolutionary advantage to trilobites, resulting in their extensive longevity and extreme diversity, abundance and geographical ubiquity (Drage 2019a). In contrast, modern arthropod groups tend to show less flexibility in their moulting behaviours (Daley & Drage 2016), and an opposing view suggests that this lack of specialization may have contributed to the decline and ultimate extinction of trilobites (Brandt 2002). Although interspecific variation can be broadly examined by looking at examples across different groups (Drage 2019a), trilobite specimens are not usually preserved in sufficient quantity and quality to allow investigations into the extent of intraspecific variability in moulting behaviour, or any influence this may have had on the evolution of their morphology, behaviour and development, or indeed the evolutionary success of Trilobita as a whole.

Estaingia bilobata Pocock, 1964, is a small ellipsocephaloid trilobite preserved as articulated carcasses and moulted exoskeletons in enormous numbers in the Cambrian (Series 2, Stage 4) Emu Bay Shale on Kangaroo Island, South Australia (Paterson *et al.* 2016). Owing to the low-energy depositional environment, exceptional preservation and extreme abundance of *E. bilobata* in the Emu Bay Shale (Holmes *et al.* 2021a, 2021b), *in situ* moult configurations are very common. Specimens of *E. bilobata* from the Emu Bay Shale were previously used by Drage *et al.* (2018) to determine the variety of moulting configurations in this species (Fig. 1). Here, we document moulting behaviour variability within a single trilobite species using almost 500 specimens of *E. bilobata* and provide the first quantitative analysis to test for a potential link between allometry and moulting behaviour in trilobites. In this study, we aim to:

1. Investigate the full extent of moult configuration variability within a single, exceptionally-preserved trilobite species;
2. Determine whether there is a link between moulting mode/configuration and allometry; and
3. Qualify the impact, using *E. bilobata* as an example, that collection and museum-accession bias (e.g. registering only interesting or unique specimens) can have on the study of trilobite moulting.

MATERIAL AND METHOD

Sample collection

236 moult configurations of *Estaingia bilobata* were identified within the Emu Bay Shale material at the South Australian Museum – Adelaide Palaeontological collection (prefix SAMA P), here termed the ‘museum sample’. 71% of these were considered ‘bycatch’, that is, moult configurations found on samples collected and accessioned for a

different fossil on the same sample. A further 243 moult configuration specimens were collected during a 2018 field study, termed the ‘field sample’, during which all *E. bilobata* specimens identified as moults were kept and described. All field and museum specimens recorded in this study were collected from a *c.* 2 m stratigraphic interval composed largely of dark grey, laminated mudstone approximately 10 m above the base of the Emu Bay Shale, exposed in Buck Quarry and the adjacent Daily Quarry (*c.* 50 m away), at Big Gully on the north coast of Kangaroo Island (see García-Bellido *et al.* (2009) and Paterson *et al.* (2016) for detailed locality information and maps). The mudstones that preserve articulated specimens of *E. bilobata* are interpreted as having been deposited in a dysoxic, pro-delta environment, to which these trilobites were likely to have been adapted (Gehling *et al.* 2011; Paterson *et al.* 2016; Jago *et al.* 2021). Although approximate stratigraphic level is recorded for collected specimens, it is not possible to study populations on single bedding planes due to the laterally-discontinuous nature of layers. However, the mudstones within the interval sampled for *E. bilobata* specimens display the same sedimentology, and the fossils exhibit the same style of preservation (see Holmes *et al.* 2021a). All specimens collected and recorded represent holaspides, with a full complement of 13 thoracic tergites and a lack of macropleural spines on the second thoracic tergite (Holmes *et al.* 2021a). Further, all included specimens are larger than the smallest early-stage holaspides of *E. bilobata* recorded by Holmes *et al.* (2021a, fig. 8). The total study sample therefore consists of 479 adult *E. bilobata* specimens.

Analysis

For each specimen, the mode of moulting employed by the individual and the resulting preserved moulting configuration were recorded, based on the designations described and figured by Drage *et al.* (2018, fig. 1). The modes of moulting described for trilobites are Salter’s mode, which involves disarticulation of the cephalothoracic joint only (Henningsmoen 1975), and the Sutural Gape mode, in which only cephalic sutures (facial and/or ventral sutures) open during moulting (Drage 2019a). The preserved moulting configurations, depending on the disarticulation and orientation of specific sclerites of the exoskeleton, include the axial shield, Harrington’s, Henningsmoen’s, McNamara’s, Nutcracker, Somersault, Salter’s and Zombie configurations, as described by Drage *et al.* (2018) and Drage (2019a, 2019b). The Zombie configuration was not originally documented in *E. bilobata* by Drage *et al.* (2018), but was later described and named by Drage (2019b, ch. 1); this configuration comprises an

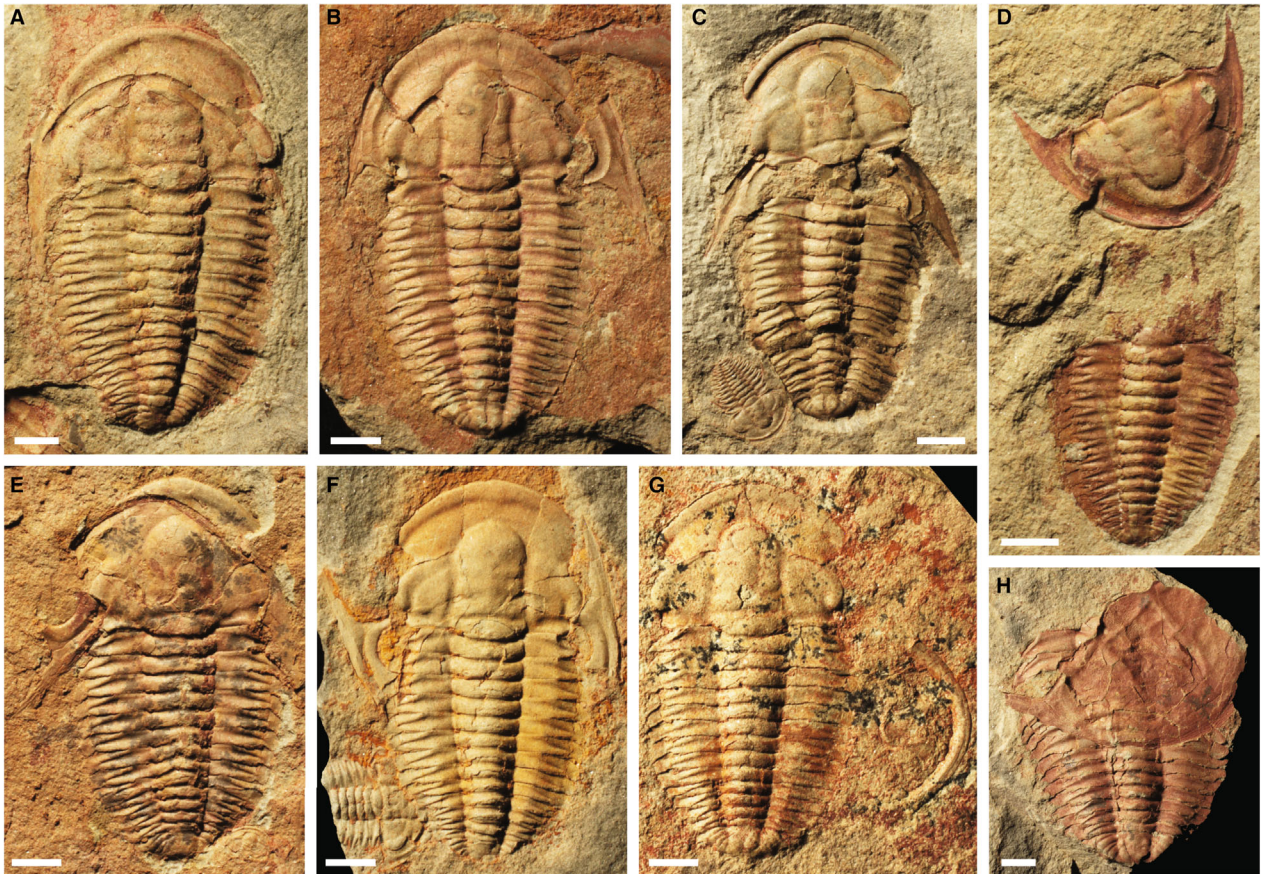


FIG. 1. The variety of moulting configurations preserved by specimens of *Estaingia bilobata* from the Emu Bay Shale. A, Harrington's configuration (SAMA P59487). B, Nutcracker configuration (SAMA P59512). C, Henningsmoen's configuration (SAMA P59513). D, Zombie configuration (SAMA P59514). E, McNamara's configuration (SAMA P54207). F, Somersault configuration (SAMA P54204). G, axial shield with hypostome and rostral plate displaced to right side of trunk (SAMA P59515). H, Salter's configuration (SAMA P52810). See Drage *et al.* (2018) and Drage (2019a) for full descriptions of moulting configurations, and Drage (2019b) for details on the Zombie configuration. All scale bars represent 5 mm.

intact cephalon disarticulated from the thoracopygon, with no inversion of the cephalon. In this study, we consider moulting configurations with clear cephalothoracic joint disarticulation but no cephalic suture operation to represent Salter's mode (i.e. Salter's and Zombie configurations), and those showing only cephalic suture use to represent the Sutural Gape mode (i.e. axial shield, Harrington's, McNamara's, Nutcracker and Somersault configurations). Henningsmoen's configuration shows both opening of the facial sutures and cephalic/thoracic disarticulation, as such representing a transitional mode. We therefore initially conducted our analysis with this configuration excluded, and for comparison then included it within either the Sutural Gape or Salter's mode.

Size measurements were taken for 431 specimens. Measurements include: total body length (sag.); maximum width (tr.) of anterior cranial border; maximum width (tr.) of thorax excluding the pleural spines; and width (tr.) of cephalothoracic joint (Fig. 2). Field specimen

measurements were taken using digital callipers at the millimetre scale. ImageJ (Schneider *et al.* 2012) was used to take all linear measurements from photos of the museum specimens. Not all measurements could be taken for every moult, being dependent on their preservation. Where the cephalon was detached, total body length (sag.) was measured by summing the lengths of the cephalon and the thoracopygon, excluding the first articulating half ring.

The proportions of different moulting configurations preserved in the *E. bilobata* samples are shown using tree-maps (Bederson *et al.* 2002), and chi-squared analyses were used to test for differences in proportions between the museum and field samples. Links between body measurements and moulting behaviour were tested for by linear regression of measurement data, and ANCOVA analyses to compare the slopes and intercepts of these linear regressions for the different moulting modes. We tested for association between body measurement data

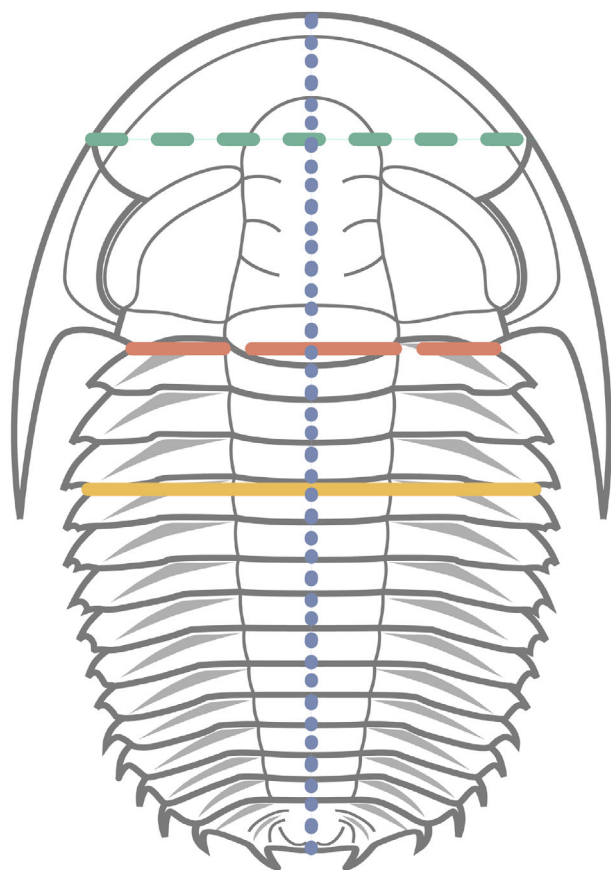


FIG. 2. Body size dimensions recorded for field and museum specimens of *Estaingia bilobata*: total body length (sag.) (dotted blue line); maximum width (tr.) of anterior cranial border (short-dashed green line); maximum width (tr.) of thorax (solid yellow line); width (tr.) of cephalothoracic joint (long-dashed red line).

and mode of moulting, rather than the moulting configuration preserved, because the segmented sample sizes for two moulting configurations were not sufficiently large to enable reliable significance testing. Body measurement data contrasted includes:

1. Maximum width (tr.) of anterior cranial border with total body length (sag.);
2. Maximum width (tr.) of thorax with total body length (sag.);
3. Maximum width (tr.) of anterior cranial border with maximum width (tr.) of thorax;
4. Width (tr.) of cephalothoracic joint with maximum width (tr.) of thorax.

These measurements were chosen because they capture relevant allometric data for the exuvial gape locations: the anterior cephalic opening between the facial sutures, and the cephalothoracic joint. F-tests were used to confirm significant links between the allometric pairings listed above, that is, whether different body proportions are positively correlated with overall growth.

Each separate body measurement (total body length, maximum width of anterior cranial border, maximum width of thorax, and width of cephalothoracic joint) was tested for association with mode of moulting through ANCOVA analyses, while controlling for its covariate, as listed above. In each case, the covariate was decided based on consideration of the body measurement that might have directly caused a change in the measurement of interest. Controlling for these covariates meant that the ANCOVA results were unaffected by changes in the covariate and only impacted by a potential association with mode of moulting. Where appropriate, ANCOVA analyses were followed by post-hoc two-sample *t*-tests to aid further interpretation of significant results.

All graphs and statistical analyses were made and conducted, respectively, using R in RStudio (v2021.09.0+351 ‘Ghost Orchid’; RStudioTeam 2020), in addition to the following packages: ggplot2 (v3.3.6; Wickham 2016); ColorBrewer (v1.1.3; Brewer *et al.* 2003); car (v3.1.1; Fox & Weisberg 2019); tidyverse (v1.3.1; Wickham *et al.* 2019); all using R v4.1.2 (R Core Team 2013). Raw data for all specimens included in this study are available in Appendix S1.

RESULTS

Moulting variability in Estaingia bilobata

Both Salter’s and the Sutural Gape mode of moulting are identifiable in the museum and field specimens of *E. bilobata*, and all types of moult configuration described by Drage *et al.* (2018) are observed. In fact, additional deviations in moult configuration preservation are occasionally observed, such as specimens characterized by inversion of the librigenae (McNamara’s and Somersault configurations) also showing slight cranial displacement. There is also a high incidence of rostral plate displacement in the total sample, including most axial shields (with missing librigenae); this was not part of the definition of an axial shield by Henningsmoen (1975) or Drage *et al.* (2018). Opening of the rostral suture is, in general, less commonly described for trilobite moult configurations. This may be because: (1) the rostral plate cannot be observed in association with the rest of the exoskeleton, especially if only the dorsal surface morphology of the trilobite is preserved or dorsal imprinting of ventral structures is lacking in mouldic or compressed specimens; (2) rostral plates are moved to a location away from the remainder of the exoskeleton either through movement of the emerging individual or by other physical processes (e.g. currents, interactions with other individuals); or (3) some lineages fused the rostral plate (Henningsmoen 1975) and others reduced, or reduced and subsequently lost, the rostral plate (Chatterton *et al.* 1994).

In the total (field and museum) sample, 341 specimens (71%) show the Sutural Gape mode, with Harrington's configuration being the most common within this mode with 205 specimens (43%) (Fig. 3). The Somersault configuration is relatively rare, with 9 specimens (2%), but more than 30 specimens show each of the other Sutural Gape configurations. Salter's mode is represented by 73 specimens (15%), with 58 specimens (12%) in Salter's configuration and the rarer Zombie configuration at 15 specimens (3%). The hybrid Henningsmoen's configuration is reasonably common, representing 65 specimens (14% of the total sample).

Body proportions and moulting

All pairings of *E. bilobata* body measurements are well explained by linear regression models, with all instances showing strong positive correlations and highly significant F-test associations (Table 1). The slope values for all specimens, and each of the regression lines for the two modes of moulting, vary little between themselves for each measurement pair (Table 1); this is also readily apparent in their scatter graphs (Fig. 4). Similarly, the intercepts are

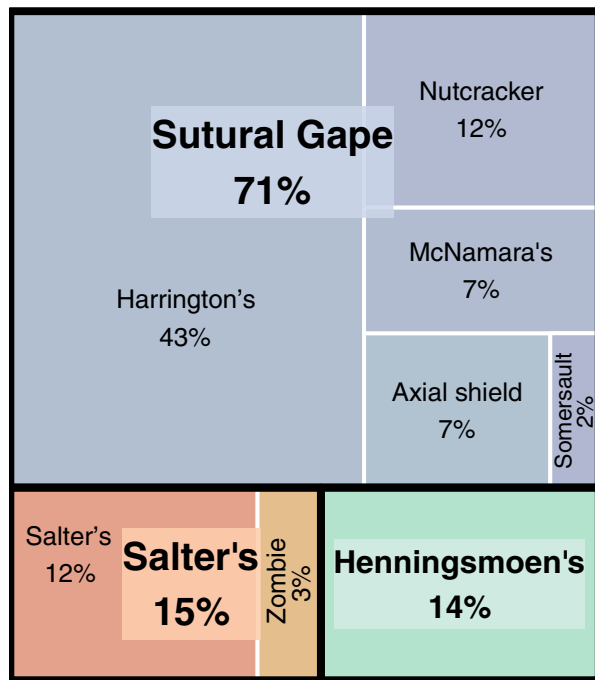


FIG. 3. Treemap (Bederson *et al.* 2002; RStudioTeam 2020) of the total sample. Percentages represent the proportion of *Estaingia bilobata* specimens that used Salter's or the Sutural Gape mode of moulting, the hybrid Henningsmoen's configuration, and preserved in each of the moulting configurations (Drage *et al.* 2018; Drage 2019a, 2019b).

very consistent for the two modes of moulting and for both combined, although they tend to show some slight differences (Table 1). However, the linear regressions for Salter's mode have slightly steeper slopes and correspondingly more negative intercepts.

Body measurements capturing the exuvial gape locations (i.e. anterior cranial width, thoracic width and cephalothoracic joint width) show little statistical association with the mode of moulting employed. ANCOVA analyses testing for differences between the specimens showing either Salter's or the Sutural Gape mode of moulting were non-significant for all these moulting location-related measurements (Table 2), except for body length. Therefore, when controlling for a covariate, mode of moulting used by an individual of *E. bilobata* had no significant association with the maximum width of the anterior cranial border ($p = 0.147$), the maximum width of the thorax ($p = 0.595$), or the cephalothoracic joint width ($p = 0.0660$) (Table 2). However, the result for cephalothoracic joint width is very close to significance and so this may be considered to indicate a weak association with moulting mode. The means suggest that specimens showing the Sutural Gape mode may have a slightly broader cephalothoracic joint at 8.18 mm on average compared to those showing Salter's at 7.33 mm. This result was maintained for these body measurements when controlling for a variety of other covariates. Only body length, when controlled for a variety of covariates, was significantly associated with the mode of moulting employed ($p = 0.0175$; Table 2). Mean body length was higher for specimens showing the Sutural Gape mode of moulting at 21.1 mm than for Salter's at 14.1 mm. A post-hoc two-sample *t*-test comparing the mean body length of specimens showing each mode of moulting was significant ($t = -4.66$, $p < 0.001$), suggesting that this difference may represent a true signal.

The results overall showed the same significance (or lack thereof) with specimens showing Henningsmoen's configuration incorporated within either the Sutural Gape or Salter's mode, or excluded entirely from the dataset (as reported in Tables 1, 2). The only difference being that the inclusion of the hybrid Henningsmoen's configuration within Salter's mode (based on sharing cephalothoracic joint disarticulation) erased the significant result from body length, and caused the cephalothoracic joint width signal to become strongly significant (see Table S1). Thus, the inclusion or exclusion of the hybrid Henningsmoen's configuration had only minor impact on the body proportion results.

Museum collection vs field sampling

In general, the proportions of moulting modes and preserved configurations for *E. bilobata* are very similar

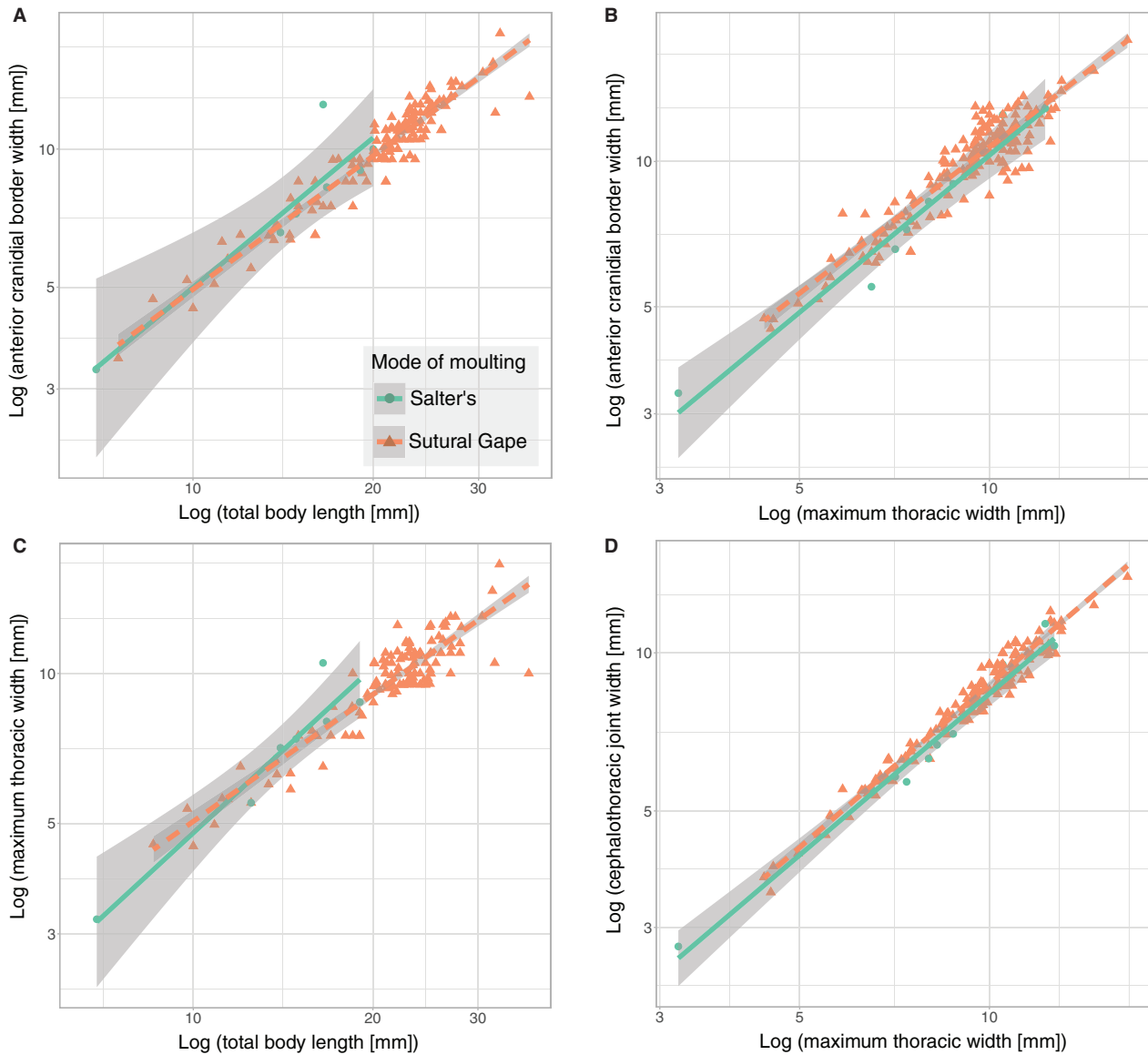


FIG. 4. Scatter graphs (RStudioTeam 2020) comparing log anterior cranial border width (tr.) to log total body length (sag.) (A) and log maximum thoracic width (tr.) (B); log maximum thoracic width (tr.) to log total body length (sag.) (C); and log cephalothoracic joint width (tr.) to log maximum thoracic width (tr.) (D), for measured specimens of *Estaingia bilobata* (Henningsmoen's configuration specimens excluded). Lines and error margins (grey) show the linear regression models for the specimens separately showing the Sutural Gape mode of moulting (orange triangle points, orange dashed line), and Salter's mode of moulting (green circle points, green solid line).

between the museum and field samples, though there are slight differences. Overall, 77% of the field sample represented the Sutural Gape mode (Fig. 5A), compared to 65% of the museum sample (Fig. 5B). Harrington's configuration was the most common in both samples, though less common in the museum sample (38%) compared to the field sample (48%) (Fig. 5). Summing both Harrington's and Henningsmoen's configurations in the two samples (with Henningsmoen's differing from Harrington's only in having an open cephalothoracic joint) gives a very similar proportion of 58% of the field sample and 55% of

the museum sample. The proportions of other moult configurations were very similar between the two samples, differing by <4%. A chi-squared test for differences in the proportions of moult configurations between the two samples was non-significant ($p = 0.219$; Table 3), suggesting that there is no non-random bias in the moulting configuration proportions observed in the museum and field samples.

To determine whether the accessioned specimens within the museum sample (equating to 29% of the total sample) were creating a bias towards the less common Salter's

TABLE 1. Results from linear regression analyses of body proportion measurements in *Estaingia bilobata* (Henningsmoen's configuration excluded).

Measurement pair	Mode of moulting	Intercept	Slope	F value	p value
Maximum width (tr.) of anterior cranial border + total body length (sag.)	Both	-0.2672	0.9633	1327	$<2.200 \times 10^{-16}$ *
	Sutural Gape	-0.2723	0.9664	1331	$<2.200 \times 10^{-16}$ *
	Salter's	-0.3855	1.084	26.52	3.615×10^{-3} *
Maximum width (tr.) of thorax + total body length (sag.)	Both	-0.1549	0.8556	779.8	$<2.200 \times 10^{-16}$ *
	Sutural Gape	-0.1438	0.8467	653.4	$<2.200 \times 10^{-16}$ *
	Salter's	-0.4243	1.104	50.89	8.404×10^{-4} *
Maximum width (tr.) of anterior cranial border + maximum width (tr.) of thorax	Both	0.005426	1.024	1436	$<2.200 \times 10^{-16}$ *
	Sutural Gape	0.02295	1.007	1220	$<2.200 \times 10^{-16}$ *
	Salter's	-0.06860	1.081	128.3	2.836×10^{-5} *
Width of cephalothoracic joint (tr.) + maximum width (tr.) of thorax	Both	-0.09584	1.035	4674	$<2.200 \times 10^{-16}$ *
	Sutural Gape	-0.09083	1.031	4136	$<2.200 \times 10^{-16}$ *
	Salter's	-0.09870	1.021	357.8	6.313×10^{-8} *

*Significant result $p < 0.05$.

TABLE 2. ANCOVA analysis results for each body measurement of *Estaingia bilobata*, testing whether moulting mode (Sutural Gape or Salter's; Henningsmoen's configuration excluded) had a significant effect on the body measurement, while controlling for a covariate.

Measurement	F value	p value
Maximum width (tr.) of anterior cranial border (total body length controlled)	2.13	0.147
Maximum width (tr.) of thorax (total body length controlled)	0.284	0.595
Total body length (sag.) (maximum width of thorax controlled)	5.79	0.0175*
Width (tr.) of cephalothoracic joint (maximum width of thorax controlled)	3.42	0.0660

*Significant result, $p < 0.05$.

mode (35%, compared to 23% in the field sample) and related configurations (Fig. 5), all registered specimens were removed and the data reanalysed. This resulted in the proportion of Salter's mode being reduced to 30% (Fig. 5C), closer in line with the field sample. Moreover, the proportions of all moult configuration types in the bycatch-only museum sample became more closely aligned with those in the field sample, particularly Harrington's and Henningsmoen's configurations (Table S1). A chi-squared test comparing the field sample and the bycatch-only museum sample remained non-significant ($p = 0.911$; Table 3), and was in fact much further from suggesting a significant difference in configuration proportions between the two samples.

DISCUSSION

Moulting variability in trilobites

The large dataset of *Estaingia bilobata* from the Emu Bay Shale demonstrates the significant variation that early Cambrian trilobites can exhibit in terms of their moulting behaviour, and the range of moult configurations that

could be preserved under exceptional preservational regimes. Based on the high prevalence of the Sutural Gape mode of moulting (plus the transitional Henningsmoen's configuration) in the dataset, *E. bilobata*, like other members of the Redlichiina, generally required the opening of the cephalic sutures to moult. Harrington's configuration is the most common for preserved moults, suggesting that, in general, opening of the cephalic sutures and relatively minor movements were sufficient for the animal to moult successfully, as previously suggested by Drage *et al.* (2018).

Other configurations resulting from the Sutural Gape mode were comparably uncommon and probably resulted from the animal making more substantial movements to free itself from the exuvium. The Somersault configuration is particularly rare, possibly because it required the greatest degree of dorsally-directed movement in order to invert the librigenae. The reasonably common occurrence of Salter's configuration, together with the presence of Somersault and McNamara's configurations, suggests that some amount of dorsal flexure or partial enrolment played an important role when opening a moulting gape in *E. bilobata* (see Whittington 1990, figs 2–6).

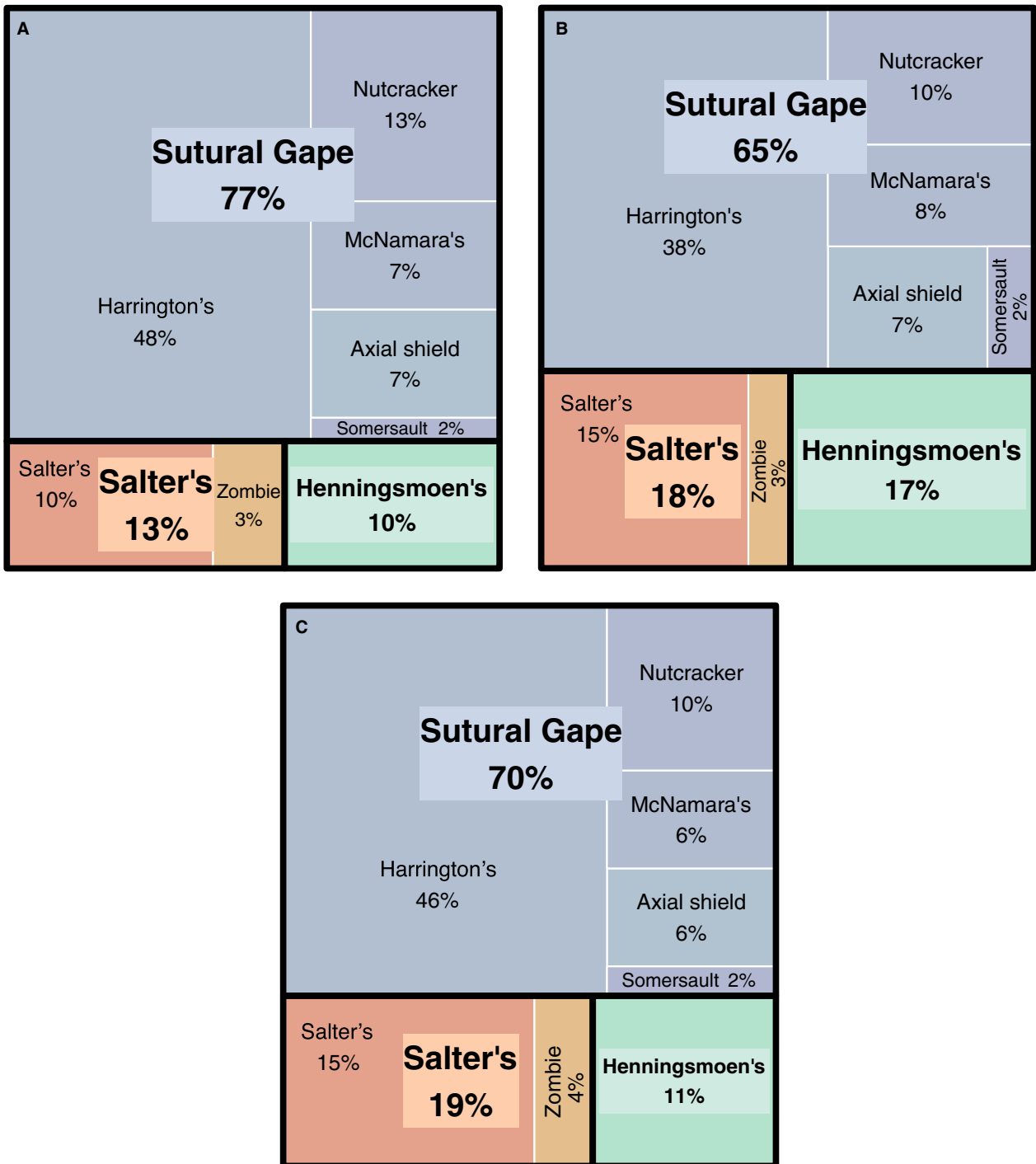


FIG. 5. Treemaps (Bederson *et al.* 2002; RStudioTeam 2020) showing the proportion of *Estaingia bilobata* specimens that used Salter's or the Sutural Gape mode of moulting, in the hybrid Henningsmoen's configuration, and preserved in each of the moulting configurations (Drage *et al.* 2018; Drage 2019a, 2019b). A, field sample. B, total museum sample. C, museum bycatch-only sample.

The rare occurrence of hybrid moulting configurations in this dataset (such as the unusual occurrence of librigenal inversions with cephalothoracic joint opening and minor displacement, as seen in one museum and two field specimens exhibiting McNamara's configuration, as

well as two field specimens in Somersault configuration) suggests that the configurations described by Drage *et al.* (2018) may represent discrete examples of a more continuous range of possibilities resulting from relatively consistent moulting movements. In other words, the

TABLE 3. Chi-squared results comparing the proportions of the different moulting configurations (Drage *et al.* 2018; Drage 2019a, 2019b) of *Estaingia bilobata* in the field and total museum samples, and the field and museum bycatch only samples.

Sample	Chi-squared value	p value
Museum/field	9.49	0.219
Museum bycatch/field	2.70	0.911

inevitable variation between each individual trilobite moulting event is likely to produce a relatively continuous spectrum of configurations, probably resulting from a greater-or-lesser amount of movement required to shed the exuvium. Unique combinations of sclerite positions and orientations resulting from these variable movements then allows the definition of the discrete configurations considered here. These range from slight disarticulation of the facial sutures suggestive of minor movements (e.g. Harrington's configuration), to those characterized by inverted sclerites and breakage of the cephalothoracic joint, suggesting more severe movements. Future work on trilobite moulting variability should take this into consideration, and use the trilobite moult configurations described to date as guidelines for interpreting moulting patterns.

Body proportions and moulting

The general paucity of significant results in the linear regression comparisons suggest that body proportions and moulting behaviour are mostly unrelated in *E. bilobata*. For example, it might be expected that individuals moulting via Salter's mode would have a wide trunk which could be difficult to extract through a librigenal sutural gape that was comparably narrower than a cephalothoracic gape, but this was not found to be the case. This suggests that moulting mode has little to do with sutural gape size in this species; rather, it may simply be a function of whether the sutures open correctly or not. The inclusion of Henningsmoen's configuration in either mode, or excluded completely, did not appear to introduce additional bias, despite its hybrid nature, with characteristics of both the Sutural Gape and Salter's modes.

The ANCOVA analyses did reveal one significant result: when controlled for thoracic width, the body length differed between individuals using Salter's mode and the Sutural Gape mode (Table 2). The mean average body lengths for the two moulting modes show that individuals using the Sutural Gape mode tend to have a body that is 50% longer on average. In addition, the cephalothoracic

joint widths were close to significance (and significant when including Henningsmoen's configuration within Salter's mode), with the Sutural Gape mode having a width that is 12% wider on average. This seems counter-intuitive from a mechanical perspective, because a larger cephalothoracic joint would provide a wider exuvial gape to simplify extraction of the trunk during moulting. Yet those individuals using the Sutural Gape mode do not use the exuvial gape created at the cephalothoracic joint, whereas those using Salter's mode must extract the trunk through this opening. These two significant (or close to significant) results may be related to the strength or development of different exoskeletal articulations and of the cuticle and underlying integument in *E. bilobata*. For example, if a broader cephalothoracic joint provides a stronger articulation, the cranidium would be less likely to disarticulate from the thorax, leading to moults with only the cephalic sutures open (Sutural Gape mode). Similarly, perhaps larger individuals of *E. bilobata* had stronger and/or more developed articulations, so that the more robust cephalothoracic joint was less likely to open incidentally during moulting movements, leaving moults of larger individuals more often showing the Sutural Gape mode. It has previously been suggested that varied strength of exoskeletal articulation impacts the trilobite moulting configurations preserved (Whittington 1990; Drage *et al.* 2018). However, this is not a strong signal in the dataset, so this observation may represent a random chance association. It is also possible that this signal is partly a result of taphonomic factors. Although disturbance of moults in the Emu Bay Shale is interpreted to be extremely minimal, and we consider the great majority of variation to result from behaviour (Drage *et al.* 2018), it is possible that the integument holding small specimens together decayed more quickly than in larger specimens, meaning that smaller moults were more likely to suffer slight disarticulations prior to, and during the burial process.

Moult configuration sampling in museums

The high congruence between the two independently-collected museum and field samples of *E. bilobata* moults suggests the museum sample is relatively unbiased, though it contains a low proportion of intentionally-collected material. However, this does not typically apply to museum collections as a whole, which can be biased towards interesting or unusual specimens, particularly for common species (pers. obs. for *E. bilobata*, all authors), or the various personal objectives of collectors (Wehi *et al.* 2012; Allmon *et al.* 2018; Whitaker & Kimmig 2020; Meineke & Daru 2021). Removing accessioned specimens from the museum sample further aligned the proportions

of the different moulting modes and configurations with those from the randomly-collected field sample, which does suggest that our total museum sample was still slightly biased by the inclusion of registered specimens. However, the proportion of Salter's mode, in other words, the non-standard mode of moulting in *E. bilobata* that produces rarer moult configurations (Drage *et al.* 2018), was higher in the total museum sample than the bycatch-only or field samples, and this does support the idea that accessioned moult specimens can be biased towards unusual specimens. Based on these findings, we suggest that future quantitative studies aiming to interpret trilobite moulting behaviour endeavour to: include bycatch museum moult specimens (if possible) and randomly-collected specimens in the field; analyse and interpret registered and non-registered specimens together and separately; and provide randomized field comparisons to reduce the potential for museum collection bias. Without a system of random sampling, or appropriate field collection comparisons, a museum sample is likely to be unrepresentative of the original population, and so should not be considered an entirely reliable basis for hypothesis testing (Wehi *et al.* 2012; Whitaker & Kimmig 2020).

CONCLUSION

The lack of a strong significant relationship between overall body proportions and moulting mode, and the relative abundance of different moult configurations, suggest that the observed variation in *Estiaingia bilobata* is most likely to have been a function of whether the facial sutures opened (Salter's vs Sutural Gape modes) and the amount of movement required to free the animal from the old exoskeleton, which explains the relatively continuous variation in configurations seen within these modes.

This study demonstrates that it is feasible to sample trilobite moults in sufficient quantity to explore broader evolutionary questions, such as whether the moulting behaviour variability of trilobites impacted their morphology (Henningmoen 1975; Brandt 2002). This is particularly achievable for sites of exceptional preservation (e.g. the Emu Bay Shale), which, due to the environmental conditions required for such preservation, often contain a high proportion of *in situ* articulated trilobites including moults. Such studies should be extended to data collection at other Palaeozoic Konservat-Lagerstätten, for example, *Elrathia kingii* from the mid-Cambrian Wheeler Shale, House Formation, USA (Gaines & Droser 2003). However, non-Konservat-Lagerstätten should also be considered for similar treatments, as it is feasible that dense assemblages of trilobite moults can occur under less-exceptional preservational regimes, assuming minor disturbance of sclerites. It is possible that this would reveal

results different to those presented here; perhaps other trilobite species did indeed show moulting behaviours related to their body proportions.

The comparisons presented here between a random field sample of trilobite moults, a museum bycatch sample, and additional registered museum specimens, reinforce the need to anticipate collection bias in quantitative palaeontological studies (Whitaker & Kimmig 2020). However, the similarities between the field and museum bycatch data suggest it is possible to ameliorate these issues. We therefore recommend that researchers should sample non-accessioned museum specimens where collections are sufficiently large enough to facilitate this. In particular, we advocate the incorporation of field samples to compare with museum-based samples in future single-species quantitative moulting studies, especially where focus on museum bycatch moults is not feasible.

Acknowledgements. This research was supported by a NERC Doctoral Training Partnership scholarship (during sample collection; NE/L002612/1) and Swiss National Science Foundation Sinergia postdoctoral funding (during analysis and writing; 198691) to HBD, Australian Research Council grants (LP0774959 and FT120100770 to JRP; FT130101329 to DCGB), and a National Geographic Society Research & Exploration grant (8991-11). This manuscript was improved through the helpful reviews of Lukáš Laibl, one anonymous reviewer, and the publishing team at the Palaeontological Association. We thank all collaborators on the Emu Bay Shale research project for their hard work and collecting efforts: R. Atkinson, M. Betts, R. Bicknell, M.-A. Binnie, G. Brock, A. Camens, A. Daley, G. Edgcombe, E. Ellis, R. Gaines, J. Gehling, M. Gemmill, T. Ireland, C. Ireland, J. Jago, K. Kenny, P. Kruse, J. Laurie, M. Lee, B. McHenry, J. Ortega-Hernández, L. Reid, and N. Schroeder. Financial assistance was provided by the South Australian Museum, Beach Energy, and SeaLink. Thanks also to landowners P. and C. Buck for generously allowing access to the field site. Open access funding provided by Université de Lausanne.

Author contributions. **Conceptualization** HB Drage (HBD), JD Holmes (JDH); **Investigation** HBD, JDH, DC García-Bellido (DCGB), JR Paterson (JRP); **Formal Analysis, Methodology & Visualization** HBD; **Writing – Original Draft Preparation** HBD; **Writing – Review & Editing** HBD, JDH, DCGB, JRP.

Editor. Javier Álvaro

SUPPORTING INFORMATION

Additional Supporting Information can be found online (<https://doi.org/10.1111/pala.12651>):

Appendix S1. The first sheet presents all data used for analyses within this study. Other sheets include Tables S1 and S2.

Table S1. Results of ANCOVA analyses that include Henningsmoen's configuration specimens in either of the two modes of moulting.

Table S2. Summary of total specimen counts for each of the sample groupings.

REFERENCES

- ALLMON, W. D., DIETL, G. P., HENDRICKS, J. R. and ROSS, R. M. 2018. Bridging the two fossil records: palaeontology's "big data" future resides in museum collections. 35–44. In ROSENBERG, G. D. and CLARY, R. M. (eds) *Museums at the forefront of the history and philosophy of geology: History made, history in the making*. Geological Society of America.
- BEDERSON, B. B., SHNEIDERMAN, B. and WATTENBERG, M. 2002. Ordered and quantum treemaps: making effective use of 2D space to display hierarchies. *ACM Transactions on Graphics*, **21**, 833–854.
- BRANDT, D. S. 2002. Ecdysial efficiency and evolutionary efficacy among marine arthropods: implications for trilobite survivorship. *Alcheringa*, **26**, 399–421.
- BREWER, C. A., HATCHARD, G. W. and HARROWER, M. A. 2003. ColorBrewer in print: a catalog of color schemes for maps. *Cartography & Geographic Information Science*, **30**, 5–32.
- CHATTERTON, B. D. E., EDGEcombe, G. E., SPEYER, S. E., HUNT, A. S. and FORTEY, R. A. 1994. Ontogeny and relationships of the Trinucleoidea (Trilobita). *Journal of Paleontology*, **68**, 523–540.
- DALEY, A. C. and DRAGE, H. B. 2016. The fossil record of ecdysis, and trends in the moulting behaviour of trilobites. *Arthropod Structure & Development*, **45**, 71–96.
- DRAGE, H. B. 2019a. Quantifying intra- and interspecific variability in trilobite moulting behaviour across the Palaeozoic. *Palaeontologia Electronica*, **22.2.34A**, 1–39.
- DRAGE, H. B. 2019b. The evolution of exoskeleton moulting in Trilobita. PhD thesis, University of Oxford. <https://ora.ox.ac.uk/objects/uuid:42af12f6-ff0c-41ca-b93c-8d1482f8c479>
- DRAGE, H. B., HOLMES, J. D., GARCÍA-BELLIDO, D. C. and DALEY, A. C. 2018. An exceptional record of Cambrian trilobite moulting behaviour preserved in the Emu Bay Shale, South Australia. *Lethaia*, **51**, 473–492.
- EWER, J. 2005. How the ecdysozoan changed its coat. *PLoS Biology*, **3**, e349.
- FOX, J. and WEISBERG, S. 2019. *An R companion to applied regression*. Third edition. Sage.
- GAINES, R. R. and DROSER, M. L. 2003. Palaeoecology of the familiar trilobite *Elrathia kingii*: an early exaerobic zone inhabitant. *Geology*, **31**, 941–944.
- GARCÍA-BELLIDO, D. C., PATERSON, J. R., EDGEcombe, G. D., JAGO, J. B., GEHLING, J. G. and LEE, M. S. Y. 2009. The bivalved arthropods *Isoxys* and *Tuzoia* with soft-part preservation from the Lower Cambrian Emu Bay Shale Lagerstätte (Kangaroo Island, Australia). *Palaeontology*, **52**, 1221–1241.
- GEHLING, J. G., JAGO, J. B., PATERSON, J. R., GARCÍA-BELLIDO, D. C. and EDGEcombe, G. D. 2011. The geological context of the Lower Cambrian (Series 2) Emu Bay Shale Lagerstätte and adjacent stratigraphic units, Kangaroo Island, South Australia. *Australian Journal of Earth Sciences*, **58**, 243–257.
- HENNINGSMOEN, G. 1975. Moulting in trilobites. *Fossils & Strata*, **4**, 179–200.
- HOLMES, J. D., PATERSON, J. R. and GARCÍA-BELLIDO, D. C. 2021a. The post-embryonic ontogeny of the early Cambrian trilobite *Estaingia bilobata* from South Australia: trunk development and phylogenetic implications. *Papers in Palaeontology*, **7**, 931–950.
- HOLMES, J. D., PATERSON, J. R. and GARCÍA-BELLIDO, D. C. 2021b. Complex axial growth patterns in an early Cambrian trilobite from South Australia. *Proceedings of the Royal Society B*, **288**, 20212131.
- JAGO, J. B., BENTLEY, C. J., PATERSON, J. R., HOLMES, J. D., LIN, T. R. and SUN, X. W. 2021. The stratigraphic significance of early Cambrian (Series 2, Stage 4) trilobites from the Smith Bay Shale near Freestone Creek, Kangaroo Island. *Australian Journal of Earth Sciences*, **68**, 204–212.
- MEINEKE, E. K. and DARU, B. H. 2021. Bias assessments to expand research harnessing biological collections. *Trends in Ecology & Evolution*, **36**, 1071–1082.
- PATERSON, J. R., GARCÍA-BELLIDO, D. C., JAGO, J. B., GEHLING, J. G., LEE, M. S. Y. and EDGEcombe, G. D. 2016. The Emu Bay Shale Konservat-Lagerstätte: a view of Cambrian life from East Gondwana. *Journal of the Geological Society*, **173**, 1–11.
- POCOCK, K. J. 1964. *Estaingia*, a new trilobite genus from the lower Cambrian of South Australia. *Palaeontology*, **7**, 458–471.
- R CORE TEAM. 2013. R: a language and environment for statistical computing. R Foundation for Statistical Computing. <https://www.R-project.org>
- RSTUDIOTEAM. 2020. RStudio: integrated development for R. RStudio, PBC, Boston, MA. <https://posit.co/products/open-source/rstudio/>
- SCHNEIDER, C. A., RASBAND, W. S. and ELICEIRI, K. W. 2012. NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*, **9**, 671–675.
- STUBBLEFIELD, C. J. 1959. Evolution in trilobites. *Quarterly Journal of the Geological Society*, **115**, 145–162.
- VEVEA, D. and HALL, K. D. 1984. The effects of water temperature on moulting and egg production following eyestalk ablation in two species of crayfish, *Orconectes rusticus* and *Orconectes propinquus*. *Bios*, **55**, 135–143.
- WANG, Y., PENG, J., WANG, D., ZHANG, H., LUO, X., SHAO, Y., SUN, Q., LING, C. and WANG, Q. 2021. Ontogenetic moulting behaviour of the Cambrian oryctocephalid trilobite *Arthrocephalites xinzhaiheensis*. *PeerJ*, **9**, e12217.
- WEHI, P. M., WHAANGA, H. and TREWICK, S. A. 2012. Artefacts, biology and bias in museum collection research. *Molecular Ecology*, **21**, 3103–3109.
- WHITAKER, A. F. and KIMMIG, J. 2020. Anthropologically introduced biases in natural history collections, with a case study on the invertebrate palaeontology collections from the

- middle Cambrian Spence Shale Lagerstätte. *Palaeontologia Electronica*, **23**(3), a58.
- WHITTINGTON, H. B. 1990. Articulation and exuviation in Cambrian trilobites. *Philosophical Transactions of the Royal Society B*, **329**, 27–46.
- WHITTINGTON, H. B., CHATTERTON, B. D. E., SPEYER, S. E., FORTEY, R. A., OWENS, R. M., CHANG, W. T., DEAN, W. T., JELL, P. A., LAURIE, J. R., PALMER, A. R. REPINA, L. N., RUSHTON, A. W. A., SHERGOLD, J. H., CLARKSON, E. N. K., WILMOT, N. V. and KELLY, S. R. A. 1997. Trilobita: Introduction, Order Agnostida, Order Redlichiida. O1–O530. In R. L. KAESLER (ed.) *Treatise on invertebrate palaeontology. Part O. Arthropoda 1 (Revised)*. Geological Society of America & University of Kansas Press.
- WICKHAM, H. 2016. *Ggplot2: Elegant graphics for data analysis*. Springer. <https://ggplot2.tidyverse.org>
- WICKHAM, H., AVERICK, M., BRYAN, J., CHANG, W., D'AGOSTINO, MCGOWAN, L., FRANÇOIS, R., GROLEMUND, G., HAYES, A., HENRY, L., HESTER, J., KUHN, M., LIN PEDERSEN, T., MILLER, E., MILTON BACHE, S., MÜLLER, K., OOMS, J., ROBINSON, D., SEIDEL, D. P., SPINU, V., TAKAHASHI, K., VAUGHAN, D., WILKE, C., WOO, K. and YUTANI, H. 2019. Welcome to the tidyverse. *Journal of Open Source Software*, **4**, 1686.