

Review Article

Emerging insights into the controls of development within an ancient arthropod, the Silurian trilobite *Aulacopleura koninckii* (Barrande 1846)

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ABSTRACT: A long-term research programme, spurred on first by Euan Clarkson, into controls upon the development of the middle Silurian trilobite *Aulacopleura koninckii* (Barrande 1846) has allowed insights into the processes by which exoskeletal segment size and shape were generatively controlled that are remarkable for a fossil of some 429 million years old. Competing hypotheses for the nature of the growth controls have been tested and resolved. These results provide palaeontological contributions to evolutionary developmental biology. Here the principal and widely scattered publications resulting from this effort are briefly summarised and reflected upon. This essay provides a contemporary, synoptic overview of our published literature on the development and life of this animal.



KEYWORDS: Evo-devo, morphometrics, ontogeny, segmentation, trunk growth

Euan Clarkson's (1986, p. 326) influential textbook *Invertebrate Palaeontology and Evolution* mentioned that the Silurian trilobite *Aulacopleura koninckii* added thoracic segments until a 'very late stage in development'. In summer 1989 I wrote to Euan requesting further details and he swiftly replied with a copy of Barrande's (1852, pl. 18, figs 1–20) plate illustrating *A. koninckii*'s ontogeny and the comment: 'I hope this is what you need – it is all I have!' A couple of years later Richard Fortey drew my attention to *A. koninckii* as a homeomorph of common Cambrian 'ptychopariids' (Fortey & Owens 1990). So began investigation into what I hoped might offer instructive insight into the developmental control of an extinct organism. Below I reflect on the series of published works that others and I have produced on *A. koninckii*, draw links between them, and consider what can be summarised about the developmental palaeobiology of this animal. Euan and Richard were both instrumental in stimulating and further encouraging this work despite repeatedly expressing astonishment that there could be 'yet another paper on *A. koninckii*'. Indeed, as far as I am aware no other trilobite species has been subjected to so exhaustive a series of studies, save arguably some with soft tissues preserved. Our work on *A. koninckii* has also spanned many topics, employs some detailed and specific analytical approaches, and has appeared across a range of journals. Accordingly, I offer this essay as an integrated introduction to our work on *A. koninckii*, omitting details on any topic but summarising what I see as key points from our 35 or so years of research into this fascinating trilobite. Furthermore, Euan himself was recently co-author of a paper on the eyes of *A. koninckii* (Schoenemann & Clarkson 2020), cementing his connection with this animal.

1. Motivation for studying *A. koninckii*

Through PhD work on the late Cambrian trilobite *Dikelocephalus minnesotensis* Owen 1852, I became interested in small-scale morphological variation and the pattern of variance within and between collections made from single beds or bedsets (*sensu* Patzkowsky & Holland 2012). Large, populous species showing evident but relatively subtle morphological variation are consistent with Darwin's (1859) expectations for successful species lineages when evolving rapidly, but at the time of publication the pattern of variation seemed relevant in additional ways. In his noted popular account of the discovery and interpretation of the middle Cambrian Burgess Shale fauna, *Wonderful Life*, Steve Gould (1989) suggested that Cambrian taxa may generally show unusually high levels of phenotypic plasticity due to their developmental pathways being less firmly entrenched than those of later organisms, leading to higher variability in characters that became stabilised during subsequent evolutionary history. The case study of *D. minnesotensis* hinted at possible support for Gould's idea at a low taxonomic level (Hughes 1991, 1994) but comparative studies of species across basal and derived clade members were required to explore this idea more rigorously.

Testing Gould's idea at low taxonomic levels presented a challenge. Comparative estimates of morphological variation among species in phylogenetic context required a focus on variation in particular attributes of morphology that were informative about aspects of the developmental bauplan of the animal. In this the number of segments in the mature trilobite trunk and their allocation to thorax or pygidium provided a promising comparative system, as McNamara (1986) and

others had suggested. This was because segments arrayed along the anterior–posterior axis are the fundamental constructional units of the trilobite (and arthropod) bodyplan and, whilst apparently stable in number in the trilobite cephalic region throughout ontogeny, vary in number in the trunk both ontogenetically and phylogenetically. Segmentation along the anterior–posterior axis thus seemed a promising study system for exploring developmental variation.

Discussions of this matter with Euan and Richard sparked the suggestion that *A. koninckii* might be of interest for several reasons. Firstly, articulated dorsal exoskeletons of *A. koninckii* are notably common from a short stratigraphic interval at the Na Černidlech site, near Loděnice in the Czech Republic, as described by the great palaeontologist Joachim Barrande in 1852. Secondly, marked variation in the number of mature thoracic segments was known to occur within this species in this interval: mature forms with anywhere from 18 to 22 thoracic segments occurred at the site. Thirdly, Na Černidlech also yielded multiple articulated juvenile (in this case meraspid) exoskeletons. Barrande (1852) had commented on all three matters which, in combination, subsequently resulted in *A. koninckii* becoming a something of a palaeontological ‘model animal’ for studying developmental control among the extinct. We could use this animal to dissect the controls on its development to a degree unknown in other Palaeozoic fossils. Below I chronicle the development of our investigations into *A. koninckii* by discussing the aims and results of the principal papers we have published on this form, along with a reflection on how I now view these works as part of a body of research on an extraordinary fossil.

2. Initial studies of variation in *A. koninckii*

2.1. Hughes & Chapman 1995, *Lethaia*

Our first paper on *A. koninckii* was based solely on well-preserved specimens available in museums in the United States and the United Kingdom, and particularly those from the Museum of Comparative Zoology at Harvard University (Hughes & Chapman 1995), of which there were 155 in total, 85 of which were assessed using a geometric morphometric approach. Ralph Chapman was a morphometrician with an early interest in geometric morphometrics and a love of trilobites. The work illustrated the developmental sequence of *A. koninckii* through specimens at different developmental stages or with different numbers of holaspid thoracic segments, along with photographs of enrolled and teratological specimens, and isolated hypostomes. It showed that Euan’s characterisation of *A. koninckii* was right in the sense that, because the number of thoracic segments in mature specimens is relatively large (18 to 22) in this species and segments are added to the thorax sequentially during the meraspid period (in which segments are released into the thorax from the meraspid pygidium), this period extended later into ontogeny than in most other trilobites. On the other hand, it was clear that a growth transition, analogous to onset of the holaspid growth stage (which is invariant in thoracic segment number and characteristic of almost all trilobite species), did occur within *A. koninckii*. This is because the number of thoracic segments among larger specimens stabilised in this form, just not at a consistent number of segments but rather across a range of mature thoracic segment numbers. The paper also presented a ‘conventional’ multivariate morphometric analysis of the species’ growth using linear measurements alongside an early landmark-based geometric morphometric approach to illustrate both ontogenetic change and shape variance. It revealed gradual changing overall morphology with growth, modest

but significant change in cranial shape throughout growth, and a marked switch in the growth dynamics of the pygidium at the onset of the holaspid phase as it transitioned from the generation of new segments to increasing the sizes of segments in the mature complement of pygidial segments.

Some of the inferences on the controls of growth of *A. koninckii* in this paper were subsequently shown to be incorrect. A claim that during holaspid growth phase segments continued to be added to the thorax, but erratically and at a much slower rate than in the meraspid phase, was soon challenged. This interpretation required transition into the holaspid phase in this species to be defined not on the basis of reaching a final and stable number of thoracic segments (as in Raw 1925), but on initiation of a sharply reduced rate of segment release from the pygidium. This was complemented by the idea that transition from meraspid to holaspid growth was determined in late-stage ontogeny, as the individual trilobite crossed a particular overall size threshold. A further claim of this (and also of the next paper in the series) was that overall body shape among mature *A. koninckii* varied relatively little despite the highly unusual degree of variation in the number of thoracic segments. This interpretation followed the logic that as all mature forms showed similar numbers of pygidial segments, forms with 22 thoracic segments should be expected to be notably longer than those with 18 segments because of the four additional segments in the posterior thorax. Observed shape variation, however, suggested that all mature morphs shared a broadly similar shape, implying compensatory regulation in trunk segment growth modes among the different morphs.

2.1.1. Reflection. A claim of this paper, that the number of thoracic segments during the holaspid phase continued to rise sporadically, was based on data that mistakenly included some forms that were probably late-stage meraspids. Although it was the next paper in the series that first attracted the attention of myriapod biologists Alessandro Minelli and Giuseppe Fusco, who were interested in the evolution of developmental controls, it was the dataset explored in this first paper that indicated to them that *A. koninckii* offered a tractable study system for the further exploration of ancient body patterning controls. Some of the observations and interpretations in this *Lethaia* paper prompted rapid corrective response, whilst others were addressed only toward the end of the research programme. This initial paper seeded various subsequent research topics pursued using this animal.

2.2. Hughes *et al.* 1999, *Evolution & Development*

Burgeoning interest in ‘evolutionary developmental biology’ in the late 1990s prompted the launch of the new journal *Evolution & Development*, in the first issue of which we published our second *Aulacopleura* paper. The study compared the degrees of holaspid shape variation among all those trilobite taxa from the Na Černidlech assemblage that yielded sufficient specimens for comparison and presented these results in both phylogenetic and phenetic context. Jonathan Adrain joined this work because of his taxonomic expertise on proetide and aulacopleuride trilobites. The main claim of the paper was that, despite the marked variability in numbers of thoracic segments (and relatively constant numbers of pygidial segments), among holaspid *A. koninckii* its overall shape variance was not elevated compared to holaspids belonging to other trilobite taxa found in the same 1.4 m interval, all of which showed a fixed number of holaspid thoracic segments. This result again hinted that some form of compensation in trunk growth mechanics was operating among *A. koninckii*. In addition, whilst mature *A. koninckii* was shown to be phenetically more similar in shape to Cambrian ‘ptychoparioid’ equivalents (some of which also varied

intraspecifically in their holaspid thoracic segment numbers) than to any other Na Černidlech assemblage species, the paper argued that such Cambrian species were not its closest relatives. Rather, *A. koninckii* shared more recent common ancestry with other Na Černidlech assemblage forms that showed stable numbers of holaspid thoracic segments. From that we concluded that the pattern of variable holaspid numbers seen in *A. koninckii* was likely to be related to its mimicking a Cambrian morphotype, presumably related to its occupation of a comparable niche (probably related to reduced oxygen availability settings, see below). This made us question Gould's claim that early metazoan species were uniquely variable due to relatively loose developmental control among the earliest metazoans that tightened up irrevocably in derived forms. In trunk segmentation in these trilobites, at least, it seemed that relatively derived Silurian species could mimic or relapse toward patterns of variability seen in Cambrian homeomorphs, despite having relatively close relatives which showed stricter control. Hence, if the control of trunk segment development tightened during metazoan evolution, it could apparently also be relaxed again.

The combined phylogenetic/phenetic approach using in this paper was partly inspired by attempts to analytically address the claims Gould had made in *Wonderful Life* (Briggs & Fortey 1989; Wills *et al.* 1994), and the paper used a resampling technique in an attempt to account for difference in sample size among the trilobite species compared. Chapman and I elaborated some points made in this study in an invited book chapter (Hughes & Chapman 2001).

2.2.1. Reflection. Higher level phylogenetic relationships among aulacopleurid/proetide trilobites remain contentious and we realised at the time that our argument would have been stronger had *A. koninckii* been nested within a morphologically distinctive post-Cambrian clade such as Phacopida or Lichida. Nor were phylogenetic software, clade support measures or ancestral character state reconstruction employed in the study. That said, conducting an informative phylogenetic analysis of the kind needed to reveal secure associations between *A. koninckii* and its Cambrian homeomorphs remains a daunting and possibly futile prospect. The abundance of *A. koninckii* allowed us to choose only the best-preserved specimens for morphometric analysis of shape: such privilege did not extend to complete specimens of rarer species so it is possible that, despite our use of subsampling to explore the effects of sample size differences, apparently similar levels of holaspid shape variation partly reflected taphonomic differences among species samples (for an exploratory attempt to consider the effect of compaction on the form of *A. koninckii* see Hughes 1999). These concerns notwithstanding, although confined to one particular aspect of morphological variation, trilobite trunk segment number and allocation, this study provided one of the few attempts published (see also Webster 2007) to specifically test Gould's prediction. In this case, his interpretation appeared wanting, but further reflections on this matter are discussed below.

3. Investigation of the geological setting of *A. koninckii*

The industry of Barrande and others at the Na Černidlech site recovered thousands of specimens of *A. koninckii*, and sizeable collections available outside the Czech Republic formed the basis of our early work. Most of these specimens were collected in the 1840s from a trench on Na Černidlech hill, above the village of Loděnice, and the majority of them remain in museums in Prague. In the Národní Muzeum we unsealed many

boxes untouched since Barrande's collecting. The original site of 'Barrande's pits' was almost completely buried – although it had been re-exposed in trenches dug during the late 1950s – and in 1995 Jiří Kříž and I re-exposed a short section that localised stratigraphically where Barrande's workers had mined the fossils.

3.1. Hughes *et al.* 2014, *Bulletin of Geosciences*

Our excavations showed that abundant *A. koninckii* were concentrated within a 1.4 m interval within the Motol Formation at Na Černidlech that was made up of over a hundred bedding surfaces upon most of which numerous fossils were preserved. Although Barrande's extensive quarrying and the dip of the beds prohibited us from exposing extensive fresh bedding plane surfaces *in situ*, it was easy to see from loose talus that the composition of the biota varied markedly from bedding plane to bedding plane, with some having a wide diversity of trilobite and other species, others almost solely dominated by *A. koninckii*, and those with no fossils evident. Furthermore, the mean sizes of complete *A. koninckii* differed from surface to surface – some with large numbers of small meraspid only, whilst other were dominated by mature forms. Although complete articulated specimens were abundant and included relatively rare enrolled examples, the majority of exoskeletons were only partially articulated and isolated sclerites were also common. The degree of articulation varied among bedding planes, with rare surfaces dominated by complete dorsal shields, most others showing dominant disarticulation and isolated sclerites common. Specimens assigned to and prepared for museum collections were clearly a select sample of the 'best' specimens, but fieldwork revealed the ambient preservational conditions of slabs from particular bedding planes, along with co-occurrence data that was lost when museum specimens were removed from their association with others on the same slab.

We interpreted the variable nature of the bedding plane assemblages to reflect repeated colonisation of the seafloor (which had little evidence of even shallow bioturbation) by diverse associations of invertebrates, some of which represented a normal Wenlockian muddy marine assemblage, others by faunas depleted in taxic diversity but dominated by abundant *A. koninckii*, and others that Richard Fortey in Ordovician sections referred to as 'nobody's home beds' for their near total absence of fossil content. The presence of different size cohorts of *A. koninckii* among bedding planes suggested to us assemblages comprised of individuals living, growing and dying contemporaneously, often followed by some degree of decay and disarticulation before final burial. The marked fluctuations in diversity among bedding surfaces and high abundance of *A. koninckii* on particular surfaces suggest that this species thrived in stressed conditions, and its conformity to Fortey & Owen's (1990) 'olenimorphic morphotype' is consistent with adaptation to conditions of low oxygen availability. Such an interpretation was also consistent with the lithology of the rocks and the lack of shallow bioturbation.

3.1.1. Reflection. An interesting recent paper by Schoenemann & Clarkson (2020), based on the analysis of the eye structure of *A. koninckii*, concluded that this animal, with a perhaps translucent exoskeleton, occupied clear waters with high light intensity and was active during the day. There is little in the sedimentary record at Na Černidlech itself to specify a particular water depth, but sedimentary structures characteristic of shallow water are lacking and biofacies relationships generally place *A. koninckii* in a relatively deep water shelf setting, occurring adjacent to the graptolitic facies. The seafloor that *A. koninckii* occupied at Na Černidlech would have been muddy at times. Hence, the setting inferred from the geological

features varies somewhat from the interpretation derived from the eye.

Records of marked fluctuation in Phanerozoic seafloor oxygen levels have become increasingly accepted among geoscientists but were treated with more caution at the time of our excavation. We considered exploring whether geochemical proxies might help independently test our hypothesised link between blooms of *A. koninckii* and variable seafloor chemistry and published some preliminary geochemical data in the 2014 paper. However, we were discouraged from taking this further by the severe weathering of the rocks yielding specimens (weathering that contributed to convenient rock splitting, but that had also probably leached the rare elements). Getting fresher rocks capturing this stratigraphic interval would require drilling a borehole away from the weathering front and for the 1.4 m interval to be identified within the resulting core. As the site was protected, Jiří felt it would be futile to ask permission for such drilling (although we did receive permission to excavate). Furthermore, we had no reason to conclude that any geochemical record obtained would reflect conditions where the organisms were living on the seafloor, rather than, say, those 10 cm beneath the sediment–water interface within the laminated, non-bioturbated sediments. The field experience made us more attentive to palaeobiological aspects of *A. koninckii*, such as the opening or otherwise of the dorsal facial sutures and its relationship to size, and the nature of exoskeletal enrolment, all of which later came to play a part in the developing understanding of *A. koninckii* as a functioning and evolving animal.

4. Collaborations with specialists in other fields: developmental regulation and growth gradients

As mentioned above, biologists Sandro Minelli and Giuseppe Fusco from the University of Padova noticed the initial work on *A. koninckii* and were kindly put in touch with me by Richard Fortey. This brought increased analytical rigour and improved evaluation of alternate hypotheses. Through visiting institutions in the Czech Republic we were able to raise the sample size for specimens included in the geometric analysis of shape from 85 to 391.

4.1. Fusco *et al.* 2004, *The American Naturalist*

This work, co-authored with Sandro and Mark Webster, showed that *A. koninckii* exhibited targeted growth: shape variance from meraspid instar to instar was limited in a way that implied a constraint of some kind. Although such a pattern of growth is quite common among living arthropods, our study provided the geologically oldest example then and possibly still now known. Secondly, the paper specifically tested the assertion made by Hughes & Chapman (1995) that initial holaspid thoracic segment number was determined late in ontogeny, when individuals reached the size associated with the meraspid–holaspid transition. This was approached in two ways. The first considered the size distributions of observed holaspid specimens compared with predictions based on the animal reaching a specific size at which transition occurred (the late determination model) versus the idea that ultimate holaspid segment number was determined beforehand, possibly either genetically from birth or environmentally but earlier in ontogeny (the early determination model). The second approach was to consider the growth dynamics of the pygidium as it changed from meraspid mode of being both a generator of new segments posteriorly and a releaser of segments from its anterior margin, to holaspid mode of simply increasing the sizes of a stable complement of segments. The late

determination model predicted this change would occur at the same cephalic size in all holaspid morphs, whereas the early determination model predicted that pygidia in those forms with fewer holaspid thoracic segments would be relatively larger because they reached holaspid earlier. Various metrics suggested that the early determination model was supported by both investigations.

4.1.1. Reflection. Although our subsequent work further expanded and refined the dataset of *A. koninckii* specimens, the main conclusions of this paper appear still sound. The study received some attention beyond arthropod biologists alone, and Sandro and Giuseppe's involvement in the 'evo-devo' community spurred opportunities to present results to a wider audience of evolutionary biologists and thus to represent palaeontology within that community. It also stimulated consideration of more general aspects of trilobite body patterning in a wider evolutionary context (e.g., Hughes 2003, 2005, 2007) along with a general review of segmental development in trilobite ontogeny with Sandro and Giuseppe (Hughes *et al.* 2006).

David Sheets is a physicist interested in dynamics and he became seriously interested in morphometrics in the 1990s, collaborating significantly with several biologists and palaeontologists. He wrote the excellent geometric morphometrics software package called the Integrated Morphometrics Package (IMP) which served as the industry standard until the rise of R programming. Paul Hong, a talented then master's degree student at UCR, carefully applied these techniques to a study of ontogeny-related shape change in *A. koninckii*.

4.2. Hong *et al.* 2014, *Journal of Paleontology*

Although the principal attraction of *A. koninckii* was the variation evident in holaspid trunk segment numbers, our interest in both individual sclerite and integrated dorsal shield shape variation, both ontogenetic and phenotypic, was enhanced when it emerged that some kind of compensatory growth was occurring among the five holaspid morphs. Furthermore, Hammer & Harper's (2006) reanalysis of a slightly modified version of the original dataset of 85 specimens revealed some differences of interpretation that we wanted to explore in more detail. The first innovation of this study was thus the assembly of the final and highest quality morphometric dataset for *A. koninckii* that comprised 352 specimens, of which 148 were definitive meraspids. This dataset was of comparable size to the previous one (391 specimens) and included many of the same specimens. The additional high preservational quality specimens were obtained from crates at the Národní Muzeum unopened since the time of Barrande's collecting them (with proof plates of his monographs used as wrapping material). The new dataset did indeed show reduced shape variance within and among meraspid instars and, when further selectivity did nothing to improve results, we concluded that this was the least taphonomically modified sample available to us. We then presented geometric morphometric investigations of both meraspid and holaspid cranial shape change, that of holaspid pygidia among the various morphs, along with analysis of changing overall exoskeletal proportions. It affirmed targeted growth in both specimen shape and size in the meraspid phase based on the improved dataset, a gradually decreasing degree of ontogenetic shape change between instars during cranial ontogeny that become largely negligible in the holaspid phase and a distinctive growth mode for the pygidium once holaspid growth initiated. As our analytical studies of *A. koninckii* variation had systematic implications, an updated synonymy and taxonomic description was an appropriate and necessary component of this study.

4.2.1. Reflection. This work was in some ways an enhanced version of the initial Hughes & Chapman (1995) contribution, partly conducted to address Hammer & Harper's (2006) findings, partly to provide a systematic description, but mostly to further explore one of the interesting conundrums of *A. koninckii*: the relationship between its remarkable variation in holaspide segment numbers and its constrained variation in shape both from meraspide instar to instar, and among the holaspide morphs. It had increasingly become clear that although *A. koninckii* was a variable trilobite, it was also one in which such variation was precisely controlled. This was far from the style of variation one might expect to result from a loosely regulated developmental system, then considered a possible cause for the riot of animal forms associated with the Cambrian explosion.

4.3. Fusco *et al.* 2014, *Proceedings of the Royal Society of London, Series B*

Using the same dataset of 352 meraspide and holaspide specimens this work explored the basis of an interesting facet of the trunk of *A. koninckii*: that in large specimens the longest trunk segment was not located at the anterior of the thorax, but lay several segments posterior of this, after which segment lengths again declined gradually toward the rear of the animal. If a segment other than the anterior was longest, this must mean that its average growth rate exceeded that of the trunk segments preceding it. This was especially notable given the pattern of hemianamorphic growth known in trilobites (Hughes *et al.* 2006), which suggests that the trunk anterior-most segments were specified before those behind them, and therefore had been growing for longer/more instars. Could a gradient of growth rates, increasing posteriorly, occur in the trunk of *A. koninckii* with the sizes of particular segments determined both by the rate at which the segment grew between instars and by when the segment first formed? Our first task was to see whether any apparent gradient derived from our observations of the lengths of segments among the different instars of *A. koninckii* could be simply modelled mathematically. As we found this was the case, we could then explain not only the length of the longest segment but also the relative lengths of those segments that preceded and followed it. We could then ask the question of whether particular segments each had a persistent, individualised growth rate, or whether the growth rates of individual segments changed progressively in relation to their position within the trunk as a unitary field of growth. Our dataset proved of sufficient quality that we could show that the latter possibility was correct. The meraspide trunk growth gradient in *A. koninckii* remained constant throughout sampled ontogeny and growth rates of particular segments were adjusted in relation to their changing position along that gradient.

4.3.1. Reflection. This paper may have been our most fundamental contribution on *A. koninckii* because of the correspondence between the simple growth gradients we described from our morphometric observations of a fossil extinct for some 429 million years and what we know of how regional growth is controlled in living arthropods. Our results were not surprising from a developmental control perspective based on extant organisms, but that information on such control could be extracted and tested in an animal so old was both remarkable and novel.

4.4. Fusco *et al.* 2016, *Paleobiology*

Building on the previous paper, we expanded the same approach to consider growth gradients not only in the trunk but also in the cephalon, and also into holaspide ontogeny for both regions (with the caveat of assuming that the persistently

steady moult increment seen in meraspide growth, confirming to Dyar's law, persisted during holaspide growth too). In doing so we found a growth gradient in the cephalon comparable to that in meraspide growth but that was flatter and in the opposite direction (i.e., higher growth rates at the front of the cephalon as opposite to the back of the pygidium) that persisted during holaspide growth. A holaspide growth gradient for the trunk was also described which differed from that of meraspide growth, but which is compatible with the mechanism of growth control inferred for the meraspide trunk. This research enabled recording generative developmental information at a node quite deep in the arthropod phylogenetic tree.

4.4.1. Reflection. This paper and the previous one formed a set of complementary papers whose approach has since been applied to other trilobites (e.g., Dai *et al.* 2021) and sometimes by workers other than us (e.g., Holmes *et al.* 2021a, 2021b; Hopkins 2020a). Control of growth via gradients in trilobites has also been applied in other directions, including building models of the types of morphologies that might result from gradients with different profiles (e.g., Hopkins 2020b). Variations in growth gradient form and profile surely underpin some of the major observed differences among trilobite bodyplans, and we hope that further development of this area of study will be fruitful.

4.5. Hughes *et al.* 2017, *Frontiers in Ecology and Evolution: Evolutionary Developmental Biology*

As a conclusion to our work on *A. koninckii*'s growth gradients, this paper used gradient modelling to reconstruct the ontogeny of the animal including all its morphs (Fig. 1) – with help from the excellent illustrative work by co-author Jin-bo Hou. As far as we are aware, this was the first time that such an approach (as opposed to reconstruction based directly on specimens observed for each developmental stage represented) has been used in trilobite ontogeny. By using the growth gradients established during the study we were able to predict the form of missing instars, and also illustrate the forms of the five different holaspide varieties at the same developmental stage, providing an instructive illustration of the compensatory growth of the trunk in morphs with fewer total numbers of segments. We also discussed what broader role palaeontology can play within evolutionary developmental biology.

4.5.1. Reflection. The purpose of this paper was to summarise and extol how palaeontological data can, in unique circumstances, provide evidence not only of the manner by which ancient organisms grew but also allows insight into the underlying processes responsible for generating these forms. In recent years much attention has been paid in palaeontology to studies of morphospace occupation, which I myself have commonly used to map the distribution of fossil form and to explore its relationships to factors such as locality, stratigraphic position or size (e.g., Srivastava & Hughes 2024; Srivastava-Losey & Hughes 2025). Our *Aulacopleura* work not only described and distinguished forms represented in the fossil record, but also provided insight into how such forms were generated. Such glimpses are rare in palaeontology, and we have been fortunate to explore them.

5. *Aulacopleura koninckii* as a functioning animal: a remaining quirk explained?

Jorge Esteve is a palaeontologist well known for, among many other talents, producing elegant digital models of trilobite exoskeletons that can be manipulated in various ways, including exoskeletal enrolment based on constraints provided

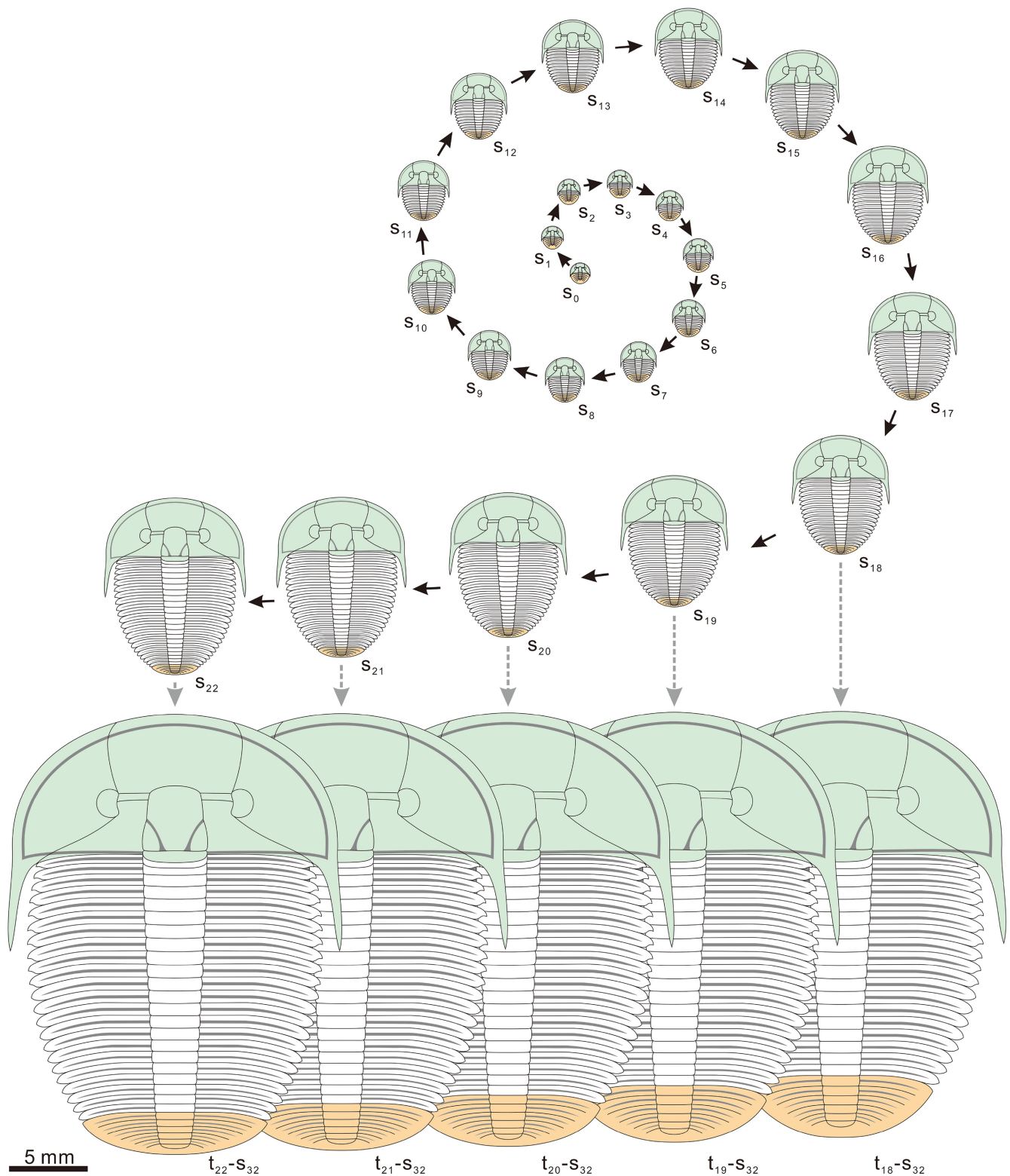


Figure 1 Meraspid and holaspid ontogeny of the five mature morphotypes of *A. koninckii*. Meraspid ontogeny of the five morphotypes extended through a different number of stages, from s_0 – s_{17} for morphotype t_{18} to s_0 – s_{21} for morphotype t_{22} . All stages are shown at the same scale with respect to body length. Note: (i) as specimens of the different morphotypes entered the holaspid period at a different stage (from s_{18} for t_{18} , to s_{22} for t_{22}) and trunk length growth rate was higher during meraspid period than in the holaspid period, at stage s_{32} morphotypes with more thoracic segments tended to have longer trunks than the morphotypes with less thoracic segments, as a consequence of a prolonged meraspid period; (ii) as specimens of the different morphotypes terminated thoracic segment release at a different stage (from s_{18} for t_{18} , to s_{22} for t_{22}) and thoracic segment release had the effect of shortening the relative length of the pygidium (overwhelming the larger growth rate of the pygidium due to the growth gradient), at stage s_{32} morphotypes with more thoracic segments tended to have relatively shorter pygidia than the morphotypes with less thoracic segments, as a consequence of more prolonged thoracic segment release. Modified from Hughes *et al.* 2017, fig. 4.

by the properties of the model components. I have worked with Jorge on other projects but was most excited to engage with him on this project because a remaining issue concerning *A. koninckii* continued to trouble me, which we addressed in the following paper:

5.1. Esteve & Hughes 2023, *Proceedings of the Royal Society of London, Series B*

Our various studies had made it clear that, although *A. koninckii* shows unique variation in holaspid thoracic segment numbers, control of the shape and size of its exoskeletal components was precise. However, variation in a single attribute, the development of an articulating joint between the terminal thoracic segment and the anterior of the pygidium (the joint forming as part of the steady transfer of segments from the meraspid pygidium into the thorax during the meraspid growth phase) was remarkably erratic only amongst specimens from latest meraspid and holaspid stages (Fig. 2) – i.e., those with at least 18 thoracic segments bearing complete sutures. Earlier in ontogeny separation of segments into the thorax from the anterior of the meraspid pygidium was clear-cut, yielding fully functional thoracic segments. But in more mature specimens the clarity of the last joint or joints in the thorax became obscured in a sizeable proportion (nearly 10 %) of otherwise excellently preserved specimens (all such specimens had been excluded from the morphometric analyses because the count of thoracic segment number was equivocal). In all other features these individuals looked typical and unremarkable.

Jorge's modelling of enrolment proved key to understanding the dilemma of why survivorship of a trilobite species that controlled segment count, size and shape so carefully could be so apparently indifferent with respect to development of this functional joint in later stage ontogeny. As mentioned above, encapsulated spherically enrolled specimens of *A. koninckii*, whilst a small percentage of the number of exoskeletons from Na Černidlech and elsewhere, are well known, but all are small specimens. Jorge showed from the modelled ontogeny from the paper discussed directly above, coupled with the z-plane dimensions derived from some uncompressed specimens found elsewhere locally, that beyond a certain size threshold, accompanied by at least 18 articulating thoracic segments, it was no longer physically possible for *A. koninckii* to enroll in an encapsulated form, and that in all large forms upon enrolment the rear of the trunk would instead have jutted beyond the anterior margin of the cephalon. Such an enrolled posture would not have required flexure of either the pygidium or the posteriormost thoracic segments because these segments lay flat against the base of the cephalon. This ontogenetic transition from sphaerical to external spiral enrolment thus offers a plausible explanation for why the selective premium for functional sutures in these, but only these, segments may have been reduced. Such an enrolled stance would have been less robust than encapsulated enrolment and thus less likely to be preserved, which may explain the absence of large enrolled specimens.

5.1.1. Reflection. The argument made immediately above, although consistent with all pertinent observations, remains speculative because no large enrolled specimens are yet known. I find it both attractive and a fitting conclusion to the study of this organism. Attractive because it offers a satisfying and plausible explanation for the variability of this one character alone – the exception that perhaps proves the rule. Fitting because *A. koninckii* was a living species, and the answers to its mysteries are most likely to be rooted in fundamental aspects of its day-to-day biology, which probably included enrolment.

6. Concluding thoughts on *A. koninckii* and prospectus for the future

As I embarked on this adventure with *A. koninckii* my assumption, encouraged by Steve Gould's notions of early metazoan plasticity, was that intracollectional variability in the species would imply lax developmental control. I anticipated that documentation of *A. koninckii*, known to be highly variable in segment numbers, would reveal that its growth was poorly regulated. Almost exactly the opposite resulted: trunk segment numbers, their allocation to thorax or pygidium, and their shape and growth rates were very precisely controlled in almost all cases, at least to the limits that the sample taphonomy allows us to explore these issues.

Studies at the section suggest that *A. koninckii*, while present within the diverse assemblages of skeletonised metazoans represented on some bedding planes in the Na Černidlech section, commonly 'bloomed' opportunistically when conditions prevailed that inhibited other benthos, although bedding planes within the section devoid of all fauna suggest episodes from which *A. koninckii* was also excluded. As trilobite exoskeletal trunk segments relate to paired biramous appendages beneath, including the respiratory exopod (Hou *et al.* 2021), variation in trunk segment number was probably closely tied to surface area for respiration. Those forms having 22 thoracic segments in maturity had more gills than their relatives with 18, but each gill was on average likely to be slightly smaller and narrower than those of the 18 thoracic segment forms. Presumably morph suitability to ambient conditions varied such that particular morphs favoured particular conditions. To date there is no evidence that specific holaspid morphs occupied assemblages exclusively – if that was indeed so it has been masked taphonomically, as individual bedding surfaces may show holaspid morphs differing by at least three segments.

In such a challenging physical environment there seems to have been little room for developmental error and targeted growth ensuring specific segment shape and size at each particular instar. The ability to produce different holaspid morphs may have proved an effective strategy for responding to varying levels of oxygen availability, and one that could be enacted even with strong constraint on size and shape variation within and among meraspid instars, part of which may have been imposed by encapsulated spherical enrolment. The one example of apparently lax developmental control – that of posterior thorax segment articulation near the meraspid–holaspid transition – is explicable in terms of what we know of the animal's biology and habits. Hence *A. koninckii*'s development and variation appears finely tuned to and limited by the challenging and variable environment it occupied. Nevertheless, if the explanation offered above concerning incomplete exosegmental separation is accepted, when functional constraints were released *A. koninckii* had the capacity to tolerate plasticity and even apparent malformity in a character apparently not subject to strong selection at this point in its ontogeny (Fig. 2).

With respect to further work on *A. koninckii*, I look forward to a future of accurately imaging fossils *in situ* without them being physically extracted or prepared. What a boon to both palaeontology and conservation that will be! Until that time, extracting core at Na Černidlech away from the weathering front might yield geochemical data that could shed significant light on the environmental context, though the concerns expressed above endure. There are also other aulacopleuriid taxa that show comparable variation in holaspid segment numbers (see Hughes *et al.* 1999) and study of these could provide further instructive results.

Finally, what of testing Gould's idea concerning early metazoan plasticity later surrendered irrevocably? The idea has found some support (Webster 2007) and continues to be considered, for

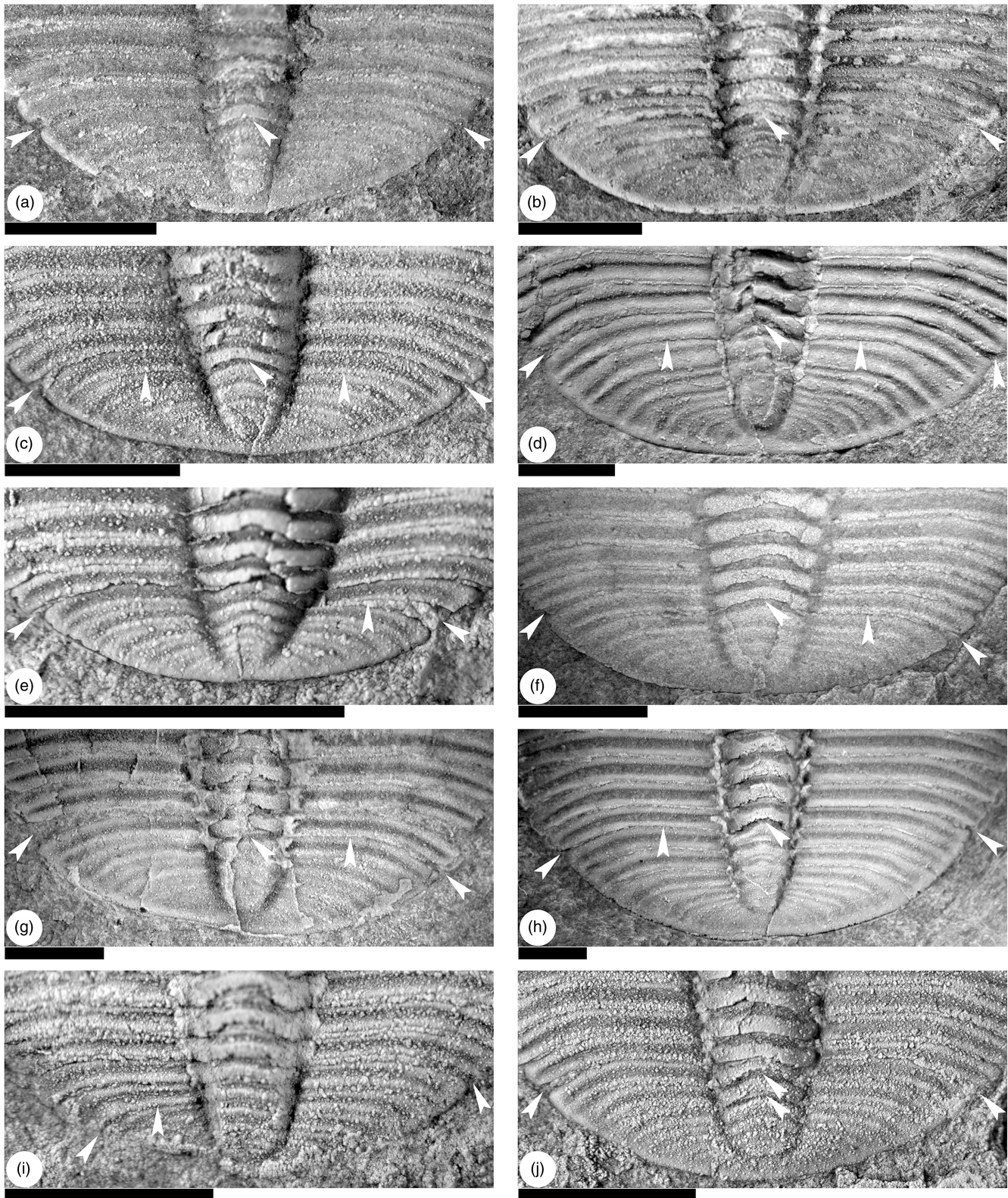


Figure 2 Incomplete release of segments from the anterior pygidium among late-stage meraspid and holaspid specimens of *A. koninckii*, showing a variety of ankyloses including: (a–d) segment fused in the pleural regions but released axially; (e–h) segment released pleurally on one side but ankylosed on the other; (i–j) intermittent and complex pattern of release, including with two segments apparently released on one side that are fused on the other. Segment number counts reflect the number of completely released thoracic segments. White arrows point to regions where a functional suture is apparent. Note also asymmetry pygidial shape and segment expression in several specimens. (a) MCZ 116088–19 segments; (b) NHM 42367(1)–19 segments; (c) MCZ 177974–19 segments; (d) MCZ 115964–20 segments; (e) MCZ 114878–18 segments; (f) CGS P2096–19 segments; (g) NMP L39401–19 segments; (h) NMP L39402–18 segments; (i) MCZ 115979–19 segments; (j) MCZ 115975–20 segments. Repositories: CGS – Czech Geological Survey, MCZ – Museum of Comparative Zoology, Harvard University, NHM – British Museum of Natural History, NPM – Národní Muzeum Praha. Scale bar is 2 mm for all images.

example recently in a review of the evolution of styles of biomineralisation (Murdock 2020), although I have more to say on that matter from other work in progress. Our 1999 paper was an effort to test the idea at that time, but the issue is a good deal more subtle than I appreciated when embarking on the *A. koninckii* adventure, or on dikelocephalids, too, for that matter. But what we have learned has been remarkable: to be able to determine that the dynamics of segment shape and size arrayed along a growing axis could be governed by simple gradients sustained across multiple instars has been most satisfying, as has finding evidence of targeted growth and early determination of mature trunk segment number. The reasoning for toleration of incompletely released trunk segments and the various holaspis morphs, if correct, has some kind of elegance. Overall, *A. koninckii* has opened a wider role for contributions from trilobite ontogeny to the field of evolutionary developmental biology. The accumulation of *A. koninckii* and other species at Na Černidlech provides a rare 'natural experiment' for insights into ancient development and I wish any future researchers in this area as much pleasure and generous company, including the like of Euan's, as I have enjoyed.

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Competing interests

The author declares none.

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