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Functional assessment of morphological homoplasy in stem-gnathostomes

Humberto G. Ferrón,^{1,2,*} Carlos Martínez-Pérez,^{1,2} Imran A. Rahman,³ Víctor Selles de Lucas,⁴ Héctor Botella,² and Philip C. J. Donoghue,^{1,*}

¹School of Earth Sciences, University of Bristol, Life Sciences Building, Tyndall Avenue, Bristol BS8 1TQ, UK.

²Instituto Cavanilles de Biodiversidad i Biología Evolutiva, Universitat de València, C/ Catedrático José Beltrán Martínez, 2, 46980 Paterna, Valencia, Spain.

³Oxford University Museum of Natural History, Parks Road, Oxford OX1 3PW, UK.

⁴School of Engineering and Computer Science, University of Hull, Cottingham Rd, Hull HU6 7RX, UK.

*Correspondence: humberto.ferron@bristol.ac.uk, phil.donoghue@bristol.ac.uk

ABSTRACT

Osteostraci and Galeaspida are stem gnathostomes, occupying a key phylogenetic position for resolving the nature of the jawless ancestor from which jawed vertebrates evolved more than 400 million years ago. Both groups are characterized by the presence of rigid headshields that share a number of common morphological traits, in some cases hindering the resolution of their interrelationships and the exact nature of their affinities with jawed vertebrates. Here, we explore the morphological and functional diversity of osteostracan and galeaspid headshields using geometric morphometrics and computational fluid dynamics to constrain the factors that promoted the evolution of their similar morphologies and informing on the ecological scenario under which jawed vertebrates emerged. Phylomorphospace, Mantel analysis and Stayton metrics demonstrate a high degree of homoplasy. Computational fluid dynamics reveals similar hydrodynamic performance among morphologically convergent species, indicating the independent acquisition of the same morphofunctional traits and, potentially, equivalent lifestyles. These results confirm that a number of the characters typically used to infer the evolutionary relationships among galeaspids, osteostracans and jawed vertebrates are convergent in nature, potentially obscuring understanding of the assembly of the gnathostome bodyplan. Ultimately, our results reveal that while the jawless relatives of the earliest jawed vertebrates were ecologically diverse, widespread convergence on the same hydrodynamic adaptations suggests they had reached the limits of their potential ecological diversity – overcome by jawed vertebrates and their later innovations.

KEY WORDS

1. Introduction

The origin of jawed vertebrates (gnathostomes) was a key step in the evolutionary history of animals, culminating in the bodyplan shared by almost all living vertebrates [1]. This episode entailed more than simply the acquisition of jaws, also including a large and diverse suite of traits that distinguish living jawless and jawed vertebrates [2]. Fossil evidence demonstrates that the morphological gap between extant forms was once bridged by a disparate assemblage of extinct jawless (stem-gnathostome) vertebrate lineages, that record the sequential assembly of gnathostome characters [3,4]. Among these, Osteostraci and Galeaspida are of greatest significance because they represent the immediate jawless relatives of jawed vertebrates. While osteostracans are widely regarded as the sister-lineage of jawed vertebrates [5,6], their phylogenetic relationships are obscured by character reversal, convergence and missing data [2,7,8], and hence the competing clades Galeaspida+Osteostraci and Galeaspida+jawed vertebrates cannot be dismissed [8]. Indeed, the bodyplans of osteostracans and galeaspids exhibit gross similarity [9] (figure 1a), including bony headshields that have similar cephalic elaborations (elongated rostra, lateral expansions or processes) and vary between oblate and prolate cephalic profiles [10–13]. Ferrón *et al.* [14] showed that in osteostracans these structures represent adaptations for passive hydrodynamic control, including lift generation. Here, we attempt to provide insight into the ecology of the gnathostome ancestor by characterising quantitatively similarities in the anatomy of galeaspids and osteostracans and, through computational fluid dynamics (CFD), determine whether these similarities have a common functional basis. We found significant convergence in both the shape and hydrodynamic performance of both groups, compatible with the interpretation of their head shapes as adaptations to comparable lifestyles. We consider the implications and potential impact of these findings on current understanding of early vertebrate evolution and the ecological scenario preceding the emergence of jawed groups.

2. Materials and Methods

We characterized headshield morphological diversity in osteostracans and galeaspids in a phylogenetic context by means of geometric morphometrics to determine whether similarities in morphology reflect convergence or common descent. We then applied computational fluid dynamics to interrogate the resulting phylomorphospace (i.e. a projection of the phylogenetic tree into morphospace) from a functional perspective and evaluate if characteristics used in cladistic studies are compatible with convergent adaptations.

(a) Geometric morphometrics

Our study is limited to species of established taxa, with analyses performed at generic level, using one specimen per genus. We considered the type species and holotype specimen where possible; when this specimen was poorly preserved, another well-defined specimen or species was used instead. We incorporated a total of 59 specimens into the analysis, including 30 osteostracans and 29 galeaspids, representing all major groups and the systematic breadth of both clades (electronic supplementary material, table S1).

We used geometric morphometrics to numerically describe the morphological diversity of osteostracan and galeaspid headshields following Ferrón *et al.* [14,15]. Landmark digitization was performed on photographic images of specimens from Ferrón *et al.* [15] and Zhu [12] using TpsDig v.2.26 [16] (electronic supplementary material, table S1). When one half of the headshield was poorly preserved or strongly deformed, the other half was mirrored; this approach has been shown to provide equivalent results to the analysis of complete unaltered structures at macroevolutionary scales [17]. When preservation issues and/or deformation were minor, no corrections were implemented in order to avoid the introduction of additional human error and given that biological signal is still maintained in those cases [18]. Our landmark configuration included a total of 6 landmarks of type I and II, and 82 landmarks of type III that were equally interpolated along the specimen outline in four different open curves (figure 1b). Generalized Procrustes analysis (GPA) was performed in MorphoJ v. 1.06d [19] to remove the variation in translation, rotation and size from the original landmark configurations. No sliding methods for Type III landmarks were implemented. We obtained Euclidean distance matrices which were subjected to principal coordinate analysis in R [20] using the packages 'cluster' [21] and 'ape' [22].

(b) Morphological homoplasy analyses

The relationship between morphospace occupation and phylogeny were explored by constructing and visualizing phylomorphospaces. These were generated using the R packages 'Phytools' [23] and 'ggplot2' [24] based on pre-ordination ancestral state reconstruction [25] through stochastic character state mapping [26] in the R package 'geomorph' [27]. Our tree is based on the phylogenetic hypotheses and stratigraphic ranges published by Zhu *et al.* [11], Gai *et al.* [13,28], Sansom [10], and Sansom *et al.* [29], after modification in Mesquite [30] and time-calibration in the R package 'paleotree' [31].

Morphological homoplasy was quantified following two different procedures. First, we determined the strength and significance of linear correlations among the distance matrices derived from morphometric and phylogenetic data (i.e., phenetic vs phylogenetic distances) by implementing Mantel tests considering the whole dataset, only osteostracans, and only galeaspids in the R package 'vegan' [32]. Distance matrices show greater decoupling and, consequently, lower correlation where homoplasy occurs. Secondly, we considered the C1

metric of Stayton [33], which quantifies the ratio of the phenotypic distance between putatively convergent taxa (D_{tip}) and the maximum phenotypic distance between any pair of ancestors (D_{max}) as:

$$C1 = 1 - (D_{tip} / D_{max})$$

C1 ranges from 0 to 1, where 1 indicates complete convergence. C1 was calculated for different osteostracan-galeaspid pairs, selecting representatives of all major clades of both groups. To test significance of the measured C1 values, we ran 1000 simulations for each pair using Brownian Motion models in order to determine if the observed C1 value is greater than expected by chance. These analyses were conducted in the R package 'convevol' [34].

(c) Three-dimensional virtual modelling

We created three dimensional digital models for eight species of osteostracans (*Boreaspis ceratops*, *Cephalaspis lyelli*, *Hemicyclaspis munchisoni*, *Hoelaspis angulata*, *Kiaeraspis auchenaspoides*, *Spatulaspis robusta*, *Stensiopelta pustulata* and *Tremataspis schmidtii*) and five species of galeaspid (*Geraspis rara*, *Macrothyraspis longicornis*, *Pentathyraspis pelta*, *Polybranchiaspis liaojiaoshanensis* and *Rhegmaspis xiphoidea*), which constitute a good representation of all occupied areas of the phylomorphospace. Digital models were built in 3D Studio Max based on published reconstructions in several views and/or photographs of fossil specimens (electronic supplementary material, table S2). For most of the selected species, well-preserved fossil headshields are known, ensuring these parts could be modelled accurately. The postcranial region was modelled for species in which it is known (i.e., *C. lyelli* [35], *H. munchisoni* [36], *T. schmidtii* [37] and *G. rara* [12]), whereas a generalized osteostracan or galeaspid morphology based on existing published reconstructions was considered for the remaining taxa. The resulting three-dimensional models were scaled to life size using Netfabb Basic and converted into NURBS surfaces in Geomagic Studio (www.geomagic.com). Osteostracan models correspond to those used in Ferrón *et al.* [14].

(d) Computational fluid dynamics analyses

Computational fluid dynamics is a computational method for simulating fluid flow (liquids or gases) and their interaction with solid surfaces, enabling large-scale comparative analyses for resolving functional and ecological hypotheses in extinct taxa [38]. We performed simulations of water flow around the three-dimensional models in COMSOL Multiphysics 5.2 (<http://www.comsol.com>), following the procedure outlined in Ferrón *et al.* [14]. Pelagic and benthic conditions were emulated in order to assess the functional constraints of homoplasy in different ecological scenarios. For the pelagic scenario, the computational domain consisted of a cylinder (1500 mm in length and 300 mm in diameter), with the three-dimensional model centrally located and positioned at eight different angles of attack (from 0° to 70°, every 10°) (figure 1c). For the benthic scenario, the computational domain consisted of a half-cylinder (1500 mm in length and 300 mm in diameter), with the three-

dimensional model positioned at 0.1, 0.5 and 1.0 body lengths above the lower surface of the domain (figure 1c).

An inlet with a normal inflow velocity boundary condition, with a turbulence intensity of 0.05, was specified at the anterior end of the domain; and an outlet with a zero pressure boundary condition was defined at the opposing end. Boundaries at the water–fossil interface were ‘solid’ (i.e., no-slip boundary condition) whereas the boundaries at the top and sides of the domain were ‘open’ (i.e., slip boundary condition). In the benthic scenario, a moving wall boundary condition with the same velocity as the inlet was assigned to the flat lower boundary of the half-cylinder. The domain was meshed in COMSOL Multiphysics using free tetrahedral elements and the ‘normal’ mesh size parameter. Previous work has demonstrated that CFD results are independent of the domain and mesh sizes under this experimental setting within the range of body sizes considered in our analysis [14].

We simulated three-dimensional incompressible flow through the domain, using a stationary solver to compute the steady-state flow patterns, considering a flow velocity of 0.30 m s^{-1} (Reynolds numbers of 17100 to 64500). This constitutes a realistic swimming velocity according to records of similar sized living fishes [39]. The shear stress transport (SST) turbulence model and a segregated solver algorithm were used to solve the Reynolds averaged Navier-Stokes (RANS) equations. The choice of the RANS SST model is based on its reduced computational requirements and potential for capturing the same general flow patterns than other more refined models (e.g., large eddy simulation) [40]. Segregated iterations terminated when the relative error was lower than the relative tolerance (0.001). We carried out additional simulations with inlet velocities of 0.65 and 1.00 m s^{-1} (Reynolds numbers of 37050 to 215000) in order to test the effect of increasing inlet velocity (electronic supplementary material, table S3).

Flow patterns over the surface of the three-dimensional models were visualized using plots of vorticity (Z-vorticity, s^{-1}) and pressure (Pa). Drag and lift forces (C_D and C_L) acting on the surfaces of the models were calculated using the headshield area as the reference area (electronic supplementary material, table S2). Heat maps of the C_D and C_L were constructed and plotted over the phylomorphospaces using the R package “akima” [41]. Correlation between headshield morphology and hydrodynamic parameters (i.e., C_D and C_L) was assessed through phylogenetic generalized least square (PGLS) analysis in MorphoJ v. 1.06d [19]. PGLS were undertaken for three pelagic (at 0° , 10° and 20°) and one benthic (0.1_{BL}) simulations, which were considered the most biologically realistic scenarios as evidenced by living fishes [42]. In order to evaluate the biological relevance of the calculated forces, the apparent weight of each taxon was inferred from the model volume, assuming a body density of 1100 kg m^{-3} [43]. This value represents a reasonable estimate for osteostracans and galeaspid in light of the body densities calculated for other jawless stem-gnathostomes where the

distribution and density of dermal bone, soft tissues and internal cavities is taken into account [44].

3. Results

(a) Geometric morphometrics and homoplasy tests

Osteostracans and galeaspids occupy similar regions of the morphospace, with most of their representatives located in positive scores on PCo1 and PCo3 (figure 2a). Only a few regions are exclusively occupied either by osteostracans or galeaspids: osteostracans with comparatively narrow headshields and poorly developed cornual processes occupy the region of morphospace that is strongly negative on PCo1 and strongly positive on PCo2; and long-snouted galeaspids with long and thin lateral cephalic processes occupy the region of morphospace that is strongly positive on PCo1 and strongly negative on PCo2 and PCo3. The phylomorphospace exhibits a large number of intersections among galeaspid and osteostracan clades and, at least, two strong branching trends (figure 2a). The trends in osteostracans occur along PCo1 and PCo3, one characterized by the reduction and eventual loss of the cornuae, and the other correlating with the acquisition of well-developed cornual processes and elongated rostra. The trends in galeaspids are captured along PCo1–3, one characterized by the elongation of the rostrum, and another correlating with the acquisition of both expanded lateral processes and long rostra (figure 2a). The results of the Mantel tests indicate significant correlation between phenetic and phylogenetic distances in both the total dataset and the galeaspid subset (p -value = 0.003 in both cases), but not in the osteostracan subset (p -value = 0.653). Mantel r statistic ranged between -0.030 and 0.313 in all analyses (figure 2b). Stayton metrics analyses reveal that 42 of the 169 tested pairs of osteostracans-galeaspids show C_1 values significantly greater than those expected under Brownian motion evolution (figure 2b and electronic supplementary material, table S4).

(b) Computational fluid dynamics

For all tested species, the highest drag coefficients (C_D) occur at higher angles of attack and in closer proximity to the substrate (electronic supplementary material, figure S1 and table S3). In the pelagic scenario, the lift coefficient (C_L) increases linearly with angle of attack, reaching a maximum at 40°–50° before attaining the stall angle of attack, from which C_L decreases abruptly (electronic supplementary material, figure S1 and table S3). Usually, absolute lift force overcomes apparent weight at realistic swimming speeds and angles of attack (between 10° and 40°) in all taxa (electronic supplementary material, table S3). Under benthic conditions, C_L increases considerably for all models when they are placed in proximity to the substrate (electronic supplementary material, figure S1 and table S3), but this phenomenon is more evident in some species with dorso-ventrally oblate headshields such as for example the osteostracans *Stensiopelta* and *Boreaspis*, or the galeaspids *Geraspis*, *Pentathyraspis* and *Polybranchiaspis*. The extra lift force generated when the models are located at 0.1 body lengths above the substrate is enough to counteract the apparent body weight of most of the

taxa (electronic supplementary material, table S3). The lift to drag ratio (L/D), considered a measure of hydrodynamic efficiency, shows important variations among the different species and tested experimental conditions (electronic supplementary material, figure S1 and table S3). In the pelagic scenario, most of the species attain maximum L/D at angles of attack between 20° and 30° (except for the osteostracan *Hoelaspis*, where it is reached at 40°). Under benthic conditions, all species attain the highest L/D when they are placed at 0.1 body lengths above the substrate. In general terms, species with dorso-ventrally prolate headshields reach the maximum L/D under pelagic conditions whereas species with dorso-ventrally oblate headshields show the peak L/D under benthic scenarios. Generally, convergent species pairs (supported by Stayton metrics) exhibit maximum L/D in the same ecological scenario, but there are exceptions to this rule (*Boreaspis* vs *Macrothyraspis*; *Hemicyclaspis*, vs *Geraspis*, *Pentathyraspis*, and *Polybranchiaspis*).

C_D and C_L heat maps plotted over phylomorphospaces reveal very similar patterns when considering osteostracans and galeaspids separately under pelagic scenarios at 0° angle of attack (figure 3a). In both cases, the highest C_D values are associated with positive scores on PCo1–3 and the highest C_L values correlate with negative and positive scores of PCo1 and PCo2, respectively. Similar patterns in the C_D and C_L heat maps derived from osteostracans and galeaspids are also evident in other tested scenarios (electronic supplementary material, data S1). Morphological aspects correlated with the generation of drag and lift show important similarities in both osteostracans and galeaspids (figure 3b). Thus, C_D is significantly correlated in both groups with the degree of development of headshield lateral expansions in benthic and pelagic scenarios at angles of attack ranging 0–20°. On the other hand, C_L correlates with headshield morphology in pelagic scenarios at low angles of attack (i.e., from 0–10° in osteostracans, and 0° in galeaspids) where species with delta-shaped headshields show the highest C_L values. Otherwise, no significant correlation is detected between C_L and headshield morphology in benthic scenarios.

The distribution of tip vortices is similar within convergent pairs of osteostracans and galeaspids (figure 4). In species whose headshields exhibit well-developed lateral processes, tip vortices form in more distal positions separated from the body, whereas in species lacking prominent lateral processes, the tip vortices remain close or attached to the body surface. In general, the intensity of these vortices is similar in convergent taxa, excepting the *Boreaspis* vs *Macrothyraspis* pairing, where they are less developed in *Macrothyraspis*. The patterns of pressure over the headshields show significant differences between the pelagic and benthic scenarios (electronic supplementary material, figure S2). In the pelagic simulations, the highest pressures occur on the snout, whereas in the benthic simulations they expand along most of the ventral surface, especially in species with dorso-ventrally oblate headshields (e.g., *Stensiopelta*, *Boreaspis*, *Geraspis*, and *Pentathyraspis*).

4. Discussion

(a) Similar galeaspid and osteostracan headshields result from widespread convergence

Our results reveal widespread convergence in the morphology of the headshields of osteostracans and galeaspids, supported both by qualitative interpretation of phylomorphospaces and quantitative metrics (figure 2). Despite significant correlation between phenetic and phylogenetic distances, the Mantel r statistic is comparatively low in all analyses, indicating that correlations are weak and suggesting widespread morphological convergence (figure 2b). Stayton metric analyses provide further support, indicating that a large number of osteostracans and galeaspids, including representatives of several major groups of both lineages, evolved to be more similar to each other than would be expected under a null evolutionary model of Brownian motion (figure 2b and electronic supplementary material, table S4). The most remarkable instances of convergence include forms with elongate rostra (e.g., *Spatulaspis* vs *Rhegaspis*), expanded lateral processes (*Cephalaspis*, *Tegaspis*, *Stensiopelta*, and *Auchenaspis* vs *Eugaleaspis*, *Geraspis*, and *Zhaotongaspis*), both elongated rostra and expanded lateral processes (e.g., *Boreaspis* vs *Pterogonaspis*, *Sanchaspis*, and *Macrothyraspis*), and more fusiform headshields (*Hemicyclaspis*, and *Kiaeraspis* vs *Pentathyraspis*, *Lopadaspis*, and *Polybranchiaspis*); in many cases, these pairs exhibit p -values < 0.01 .

(b) Convergence generally correlates with hydrodynamic performance

Exploration of the hydrodynamic properties of the morphospace circumscribed by osteostracan and galeaspid headshields revealed that morphological convergence correlates with morphofunctional traits and, potentially, equivalent lifestyles and ecologies. Discrete regions of the morphospace are occupied by osteostracans and galeaspids that show comparable hydrodynamic performance (figure 3a and electronic supplementary material, data S1). In fact, we detected significant correlations between hydrodynamic forces (i.e., C_D and C_L) and equivalent headshield structures in both groups (figure 3b). Morphologically comparable osteostracan and galeaspid headshields exhibit remarkably similar patterns of hydrodynamic flow, as revealed by vorticity and pressure visualizations (figure 4 and electronic supplementary material, figure S2). The distribution and intensity of tip vortices is similar in most of the convergent species pairs (figure 4). These vortices originate at the tips of cornual or inner cornual processes, similar to aircraft wingtips [45]. In general terms, species with high aspect ratios (e.g., *Boreaspis* and *Machairaspis*) exhibit less intense tip vortices because of less important tip losses (i.e., fluid leakage between the lower and upper surfaces), which generate smaller induced drag. However, high aspect ratios result in lower manoeuvrability due to a larger moment of inertia [45]. Similar vorticity patterns have been reported previously in other groups of stem-gnathostomes [46]. Likewise, the distribution of pressure around the models suggest some common biomechanical properties in osteostracan and galeaspids with similar shaped headshields (electronic supplementary material, figure S2). Thus, headshields that are oblate in cross-section exhibit a greater increase in pressure on the ventral surface and considerably higher lift generation when they are placed close to the substrate (electronic supplementary material, figures S1 and S2 and table S3). This is

indicative of ground effect, a phenomenon that many living benthic species benefit from, which entails modification of fluid flow below the body, increasing lift and facilitating enhanced hydrodynamic efficiency when moving close to the substrate [47].

Among the convergent groupings of osteostracans and galeapids, only *Boreaspis* and *Macrothyraspis*, characterized by long rostra and lateral expansions in the headshields, exhibit significant differences in hydrodynamic performance. When compared to *Boreaspis*, *Macrothyraspis* exhibits much more poorly-developed vortices (figure 4) and a less intense ground effect (electronic supplementary material, figures S1 and S2), both of which result in very distinct trends in C_D and C_L in most of the tested scenarios (electronic supplementary material, figure S1 and table S3). Thus, this particular instance of convergence cannot be correlated with hydrodynamic performance; rather, the acquisition of long rostra and lateral expansions must have been driven by other factors, including predator deterrence [48], housing sense organs [49], substrate anchoring [50], or specialised feeding strategies [51].

(c) Implications for phylogenetic analyses of early vertebrate evolution

Our study identifies a number of convergent traits in the headshields of galeaspids and osteostracans. This is of concern since the headshield comprises the character complex from which the vast majority of phylogenetic characters are derived [15] and competing hypotheses on the phylogenetic relationships of galeaspids, osteostracans and jawed vertebrates are discriminated by few characters [5,52]. Characteristics including the cross-sectional (oblate versus prolate) and dorso-ventral profiles of the headshield, presence or absence of cornual/corner processes, rostral processes, serrate versus entire margin of the headshield, have all been used to resolve the phylogeny of galeaspids and osteostracans [6,10,11,52–54] and yet they are clearly convergent, correlating with hydrodynamic properties. Furthermore, while we have considered only galeaspids and osteostracans, other stem-gnathostome clades, including thelodonts [55,56] and pteraspidomorphs [57,58], exhibit anatomical features compatible with hydrodynamic adaptation. The exclusion of these characters, together with inclusion of recently identified similarities between galeaspids and jawed vertebrates [7,8], is likely to lead to a shake-up of received wisdom concerning the relationships among stem-gnathostomes, changing perceptions of character evolution in the assembly of the gnathostome bodyplan.

(d) Implications for the ecological scenario preceding the emergence of jawed vertebrates

Our results provide additional support for the view that the immediate jawless relatives of the earliest jawed vertebrates were already ecologically diverse [14], showing a range of adaptations for passively controlling flow around the body. These adaptations could have provided stem-gnathostomes with higher manoeuvrability and versatility for adopting a greater diversity of locomotory strategies than previously thought (conventional interpretations view stem-gnathostomes as benthic mud-grubbers with poor swimming capabilities [59–62]). Among these convergent adaptations, cornual processes have

sometimes been considered as structures to protect the pectoral fins in osteostracans [63]. However, given the lack of paired appendages in galeaspids, we propose that cornual processes represent independent adaptations to generating lift, later enhanced by the acquisition of pectoral fins in osteostracans, and eventually rendered redundant by the more flexible and muscular fins of jawed vertebrates. The high degree of morphological homoplasy among galeaspids and osteostracans, with the evolution of largely the same set of hydrodynamic adaptations in both groups, might imply that they had approached the limits of ecological diversity that could be achieved by the bodyplan of jawless vertebrates [64]. The evolution of jawed vertebrates built upon the bodyplan and anatomical constraints that galeaspids and osteostracans manifest. Key innovations including paired pelvic fins, a mineralised vertebral skeleton, and a pectoral girdle distinct from the head and jaws, all served to release these constraints, allowing jawed vertebrates to assume greater ecological diversity and, thus, dominance over their jawless kin [65–67].

5. Conclusions

The headshields of osteostracans and galeaspids show a high degree of morphological homoplasy, as suggested by geometric morphometrics, phylomorphospace analysis and different quantitative metrics of convergence. Computational fluid dynamics analyses reveal that most of the detected convergent traits are compatible with morphofunctional adaptations, thus suggesting that the acquisition of similar morphologies in both groups may relate to the evolution of similar lifestyles. The exclusion of these characters in future phylogenetic analyses, and the consideration of recently identified similarities between galeaspids and jawed vertebrates, may change our current perception on the relationships among stem-gnathostomes and on the evolution of the gnathostome bodyplan. Ultimately, this finding further supports ecological diversification among the immediate jawless relatives of all jawed vertebrates and the prevalence of adaptations for passively controlling the flow around the body in these groups, which may have conferred them greater manoeuvrability and locomotory capabilities than previously thought.

Data accessibility

Data and R code are available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.9s4mw6mfd>.

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FIGURE AND TABLE CAPTIONS

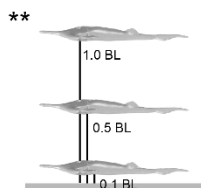
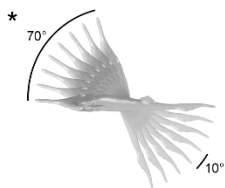
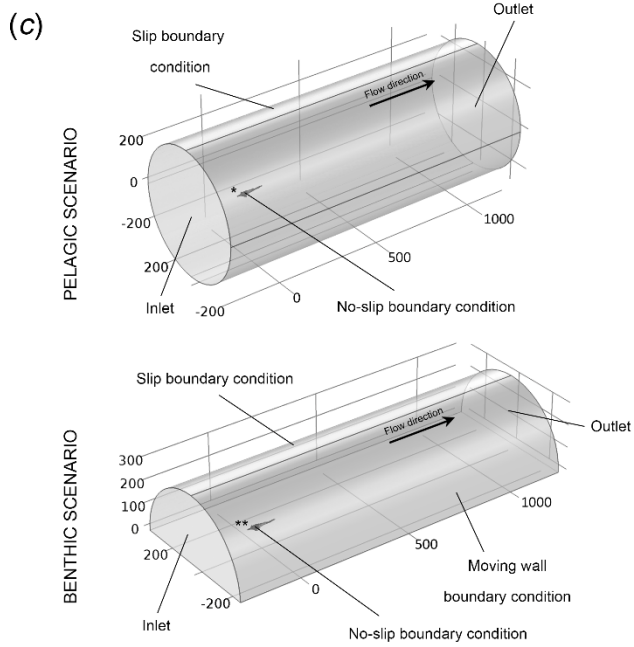
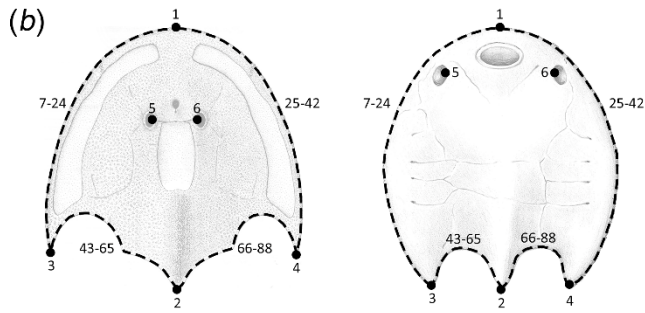
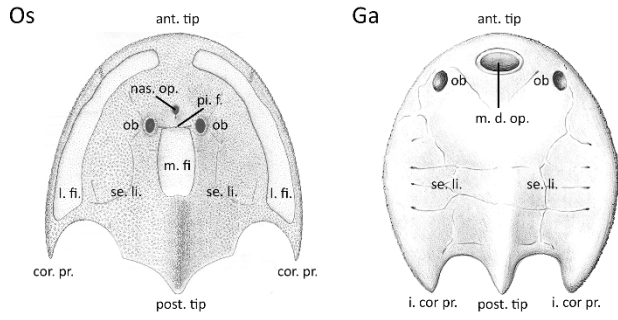
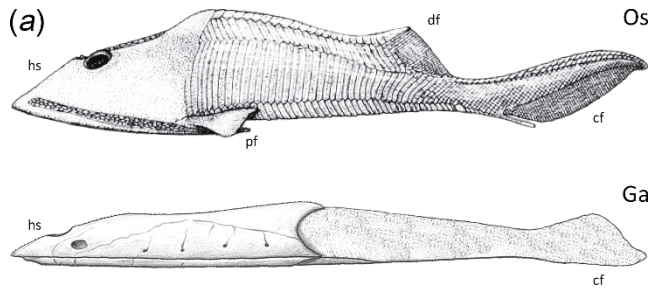
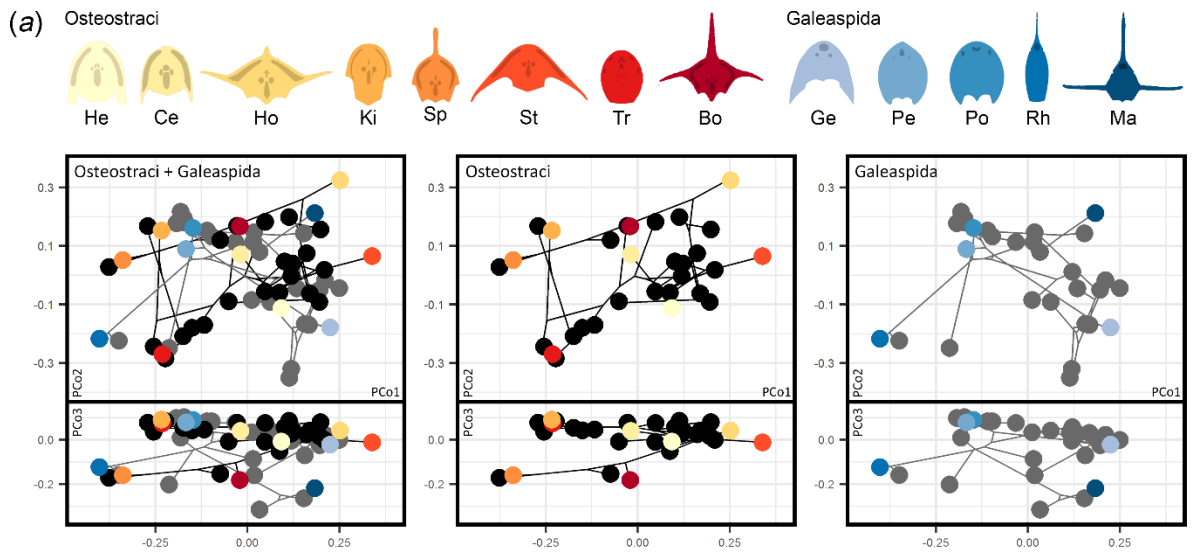


Figure 1. General morphology of osteostracans and galeaspids and experimental setup used in computational fluid dynamics simulations. (a) Anatomy of the body and headshield of a well-known osteostracan (Os) and galeaspid (Ga), showing the location of the pectoral fins (pf), dorsal fin (df), caudal fin (cf), headshield (hs), anterior and posterior tips of the headshield (ant. tip. and post. tip, respectively), cornual processes (cor. pr.), inner cornual processes (i. cor. pr.), sensory lines (se. li.), eye orbits (ob.), nasohypophysial opening (nas. op.), pineal foramen (pi. f.), median and lateral fields (m. fi. and l. fi. respectively), and median dorsal opening (m. d. op.). Osteostracan whole body and galeaspid drawings modified from Janvier [6]: figs. 4.14A1, 4.19A1 and 4.19A2. Osteostracan headshield drawing modified from Janvier [50]: fig. 99. (b) Landmark configuration used in the geometric morphometric analysis. Landmark 1, anterior tip of the headshield or the rostral process. Landmark 2, posterior tip of the headshield. Landmarks 3 and 4, left and right most distal points of the cornual processes, respectively. Inner cornual processes were considered in galeaspids lacking cornual processes. Landmarks 5 and 6, most medial points of the eye orbits (ob.). Landmarks 7–42, type III landmarks situated between Landmarks 1 and 3 and between Landmarks 1 and 4. Landmarks 43–88, type III landmarks situated between Landmarks 2 and 3 and between Landmarks 2 and 4. (c) Diagram of the computational domains used in CFD simulations for pelagic (upper) and benthic (lower) scenarios where models were placed at different angles of attack (bottom left) and different distances above the lower surface of the domain (bottom right). All measurements in mm. Modified from Ferrón *et al.* [14]: fig. 1D.



(b) Mantel test (Mantel statistic r : 0.149; p -value: 0.003) Mantel test (Mantel statistic r : -0.030; p -value: 0.653) Mantel test (Mantel statistic r : 0.313; p -value: 0.003)

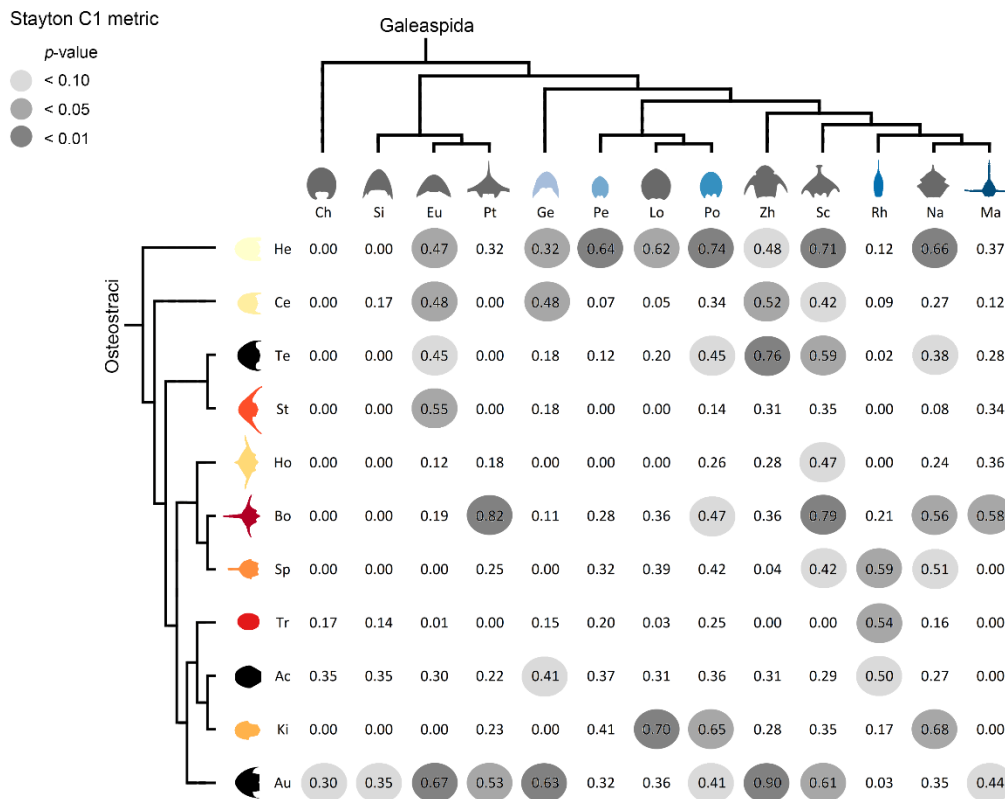


Figure 2. Geometric morphometrics and convergence analysis results. (a) Phylomorphospace summarizing the morphological disparity of osteostracan and galeaspid headshields. Ordination of each group is also shown in separate plots to facilitate comparison, but results correspond to a single analysis considering representatives of both groups together. The proportion of variance explained by PCo1–3 is 43.82%, 32.11% and 11.87%, respectively. (b) Mantel test results for each of the datasets (upper) and Stayton C1 metric for several pairs of osteostracans vs galeaspids (lower), indicating different degrees of statistical significance in distinct grey tones. Phylogenetic trees based on Zhu *et al.* [11], Gai *et al.* [13,28], Sansom [10], and Sansom *et al.* [29]. Taxa included in CFD analyses are shown in colour, whereas the rest

of osteostracans and galeaspids are in black and grey, respectively. Osteostraci: non-cornuates (*Hemicyclaspis*, He), Cephalaspida (*Cephalaspis*, Ce), Zenaspidida (*Tegaspis*, Te; *Stensiopelta*, St), Benneviaspidida (*Hoelaspis*, Ho: *Boreaspis*, Bo; *Spatulaspis*, Sp), Kiaeraspidida (*Acrotomaspis*, Ac; *Kiaeraspis*, Ki), and Thyestiida (*Auchenaspis*, Au; *Tremataspis*, Tr). Galeaspida: Xiushuiaspididae (*Changxingaspis*, Ch), Sinogaleaspidae (*Sinogaleaspis*, Si), Eugaleaspidae (*Eugaleaspis*, Eu; *Pterogonaspis*, Pt), Geraspididae (*Geraspis*, Ge), Pentathyraspidae (*Pentathyraspis*, Pe), Duyunolepidae (*Lopadaspis*, Lo), Polybranchiaspididae (*Polybranchiaspis*, Po), Zhaotongaspididae (*Zhaotongaspis*, Zh), Sanchaspidae (*Sanchaspis*, Sc), Gantarostrataspidae (*Rhegmaspis*, Rh), and Huananaspidae (*Nanpanaspis*, Na; *Macrothyraspis*, Ma).

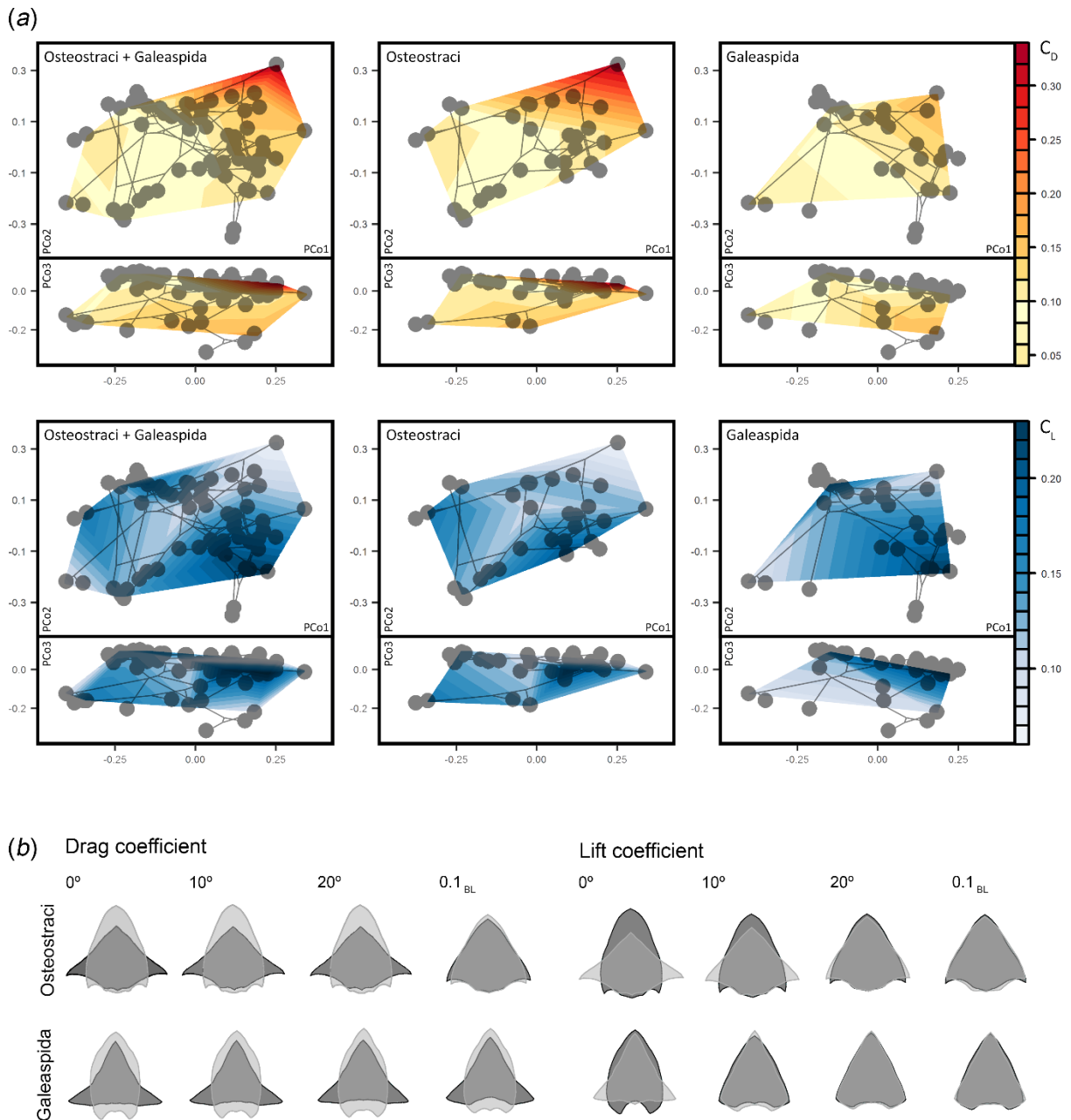


Figure 3. Morphological and biomechanical correlates. (a) Drag (C_D) and lift (C_L) coefficient heat maps plotted over the phylomorphospaces of the whole dataset, osteostracans and galeaspids. Force values correspond to pelagic scenarios considering an angle of attack of 10° with an inlet velocity of 0.3 m s^{-1} . (b) Correlations between headshield morphology and hydrodynamic force coefficients (C_D and C_L) calculated under different experimental conditions (i.e., pelagic scenario at angles of attack of 0° – 20° and benthic scenario at 0.1 BL above the substrate, with an inlet velocity of 0.3 m s^{-1}). Pairs of overlapping outlines (landmark wireframe configurations) represent the headshield morphologies that generate the highest and lowest force coefficients (in darker and lighter grey, respectively) in each scenario. Correlation results of Osteostraci: C_D (0°): % predicted = 46.53, p -value = 0.0005; C_D (10°): % predicted = 43.36, p -value = 0.0006; C_D (20°): % predicted = 40.58, p -value = 0.0014; C_D (0.1_{BL}): % predicted = 35.84, p -value = 0.0056; C_L (0°): % predicted = 52.69, p -value = 0.0001; C_L (10°): % predicted = 25.64, p -value = 0.0317; C_L (20°): % predicted = 4.17,

p -value = 0.5488; C_L (0.1_{BL}): % predicted = 5.39, p -value = 0.4808. Correlation results of Galeaspida: C_D (0°): % predicted = 59.09, p -value = 0.0038; C_D (10°): % predicted = 57.15, p -value = 0.0053; C_D (20°): % predicted = 63.35, p -value = 0.0011; C_D (0.1_{BL}): % predicted = 38.59, p -value = 0.0346; C_L (0°): % predicted = 29.22, p -value = 0.1064; C_L (10°): % predicted = 10.87, p -value = 0.5109; C_L (20°): % predicted = 11.61, p -value = 0.4875; C_L (0.1_{BL}): % predicted = 10.92, p -value = 0.5593.

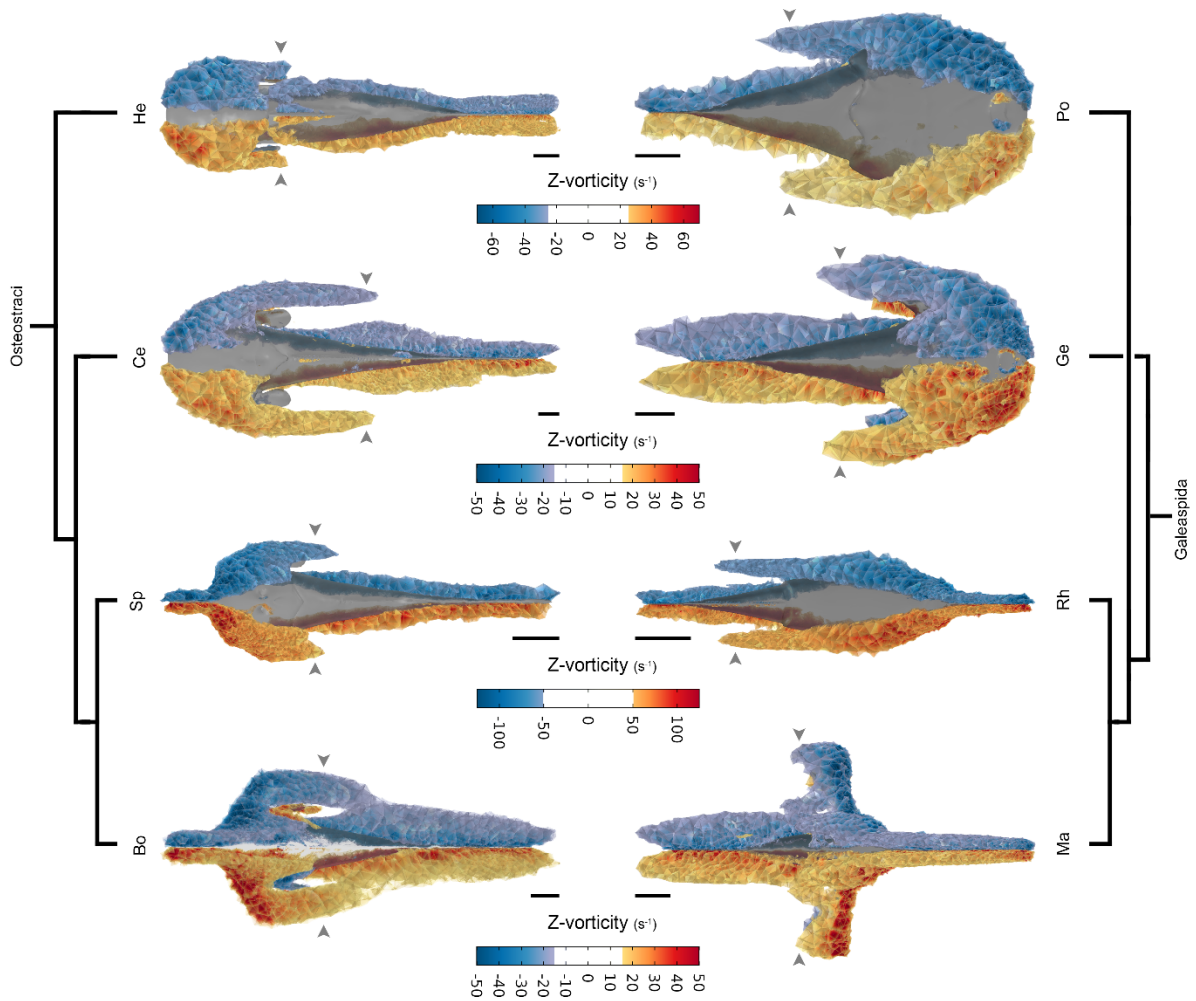


Figure 4. Vorticity patterns over osteostracan and galeaspid models under pelagic scenarios considering an angle of attack of 20° . Arrows indicate the position of tip vortices. Species are associated by homoplastic pairs with the highest Stayton C1 values for comparison. Bo, *Boreaspis*; Ce, *Cephalaspis*; Ge, *Geraspis*; He, *Hemicyclaspis*; Ma, *Macrothyraspis*; Po, *Polybranchiaspis*; Rh, *Rhegmaspis*; Sp, *Spatulaspis*. Phylogenetic trees based on Zhu *et al.* [11], Gai *et al.* [13,28], Sansom [10], and Sansom *et al.* [29]. Scale bar 1 cm.