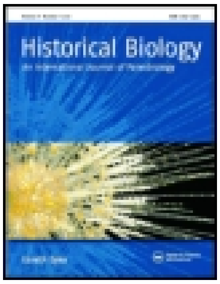
This article was downloaded by: [Max Planck Inst fuer Evolutionsbiologie]
On: 03 September 2013, At: 07:01
Publisher: Taylor & Francis
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Historical Biology: An International Journal of Paleobiology
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/ghbi20

Using information in taxonomists' heads to resolve hagfish and lamprey relationships and recapitulate craniate-vertebrate phylogenetic history

Maria Abou Chakra [a] , Brian Keith Hall [b] & Johnny Ricky Stone [a b c d]
[a] Department of Biology , McMaster University , Hamilton , Canada
[b] Department of Biology , Dalhousie University , Halifax , Canada
[c] Origins Institute, McMaster University , Hamilton , Canada
[d] SHARCNet, McMaster University , Hamilton , Canada

Published online: 02 Sep 2013.

To cite this article: Historical Biology (2013): Using information in taxonomists' heads to resolve hagfish and lamprey relationships and recapitulate craniate-vertebrate phylogenetic history, Historical Biology: An International Journal of Paleobiology

To link to this article: http://dx.doi.org/10.1080/08912963.2013.825792

PLEASE SCROLL DOWN FOR ARTICLE

Taylor & Francis makes every effort to ensure the accuracy of all the information (the "Content") contained in the publications on our platform. However, Taylor & Francis, our agents, and our licensors make no representations or warranties whatsoever as to the accuracy, completeness, or suitability for any purpose of the Content. Any opinions and views expressed in this publication are the opinions and views of the authors, and are not the views of or endorsed by Taylor & Francis. The accuracy of the Content should not be relied upon and should be independently verified with primary sources of information. Taylor and Francis shall not be liable for any losses, actions, claims, proceedings, demands, costs, expenses, damages, and other liabilities whatsoever or howsoever caused arising directly or indirectly in connection with, in relation to or arising out of the use of the Content.

This article may be used for research, teaching, and private study purposes. Any substantial or systematic reproduction, redistribution, reselling, loan, sub-licensing, systematic supply, or distribution in any form to anyone is expressly forbidden. Terms & Conditions of access and use can be found at http://www.tandfonline.com/page/terms-and-conditions

Historical Biology, 2013
http://dx.doi.org/10.1080/08912963.2013.825792

Using information in taxonomists' heads to resolve hagfish and lamprey relationships and recapitulate craniate–vertebrate phylogenetic history

Maria Abou Chakra[a], Brian Keith Hall[b] and Johnny Ricky Stone[a,b,c,d]*

[a]*Department of Biology, McMaster University, Hamilton, Canada;* [b]*Department of Biology, Dalhousie University, Halifax, Canada;* [c]*Origins Institute, McMaster University, Hamilton, Canada;* [d]*SHARCNet, McMaster University, Hamilton, Canada*

(*Received 3 June 2013; final version received 13 July 2013*)

In 1806, a hypothesis in which hagfishes and lampreys were classified as the taxon Cyclostomi was proposed on the basis of shared morphological traits. That 'monophyletic cyclostome' classification prevailed into the twentieth century and has persisted until the present. In 1958, a study involving coordinate grid transformations to analyse head ontogenies for living and fossil craniates was published. Results obtained in that evolutionary–developmental analysis revealed that extant hagfishes and extinct heterostracans developed substantially differently from closely related extant and extinct agnathans and warranted recognition as a distinct lineage. In 1977, a classification in which lampreys and jawed vertebrates formed a group exclusively from hagfishes was proposed on the basis of neontological, morphological and molecular traits. This 'paraphyletic cyclostome' classification garnered acceptance among some taxonomists and has persisted alongside the monophyletic cyclostome classification until the present. We applied geometric morphometrics to data obtained from the 1958 evolutionary–developmental analysis, to objectively test and confirm these overlooked and underappreciated results. We demonstrated that the paraphyletic cyclostome classification was conceived at least 19 years earlier than usually acknowledged. Our reanalysis emphasises that the debate on whether the Cyclostomata is monophyletic or paraphyletic must be resolved formally on the basis of principles and practices for phylogenetic systematic analysis including fossil data.

Keywords: coordinate grid transformation; cyclostome classification; evolutionary developmental biology; geometric morphometrics; history of ideas; systematics

1. Introduction

Scientists' practices are guided by intuition. Occasionally, hypotheses, predictions or theories derived from studying a particular subject for years can be scrutinised effectively only after experimental or technological innovation provides a means for testing with new observations, data or information; sometimes, circumstance is a factor – Eddington's confirming Einstein's General Theory of Relativity by measuring light deflection by the sun during a total eclipse in 1919 provides a most celebrated instance (Dyson et al. 1920). Less occasionally, approaches, records or interpretations that were underappreciated upon their initial publication remain available and amenable to re-examination using new methods and techniques, and scientists may be considered retrospectively as having contributed to specific research fields; population geneticists' (i.e. Hugo de Vries, Carl Correns and Erich von Tschermak) rediscovering Mendel's laws provides a remarkable instance (Henig 2009) and Telford's (2013) reanalysing data from Field et al. (1988) provide a more recent instance. Herein, we use geometric morphometrics to quantitatively analyse data that were produced and used in a theoretical analysis conducted by Strahan (1958), celebrated Australian academic, educator, author and zoological park director. Strahan, who was considered a world authority on extinct agnathans, was among the first researchers to use fossil and evolutionary–developmental information to describe craniate phylogeny in a manner that pre-dated more contemporary classifications; Strahan also ardently advocated museums and their collections as cultural institutions and contributed to books on Australian birds, mammals and museums (Strahan and Branagan 1977).

1.1 Craniate–vertebrate and cyclostome classification

The taxa Craniata and Vertebrata were erected by Linnaeus in 1798 (Janvier 1996, 1997; Nielsen 2012). In modern parlance, craniates constitute a taxon diagnosed by the 'synapomorphic', or shared derived, character state 'possessing a cartilaginous or bony skull' and, thereby, technically include Hyperotreti (hagfishes), Hyperoartia (lampreys), extinct agnathan (jawless vertebrate) groups and Gnathostomata (jawed vertebrates). A classic and long-standing dispute among taxonomists concerns relationships among the hagfishes, lampreys, and jawed vertebrates (Janvier 1997, 2013).

Traditionally, hagfishes and lampreys were grouped together as the taxon Cyclostomi ('round mouths'), currently Cyclostomata, which was considered to be allied

---

*Corresponding author. Email: jstoner@mcmaster.ca

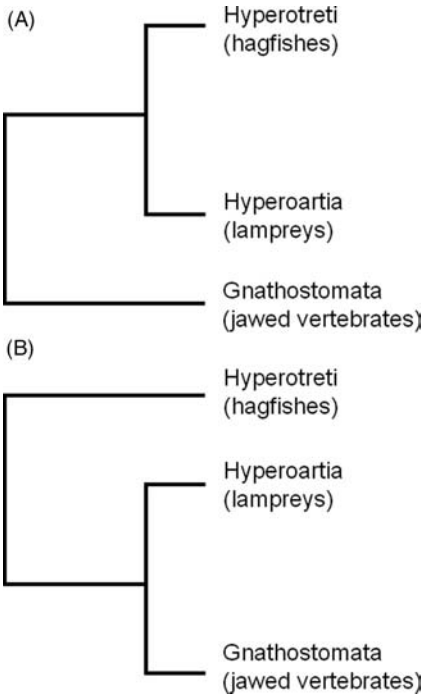


Figure 1. Extant craniate phylogenies, as inferred on the basis of two different cladograms: (A) monophyletic cyclostome classification, wherein hagfishes and lampreys are grouped together as the taxon Cyclostomata; (B) paraphyletic cyclostome classification, wherein hagfishes are allied to a group containing lampreys and gnathostomes.

with the jawed vertebrates (Figure 1(A)). Duméril (1806), French zoologist, was the first scientist to erect this classification, which he formulated on the basis of shared morphological traits, such as keratinous teeth borne on a tongue-like apparatus, a large notochord and pouch-shaped gills (Janvier 1996, 1997). Cope (1889), American paleontologist, coined the name 'Agnatha' to christen a group that included the cyclostomes and some jawless fossil taxa (Janvier 1996, 1997). Thenceforth and well into the twentieth century, taxonomists divided the vertebrates into two major 'sister', or nearest-classified, groups, the Agnatha and the Gnathostomata. Stensiö (1927), Swedish paleozoologist, suggested that living agnathans were 'diphyletic', with hagfishes and lampreys arising separately from two now-fossilised, armoured agnathan lineages, but retained the opinion that agnathans constituted a 'monophyletic group' (i.e. a valid taxonomic unit including all species that may be inferred to have descended from a common ancestor). This 'monophyletic cyclostome' classification (Figure 1(A)) has been supported recently by information obtained from morphological data (e.g. see Delarbre et al. 2000 for a discussion; Janvier 2013; Oisi et al. 2013) and molecular data (e.g. rRNA gene sequences: Mallat and Sullivan 1998; Mallat et al. 2001; Zardoya and Meyer 2001; nuclear gene sequences: Kuraku et al. 1999; Takezaki et al. 2003; microRNA sequences: Heimberg et al. 2010; mitochondrial DNA sequences: Delarbre et al. 2002; protein sequences: Furlong and Holland 2002).

An alternative scheme wherein hagfishes were considered as the sister group to a group comprising lampreys and jawed vertebrates was proposed by Løvtrup (1977), Swedish developmental biologist and systematist. Løvtrup showed that lampreys and gnathostomes share morphological traits absent in hagfishes and, thereby, proposed that the cyclostomes constituted a 'paraphyletic assemblage' [i.e. an invalid taxonomic unit including only some species that may be inferred to have descended from a common ancestor; in the paraphyletic cyclostome classification, gnathostomes are excluded; the shared morphological traits included arcualia (serially arranged, paired, supra-notochordal cartilaginous, neural arches), an adenohypophysis (a differentiated anterior lobe in the pituitary gland) and a typhlosole (a spirally coiled valve within the intestinal wall), which have been received with caution (Schaeffer and Thomson 1980); Janvier 1996, 1997]. To recognise that hagfishes, lampreys and gnathostomes possess a skull whereas only lampreys and gnathostomes possess vertebral elements, Janvier (1978) proposed that the names that had been erected originally by Linnaeus (1798; Janvier 1996), Craniata and Vertebrata, be used to designate the two nested taxa that are defined by those traits. This 'paraphyletic cyclostome' classification (Figure 1(B)) has been supported recently by information obtained from novel morphological data (e.g. Gagnier 1993; Forey and Janvier 1994; Janvier 1996), reanalysed morphological data (e.g. Heimberg et al. 2010) and molecular data [e.g. mitochondrial protein-coding genes: Rasmussen et al. (1998); Hox gene sequences and MHC protein gene sequences: Escriva et al. (2002)].

While the debate as to whether the Cyclostomata is monophyletic or paraphyletic continues (Zardoya and Meyer 2003; Heimberg et al. 2010), taxonomists are establishing consensus toward the original, monophyletic cyclostome classification (Shimeld and Donoghue 2012; Janvier 2013; Oisi et al. 2013; Figure 1(A)). We hereby contribute constructively to the debate by reanalysing data from a classic but underappreciated paper (Strahan 1958). We demonstrate that data obtained from fossils may be included in evolutionary–developmental as well as geometric morphometric analyses. Such inclusion yields information that should be considered in concert with copious other taxonomically relevant data, especially if the fossil information proves to be incongruous with and challenging to information yielded by the other data. In particular, we emphasise that, ultimately, the cyclostome classification must be determined strictly on the basis of phylogenetic systematic analysis including fossil data.

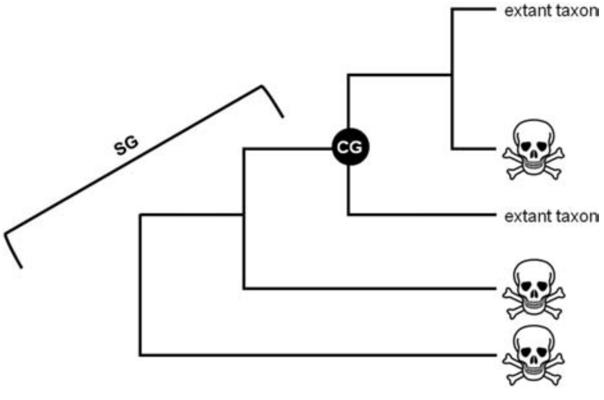


Figure 2. Crown and stem groups. A crown group is the smallest monophyletic group that includes all extant taxa, which may be inferred to have descended from a common ancestor (i.e. as originating at the node "CG"). A stem group comprises the extinct taxa 'topologically outside' the crown group (i.e. the two bottommost 'skull and crossbones' terminal nodes). *Source*: Adapted from Budd and Jensen (2000).

1.2 Crown and stem groups

Budd and Jensen (2000) emphasised how fossil taxa and the related concepts 'crown group' and 'stem group' can and should guide taxonomists in devising classifications and elucidating evolutionary patterns, by forcing phylogenetic systematists to consider explicitly that traits originate and evolve and how traits originate and evolve (i. e. character states appear and transform on cladograms; Figure 2). Craniates are no exception. Indeed, fossil data have provided information useful for constructing early craniate classifications, from research in the nineteenth century by Hugh Miller, John Grant Malcolmson, Eliza Maria Gordon Cumming and Ramsay Heathley Traquair (reviewed in Janvier 1996), through classical work by Agassiz (1857), Huxley (1858), Lankester (1864) and Stensiö (1927, 1968), to modern studies by Nelson (1969, 1989) and Janvier (1996), and with fossil discoveries by Shu et al. (1999) and Chen et al. (1999). Analyses conducted on fossil specimens (e.g. conodonts, Lower Cambrian Chengjiang *Haikouella* and *Yunnanozoon*, and Middle Cambrian Burgess Shale *Pikaia* and *Nectocaris*) have yielded information useful in functional analyses (e.g. Donoghue 2009) and classifications (e.g. Janvier 2013).

1.3 Evolutionary–developmental data and coordinate grid transformations

In contrast to the aforementioned molecular and morphological data, developmental data, which also provide information useful in phylogenetic systematics, have been underutilised in establishing craniate classifications. Schaeffer and Thomson (1980) proposed a notable exception to prevailing perspectives on agnathan–gnathostome affinities and cyclostome classification; the authors discussed such fundamental issues such as vertebrate monophyly, reviewing and assessing the evidence – including embryological – available at the time for hagfishes, lampreys and gnathostomes.

One historical explanation for this deficiency involves the rarity with which embryonic studies have been conducted on 'basal' craniates, such as hagfishes and lampreys. The hagfish collection established and analysed by Bashford Dean in 1896 (published in 1899) and related specimens used by Jesse Leroy Conel in 1942 constituted the first among only a few living embryo studies ever conducted (Wicht and Northcutt 1995; Gorbman 1997; Wicht and Tusch 1998; Hall 1999, 2009); researchers only recently have conducted additional embryological analyses (Ota et al. 2007; Ota and Kuratani 2008; Oisi et al. 2013). All published lamprey embryo studies involved fertilised eggs obtained from wild populations (Piavis 1971; Tahara 1988; Nikitina et al. 2009); culturing embryos using captive lampreys never has been achieved. Recognising the utility in analysing developmental information in an evolutionary context and lacking embryogenesis data for basal taxa, in 1958, Ronald Strahan applied the coordinate grid transformation technique that had been championed previously by Thompson (1917; Dürer 1613), 'the most influential biologist ever left on the fringes of legitimate science' (Gleick 1987), to infer ontogenetic stages for extinct and extant craniates.

In this technique, a coordinate grid is imagined to be superimposed over one specimen; homologous landmarks on this specimen and a second specimen are identified, and the coordinate grid is deformed so that landmarks on the first specimen are transformed to assume the same configuration as do homologous landmarks on the second specimen. The manner in which, and the extent to which, coordinate grids must be deformed provides visual depictions for morphological transformations. Thompson (1917) interpreted these primarily from a geometric perspective. However, if the taxa being compared may be inferred to be related closely (i.e. contemporarily, on the basis of a phylogenetic systematic analysis), then the morphological transformation may be considered as representing the changes incurred by the representative species through phenotypic evolution. As Thompson (1917) stated, coordinate grid transformations provide

> a means of comparing one known structure with another. But it is obvious, … that it may also be employed for drawing hypothetical structures, on the assumption that they have varied from a known form in some definite way. And this process may be especially useful, and will be most obviously legitimate, when we apply it to the particular case of representing intermediate stages between two forms which are actually known to exist, in

> other words, of reconstructing the transitional stages through which the course of evolution must have successively travelled if it has brought about the change from some ancestral type to its presumed descendant.

Strahan (1958, p. 84) defined a hypothetical 'basic agnathan embryo' and used it as an initial form from which representative *Myxine* (Hyperotreti), *Lampetra* (Hyperoartia), *Pterygolepis* (Anaspida), an unidentified genus in the Heterostraci (Pteraspidomorphi) and *Kieraspis* (Cephalaspidida) specimens could be derived (the latter three specimens representing stem group taxa for extant jawed craniates, as a crown group taxon). Strahan created the basic agnathan embryo on the basis of lamprey embryos and Goodrich's (1930) diagram depicting segmented heads in dogfish, entailing that the basic agnathan embryo possessed three pro-otic somites, gill pouches between neighbouring somites (including premandibular, mandibular and hyoid), a short stomodeum, a velum arising from the oral plate and a hypophysis inside the buccal cavity, with nostrils positioned anteriorly.

Strahan (1958) used *Myxine glutinosa* as the representative for hagfishes, citing Dean's (1899) embryological research on gill pouches in *Epiatretus* and Holmgren (1946) embryological research on gill pouches in *Myxine*. Strahan used tracings to construct the representative for lampreys, citing Damas' (1944) developmental research on *Lampetra fluviatilis*. Strahan used the taxon name *Pterolepis* in determining the representative for anaspids, citing Stensiö's (1958) pterygolepid reconstruction (*Pterolepis* Kiaer, 1911 was preoccupied and replaced by *Pterygolepis* Cossman, 1920). Strahan used the colloquial term 'Heterostracan' in determining the representative for Heterostraci, citing Stensiö's (1958) pteraspid reconstruction. Strahan used figures to determine the representative for cephalaspids, citing Stensiö's (1958) kieraspid reconstruction.

Strahan constructed stages between the hypothetical agnathan embryo and adults for the five agnathan taxa, by assuming four intermediate ontogenetic stages equidistant from one another and interpolating coordinate grid transformations between them. The intermediate forms bore similarities to actual developmental stages manifested by extant forms (e.g. lampreys; Strahan 1958, fig. 4).

Using coordinate grids in this manner constituted a fascinating, innovative and still underutilised application, despite the previously quoted suggestion from Thompson (1917). In justifying the application, Strahan (1958) cited Medawar's (1945) observation that coordinate grid transformations are applicable to a continuous change and so more relevant to developmental than evolutionary analysis. We interpret the relevance from a contemporary perspective as being more balanced, a *bona fide* application to 'evolutionary developmental biology' (Dalton 2000; Goodman and Coughlin 2000; Raff 2000; Raff and Love 2004): transforming a hypothetical, ammocoete-like-stage agnathan to adult hagfish, lamprey, anaspid, heterostracan and cephalaspid specimens.

1.4 Geometric morphometrics

Since 1958, coordinate grid transformation analyses, themselves, have been transformed into an influential subculture in biological statistics, known as 'geometric morphometrics' (Rohlf and Marcus 1993; Adams et al. 2004). Geometric morphometrics comprises methods for analysing size (especially, by eliminating size differences among specimens) and shape while preserving the relative positional arrangements among landmark data. The techniques have been described extensively (e.g. Bookstein et al. 1985; Rohlf and Bookstein 1990; Bookstein 1991; Marcus et al. 1993; Rohlf and Marcus 1993), so are recapitulated only briefly herein and for only relative warp analysis.

First, a reference landmark configuration is identified; this could constitute the mean or generalised Procrustes analysis fit among landmark coordinate positions for specimens, an outgroup taxon or a developmental stage [e.g. Strahan (1958) used the hypothetical agnathan embryo]. Next, a matrix containing squared inter-landmark distances for the reference landmark configuration is created; this matrix is known as partitioned matrix L. Then, the upper left $p \times p$ block (where p enumerates landmarks) in L^{-1} is determined; this is known as the bending energy matrix. Eigenvalues for the bending energy matrix yield eigenvectors known as principal warps. Eigenvalue magnitudes are weighted inversely with scale, with large eigenvalues describing small-scale bending (i.e. deformation for landmarks in close proximity to one another) and the converse. Three eigenvalues equal zero (although computer software might identify as zero additional eigenvalues that reside within a prescribed error range from zero); these correspond to affine components (translation, rotation and dilation), which are infinite in scale. Penultimately, landmark coordinate positions for specimens are projected onto principal warps (e.g. into 'x' and 'y' components), yielding partial warps. Partial warps provide p-3 orthogonal components into which non-affine transformations may be resolved at different scales (the infinite-scale, affine transformation also may be represented by a single partial warp). Finally, a principal components analysis is performed, using a covariance matrix obtained from partial warp scores, to obtain relative warps.

The transformation from the reference landmark configuration into landmark coordinate positions for specimens can be displayed conveniently, using a thin-plate spline as a physical metaphor: the reference landmark configuration is analogised to points on an

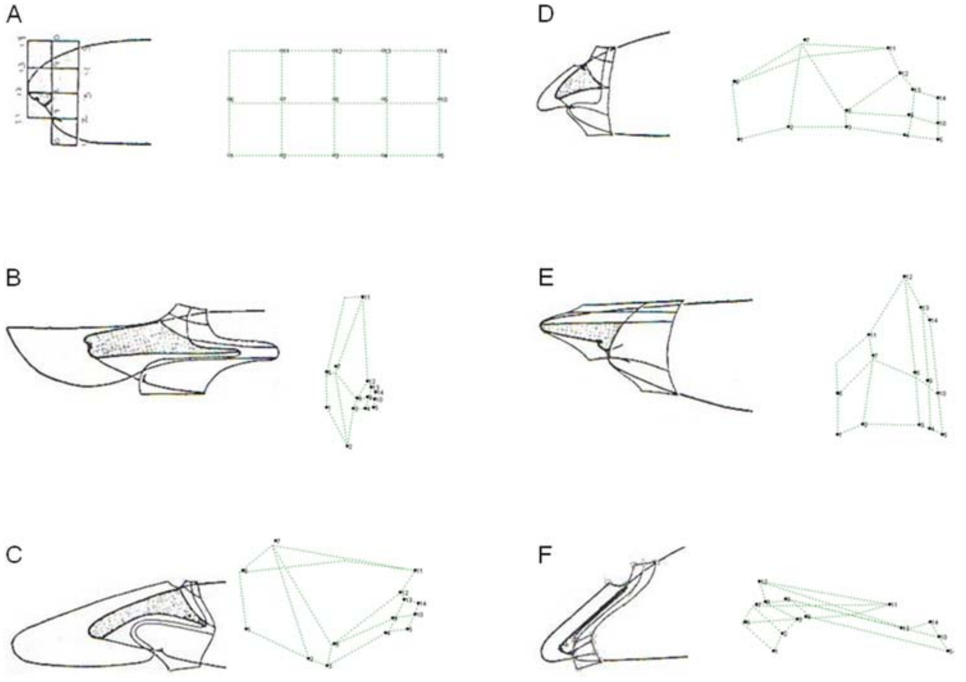


Figure 3. Strahan's (1958) coordinate grid transformation analysis for craniates (left in each part) accompanied by new thin-plate spline graphics representing a transformation from the basic agnathan embryo, rotated 90° 'clockwise' (right in each part). Strahan defined (A) a hypothetical 'basic agnathan embryo' and used it as an initial form from which representative (B) *Myxine* (Hyperotreti), (C) *Lampetra* (Hyperoartia), (D) *Pterolepis* (Anaspida), (E) heterostracan (Pteraspidomorphi) and (F) *Kieraspis* (Cephalaspidida) specimens could be derived (coordinate grid transformation graphics reproduced from Strahan 1958, fig. 11).

infinitely thin, uniform metal plate constrained at those reference landmark configuration points, but otherwise free to adopt the form that minimises bending energy, and relative warps transform the reference landmark configuration points on this plate into homologous landmark coordinate positions for the specimens. Specimens also may be represented as points in the relative warp space on the basis of the scores.

2. Data and methods

We used the software tpsSpline (Rohlf 2004a) and tpsRelw (Rohlf 2004b) to relate the basic agnathan embryo to the representative specimens for hagfishes, lampreys, anaspids, heterostracans and cephalaspids, as depicted by Strahan (1958, fig. 11; Figure 3). Images for the specimens were enlarged to 1.8 magnification using a Canon© NP 6551 photocopy machine. We identified homologous landmarks by noting correspondences among the coordinates (coordinate grids were included in the original published figure). Landmark coordinate positions were determined by superimposing the enlarged images onto a transparent graph paper. We calculated bending energies, visualised coordinate grid transformations (using partial warps) with tpsSpline and obtained relative warp scores with tpsRelw (for subsequent plotting).

3. Results

Among the transformations that were presented in Strahan (1958, fig. 11), deformation to the hagfish specimen required the greatest bending energy, almost twice that for any other specimen (Table 1). The transformations depicted as thin-plate splines indicated that landmarks 1 and 2, and 6 and 7 became compressed in the anterior direction for the hagfish and heterostracan specimens relative to the lamprey, anaspid and cephalaspid specimens; landmarks 3 and 4, and 8 and 9 compressed for the hagfish, heterostracan and cephalaspid specimens, expanded for the lamprey and anaspid specimens, and inverted for the hagfish specimen; landmarks 4 and 5 expanded for the hagfish, heterostracan and cephalaspid specimens and compressed for the lamprey and anaspid

Table 1. Bending energies for coordinate grid transformations between a 'basic agnathan embryo' and representative specimens for hagfishes, lampreys, heterostracans, anaspids and cephalaspids, on the basis of the data from Strahan (1958).

Specimen	Bending energy
(B) *Myxine* (Hyperotreti)	17.08
(C) *Lampetra* (Hyperoartia)	5.00
(D) *Pterolepis* (Anaspida)	1.36
(E) Heterostracan (Pteraspidomorphi)	2.69
(F) *Kieraspis* (Cephalaspidida)	8.65

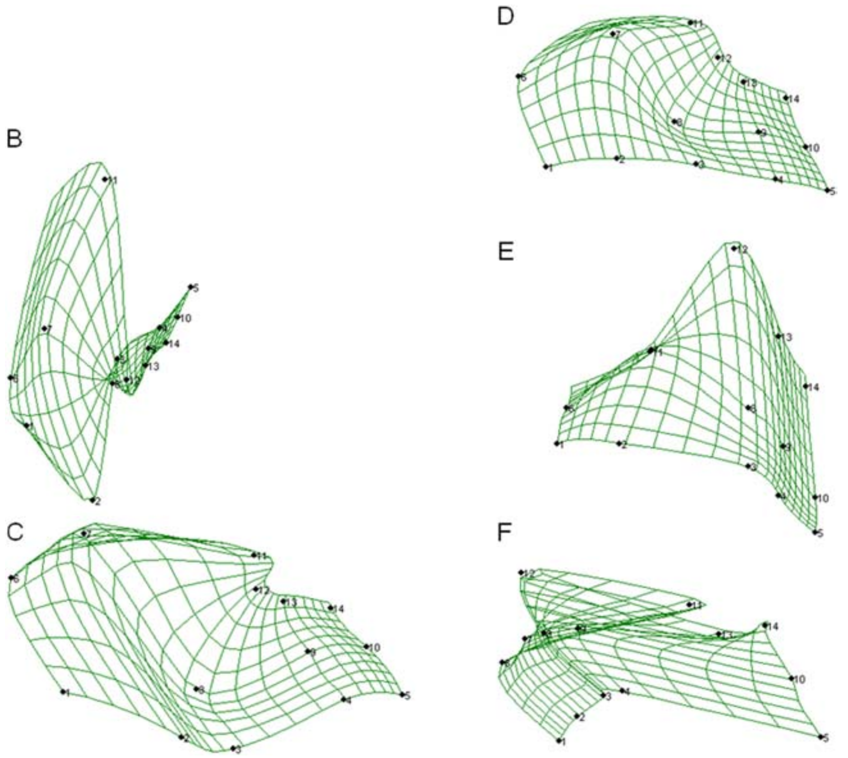


Figure 4. Transformations depicted by visualising partial warps as thin-plate splines for representative (B) *Myxine* (Hyperotreti), (C) *Lampetra* (Hyperoartia), (D) *Pterolepis* (Anaspida), (E) heterostracan (Pteraspidomorphi) and (F) *Kieraspis* (Cephalaspidida) specimens (specimen graphic identifications and orientations correspond to those in Figure 3).

specimens; and landmarks 11–14 compressed for the hagfish, lamprey and cephalaspid specimens and expanded for the anaspid and heterostracan specimens (Figure 4). The hagfish specimen occupied a different region in the relative warp space from that occupied by the other specimens, scoring strongly negatively on relative warp 1, as did the heterostracan specimen, and relative warp 2, uniquely (Figure 5).

4. Discussion

Strahan (1958) applied the coordinate grid transformation technique to compare ontogenetic sequences for five agnathan taxa and concluded that hagfishes and heterostracans develop substantially differently from the other three specimens. He summarised the major differences graphically (Strahan 1958, fig. 11; Figure 3) and descriptively: hagfishes exhibit extended pre- and post-hypophyseal folds; lampreys, anaspids and cephalaspids are characterised by anteriorly extended post-hypophysial folds; and heterostraci are characterised by an extended prenasal or rostral region in the head. Strahan (1958, pp. 93–94) reasoned that apparently closely related groups that differ ‘phenetically’ (i.e. phenotypically) either must have diverged from one another early in their shared evolutionary history or represent independent evolutionary lines (Hall 1999, 2009), and concluded on the basis of the observed differences that hagfishes and heterostracans represent a separate evolutionary lineage; he interpreted the dorsal monorhinal ammocoete-like condition that prevails in lampreys, anaspids and cephalaspids as a trait that was inherited from a common ancestor and so craniate phylogeny as hagfishes and vertebrates (i.e. lamprey + gnathostome) as sister group lineages (Figure 1(B)).

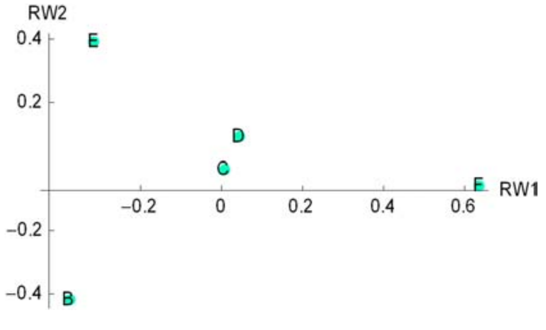


Figure 5. Agnathan specimen distribution in relative warp space: (B) *Myxine* (Hyperotreti), (C) *Lampetra* (Hyperoartia), (D) *Pterolepis* (Anaspida), (E) heterostracan (Pteraspidomorphi) and (F) *Kieraspis* (Cephalaspidida) specimens. The hagfish specimen occupies uniquely one quadrant, scoring strongly negatively on relative warps (RW) 1 and 2 (specimen graphic identifications and orientations correspond to those in Figures 3 and 4).

We considered geometric morphometrics (i.e. thin-plate spline relative warp analysis) as a means for quantifying the developmental intricacies that the keen-eyed Strahan (1958) was able to discern observationally, and found that the bending energy and thin-plate spline for the transformation from the basic agnathan embryo to the hagfish specimen and, to a lesser extent, heterostracan specimen were greater and more dynamic than were the bending energies and thin-plate splines for transformations to the other agnathan taxa that Strahan had examined (Table 1). On the basis of these results, we objectively confirm Strahan's observations and substantiate why Strahan (1958, p. 94) drew the conclusion that '[t]he anaspids are the only fossil agnathans which could have possessed an ammocoete-like larva. The cephalaspids, anaspids and lampreys form a natural group which does not include the [hagfishes]. The [hagfishes] may be derived independently from the [heterostracans]'. The distribution for points in the relative warp space confirms the developmental differences that Strahan noted: hagfishes occupy a region that is distinct from the regions that are occupied by the other taxa, similarly negative to heterostracans along relative warp 1 and uniquely negative with respect to lampreys, anaspids, heterostracans and cephalaspids along relative warp 2 (Figure 5). These differences can be visualised by the unique, dynamic transformations for landmarks (Figure 4), which correspond morphologically to the aforementioned extended pre-hypophyseal folds and the prenasal or rostral region.

Recently, Ota et al. (2011) showed that development in the hagfish species *Eptatretus burgeri* involves a sclerotome compartment in which small, axial, cartilaginous elements form at all axial levels in a manner that is similar to how lamprey axial cartilaginous nodules (arcualia) form. On the basis of this observation, researchers have interpreted lamprey arcualia and gnathostome vertebrae as anatomical homologues (Ota et al. 2011) and the Craniata and Vertebrata as taxonomic synonyms (Shimeld and Donoghue 2012; Figure 1(A)). Oisi et al. (2013) showed that the adenohypophysis arises ectodermally during development in *E. burgeri* and embryos are characterised by a median nasohypophyseal plate (containing one olfactory and one adenohypophyseal placode). On the basis of these observations, researchers have interpreted median nasohypophyseal plates as homologous among cyclostomes (Oisi et al. 2013) and the Craniata and Vertebrata as taxonomic synonyms (Shimeld and Donoghue 2012; Figure 1(A)). With consensus about early craniate–vertebrate taxonomic relationships emerging toward the monophyletic cyclostome classification (Figure 6), proposing the paraphyletic cyclostome classification may be viewed retrospectively as a crucial historical step in advancing craniate phylogenetic systematics. Given that gnathostome embryos are characterised separately by two olfactory placodes and one adenohypophyseal placode, researchers now are charged to re-examine fossil taxa, to infer character states therein [e.g. anaspids and cephalaspids (representative for osteostracans in Figure 6) are characterised by a small dorsal nostril in a nasohypophyseal plate similar to the condition in cyclostomes, whereas heterostracans (and galeaspids) are characterised by paired olfactory organs and a large shared nostril similar to the condition in gnathostomes; Janvier 2008, 2013]. Strahan's (1958) keen-eyed observations and morphological analysis were prescient and underappreciated. Taxonomically, considered from a contemporary perspective, he might 'have had his heads in the wrong place'. Nevertheless, dissenting perspectives often have provided impetus for additional research effort in systematic communities (Hull 1988; e.g. Nikaido et al. 1999), and results obtained from fossil and evolutionary–developmental as well as geometric morphometric analyses generally may contribute to taxonomy.

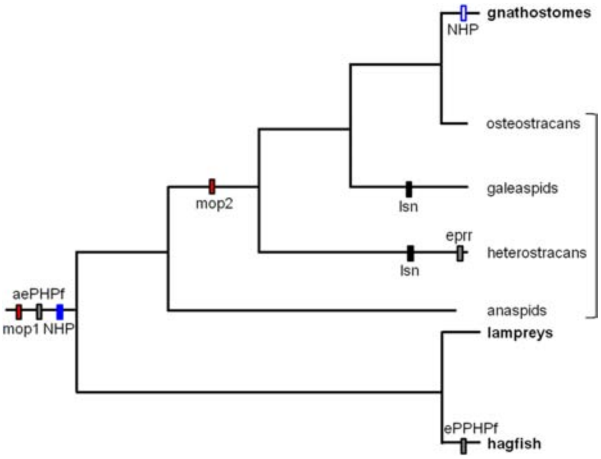


Figure 6. Cladogram depicting consensus emerging about craniate–vertebrate taxonomic relationships (adapted on the basis of cladograms and data from Janvier 2008, 2013). Emboldened names represent extant taxa, whereas unemboldened names represent extinct taxa. The topology is consistent with the monophyletic cyclostome classification (Figure 1(A)). Bars on the internodes represent character states, distributed most parsimoniously: one median olfactory placode (mop1) or two median olfactory placodes (mop2); anteriorly extended post-hypophysial folds (aePHPf), extended pre- and post-hypophysial folds (ePPHPf) and extended prenasal or rostral region in the head (eprr), nasohypophyseal plate (NHP), in which the median olfactory and adenohypophyseal placodes are united (the empty box in the gnathostomes represents separate olfactory and adenohypophyseal development); large, shared nostril (lsn; a small nostril is the assumed ancestral state for the entire clade, and only one parsimonious distribution is depicted). The paraphyletic group 'ostracoderms' is indicated by the square bracket.

Classifications must accord with cladograms, and fossils can play an important role in generating and corroborating cladograms (e.g. Hulsenbeck 1991). For early craniate systematics specifically, data extracted from fossils and implemented in 'evo-devo' studies, when combined with 'geo-morpho' analyses like those considered and performed by Strahan, yield information that should be considered along with the abundant information that has been gleaned from other morphological as well as molecular data and analyses. Objectively reconsidering Strahan's theoretical analysis and situating it in its proper historical perspective emphasises that the debate about cyclostome monophyly or paraphyly (and whether a taxonomic distinction between craniates and vertebrates can be maintained) ultimately must be determined rigorously using protocols associated with principles and practices for phylogenetic systematic analysis including fossil data.

Acknowledgements

This study was developed with financial support from the Canadian Institutes of Health Research (postdoctoral fellowship to JRS), the Natural Sciences and Engineering Research Council of Canada (Discovery Grants 261590 to JRS and A5056 to BKH) and the resources from the Department of Biology, Origins Institute and Shared Hierarchical Academic Research Computing Network at McMaster University. The authors thank D. A. Elliott, anonymous Reviewer 2, D. Cantelmi, T. Domladovac, S. Faheim, W. Mok and M. Zdelar for intellectual assistance, M. G. B. Colangelo for overseeing and B. Pratt and Luz de Lourdes Vasquez Paz for inspiration.

References

Adams DC, Rohlf FJ, Slice DE. 2004. Geometric morphometrics: ten years of progress following the 'revolution'. Ital J Zool. 71:5–16.

Agassiz JLR. 1857. Classification of fishes. Proc Am Acad Arts Sci. 4:8–9.

Bookstein FL. 1991. Morphometric tools for landmark data. Geometry and biology. Ann Arbor (MI): University of Michigan.

Bookstein FL, Chernoff B, Elder R, Humphries J, Smith G, Strauss R. 1985. Morphometrics in evolutionary biology: the geometry of size and shape change, with examples from fishes. Philadelphia, PA: Academy of Natural Sciences.

Budd GE, Jensen S. 2000. A critical reappraisal of the fossil record of the bilaterian phyla. Biol Rev. 75:253–295.

Chen J-Y, Huang D-Y, Li C-W. 1999. An early Cambrian craniate-like chordate. Nature. 402:518–522.

Conel JL. 1942. The origin of the neural crest. J Comp Neurol. 76(2): 191–215.

Cope ED. 1889. Synopsis of the families of Vertebrata. Am Nat. 23:1–29.

Dalton R. 2000. Biologists flock to 'evo-devo' in a quest to read the recipe for life. Nature. 403:125.

Damas H. 1944. Recherches sur le développement de Lampetra fluviatilis. Contributions à l'étude de la céphalogenèse des vertébrés. Arch Biol. 55(3):3–82.

Dean B. 1899. On the embryology of Bdellostomata stouti. A general account of myxinoid development from the egg and segmentation to hatching. Jena: G. Fischer. p. 221–277.

Delarbre C, Escriva H, Gallut C, Barriel V, Kourilsky P, Janvier P, Laudet V. 2000. The complete nucleotide sequence of the mitochondrial DNA of the agnathan *Lampetra fluviatilis*: bearings on the phylogeny of cyclostomes. Mol Biol Evol. 17:519–529.

Delarbre C, Gallut C, Barriel C, Janvier P, Gachelin G. 2002. Complete mitochondrial CAN of the hagfish, *Eptatretus burger*: the comparative analysis of mitochondrial DNA sequences strongly supports the cyclostome monophyly. Mol Phyl Evol. 22:184–192.

Donoghue PCJ. 2009. Origin of vertebrates. In: Evolution: the first four billion years. Cambridge: Belknap. p. 788–792.

Duméril AMC. 1806. Zoologie Analytique, Ou Méthode Naturelle de Classification des Animaux. Paris: Didot.

Dürer A. 1613. Les quatres livres d'Albert Durer de la proportion des parties et pourtraicts des corps humains. Paris: Charles Perier.

Dyson FW, Eddington AS, Davidson C. 1920. A determination of the deflection of light by the Sun's gravitational field, from observations made at the total eclipse of May 29, 191. Phil Trans R Soc Lond Ser A. 332:291–333.

Escriva H, Manzon L, Youson J, Laudet V. 2002. Analysis of lamprey and hagfish genes reveals a complex history of gene duplications during early vertebrate evolution. Mol Biol Evol. 19:1440–1450.

Field KG, Olsen GJ, Lane DJ, Giovannoni SJ, Ghiselin MT, Raff EC, Pace NR, Raff RA. 1988. Molecular phylogeny of the animal kingdom. Science. 239:748–753.

Forey PL, Janvier P. 1994. Evolution of the early vertebrates. Am Sci. 82: 554–565.

Furlong R, Holland PWH. 2002. Bayesian phylogenetic analysis supports monophyly of Ambulacraria and Cyclostomes. Zool Sci. 19: 593–599.

Gagnier PY. 1993. *Sacabambaspis janvieri*, Vertébré ordovicien de Bolivie. 2. Analyse phylogénétique. Ann Paléontol. 79:119–166.

Gleick J. 1987. Chaos: making a new science. New York: Penguin.

Goodman CS, Coughlin BC. 2000. The evolution of evo-devo biology. Proc Natl Acad Sci USA. 97:4424–4425.

Goodrich ES. 1930. Studies on the structure and development of vertebrates. London: MacMillan.

Gorbman A. 1997. Hagfish development. Zool Sci. 14:375–390.

Hall BK. 1999. The neural crest in development and evolution. New York: Springer.

Hall BK. 2009. The neural crest and neural crest cells in vertebrate development and evolution. New York: Springer.

Heimberg AM, Cowper-Sallari R, Sémon M, Donoghue PCJ, Peterson KJ. 2010. MicroRNAs reveal the interrelationships of hagfish, lampreys, and gnathostomes and the nature of the ancestral vertebrate. Proc Natl Acad Sci USA. 107:19379–19383.

Henig RM. 2009. The monk in the garden: the lost and found genius of Gregor Mendel, the father of modern genetics. Boston (MA): Houghton Mifflin.

Holmgren N. 1946. On two embryos of Myxine glutinosa. Acta Zool. 27 (1):1–90.

Hull D. 1988. Science as a process: an evolutionary account of the social and conceptual development of science. Chicago (IL): University of Chicago.

Hulsenbeck JP. 1991. When are fossils better than extant taxa in phylogenetic analysis? Syst Zool. 40:458–469.

Huxley TH. 1858. The Croonian lecture. On the theory of the vertebrate skull. Proc R Soc Lond. 9:381–457.

Janvier P. 1978. Les nageoires paires des osteostracés et la position systématique des céphalaspidomorphes. Ann Paléontol. 64:113–142.

Janvier P. 1996. Early vertebrates. Oxford: Clarendon.

Janvier P. 1997. Craniata. Animals with skulls [Internet]. Version 01 January 1997. Available from: *The Tree of Life Web Project.* http://tolweb.org/tree?group=Craniata

Janvier P. 2008. Early jawless vertebrates and cyclostome origins. Zool Sci. 25(10):1045–1056.

Janvier P. 2013. Developmental biology: led by the nose. Nature. 493: 169–170.

Kuraku S, Hoshiyama D, Katoh K, Suga H, Miyata T. 1999. Monophyly of lampreys and hagfishes supported by nuclear DNA-coded genes. J Mol Evol. 49:729–735.

Lankester ER. 1864. On the discovery of the scales of pteraspis, with some remarks on the cephalic shield of that fish. Quart J Geol Soc. 20:194–197.

Løvtrup S. 1977. The phylogeny of vertebrata. New York (NY): Wiley.

Mallat J, Sullivan J. 1998. 28S and 28S rDNA sequences support the monophyly of lampreys and hagfishes. Mol Biol Evol. 15(12): 1706–1718.

Mallat J, Sullivan J, Winchell CJ. 2001. Major events in early vertebrate evolution: palaeontology, phylogeny, and development. London: Taylor and Francis. Chapter 7, The relationship of lampreys to hagfishes: a spectral analysis of ribosomal DNA sequences; p. 106–118.

Marcus LF, Bello E, García-Valdecasas A. 1993. Contributions to morphometrics. Madrid: Museo Nacional de Ciencias Naturales; p. 106–118.

Medawar PB. 1945. Essays on growth and form presented to D'Arcy Wentworth Thompson. Oxford: Clarendon. Chapter 5, Size, shape, and age; p. 157–187.

Nelson GJ. 1969. Gill arches and the phylogeny of fishes, with notes on the classification of vertebrates. Bull Am Mus Nat Hist. 141:475–552.

Nelson GJ. 1989. The hierarchy of life. Amsterdam: Excerpta Medica. Chapter 15, Phylogeny of major fish groups; p. 325–336.

Nielsen C. 2012. The authorship of higher chordate taxa. Zool Scripta. 41:435–436.

Nikaido M, Rooney AP, Okada N. 1999. Phylogenetic relationships among cetartiodactyls based on insertions of short and long interpersed elements: hippopotamuses are the closest extant relatives of whales. Proc Natl Acad Sci USA. 96:10261–10266.

Nikitina N, Bronner-Fraser M, Sauka-Spengler T. 2009. Emerging Model Organisms: A Laboratory Manual, Vol. 1. New York: Cold Spring Harbor Laboratory Press. Chapter 16, The sea lamprey *Petromyzon marinus*; p. 405–429.

Oisi Y, Ota KG, Kuraku S, Fujimoto S, Kuratani S. 2013. Craniofacial development of hagfishes and the evolution of vertebrates. Nature. 493:175–181.

Ota KG, Fujimoto S, Oisi Y, Kuratani S. 2011. Identification of vertebra-like elements and their possible differentiation from sclerotomes in the hagfish. Nat Commun. 2:373.

Ota KG, Kuraku S, Kuratani S. 2007. Hagfish embryology with reference to the evolution of the neural crest. Nature. 446:672–675.

Ota KG, Kuratani S. 2008. Developmental biology of hagfishes, with a report on newly obtained embryos of the Japanese inshore hagfish, *Eptatretus burgeri*. Zool Sci. 25:999–1011.

Piavis GW. 1971. The biology of lampreys, Vol. 1. New York: Academic. Chapter 15, Embryology.

Raff R. 2000. Evo-devo: the evolution of a new discipline. Nat Rev Gen. 1:74–79.

Raff R, Love A. 2004. Kowalevsky, comparative evolutionary embryology, and the intellectual lineage of evo-devo. J Exp Zool Mol Dev Evol. 302B(1):19–34.

Rasmussen AS, Janke A, Arnason A. 1998. The mitochondrial DNA molecule of the hagfish (*Myxine glutinosa*) and vertebrate phylogeny. J Mol Evol. 46:382–388.

Rohlf FJ. 2004a. tpsSpline, thin-plate spline, version 1.20. Department of Ecology and Evolution, State University of New York, Stony Brook.

Rohlf FJ. 2004b. tpsRelw, relative warp analysis, version 1.42. Department of Ecology and Evolution, State University of New York, Stony Brook.

Rohlf FJ, Bookstein FL. 1990. Proceedings of the Michigan morphometrics workshop. Special Publication No. 2 Ann Arbor (MI): University of Michigan Museum of Zoology.

Rohlf FJ, Marcus L. 1993. A revolution in morphometrics. TREE. 8:129–132.

Schaeffer B, Thomson KS. 1980. Aspects of vertebrate history. Flagstaff: Museum of Northern Arizona. Chapter 2, Reflections on agnathan–cyclostome relationships.

Shimeld MS, Donoghue PCJ. 2012. Evolutionary crossroads in developmental biology: cyclostomes (lamprey and hagfish). Development. 139:2091–2099.

Shu D-G, Luo H-L, Morris SC, Zhang X-L, Hu S-X, Chen L, Han J, Zhu M, Li M, Chen L-Z. 1999. Lower Cambrian vertebrates from south China. Nature. 402:42–46.

Stensiö E. 1958. Les cyclostomes fossils. Grasse Traite de Zoologie. Paris: Masson.

Stensiö EA. 1927. The Devonian and Downtonian vertebrates of Spitsbergen. 1. Family Cephalaspidae. Skr Sval Isha. 12:1–391.

Stensiö EA. 1968. Current problems of lower vertebrate phylogeny: Proceedings of the Fourth Nobel Symposium. Stockholm: Interscience. Chapter 2, The cyclostomes with special reference to the diphyletic origin of the Petromyzontidae and Myxinoidea; p. 13–71.

Strahan R. 1958. Proceedings of the centenary and bicentenary congress of biology. Singapore: University of Malaya. Chapter 5, Speculations on the evolution of the agnathan head; p. 83–94.

Strahan R, Branagan DF. 1977. Rare and curious specimens: an illustrated history of the Australian Museum, 1827–1979. Sydney: National government publication.

Tahara Y. 1988. Normal stages of development in the lamprey, *Lampetra reissner* (Dybowski). Zool Sci. 5:109–118.

Takezaki N, Figueroa F, Zaleska-Rutczynska Z, Klein J. 2003. Molecular phylogeny of early vertebrates: monophyly of the agnathans as revealed by sequences of 35 genes. Mol Biol Evol. 20:287–292.

Telford MJ. 2013. Field *et al.* redux. Evo Devo. 4:5–10.

Thompson DW. 1917. On growth and form. Cambridge: Cambridge University.

Wicht H, Northcutt RG. 1995. Ontogeny of the head of the Pacific hagfish (*Eptatretus stouti*, Myxinoidea): development of the lateral line system. Phil Trans R Soc Lond Ser B. 349:119–134.

Wicht H, Tusch U. 1998. The biology of hagfishes. London: Chapman and Hall. Chapter 20, Ontogeny of the head of nervous system of myxinoids; p. 431–451.

Zardoya R, Meyer A. 2001. Major events in early vertebrate evolution. London: Taylor and Francis. Chapter 7, Vertebrate phylogeny: limits of inference of mitochondrial genome and nuclear rDNA sequence data due to an adverse phylogenetic signal/noise ratio; p. 135–155.

Zardoya R, Meyer A. 2003. Recent advances in the (molecular) phylogeny of vertebrates. Ann Rev Eco Evol Syst. 34:311–338.