

## No support for Heincke's law in hagfish (Myxinidae): lack of an association between body size and the depth of species occurrence

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This study tests for interspecific evidence of Heincke's law among hagfishes and advances the field of research on body size and depth of occurrence in fishes by including a phylogenetic correction and by examining depth in four ways: maximum depth, minimum depth, mean depth of recorded specimens and the average of maximum and minimum depths of occurrence. Results yield no evidence for Heincke's law in hagfishes, no phylogenetic signal for the depth at which species occur, but moderate to weak phylogenetic signal for body size, suggesting that phylogeny may play a role in determining body size in this group.

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Key words: body size evolution; ecomorphology; Myxinidae; phylogenetic inertia; phylogenetic signal.

### INTRODUCTION

Body size is one of the most important morphological features that affects how an organism interacts with its environment (Haldane, 1928; Thiel, 1975; Peters, 1983; Schmidt-Nielsen, 1984; Rex & Etter, 1998). Body size has been shown in previous work to be associated with, or influenced by, latitude (Jordan, 1892), temperature (Atkinson & Sibly, 1997), anguilliform swimming (Neat & Campbell, 2013), diet (Warburton, 1989; Kulbicki *et al.*, 2005), evolutionary history (Warburton, 1989), the abundance of food (Thiel, 1975), physiological constraints (Pauly, 1997; Chapelle & Peck, 1999) and in fishes, the depth at which species live (Smith & Brown, 2002). As with most morphological variables, no single ecological correlate explains all, or even a majority, of the variation in fish body size. Ecological and life-history correlations often vary within and among lineages or functional groups (Rex & Etter, 1998; Smith & Brown, 2002; Albert & Johnson, 2012; Drazen & Haedrich, 2012). One of the most

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widely cited and debated trends in body size and shape, first observed in the fossil record of mammals, is Cope's rule, which posits a progression to larger body sizes in a given lineage over macroevolutionary timescales (Cope, 1877; Maurer *et al.*, 1992). In many terrestrial species, there is a positive correlation between body size and range size (Lindstedt *et al.*, 1986), but this pattern does not seem to hold for fishes (Warburton, 1989; Jacquemin & Doll, 2014). In marine fishes, a similar pattern known as Heincke's law (Heincke, 1913) describes the pattern of increasing body size in species that live at greater depth; however, this relationship is not universal. Elasmobranchs, *e.g.* exhibit an inverse correlation between size and depth (Smith & Brown, 2002; Drazen & Haedrich, 2012).

A variety of mechanisms potentially explain the general pattern of increasing size with depth in marine fishes and these explanations may vary among lineages (Smith & Brown, 2002). A widely accepted explanation is that large fish can benefit from a significantly lowered metabolism in deeper, colder waters due to decreased respiration rate and enzymatic activity (Smith, 1978; Childress & Somero, 1979). One alternative explanation suggests benefits of large size for scavenging species that live at great depth because larger species can swim over longer distances with a lower mass-specific metabolic rate, which is beneficial when food items are sporadically available (Collins *et al.*, 2005). Another explanation is that large species tend to have elongate bodies and elongation may provide a kinematic advantage to swimming under the high hydrostatic pressure found in the deep ocean (Neat & Campbell, 2013). Several elongate species have been shown to consume less oxygen than non-elongate fishes while swimming at a constant rate as water pressure increases (Sébert *et al.*, 2009; Neat & Campbell, 2013).

Hagfishes (Myxiniidae) are a successful lineage of elongate demersal fishes that inhabit nearly all of the world's oceans and their ecomorphology, including size–depth relationships, is poorly understood. There are 78 recognized species of hagfishes, which vary substantially in maximum body length from 18 cm total length ( $L_T$ ) in the dwarf hagfish *Myxine pequenoi* Wisner & McMillan 1995 to 127 cm total length in the goliath hagfish *Eptatretus goliath* Mincarone & Stewart, 2006 and live at depths ranging from 30 to 5000 m (Fernholm, 1998; Martini, 1998; Mincarone & Stewart, 2006). Hagfishes lack several phenotypic traits occurring in most other fish taxa (*e.g.* dermal scales, image-forming eyes, jaws and vertebrae, but see Ota *et al.*, 2013; Miyashita & Coates, 2015) (Hart, 1973; Fernholm, 1998). They are also known for possessing highly plesiomorphic features: their ability to produce huge quantities of thick, proteinaceous mucus when agitated (Fudge *et al.*, 2005) and the ability to tie themselves into overhand, figure-of-eight, or even more complicated knots depending on the species (Jørgensen *et al.*, 1998; Clark & Summers, 2012; Uyeno & Clark, 2015). Hagfishes are soft animals possessing only a few poorly articulated unmineralized cartilages within the cranial region and tail tip. Hagfishes are primarily recognized as opportunistic scavengers capable of feeding on exceedingly large marine carcasses as well as live prey subdued by other animals (Auster & Barber, 2006). Hagfishes (*e.g.* *Neomyxine* Richardson 1953), however, may take on predatory behaviours by which they actively seek, capture and feed on free-swimming prey (Martini, 1998; Zintzen *et al.*, 2011). Despite their jawless condition, hagfishes use a rapacious feeding mechanism involving three-dimensionally complex arrangements of muscle and connective tissues that power dynamic toothplates with dentition that can be forcefully driven into large food items (Dawson, 1963; Clark & Summers, 2007). Hagfishes initially rely on olfaction for finding food items and then once close enough

to contact the food, will use their barbels for sensing the physical qualities of the food (*e.g.* size, texture and shape) before biting into it (Jensen, 1966). Little is known about the life history of hagfishes, but many species are thought to spawn year round and deposit large eggs (Barss, 1993; Grant, 2006; Ota & Kuratani, 2006). Between 2000 and 2008, commercial harvests of hagfishes increased dramatically, especially in the North Atlantic Ocean (Grant, 2006), but recently the market has shown signs of contracting (Grant, 2015). Several species of *Myxine* are also harvested for their skin, which makes tough, supple leather often marketed as eel leather and for Asian markets where both the fish and their slime are consumed (Gorbman *et al.*, 1990; Barss, 1993).

New species of hagfish are described annually, including 19 new species described between 1998 (Fernholm, 1998) and 2015 (Froese & Pauly, 2015). This influx of new taxa presents problems for determining the taxonomy of the Myxinidae, but recent genetic evaluations of this lineage have resolved some of the interrelationships amongst the six genera. A study by Fernholm *et al.* (2013) compared 32 unique taxa by using two mitochondrial genes and Bayesian phylogenetic methods to clarify the placement of genera and provide support for the newly described genus, *Rubicundus* (Fernholm *et al.*, 2013). The goal of the present study is to determine if species of hagfishes conform to an interspecific pattern consistent with Heincke's law. Phylogenetic relationships were reconstructed from the sequences used by Fernholm *et al.* (2013) to test for associations between depth and body size. This study is novel in three ways. First, this study tests for evidence of Heincke's law in a lineage of elongate fishes that includes species of large and small body size and that inhabit depths from shallow to extremely deep environs. Second, this test was executed with and without a phylogenetic correction, an approach that is lacking in many similar studies despite the importance of accounting for phylogeny in ecomorphological studies (Garland *et al.*, 1992; Freckleton, 2000; Alfaro *et al.*, 2009; Revell, 2009, 2010; Reece & Mehta, 2013). Third, in response to criticisms that tests of this type often oversimplify depth as a single value, when for many species it may be more complicated, association tests were executed using a variety of metrics of the depth at which each species lives.

## MATERIALS AND METHODS

### PHYLOGENETIC CONSTRUCTION

To investigate the relationship of the depth inhabited and the maximum body length among species of hagfishes, sequences were downloaded from GenBank for 652 bp of cytochrome c oxidase subunit I (*col*) and 565 bp of *16s* (sometimes referred to as the small subunit, *ssu*) regions of mitochondrial DNA for 27 of the 78 known species of hagfishes that have sequences available on GenBank ([www.ncbi.nlm.nih.gov/genbank](http://www.ncbi.nlm.nih.gov/genbank)). The analysis consisted of a total of 99 individuals from 27 species, with one to twelve representatives of each species; *col* analysis included 61 individuals with one to nine representatives of each of 17 species, while *16s* had 70 individuals with one to 10 representatives of each of 26 species. Fernholm *et al.* (2013) included 35 species-level entities, but five of these were only identified to genus. A total of 27 named species was included in the current combined dataset of *16s* and *col*. Sequences were manually aligned using GeneDoc (Nicholas & Nicholas, 1997) to ensure that homologous nucleotide positions were being used to infer patterns of ancestry and that *col* formed an open reading frame. jModelTest (Darriba *et al.*, 2012) was used to determine the model of nucleotide evolution *via* Akaike information criterion for finite samples (AICc) scores. This analysis identifies the pattern and complexity of the nucleotide evolution model that should be used to reconstruct the evolutionary history of different lineages based on DNA sequence data. The programme

BEAST 1.8.2 (Drummond *et al.*, 2012) was used to generate the tree after formatting the run in BEAUti (Drummond *et al.*, 2012). Both separate gene trees and a concatenated dataset for *coI* and *16s* Markov chain Monte-Carlo runs consisted of 200 million chain lengths with a sampling interval every 10 000 trees. All phylogenies were constructed with a relaxed, uncorrelated lognormal clock and the Yule speciation process (typical for interspecific phylogenetic reconstructions; Drummond *et al.*, 2012). In both the gene tree and concatenated analyses the *coI* model of nucleotide evolution was partitioned by codon position. In the concatenated analyses substitution and clock models were unlinked and trees were linked. The phylogeny was rooted with three closely related out-group species: the lancelet *Branchiostoma lanceolatum*, European river lamprey *Lampetra fluviatilis* (L. 1758) and sea lamprey *Petromyzon marinus* L. 1758. The output file generated by BEAST was viewed using Tracer 1.6 (Rambaut *et al.*, 2014) to determine whether phylogenetic reconstructions were consistently producing reliable and equally likely trees. Runs were considered consistent when all estimated parameters had an effective sample size value greater than 200. All BEAST runs were duplicated to ensure reliable results. Replicate runs were combined using LogCombiner (also in the BEAST package) and a single Bayesian maximum clade credibility tree was then selected by Tree Annotator (Drummond *et al.*, 2012) from all trees generated after a burn in of 10%. The constructed phylogeny was then viewed in FigTree 1.4.2 (<http://tree.bio.ed.ac.uk/software/figtree/>).

## MODEL FITTING AND PGLS

Data for depth (m) and maximum body length ( $L_T$ , cm) were obtained from the Global Biodiversity Information Facility ([www.gbif.org](http://www.gbif.org)) and FishBase (Froese & Pauly, 2015). When data on individual specimens was available from museum specimens, the exact depth records were used (when a range of depths was reported for a specimen from a trawl, *e.g.* the mean of that range was used). When no specimen data were available from museums, the depth range reported in FishBase was used. For each species, depth was calculated in four ways: average depth of occurrence for museum specimens; minimum depth; maximum depth; the mean of maximum and minimum depths. Shapiro–Wilk tests (utilizing the nlme package in R; [www.r-project.org](http://www.r-project.org); Pinheiro *et al.*, 2015) determine the normality of values for maximum  $L_T$  ( $P > 0.05$ ). Values for maximum depth were not normally distributed ( $P < 0.05$ ) due to some very large values for deep-water species, but were normally distributed after being log-transformed ( $P > 0.05$ ). Both the greatest  $L_T$  and maximum body depth were log-transformed in all subsequent analyses to ensure normality of the data and comparability of depth with maximum  $L_T$  data recorded for each species. To determine the most likely model of evolution for depth inhabited and maximum  $L_T$  across the phylogeny, packages geiger (Harmon *et al.*, 2008) and ape (Paradis *et al.*, 2004) were used in R to compare the following models [Brownian motion (BM), lambda, kappa, drift, Ornstein–Uhlenbeck (OU) and white noise] and select the best-fit model based upon AICc scores. Owing to the phylogenetic signal indicated in model analyses, a phylogenetic generalized least square (PGLS) regression was used to determine the degree to which phylogenetic relatedness explained the evolution of maximum  $L_T$  and maximum depth (analyses repeated for each of the four metrics of depth), using the caper library (Orme *et al.*, 2015) in R. To address any concerns about species with few specimen records, all analyses were rerun on a dataset reduced to those species with data from five or more specimens.

## RESULTS

### PHYLOGENETIC RECONSTRUCTION

The best model of nucleotide evolution for *coI* and *16s* was identified as GTR (general time reversible) + I (invariant sites) + G (discretized  $\gamma$ -distribution) (Table I). This model allows for certain nucleotide positions to have changed due to multiple mutations, for other positions to have had no mutations and for certain nucleotide positions to evolve at higher or lower rates than others. The gene trees for *coI* and *16s* are largely

TABLE I. Models of hagfish nucleotide evolution for *col* and *16s* genes evaluated using jModelTest. The lowest value for the negative log-likelihood score ( $-\ln L$ ) represents the preferred model

Model ( <i>col</i> )	$-\ln L$ ( <i>col</i> )	Model ( <i>16s</i> )	$-\ln L$ ( <i>16s</i> )
GTR + I + G	5763	GTR + I + G	4011.7
TIM2 + I + G	5764	TIM3 + I + G	4012.2
TIM1 + I + G	5765.9	TIM3 + G	4012.3
TIM3 + I + G	5766.1	GTR + G	4012.7
TrN + I + G	5766.2	TIM1 + I + G	4016.9

GTR, general time reversible; I, invariant sites; G, discretized  $\gamma$ -distribution; TIM, transition model with 1, 2, or 3 transversion rates; TrN, Tamura-Nei model.

concordant (Figs S1 and S2, Supporting information). The phylogeny generated from the concatenated *col* and *16s* genes (Fig. 1) places the genus *Rubicundus* as sister to the Eptatretinae (Bayesian posterior probability, Bpp 0.58) with low support (alternative topologies place *Rubicundus* as sister to Eptatretinae and Myxiniinae), while *Neomyxine* is determined to be the earliest branching point within the family Myxiniidae (Bpp 1.0). *Eptatretus* and *Myxine* are placed as sister to each other (Bpp 1.0) and *Notomyxine* is placed as a branching point within the clade containing *Myxine* (Bpp 0.53). This reconstruction differs from that of Fernholm *et al.* (2013) only in the placement of *Neomyxine*. While not shown here, species-tree reconstruction using \*BEAST to address any differences between gene-tree topologies yielded qualitatively similar results for all phylogenetic analyses.

## DEPTH AND BODY SIZE

Maximum  $L_T$  and maximum depth at which each species can be found are given in Table II. Information was assimilated for 2910 unique specimens, collected from 541 different depths; this information was compiled from museum records with an average of 108 specimens per species (median = 9; some species were very heavily represented). Maximum  $L_T$  ranged from 20 to 85 cm (average = 52 cm) and maximum collection depths ranged from 10 to 2743 m. Often, the average depth at which a species was collected is quite different from the average of the minimum and maximum depths at which that species had been recorded. As such, all four metrics were used in subsequent tests (reported below), although there is no change in the significance of any test, no matter which metric of depth was used. No qualitative differences were found in significance for any test rerun on a dataset reduced to those species with five or more specimen records, which included 16 species.

## MODEL FITTING AND PGLS

Model fitting determines white-noise and lambda ( $\lambda = 0.58$ ) to be the best models of evolution for maximum  $L_T$ , suggesting moderate to weak effects of phylogeny on body size. Models of BM, drift, OU and kappa are all refuted (Table III). The best model for maximum depth inhabited is also white-noise. The white-noise model fit is consistent across all four metrics of depth (average depth of specimens collected, minimum depth, maximum depth and the average of minimum and maximum depths).

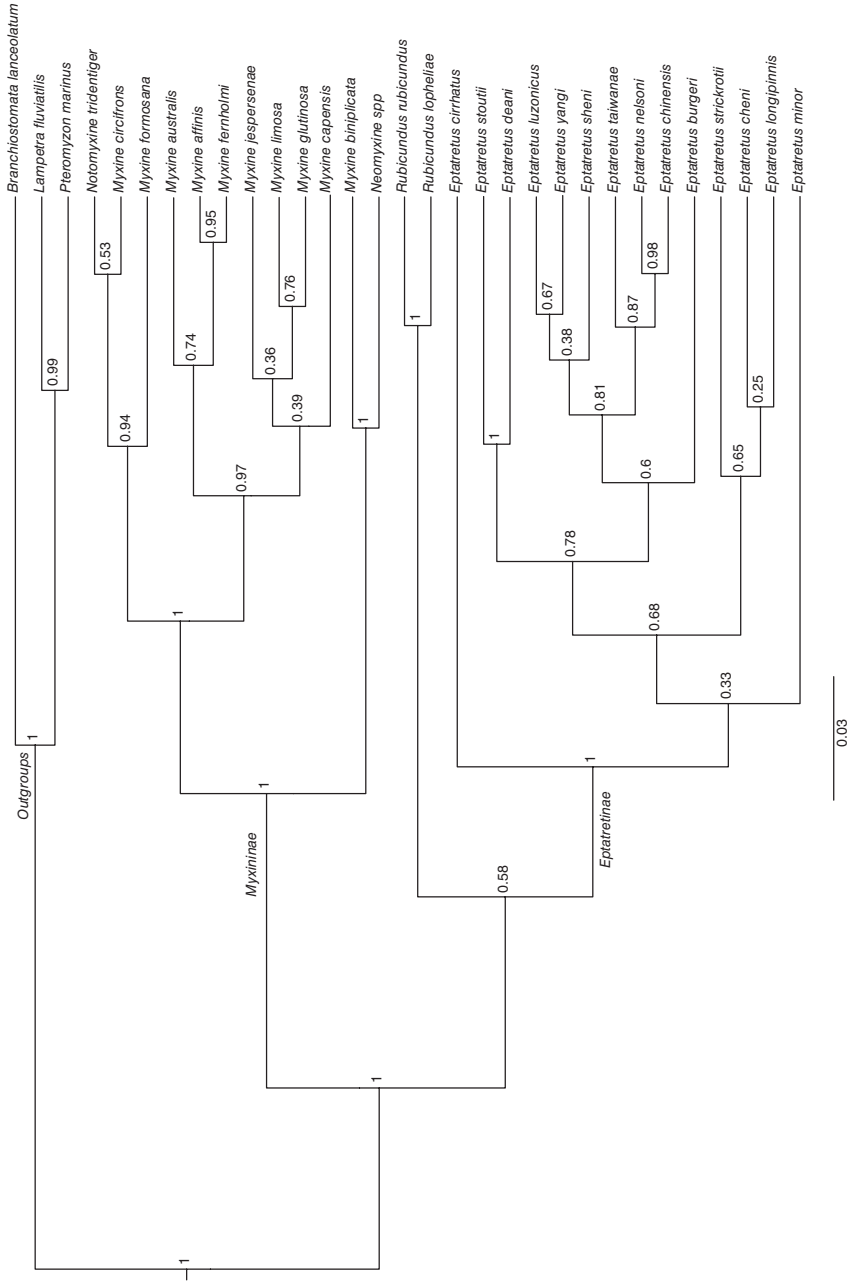


FIG. 1. Phylogeny of hagfishes created using concatenated *coI* and *16s* gene sequences. Node labels are Bayesian posterior probability values.



TABLE II. Numbers of individual hagfish sampled ( $n$ ), independent depth records, total body length ( $L_T$ ), and four metrics of depth of occurrence: calculated average depth from specimen records ( $D_C \pm$  s.d.); minimum depth from specimen records ( $D_{\min}$ ); maximum depth from specimen records ( $D_{\max}$ ); the mean of the maximum and minimum recorded depths for that species ( $D_{\min:\max}$ )

Species	$n$	Depth records	$L_T$ (cm)	$D_C$ (m)	$D_{\min}$ (m)	$D_{\max}$ (m)	$D_{\min:\max}$ (m)
<i>Eptatretus burgeri</i>	15	15	60	156.5 ± 158.9	10	270	140
<i>Eptatretus luzonicus</i>	136	5	29.5	464.4 ± 90.6	200	400	300
<i>Eptatretus cheni</i>	312	8	71.4	229 ± 34.4	156	260	208
<i>Eptatretus chinensis</i>	1	1	37.5	590	–	600	–
<i>Eptatretus cirrhatus</i>	25	25	83	416.6 ± 149.9	–	1100	–
<i>Eptatretus deani</i>	43	43	63.5	606.2 ± 339.7	103	2743	1423
<i>Eptatretus longipinnis</i>	0	0	62.7	–	14	40	27
<i>Eptatretus minor</i>	10	10	39.5	371.6 ± 49.7	300	400	350
<i>Eptatretus nelsoni</i>	9	2	25.9	184 ± 7.8	50	250	150
<i>Eptatretus sheni</i>	394	9	43.6	466.7 ± 130.3	200	800	500
<i>Eptatretus stoutii</i>	31	31	63.5	235.2 ± 230.2	16	966	491
<i>Eptatretus strickrotti</i>	0	0	31.4	–	2100	2300	2200
<i>Eptatretus taiwanae</i>	154	3	33.4	243.3 ± 162.1	20	427	223.5
<i>Eptatretus yangi</i>	1401	10	29.6	240.4 ± 116.6	20	547	283.5
<i>Myxine affinis</i>	0	0	65.9	–	30	150	90
<i>Myxine australis</i>	14	14	60	106.3 ± 35.7	10	100	55
<i>Myxine capensis</i>	170	170	40	342.5 ± 167.7	175	460	317.5
<i>Myxine circifrons</i>	7	7	65	1310.0 ± 215.6	700	1860	1280
<i>Myxine fernholmi</i>	4	4	84.6	219.0 ± 125.4	135	1480	807.5
<i>Myxine formosana</i>	2	2	76.8	839.5 ± 4.9	588	1500	1044
<i>Myxine glutinosa</i>	170	170	80	112.3 ± 63.2	30	1200	615
<i>Myxine jespersenae</i>	1	1	49.8	905.0	752	1556	1154
<i>Myxine limosa</i>	1	1	51	932.7	75	1006	540.5
<i>Neomyxine biniplicata</i>	4	4	41.2	509.3 ± 303.7	–	73	–
<i>Notomyxine tridentiger</i>	6	6	57.5	137.6 ± 25.1	11	106	58.5
<i>Rubicundus lopheliae</i>	0	0	20.1	–	382	700	541
<i>Rubicundus rubicundus</i>	0		46.4	–	–	800	–

This model is also consistently identified as the best-fit model in the dataset of 16 species that all have five or more specimen records. These results imply that, for both maximum depth inhabited and for maximum  $L_T$ , evolution in trait values as recorded here are best described by random changes irrespective of phylogeny (white-noise). It is not possible, however, to rule out a moderate to weak phylogenetic signal only for maximum  $L_T$  ( $\lambda = 0.58$ ), where  $\lambda = 0$  implies no phylogenetic signal and a  $\lambda = 1$  implies strong phylogenetic signal.

A generalized least-squares (GLS) regression shows no correlation between maximum  $L_T$  and maximum depth inhabited ( $P > 0.05$ , d.f. 27). Again, these results are consistent across all four metrics of depth. Because body size displays some phylogenetic signal ( $\lambda = 0.58$ ), it is necessary to determine whether or not any correlation between body size and depth also shows phylogenetic signal. Accordingly, a phylogenetically corrected GLS regression was performed. The results of this analysis are also non-significant ( $P > 0.05$ , d.f. 25; Fig. 2) for all four metrics of depth and when

TABLE III. Size-corrected Akaike information criterion (AIC) scores (AICc) and size-corrected  $\Delta$ AIC scores ( $\Delta$ AICc) for maximum depth inhabited and total body length of hagfishes. A score for  $\Delta$ AICc of 0 indicates the best model. Models are statistically indistinguishable when less than two units apart

	BM	Drift	Lambda	Kappa	OU	White noise
Maximum depth inhabited						
AICc	54.6	57.1	42.7	53.2	54.1	40.2
$\Delta$ AICc	14.4	16.9	2.5	13.0	13.9	0
Total body length						
AICc	-14.6	-12.0	-16.9	-13.4	-14.4	-16.2
$\Delta$ AICc	2.3	4.8	0.0	3.5	2.5	0.7

BM, Brownian motion; OU, Ornstein–Uhlenbeck.

repeated on the reduced dataset of 16 species with five or more specimen records. The same tests executed on the phylogeny in Fernholm *et al.* (2013), which differ only in the placement of the genus *Neomyxine*, are also not qualitatively different.

## DISCUSSION

Variation in maximum  $L_T$  across species of hagfishes appears to evolve independent of ocean depth and thus hagfishes do not follow Heincke's law. This finding

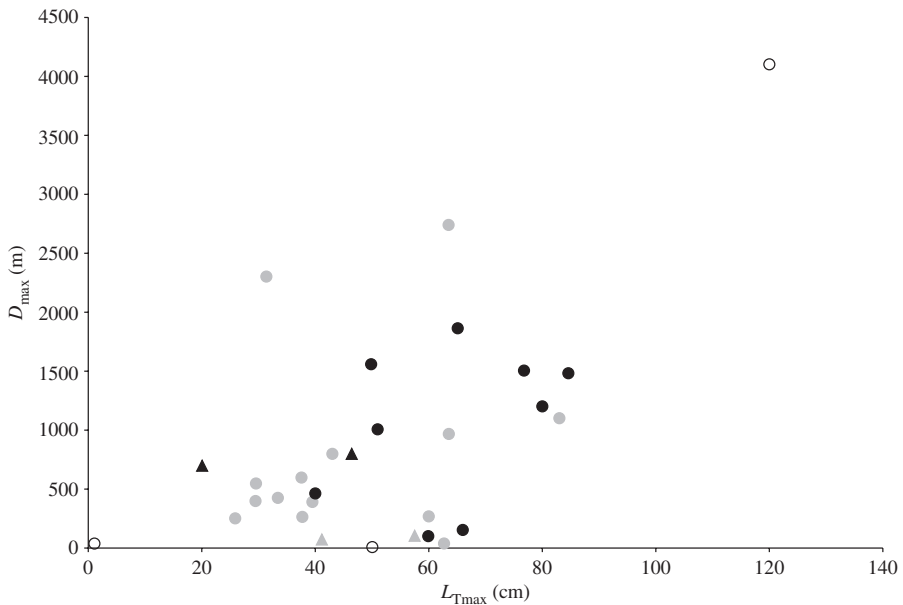


FIG. 2. Relationship between hagfish maximum total length ( $L_{Tmax}$ ) and maximum depth inhabited ( $D_{max}$ ) split into in-group and out-group genera. Generalized least-squares regression revealed no correlation ( $P > 0.05$ , d.f. 27). A phylogenetic generalized least-squares regression was also non-significant ( $P > 0.05$ , d.f. 25). O, Out-group;  $\blacktriangle$ , *Neomyxine*;  $\blacktriangle$ , *Rubicundus*;  $\bullet$ , *Eptatretus*;  $\bullet$ , *Myxine*.



was robust to corrections for phylogenetic relationships among species and to several metrics of depth of occurrence. No effect of phylogeny on depth of occurrence was found, but a moderate to weak influence of phylogeny was found on maximum body  $L_T$  ( $\lambda = 0.58$ ). These results add to a growing body of work that demonstrates that Heincke's law (Heincke, 1913) is a failed, or at best an oversimplified, paradigm for explaining variation in body size for marine fishes. Heincke's law holds true for some teleosts including Aulopiformes, Lophiiformes, Stephanoberyciformes and Pleuronectiformes (Smith & Brown, 2002), demersal scavenging teleosts (Collins *et al.*, 2005) and deep-sea gastropods (Rex *et al.*, 1999). In Perciformes, Beryciformes, Nototheniiformes, Salmoniformes and Zeiformes, however, there is no significant relationship (Drazen & Haedrich, 2012), or the weak correlation (Gadiformes) was disregarded as being coincidental (Aubry *et al.*, 2009). Elasmobranchs (Smith & Brown, 2002) and predatory deep sea teleosts (Collins *et al.*, 2005) show the inverse of Heincke's law. The current findings add the Myxiniiformes to the list of lineages that do not conform to Heincke's law.

Factors other than depth that might explain fish body size variation include phylogeny (Steele & López-Fernández, 2014), body shape and the relationship between shape and habitat depth (Sébert *et al.*, 2009; Neat & Campbell, 2013), the energetics of reproduction (Smith & Brown, 2002; Drazen & Haedrich, 2012), temperature (Ashton, 2001; Hunt & Roy, 2006; Roy, 2008), or food availability (Collins *et al.*, 2005). It is also important to consider how depth and size are measured. Maximum standard length, mass, or combinations of these and other measurements are all commonly used to determine the presence of a size–depth relationship (Smith & Brown, 2002; Grant, 2006; Neat & Campbell, 2013), but these metrics may ignore potentially important components of body size evolution such as sexual dimorphism, average size, or geographic or bathymetric variation in size. Depth of occurrence is often reported only as maximum depth (Collins *et al.*, 2005) at which a species has been sampled (or worse, the maximum depth at which a trawl was conducted that happened to sample a species). Body size and the depth at which species occur are both probably influenced by numerous factors and should be measured in multiple dimensions while also accounting for potential impacts of phylogeny (Steele & López-Fernández, 2014).

There are several potential explanations for why hagfishes do not conform to Heincke's law. Knowledge of wild hagfishes is limited, especially in terms of their occurrences at variable depths. While the present findings were robust to several measures of depth, there are numerous other dimensions that remain to be explored. The depths at which specimens of the inshore hagfish *Eptatretus burgeri* (Girard 1855) can occur range from 10 to 100 m depending on the time of the year (Fernholm, 1974). Sex ratios vary by depth in the Pacific hagfish *Eptatretus stoutii* (Lockington 1878). In the black hagfish *Eptatretus deani* (Evermann & Goldsborough 1907), body size varies by depth (Johnson, 1994; Martini, 1998). It is not currently known which, if any, species of hagfishes spend their entire adult lives at a single depth, or what the range of depths inhabited is for each species. Grant (2006) found that individuals of the Atlantic hagfish *Myxine glutinosa* L. 1758 caught at greater depths tended to be larger in body size. Intraspecific variation in depth occurrence is a particularly interesting issue as representative species of hagfishes can show a large ontogenetic range in  $L_T$  (Clark & Summers, 2012). Sampling error due to variation in the number of records available for each species is not likely to influence the results presented here. When the current analyses (data available upon request) were re-run using only

species with five or more records ( $N = 16$  species), the results were qualitatively similar across all tests. The influence of temperature as a function of depth may also affect body size evolution in hagfishes, as it does in deep-sea ostracods (Hunt & Roy, 2006). The effect of temperature, however, may be increasingly complex for hagfishes. Because temperatures remain fairly stable below 1000 m (Zelle *et al.*, 2004) and many species of hagfishes have ranges that extend well beyond that depth, any potential relationship between morphology and depth may be different in shallow waters than in deeper waters, even within a single species. Future evaluations of depth and body size should include intraspecific evaluations with thorough intraspecific geographic and depth sampling. Lastly, the evaluation of hagfishes presented here does not include all species of hagfishes and may not reflect the full spectrum of body size and depth preferences in this lineage, especially given the fact that many species were only recently described and inhabit understudied deep ocean environments (Mincarone & Stewart, 2006).

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### Supporting Information

Supporting Information may be found in the online version of this paper:

**Fig. S1.** Hagfish phylogenetic relationships based only on the *col* gene. Bayesian posterior support values are given at each node.

**Fig. S2.** Hagfish phylogenetic based only on the *I6s* gene. Bayesian posterior support values are given at each node.

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