

Discovery of fossil lamprey larva from the Lower Cretaceous reveals its three-phased life cycle

Mee-mann Chang^{a,b,1}, Feixiang Wu^a, Desui Miao^c, and Jianguo Zhang^a

^aKey Laboratory of Vertebrate Evolution and Human Origins of Chinese Academy of Sciences, Institute of Vertebrate Paleontology and Paleoanthropology, Chinese Academy of Sciences, Beijing 100044, China; ^bSchool of Earth and Space Sciences, Peking University, Beijing 100871, China; and ^cBiodiversity Institute, University of Kansas, Lawrence, KS 66045

Edited by Neil H. Shubin, The University of Chicago, Chicago, IL, and approved September 17, 2014 (received for review August 20, 2014)

Lampreys are one of the two surviving jawless vertebrate groups and one of a few vertebrate groups with the best exemplified metamorphosis during their life cycle, which consists of a long-lasting larval stage, a peculiar metamorphosis, and a relatively short adulthood with a markedly different anatomy. Although the fossil records have revealed that many general features of extant lamprey adults were already formed by the Late Devonian (ca. 360 Ma), little is known about the life cycle of the fossil lampreys because of the lack of fossilized lamprey larvae or transformers. Here we report the first to our knowledge discovery of exceptionally preserved premetamorphic and metamorphosing larvae of the fossil lamprey *Mesomyzon mengae* from the Lower Cretaceous of Inner Mongolia, China. These fossil ammocoetes look surprisingly modern in having an eel-like body with tiny eyes, oral hood and lower lip, anteriorly positioned branchial region, and a continuous dorsal skin fin fold and in sharing a similar feeding habit, as judged from the detritus left in the gut. In contrast, the larger metamorphosing individuals have slightly enlarged eyes relative to large otic capsules, thickened oral hood or pointed snout, and discernible radials but still anteriorly extended branchial area and lack a suctorial oral disk, which characterize the early stages of the metamorphosis of extant lampreys. Our discovery not only documents the larval conditions of fossil lampreys but also indicates the three-phased life cycle in lampreys emerged essentially in their present mode no later than the Early Cretaceous.

fossil lamprey larva | Lower Cretaceous | three-phased life cycle

The larva of lamprey was at first mistaken for a different agnathan adult animal because of its distinctive appearance and was given a generic name, *Ammocoetes*, by Duméril (1). The name of *Ammocoetes* came from the Greek “ammos” (sand) and “koites” (dwelling). Nearly a dozen species of *Ammocoetes* were erected before, some even after, its identity of lamprey larva was established by Müller (2) (*SI Text*). Müller (2) observed the “small lampreys” in the fresh waters near Berlin and was the first to conclude that the so-called *Ammocoetes* are, in fact, the larvae of the lamprey *Petromyzon*. All of those species referred to *Ammocoetes* were later recognized as the larvae of several genera in the lamprey families Petromyzontidae and Geotriidae (3). In fact, the larval stage lasts for up to 7 y or longer before its metamorphosis to become an adult, which usually has a much shorter lifespan than the larva (4, 5). After spawning in streams with relatively clean water, ammocoetes’ parents die, and the ammocoetes bury themselves in U-shaped or oblique burrows in the silt or sand on the stream bottom, leaving only their mouths in small pits on the surface of the deposits (6, 7). Thus, without disturbances, they rarely emerge from the burrows, although they occasionally come out at night and passively migrate downstream during the larval period. Owing to their great number and living habitat of relatively stable silt and sandy beds with slack current, there should be a chance of finding ammocoetes in the fossil state, yet none of undoubted ammocoete has been described so far. The only exception may be a small lamprey of about 50 mm long which was described from the same locality as the Early

Carboniferous lamprey *Hardistiella montanensis*. The small lamprey shows a lozenge-shaped impression dorsal to the possible eyeballs, which was interpreted by the authors as the loop of the trabecles as in recent larval lamprey, and thus it was suggested as a larval lamprey (8). However, the poor preservation of the specimen made a definitive identification nearly impossible. Consequently, although the fossil records have shown the emergence of many general features of extant lamprey adults as early as the Late Devonian (9), the origin of the three-phased life cycle of the fossil lampreys still eludes us because we know little about fossilized lamprey larvae or transformers.

Once again, the Mesozoic Jehol Lagerstätten in China has delivered to us another one of its paleontological rarities: numerous specimens of ammocoetes from the same locality and horizon where *Mesomyzon mengae* was discovered, i.e., the Lower Cretaceous Yixian Formation, Liutiaogou Village, Ningcheng County, Inner Mongolia, China (10). The matrix is light yellow mud stone, with some thin layers containing coarser grains, whereas the remains of the ammocoetes are of light brown color. Studied in this paper are 12 comparatively well-preserved specimens (IVPP V15029, 15030, 15032, 15034, 15114.2, 15114.4–15114.7, 15165.2, 15506, 15681), among which 10 consist of part and counterpart specimens. Seven specimens are each with a total length less than 67 mm, showing many important features of ammocoete, whereas five are longer than 82 mm and show several features of early transformers.

Description and Comparison

Seven specimens are small, ranging in body length from 39.7 to 67 mm (Table S1), much shorter than the average length of extant larval lampreys during metamorphosis (4) and obviously

Significance

Lampreys are one of the two surviving jawless vertebrate groups that hold the key to our understanding of early vertebrate evolution. Although the fossil records have shown the emergence of many general features of extant lamprey adults as early as the Late Devonian, the origin of the three-phased life cycle in lampreys still eludes us because we know little about fossilized lamprey larvae or transformers. This paper reports the first to our knowledge discovery of exceptionally preserved premetamorphic and metamorphosing larvae of the fossil lamprey *Mesomyzon mengae* from the Lower Cretaceous of Inner Mongolia, China. These fossil ammocoetes look surprisingly modern and show the three-phased life cycle emerged essentially in their present mode no later than the Early Cretaceous.

Author contributions: M.-m.C. designed research; M.-m.C., F.W., D.M., and J.Z. performed research; M.-m.C. wrote the paper; F.W. and D.M. participated in major aspects of research and writing; and J.Z. assisted in some aspects of research and writing.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

¹To whom correspondence should be addressed. Email: zhangmiman@ivpp.ac.cn.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1415716111/-DCSupplemental.

shorter than those we previously described (10). Their most striking characteristic is the rudimentary, tiny eyes, in sharp contrast to the large otic capsules. In extant ammocoetes, the small eyes are covered by comparatively thick skin, and normally only whitish spots in the skin are visible (2, 7, 11), similar to our personal observations on larval specimens of *Lethenteron reissneri* (Dybowski 1869) (3), IVPP 1617, 1622. In our fossil specimens, where the skin is not preserved, the eyes are round, dark brown dots (Figs. 1 *A–I* and 2 *A, B*, and *D–G*) and seem most probably to not be very functional. The diameter of the eye varies from 0.22 to 0.9 mm in specimens of different sizes. The otic capsules are oval, with their major axis around 1.1 to 1.7 mm (Figs. 1 *A–I* and 2 *D–G*). Thus, the long axis of the otic capsule is from 1.8 to 4.3 times the eye axis. The eyes seemingly tend to grow larger in comparison with the otic capsule during the growth of the animal.

In the specimens where the tip of the snout is comparatively well preserved, we are able to see the oral hood, and even the lower lip (Figs. 1 *C–I* and 2 *A, B*, and *D–G* and Figs. S1 *A–C* and S2 *A* and *E*). In a number of small individuals, the oral hood seems rather elongated and extended forward (Fig. 1 *C–I* and Fig. S1 *A–C*). This is probably because the lateral lips are thin, and when the mouth is open the hood is separated from the lower, transverse lip by deep notches, as in the extant ammocoetes (see table 1 in ref. 4 and fig. 3 in ref. 12). The branchial region is preserved best in IVPP V15114.6 (Fig. 1 *A* and *B*). The seven branchial pouches are clearly discernible in this specimen, and the first branchial arch and pouch are situated under the posterior half of the otic capsule, as in many other specimens (Figs. 1 *C, D, H*, and *I* and 2 *D–G* and Fig. S2 *A, E*, and *F*); in

other words, the first branchial arch and pouch are situated more anteriorly than those in adult lampreys, which is also the case with extant ammocoetes, as noted by Damas (13), Strahan (14), and Hardisty (7). According to Damas (13) and Strahan (14), the branchial region of the lamprey shifted posteriorly during ontogenesis. The branchial region is much longer and deeper than the prebranchial region (Table S1). No obvious change of the ratio between the lengths of the two regions is detected with the increase of size in any of our specimens. Gill openings are traced in several specimens in their lateral wall of the branchial region (Figs. 1 *H* and *I* and 2 *F* and *G*), as in *L. reissneri* (Dybowski 1869) (personal observations on specimens IVPP 1617, 1622) and other extant ammocoetes (see fig. 15), although the shape of the openings is not clear. A reddish stain is often found slightly behind the branchial region, probably indicative of the position for the iron-concentrated liver (16).

The median dorsal aorta, along with the paired anterior cardinal vein and probably the subchordal rod, left a dark brown pigmented stripe above the branchial region, sometimes slightly crimped and broken between the gill pouches (12, 17), and then the blood vessels continue to run posteriorly between the abdomen and the dorsal part of the body till the end of the tail (Figs. 1 *A–I* and 2 *A* and *B* and Figs. S1 and S2 *A–C*). Over the blood vessels the notochord stretches from the level of the otic capsule backward to the end of the body. The spinal cord, situated above the notochord, can hardly be distinguished from the notochord in the fossil specimens. In IVPP V15114.6, just above the blood vessels, a band with slightly darker color than the area on top of it, in certain places with striae, may represent the

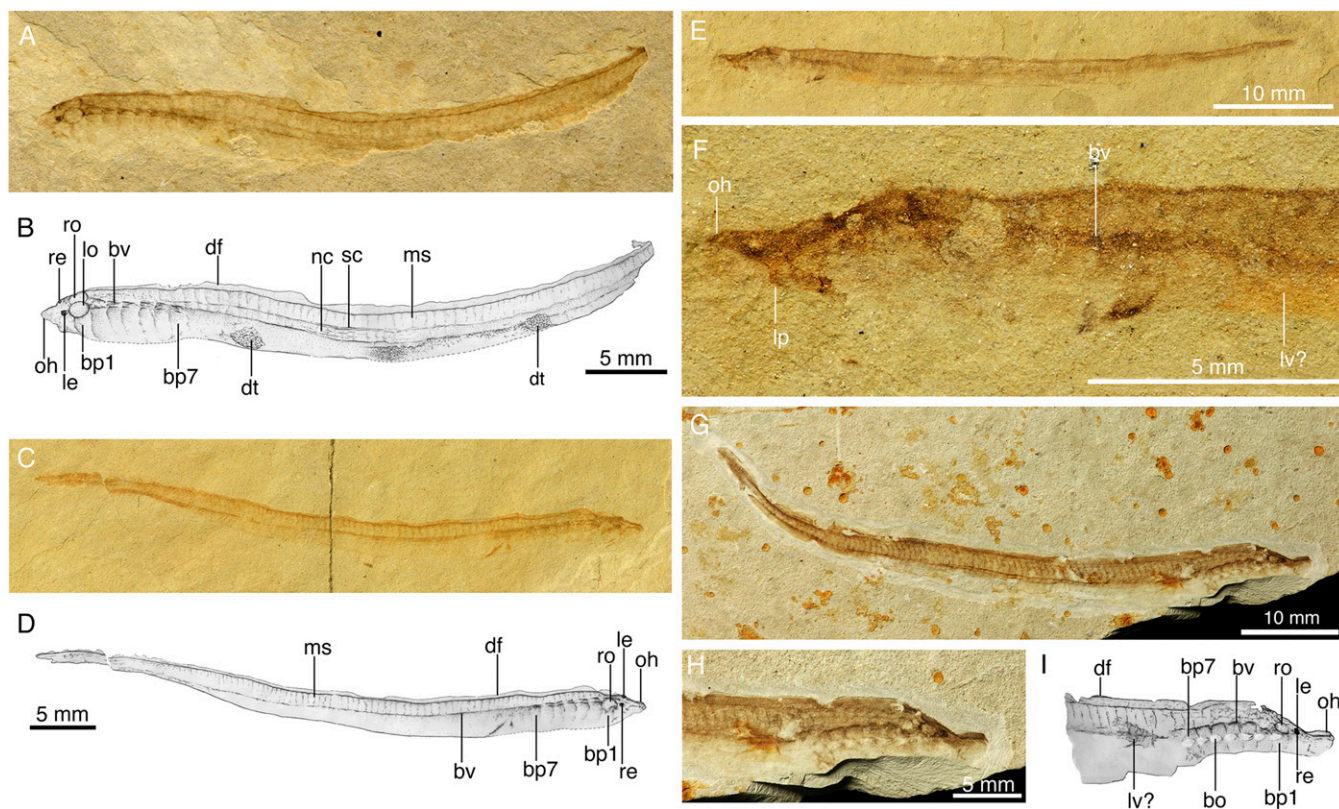


Fig. 1. Larvae of *M. mengae* from the Lower Cretaceous of China. These 125-My-old ammocoetes are the earliest known examples of lamprey larvae in the fossil records, showing almost identical anatomy and life cycle with their living counterparts. (A) Photograph and (B) drawing of IVPP V15114.6A in left view. (C) Photograph and (D) drawing of IVPP V15165.2A in right view. (E) Photograph of IVPP V15114.5B in left view and (F) that of the head and anterior part of the body of E. (G) Photograph of IVPP V15681A in right view. (H) Photograph and (I) drawing of head and anterior part of body of G. (bv, blood vessels; cf, caudal fin; df, dorsal fin; dt, detrital remains in digestive tract; bo, external branchial opening; bp1, bp7, first and seventh branchial pouch; le, left eye; lo, left otic capsule; lp, lower lip; lv?, possible remains of liver; ms, myoseptum; nc, notochord; oh, oral hood; re, right eye; ro, right otic capsule; sc, spinal cord).

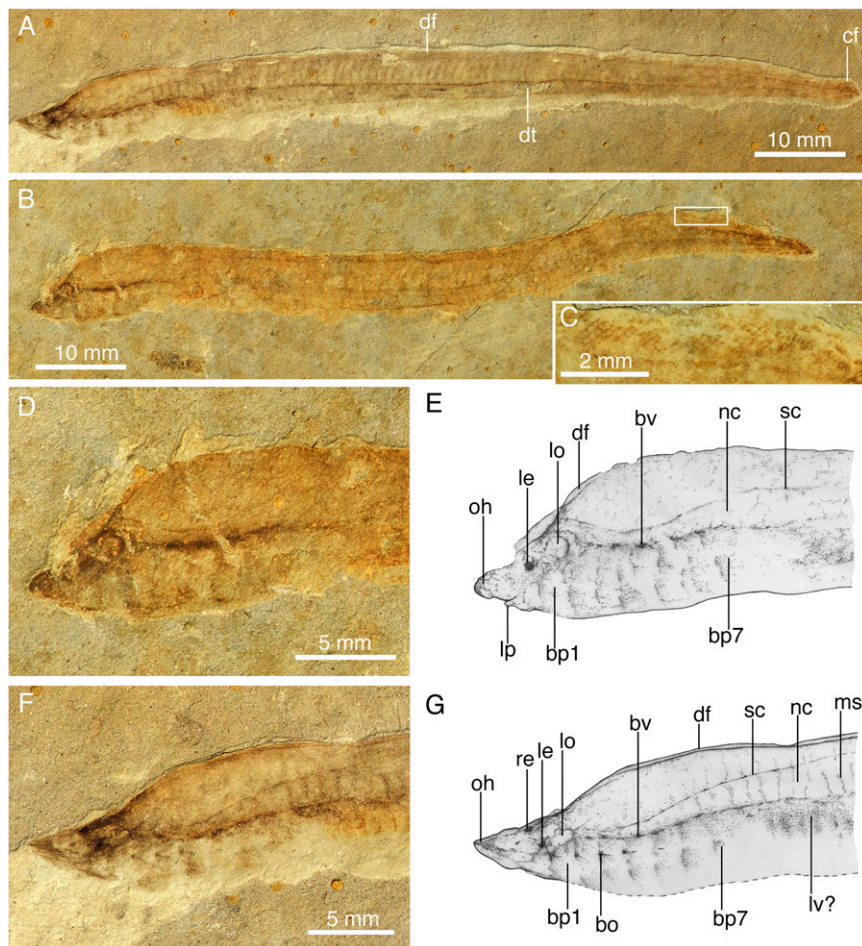


Fig. 2. Early transformers of *M. mengae*. (A) Photograph of IVPP V15030 in left view. (B) Photograph of IVPP V15032 in left view. (C) Box area in B in higher magnification, showing the radials. (D) Photograph and (E) drawing of the head and anterior part of the body of A. (F) Photograph and (G) drawing of the head and anterior part of the body of B. (For abbreviations, see Fig. 1).

notochord; and in this specimen, the spinal cord lies comparatively flat above the notochord (Fig. 1 *A* and *B*). Lateral to the notochord and spinal cord, and in the dorsal part of the body, clear myosepta are discerned, and ~90 trunk myomeres can be counted from the end of the branchial region to the end of the preserved posterior part of the animal in IVPP V15114.4 (Fig. S2 *B* and *C*).

In several small individuals, the long, low, and continuous dorsal fin fold extends all of the way along the dorsal side of the body from the end of the tail almost to the point above the back of the otic capsule (Fig. 1 *A–D*). The dorsal fin is a thin, membrane-like structure, without radials. There are definitely no paired fins. The tail in small individuals tapers at the end. In the abdomen a few lengthened patches of relatively coarse sediments are clearly shown (Figs. 1 *A* and *B* and 2*A* and Fig. S2 *B* and *C*). They must be the detrital remains in the intestine, indicating the animals were filter feeders (Fig. 3 *A*, *a2*), just like the living ammocoetes who fed on microscopic plant and animal particles together with a large amount of detritus (18). In the parasitic adult lamprey, there is no detritus in the intestine, whereas in the nonparasitic adult lamprey, the intestine is degenerated. Incidentally, our previous interpretations of the gonads and the digestive tract in the holotype of *M. mengae* (10) appear to be incorrect in the light of this discovery. The so-called “gonads” might be detrital remains in the intestine [as one of the reviewers of ref. 10 then correctly pointed out], and their circular shape may have reflected the insertion lines of the typhlosole, which is for lengthening the time

when food travels through the intestine, providing additional epithelial surface for absorption. The thin dark band in the middle of the body, labeled as “dt?” (“possible digestive tract”) in that specimen, must be the blood vessels as we interpret here.

With all the characteristics mentioned above, these seven small specimens, i.e., IVPP V15034, 15165.2, 15114.2, 15114.5–7, and 15681, are undoubtedly ammocoetes. There is almost no distinction between these specimens and the ammocoetes of extant lampreys.

Nevertheless, in addition to ammocoete characters, the five larger specimens also show the characters that normally occur during metamorphosis (Fig. 2 *A–G* and Fig. S2 *A–C*, *E*, and *F*). These specimens have the body length comparable to our previously described specimens (10). In IVPP V15032 and 15506 the oral hood seems much thickened, giving the snout a more rounded appearance (Figs. 2 *D* and *E* and 3*B* and Fig. S2*E*). These five larger ammocoetes must be already at the initial stages of metamorphosis (4). In the largest specimen, IVPP V15030 with a length of 93.5 mm, the snout is pointed with a small rounded tip, yet its first branchial arch and opening are still under the otic capsule (Fig. 2 *A*, *F*, and *G*). No actual oral disk has been detected in any of the specimens. The anterior portion of the notochord is clearly shown in IVPP V15030 and 15032, and the extensive space above this portion must have housed the brain (Fig. 2 *D–G*). In these larger specimens the posterior part of the dorsal fin is obviously higher than the rest of the skin fold and has radials (Fig. 2*C* and Fig. S2*D*). In IVPP V15030 a small caudal fin

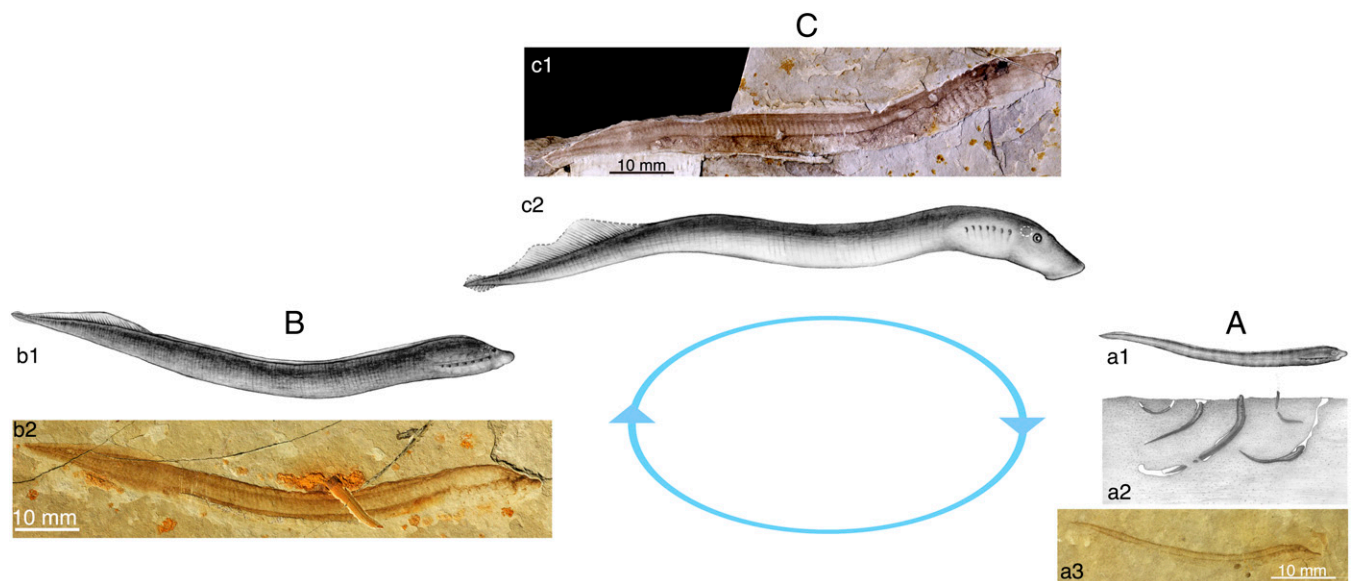


Fig. 3. Specimens and restorations of larva, early transformer, and adult of *M. mengae*, showing its three-phased life cycle, which emerged at least 125 Ma. (A) Larva (ammocoete) of *M. mengae*. a1, restoration of larval *M. mengae*; a2, restoration of burrowing larvae of *M. mengae* (cf. fig. 4.1 in ref. 7, not to scale); a3, photograph of IVPP V15034B in right view. (B) Early transformer of *M. mengae*. b1, restoration; b2, photograph of IVPP V15506B in right view (original photo horizontally flipped). (C) Adult or late transformer of *M. mengae*. c1, photograph of holotype (IVPP V14719) of *M. mengae* in right view; and c2, its restoration. (the circle in white dotted line in a1, b1, and c1 representing the position of otic capsule).

is observed (Fig. 2A). These changes occur in early stages of the seven stages of metamorphosis of living lampreys (4, 19). Consequently, we consider these five larger individuals, with a number of ammocoete characters as well as the characters only appearing in early stages of metamorphosis, as early transformers.

Discussion

When we described *M. mengae* in our previous paper (10), we did suspect that the two specimens of *M. mengae* might be “late transformers,” judging mainly from the small body length. The eyes in the holotype of *M. mengae* are already relatively large, just slightly smaller than the otic capsules; its posterior part of the dorsal fin contains radials; its first branchial pouch is situated behind the otic capsule—even the piston cartilage is developed—and the oral opening is surrounded by radiating rectangular depressed areas, presumably covered by tooth plates, forming the sucking disk (10 and Fig. 3C). Compared with the five larger specimens here, the holotype of *M. mengae* seems much more advanced developmentally in having the characters that normally occur in late stages of metamorphosis, or even in adults. As is well known, when the ammocoetes of living lampreys grow to a certain size [~100 mm (4)], they reach the time of metamorphosis, and the total body length does not alter much during metamorphosis (4). The mean length of metamorphosing stages 1–7 has been examined for several representative forms, which is no longer than 129 mm (4). However, the size at the onset of metamorphosis varies in different species and individuals. Müller (2, p. 301) examined a 6-inch (= 152 mm) long ammocoete of *Petromyzon*, which “showed no traces of metamorphosis.” Thus, the differences of size between the holotype of *M. mengae* (85 mm, as an adult or late transformer) and those we described here (82–93.5 mm, as early transformers) are considered ontogenetic, and hence intraspecific, variability. In fact, as the ammocoetes we described here are found from the same locality and strata where *M. mengae* was discovered, it is reasonable to assume that these ammocoetes are the larvae of *M. mengae* (10).

The sizes of *Mayomyzon pieckoensis* (20) and *H. montanensis* (21) from the Carboniferous [some 300–330 Ma (22)] of North America are also small (33–61 mm and less than 100 mm in length,

respectively). They also possess a number of larval characters, e.g., no oral sucker, no teeth, a long dorsal fin, and the first two branchial pouches under the otic capsule (in *M. pieckoensis* only). At the same time, they show the characteristics comparable to those found in adult lampreys, e.g., cranial cartilage, piston cartilage (in *M. pieckoensis* only), and large eyes, etc. (20, 23). Similarly, the Late Devonian (about 360 Ma) fossil lamprey, *Priscoomyzon riniensis* from South Africa (9), is also very small, only 42 mm long, and shows the larval character in its forward placed branchial apparatus and a long and continuous dorsal fin. However, *P. riniensis* possesses a large oral disk with circumoral teeth, undoubtedly an adult character. Moreover, the Paleozoic fossil lampreys were all found in marine deposits, and there is no evidence of their occurrence in fresh water. On the contrary, the extant lamprey larvae are all restricted to fresh water, and they cannot even survive in the estuarine environment (24). It has been suggested that just like the other jawless group, the hagfish, these Paleozoic fossil lampreys grew directly from a hatched embryo into an adult in the sea (7). The alternative interpretation is that the earliest lampreys were very small and larva-like or paedomorphic, and metamorphosis developed only after lampreys moved into fresh water (24, 25).

Our materials, found in the freshwater deposits, show clearly that about 125 Ma lampreys not only were almost identical to their present counterparts morphologically, but also had a very similar life cycle, consisting of a larval, a metamorphosis, and an adult stage, except that their bodies were smaller (Fig. 3).

Lampreys have been extensively studied for nearly two centuries, and the last two decades have witnessed an accelerated progress on lamprey studies, especially in areas such as chordate phylogeny (26–28), metamorphosis in vertebrates (29), and molecular and developmental biology (30, 31). Although “lampreys enter the genomic era” (25, p. 223), their fossils only begin to emerge in an unprecedented rate and quality. As the body sizes of the transformers we described here (Figs. 2 and 3B and Fig. S2) are generally larger than the earlier Paleozoic fossil lampreys but smaller than their living counterparts, this seems to support the notion that there is indeed a trend to lengthen larval life during the evolution of lampreys (7). Additionally, the lengthening of

larval life and the introduction of metamorphosis into the life cycle consequently postponed reproductive maturation, which may have some selective advantages such as more optimized reproductive behavior, changed egg size, and increased fecundity (24). Our discovery also demonstrates that such a developmental innovation in lampreys occurred no later than the Early Cretaceous, and this type of metamorphic mode has virtually unchanged for about 125 My.

ACKNOWLEDGMENTS. We thank X. Wang for providing the fossils and relevant information; F. Zhang for providing recent ammocoetes for comparison and discussions; Z. Gai for discussions; Y. Cao and M. Zhao for finding old literature; Z. Wang for preparation; and W. Gao for photography. We also thank R. G. Manzon for relevant literature and photos. We especially thank E. M. Friis for helpful suggestions. We are grateful to two anonymous reviewers and the editor for their comments. This study was supported by the National Basic Research Program of China (Grant 2012CB821900) and the Asian–Swedish Research Partnership Program of the Swedish Research Council.

- Duméril AMC (1812) *Dissertation sur les Poissons qui se Rapprochent le Plus des Animaux Sans Vertèbres* (Didot, Paris).
- Müller A (1856) On the development of the lampreys. *Ann Mag Nat Hist* 18:298–301.
- Renaud CB (2011) *Lampreys of the World, an Annotated and Illustrated Catalogue of Lamprey Species Known to Date* (Food and Agric Org of the UN, Rome).
- Potter IC, Hilliard RW, Bird DJ (1982) *The Biology of Lampreys*, eds Hardisty MW, Potter IC (Academic, London), Vol. 4B, pp 137–164.
- Janvier P (1996) *Early Vertebrates* (Clarendon, Oxford, UK).
- Hardisty MW, Potter IC (1971) *The Biology of Lampreys*, eds Hardisty MW, Potter IC (Academic, London), Vol 1, pp 85–125.
- Hardisty MW (1979) *Biology of the Cyclostomes* (Chapman and Hall, London).
- Lund R, Janvier P (1986) A second lamprey from the Lower Carboniferous (Namurian) of Bear Gulch, Montana (U.S.A.). *Geobios* 19(5):647–652.
- Gess RW, Coates MI, Rubidge BS (2006) A lamprey from the Devonian period of South Africa. *Nature* 443(7114):981–984.
- Chang MM, Zhang J, Miao D (2006) A lamprey from the Cretaceous Jehol biota of China. *Nature* 441(7096):972–974.
- Kleerekoper H (1972) *The Biology of Lampreys*, eds Hardisty MW, Potter IC (Academic, London), Vol 2, pp 373–404.
- Hardisty MW (1981) *The Biology of Lampreys*, eds Hardisty MW, Potter IC (Academic, London), Vol 3, pp 333–376.
- Damas H (1944) Recherches sur le développement de *Lampetra fluviatilis* L. Contribution à l'étude de la céphalogenèse des Vertébrés. *Arch Biol (Liege)* 55:1–285.
- Strahan R (1960) *Proceedings of the Centenary and Bicentenary Congress of Biology*, ed Purchon RD (Malaya Univ Press, Singapore), pp 83–94.
- Whiting HP (1972) Cranial anatomy of the ostracoderms in relation to the organization of larval lampreys. *Studies in Vertebrate Evolution*, eds Joysey KA, Kemp TS (Oliver & Boyd, Edinburgh), pp 1–20.
- Youson JH, Manzon RG (2012) *Metamorphosis in Fish*, eds Doufour S, Rousseau K, Kapoor BG (Science Publ, Enfield, NH), pp 12–75.
- Randall DJ (1972) *The Biology of Lampreys*, eds Hardisty MW, Potter IC (Academic, London), Vol 2, pp 287–306.
- Youson JH (1981) *The Biology of Lampreys*, eds Hardisty MW, Potter IC (Academic, London), Vol 3, pp 95–189.
- Youson JH, Potter IC (1979) Description of the stages in the metamorphosis of the anadromous sea lamprey, *Petromyzon marinus* L. *Can J Zool* 57(9):1808–1817.
- Bardack D, Zangerl R (1968) First fossil lamprey: A record from the Pennsylvanian of Illinois. *Science* 162(3859):1265–1267.
- Janvier P, Lund R (1983) *Hardistiella montanensis* n. gen. et sp. (Petromyzontida) from the Lower Carboniferous of Montana, with remarks on the affinities of the lampreys. *J Vertebr Paleontol* 2:407–413.
- Janvier P (2006) Palaeontology: Modern look for ancient lamprey. *Nature* 443(7114):921–924.
- Bardack D, Zangerl R (1971) *The Biology of Lampreys*, eds Hardisty MW, Potter IC (Academic, London), Vol 1, pp 67–84.
- Youson JH, Sower SA (2001) Theory on the evolutionary history of lamprey metamorphosis: Role of reproductive and thyroid axes. *Comp Biochem Physiol B Biochem Mol Biol* 129(2–3):337–345.
- Osório J, Rétaux S (2008) The lamprey in evolutionary studies. *Dev Genes Evol* 218(5):221–235.
- Donoghue PCJ, Sansom IJ (2002) Origin and early evolution of vertebrate skeletonization. *Microsc Res Tech* 59(5):352–372.
- 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(7431):169–170.
- Laudet V (2011) The origins and evolution of vertebrate metamorphosis. *Curr Biol* 21(18):R726–R737.
- Richardson MK, Admiraal J, Wright GM (2010) Developmental anatomy of lampreys. *Biol Rev Camb Philos Soc* 85(1):1–33.
- Oisi Y, Ota KG, Kuraku S, Fujimoto S, Kuratani S (2013) Craniofacial development of hagfishes and the evolution of vertebrates. *Nature* 493(7431):175–180.