

The sudden appearance of diverse animal body plans during the Cambrian explosion

JUN-YUAN CHEN*

LPS of Nanjing Institute of Geology and Paleontology, Institute of Evo/Developmental Biology and State Key Laboratory of Pharmaceutical Biotechnology, Nanjing University, Nanjing, China

ABSTRACT Beautifully preserved organisms from the Lower Cambrian Maotianshan Shale in central Yunnan, southern China, document the sudden appearance of diverse metazoan body plans at phylum or subphylum levels, which were either short-lived or have continued to the present day. These 530 million year old fossil representatives of living animal groups provide us with unique insight into the foundations of living animal groups at their evolutionary roots. Among these diverse animal groups, many are conservative, changing very little since the Early Cambrian. Others, especially Panarthropoda (superphylum), however, evolved rapidly, with origination of novel body plans representing different evolutionary stages one after another in a very short geological period of Early Cambrian time. These nested body plans portray a novel big picture of pararthropod evolution as a progression of step-wise changes both in the head and the appendages. The evolution of the pararthropods displays how the head/trunk boundary progressively shifted to the posterior, and how the simple annulated soft uniramous appendages progressively changed into stalked eyes in the first head appendages, into whip-like sensorial and grasping organs in the second appendage, and into jointed and biramous bipartite limbs in the post-antennal appendages. *Haikouella* is one of most remarkable fossils representing the origin body plan of Crustozoa, or «crest animals» (procraniates+craniates). The anatomy of Early Cambrian crest animals, including *Haikouella* and *Yunnanozoon*, contributes to novel understanding and discussion for the origins of the vertebrate brain, neural crest cells, branchial system and vertebrae.

KEY WORDS: *body plan, head segmentation, evolution, appendage, brain, Cambrian explosion*

The incredible 580 million year old Weng'an biota from Precambrian Doushantuo phosphate deposits reveals the presence of diverse metazoans including sponges (Li *et al.*, 1998), cnidarians (Chen *et al.*, 2000 and 2002; Xiao *et al.*, 2000 and 2002a) and bilateral animals and related embryos (Chen *et al.*, 2004a; Chen *et al.*, 2006). Animal body organization patterns, especially in bilaterian animals, were diverse until the Early Cambrian (Chen 2004). However, early animals were mostly soft-bodied, and never left their mark on the fossil record except for the rare deposits of exceptional preservation, which preserve both soft tissues and the articulated complete skeletons.

The exceptionally preserved Cambrian fossils, especially the beautifully preserved fossils from the Lower Cambrian Maotianshan Shale deposits in areas (Chengjiang, and Haikou and Anning) adjacent to Kunming, China, document a sudden appearance of a diversity of metazoans that were not only

remarkably diverse at the species level, consisting of over 100 species, but that show morphological disparity among them that provides the first manifest evidence for the appearance of the diversity of metazoan body plans, which were either short-lived or have continued to the present day (Hou *et al.*, 2004; Chen 2004).

Body plan (or body form) among animals is essentially the blueprint for the way the body of an organism is laid out (Raff, 1996; Hall, 1998). The blueprint is shared by a group of animals ranked at higher taxonomical levels (phylum, subphyla, class or order). It is characteristic of a unique anatomical body organization, is evolutionarily conserved, and deeply rooted.

The Lower Cambrian Maotianshan Shale lies at the heart of Cambrian explosive evolutionary event which gave rise to progenitors of living bilaterian animal groups including vertebrates, ascidians, priapulids, sipunculans, arrow worms, possible mol-

*Address correspondence to: Jun-Yuan Chen. LPS of Nanjing Institute of Geology and Paleontology; Institute of Evo/Developmental Biology, and State Key Laboratory of Pharmaceutical Biotechnology, Nanjing University, Nanjing, 210093, China. Fax: 0779-389-5646. e-mail: chenjunyuan@163.net

lusks, possible annelids, phoronida, and brachiopods (Chen, 2004; Hou *et al.*, 2004). Among them, many have changed little since then (for instance, the ctenophores, priapulids, sipunculans, chaetognaths, tunicates and inarticulate brachiopods). On the contrary, limb-bearing segmented animals known as panarthropods are not only diverse and abundant since the Early Cambrian, but also embrace diverse nested body plans that represent different evolutionary stages. Most of these different body plans appeared in the Early Cambrian. The emergence of novel body plans representing different evolutionary stages one after another near the root of the evolutionary history of the panarthropods are a remarkable and fairly unusual phenomenon, shedding light on the mysterious field of the evolutionary origin of various aspects of the arthropods, including head, limb, and brain evolution.

The origin of the neural crest cells is one of the most critical innovations in the evolutionary origin of the vertebrates. Holland and Chen recently coined the term *Cristozoa*, e.g. crest animals, referring to craniates and their immediate precursors (Holland and Chen 2001). Among the fossil representatives of the first crest animals, *Haikouella lanceolata* (Chen *et al.*, 1999; Mallett and Chen, 2003; Chen, 2004, 2008) is the most remarkable one—because of its key evolutionary position at the very beginning of cristozoan history and by having extraordinary preservation of anatomical details. *H. lanceolata*, together with a slightly more advanced species *Yunnanozoon lividum* (Hou *et al.*, 1991; Chen *et al.*, 1995a; Chen 2004, 2008), constitutes a wonderful portrait for the original body plan of the crest animals, contributing novel understanding of the evolutionary origin of the vertebrate brain, head sensorial organs, branchial arches, backbones and feeding behavior. The *Cristozoa* hypothesis suggests the presence of a pre-craniate evolutionary history, which is marked by the innovation of a set of vertebrate characters, including: lateral eyes, possible nostrils, large differentiated brain (but lacking a telencephalon), protovertebrae and vertebrate type of branchial system, and especially definite neural crest cells. The neural crest cells that predate the first appearance of the telencephalon provide a possible answer to why neural crest cells are not formed in the developing telencephalon.

The affinities of numerous Cambrian animals remain controversial. The body plans of these animal groups, especially stalked sessile and pelagic medusiform lophophore-bearing animals and vetulicolian organisms yield clues to their possible affinities with extant animals.

The evolution of animal body plans provides us with interesting suggestions of how the genome architecture and develop-

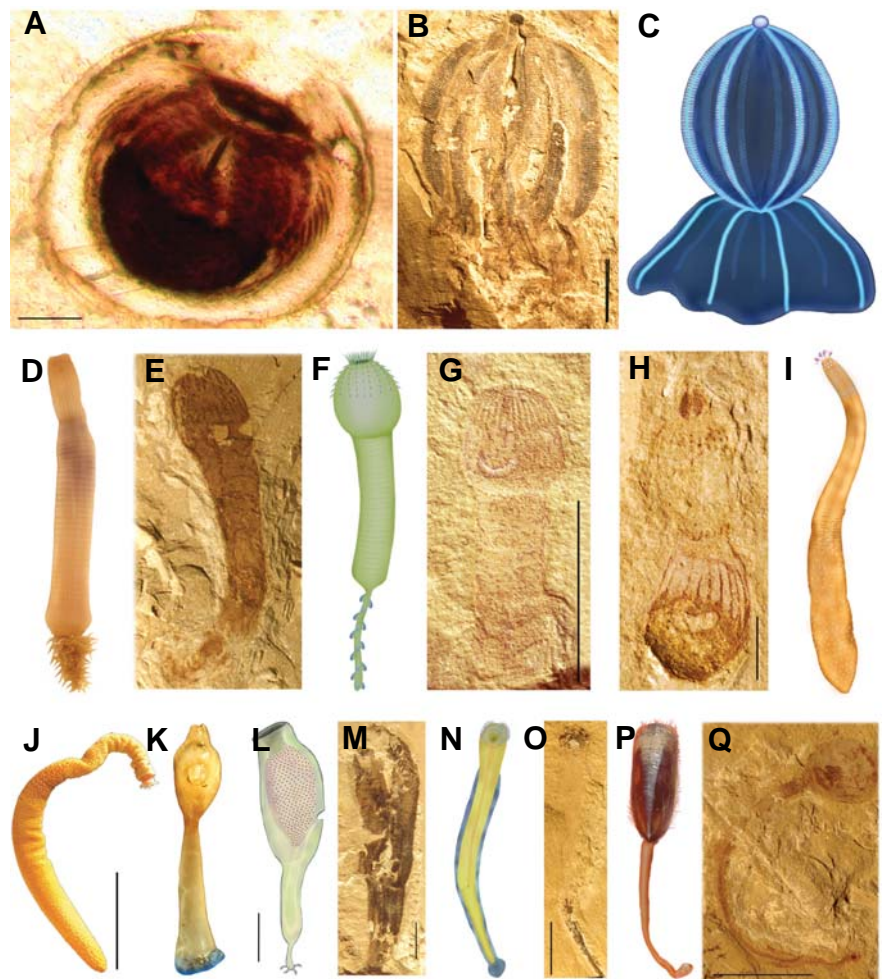


Fig. 1. Animal groups with conserved body plan. (A) Optical image of late developing ctenophore embryos from basal strata of Lower Cambrian, from Ningqiang, Shaanxi, China; (B-C) photograph (B) and restoration (C) of an adult ctenophore (*Maotianoascus octonarius*) from Lower Cambrian Maotianshan shale deposits, Chengjiang, Yunnan (southern China); D-H, (D) Living (*Priapulus caudatus*) and (E-H) fossil priapulids including *Xiaohaiqingella* (E-G) and *Palaopriapulites* (H), from Lower Cambrian Maotianshan shale deposits, Chengjiang, Yunnan (southern China); (I-J), restoration of the Lower Cambrian sipunculan (*Cambrosipunculus tentaculatus*) from Chengjiang, Yunnan (southern China) (I) and a living sipunculan (*Phascolosoma granulatum*) from Biscay bay, Spain (J) (provided by Drs. J. Vannier and D.Y. Huang); (K-M) tunicates, a living tunicate (K) from Atlantic ocean (provided by Dr. B. Weber), and restoration (L) and photograph (M) of Lower Cambrian tunicate (*Shankouclava anningense*) from Anning, near Kunming (southern China); (N-O) restoration (N) and photograph (O) of Early Cambrian arrow worm *Eognathacantha ercainella*; (P-Q) living (P) and Lower Cambrian *Lingula* (Q). Scale bars: A, 25 μ m; 1 cm in B, J, and Q; 0.5 cm in E and G and O; 0.25 mm in H, L and M.

mental processes lead the course of evolution (Wang and Chen, 2004).

Scarce animals with conservative body plans

Maotianshan Shale deposits preserve fossil representatives of modern animal groups including ctenophores, priapulids, sipunculans, arrow worms, at phyla level, ascidians at subphylum level, and *Lingula* at order level. They are not only numerically scarce, but anatomical studies reveal that they are evolutionarily conservative. The evolutionary conservatism and rarity of these

body organization patterns may imply the presence of underlying unknown evolutionary and developmental bottlenecks.

Ctenophores are a phylum of primitive triploblastic animals that have lived exclusively in marine settings since the Cambrian, representing one of the animal groups that are scarce and morphologically conservative except for some species that secondarily evolved to have 16 and 24 comb rows in the Middle Cambrian (Conway Morris and Collins, 1996), or rather bizarre body forms in some modern species (Brusca and Brusca, 1990). The ctenophore body plan as exemplified by both a 540 million years old, late developing embryo from basal strata of Lower Cambrian (Chen *et al.*, 2007b) (Fig. 1A) and by 530 million years old adult forms from Maotianshan Shale deposits (Chen and Zhou, 1997; Chen, 2004) (Fig. 1 B,C), is almost identical to the body plan in extant ctenophores, and is characterized by having a spheroidal body that bears eight comb rows but lacks tentacles. Like some extant ctenophores, the mouth in adult ctenophores is surrounded by a broad skirt-like structure known as oral lobe.

Priapulids represent a minor phylum consisting only of 18 living species (Fig. 1D), exclusively of marine animals living in sand and soft mud (Ruppert *et al.*, 2004). They first appeared in the Early Cambrian and were dominant animals in the endobenthic community both in the Lower Cambrian Maotianshan Shale (Chen, 2004; Huang, 2004a,b; Maas *et al.*, 2007; Dornbos and Chen, 2008) and Middle Cambrian Burgess Shale fauna (Conway Morris, 1977), and are comprised of at least 11 species in Maotianshan Shale (Huang, 2006) and 3 species in Burgess Shale (Conway Morris, 1979). The body plan as exemplified by Cambrian adult priapulids is characterized by having an elongated body that is divided into a large trunk with an anterior introvert, and one or two caudal appendages (Fig. 1 E,F). The body wall is covered with chitinous cuticle that bears of many types of scalids including setae, scales, and spines. The scalids are most abundant and stout on the introvert, where they are usually arranged in longitudinal rows. Interestingly, some Early Cambrian species (*Xiaoheiqingella peculiaris*; *Yunanpriapululus halteriformis*; and *Sicyophorus rara*) (Fig. 1, G-H) retained a body form resembling priapulid larvae with a thick cuticular lorica. The lorica in modern species disappears during metamorphism. It is a case of paedomorphosis, retaining the larval characters into the adult. Priapulids are not only scarce in the post-Cambrian, but also evolutionarily conservative, having changed little since the Early Cambrian.

The sipunculans are a minor phylum, consisting of only of 250 living species and 25 genera. The body plan is characterized by having a non-segmental body that consists of an elongate, anterior introvert and a swollen posterior trunk. The anterior introvert is slender, in a length ranging one-half to several times the length of the trunk, ending with a mouth opening surrounded with tentacles. The digestive tract is J-shaped. They represent an scarce animal group with the body plan as exemplified by *Archaeogolfingia* and *Cambrosipunculus* from Maotianshan Shale deposits (Huang *et al.*, 2004) that shows little change since the Early Cambrian (Fig. 1, I-J).

Chaetognatha are a minor phylum of pelagic marine animals, consisting of only about 150 living species. Their body form is reminiscent of an arrow; hence they are usually called arrow worms. Their body plan is characterized by having a small slender body with a strikingly perfect bilateral symmetry and divided into a distinct head, finned cylindrical trunk and a dorso-ventrally-

flattened, finned post-anal tail. The head is ovoid, bearing a depression that leads to a ventral mouth. The vestibule is flanked laterally with prey-seizing grasping spines. *Eognathacantha* (Chen and Huang 2002) and *Protosagitta* (Chen *et al.*, 2002, 2008) are the fossil representatives of the first arrow worms from the Lower Cambrian Maotianshan Shale. The body form of this group as exemplified by the Lower Cambrian species (Fig. 1, N-O) has changed little since the Early Cambrian.

Tunicates of the subphylum Urochordata represent a basal branch of the known chordates. *Shankouclava shankouense* (Chen *et al.*, 2003) from the Maotianshan Shale represents a solitary sessile adult tunicate, with a body consisting of a barrel-shaped anterior thoracic region containing a large pharyngeal basket, an elongated, triangular abdominal region containing a U-shaped digestive tract, and post-abdominal attaching stolons (Fig. 1, L-M). An oral siphon opens subventrally, containing oral tentacles at its base. An endostyle band extends along the mid-ventral part of the pharynx, and a sac-like atrium surrounds the pharyngeal basket. The striking resemblance of *Shankouclava* (Fig. 1, L-M) with extant tunicates (Fig. 1, K), suggests that tunicates have changed little since the Early Cambrian.

Living *Lingula*, of the phosphate-shelled inarticulate brachiopoda, lives in brackish to intertidal sandy substrata and has persisted since the Early Cambrian (Jin *et al.*, 1993) with few changes (Chen, 2004). The body plan of *Lingula* is characterized by having gently convex valves, and an elongated pedicle extending from the valves (Fig. 1, P-Q). The animal lives in a vertical position within a burrow. The two valves of *Lingula* close by the use of adductor muscles.

Panarthropod body plan evolution

Panarthropods are likely a monophyletic taxon, sharing a body pattern which includes the following several important characteristics: a segmented body, paired appendages, and a chitinous cuticle that they molt during ecdysis (Ruppert *et al.*, 2004). The presence of pyramid-yolk embryos (Chen *et al.*, 2004c) and late developing embryos with segmented germ band (Chen, 2004; Steiner *et al.*, 2004) at very basal part of the Lower Cambrian Meishucun phosphate deposits suggest that they were deeply rooted at very beginning of Cambrian about 542 million years ago or even before. The exceptionally well-preserved fossil fauna from the 530 million years old Maotianshan Shale deposits provides a fossil record of remarkably diverse arthropods that embrace a number of body organizations representing of different evolutionary stages, including worm-like stem lineage panarthropods referred to as Tardiopolygota (Chen and Zhou, 1997); stem lineage arthropods (proarthropods) (Chen, 2004; Waloszeck *et al.*, 2005); stem lineage euarthropods; and the possible ancestral form of the two major extant euarthropod groups, e.g. Chelicerata (Chen *et al.*, 2004b) and Mandibulata (Chen *et al.*, 2001). These nested body plans of different evolutionary stages suggest the presence of step-wise and condensed evolutionary events, which led in the line of evolution from worm-like ancestor to euarthropods within a short geological time of about 12 million years in the Early Cambrian.

Body plans of the stem groups of the Panarthropoda

Among the Panarthropoda, worm-like organisms with unjointed

limbs from the Lower Cambrian referred to as Tardipolypoda (Chen and Zhou, 1997) are least complex, and are likely the fossil representatives of the first panarthropods. They are diverse, composed of *Luolishania* (Hou and Chen, 1989), *Microdictyon sinicum* (Chen *et al.*, 1989; Ramsköld and Chen, 1998), *Cardiodictyon catenulum* (Hou *et al.*, 1991), *Onychodictyon ferox* (Hou *et al.*, 1991), *Paucipoda inermis* (Chen *et al.*, 1995d) and *Hallucigenia fortis* (Hou and Bergström, 1995; Ramsköld and Chen, 1998) in the Maotianshan Shale fauna. These taxa share several important characteristics, including: segmental soft body with paired appendages; an undifferentiated head, which is either segmented, or of non-segmental origin; and mouth and anus situated at each of the terminal ends (Ramsköld and Chen, 1998; Ramsköld, 1992). The segmented head consists of one or two pairs of head appendages.

The anatomical analysis of these tardipolypods reveals that they are separated by morphological gaps into several different groups, which testify to the presence of an initial and very rapid period of evolution at the base of the Panarthropoda. Their head contains the following three different types of organization, e.g.: non-segmental head (*Microdictyon sinicum*, *Paucipoda inermis* and *Luolishania longicuris*); segmented head with a pair of head appendages (*Onychodictyon ferox*); segmented head with two pairs of head appendages (*Cardiodictyon catenulum* and *Hallucigenia fortis*) (Fig. 2). The species within the non-segmented head group display great morphological variation and their trunk segments varied from only 6 to up to 16. Their body wall was either entirely soft and flexible as in *P. inermis*, or had pairs of composite-eye-like sclerites as in the trunk of *M. sinicum* and 2-3 small hardened sclerites on the dorsal side of each trunk segment as in *L. longicuris*. The head in *L. longicuris* was interpreted to bear a pair of “head appendages” (Chen, 2004), which are here reinter-

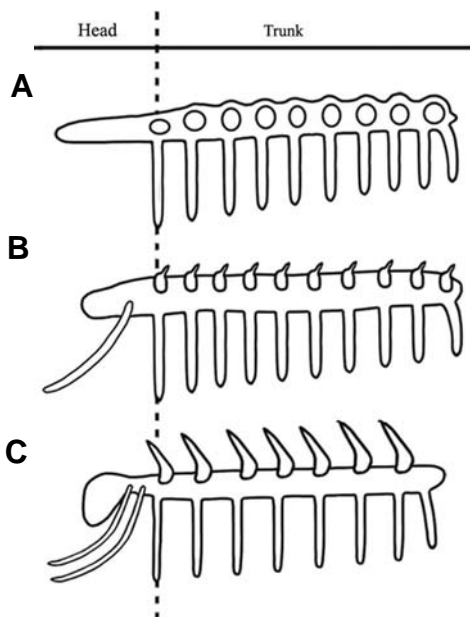


Fig. 2. The body organizations in stem groups of Panarthropoda. (A) Non-segmented head, exemplified by *Microdictyon sinicum*; (B) one-segmental head with a pair of head appendages, exemplified by *Onychodictyon ferox*; (C) two-segmental head with two pairs of head appendages, exemplified by *Hallucigenia fortis*.

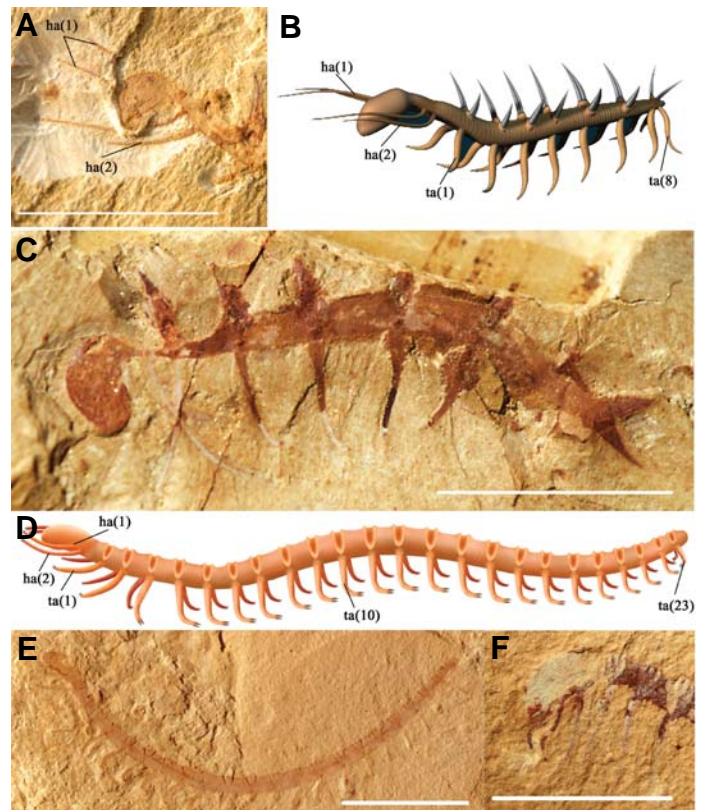


Fig. 3. The two-segmental head worm-like organisms from Lower Cambrian Maotianshan Shale deposits. (A-C) Photographs (A, C) and restoration (B) of *Hallucigenia fortis*. (D-F) Restoration (D) and photographs (E, F) of *Cardiodictyon catenulum*. Abbreviations: ha, head appendage; ta, trunk appendage. Scale bars are 1 cm in (A, C, E); 0.5 cm in (F).

preted as the first trunk appendage because of the presence of hardened sclerites. Liu and her collaborators (Liu *et al.*, 2004) recently erected a new lobopodian *Miraluolishania haikouensis* that represents an intermediate between lobopodians and arthropods. This animal preserves paired small hardened sclerites dorsally on each pair of appendages and the “possible dorsal sessile eyes” seemly represent the anterior-most paired sclerites. *M. haikouensis* has shared features with *Luolishania*. These features include; body form, number of appendages, and dorsally-situated small hardened sclerites. I reinterpret *M. haikouensis* as a possible synonym or sister species of *L. longicuris*.

The one-segmented head body plan is represented only by a single species *Onychodictyon ferox*. Its trunk bears 10 pairs of hard sclerites on each side. The head bears a pair of appendages in a close similarity with the trunk appendages but more laterally situated. Among the three types of tardipolypod body plans, the two-segmented head type yields remarkable interest for the understanding of the evolutionary origin of Arthropoda. The resemblance of this two-segmented head with that in the first arthropods known as proarthropods argues for the origin of arthropods, which may have evolved from an ancestor with a close resemblance to the two segmented head tardipolypod clade. The body plan of the two segmented head tardipolypod clade as exemplified by *Cardiodictyon catenulum* (Fig. 3, D-F) and *Hallucigenia fortis* (Fig. 1, A-C) had a two segmented head

that was in an elongated ovary form with mouth on its ventro-anterial terminal and a pair of appendages in each of head segments. The trunk bore pairs of hardened sclerites on lateral (*C. catenulum*) or on dorso-lateral sides (*H. fortis*) of trunk segments.

Body plan of the Arthropoda

Among the fossil assemblage of arthropods from Maotianshan Shale deposits, a group of arthropods were defined recently as stem group of arthropods, called proarthropods (Chen, 2004), which include *Fuxianghuia protensa* (Hou, 1987), *Shankouia zhenghei* (Waloszek *et al.*, 2005 and 2007) and *Chengjiangocaris longiformis* (Hou & Bergström, 1991). Among these 3 species, *F. protensa* is the first one being described at a basal position among Euarthropoda (Chen *et al.* 1995b), having a head made of two tergite-bearing segments (Chen *et al.* 1995b; Hou and Bergström, 1997) (Fig. 4, A,B; Fig. 5). In their interpretation, the second tergite was interpreted to bear two pairs of appendages. The "second pair of limbs" is however denied by evidence of their internal location between dorsal and ventral cuticles (Waloszek *et al.*, 2005). The authors reinterpret them as a pair

of diverticula in the anterior region of the digestive track.

The discovery of two-segmental head (Fig. 4, A,B; Fig. 5) together with lobopod-like trunk limbs among the Proarthropods (Fig. 4, C,D) is of great interest for the novel understanding of the evolutionary origin of arthropods. The anatomical characters of this animal group establish a bridge linking the tardipolypods and euarthropods. The two segmental head of the first arthropods suggest that the arthropod ancestor lies among limb-bearing worm-like organisms resembling the *Hallucigenia*+*Cardiodictyon* clade.

Whereas segmented and paired sclerites occurred widely among tardipolypods, the development of the jointed, segmental sclerites, called sclerotization, was one of the evolutionary hallmarks that led to the origination of arthropods. The presence of jointed sclerites has had far-reaching effects on evolutionary changes in anatomy, physiology, ecology and behavior for the arthropods (Ruppert *et al.*, 2004), opening great potentiality for skeleton-space expansion and morphological diversity.

The transition of the two-segmental lobopodian head into the first arthropod head required a quantum leap through multiple, synchronous events, including: transition of the first head appendage into the stalked eyes; specialization of the second head appendage into sensorial organ known as antennae; and displacement of the mouth into a ventral position of the antennal segment.

Image-forming eyes apparently had not yet appeared in tardipolypods, which may only have had a simple type of visual system, known as eye-spots. The appearance of compound eyes in all the proarthropods (Fig. 4, A,B; Fig. 5) suggests that compound eyes are apomorphic in arthropods. Whether the original compound eyes were stalked or sessile, is a long-lasting controversy. The eyes in all proarthropods have a clear, short tabular stalk. The compound, stalked eyes

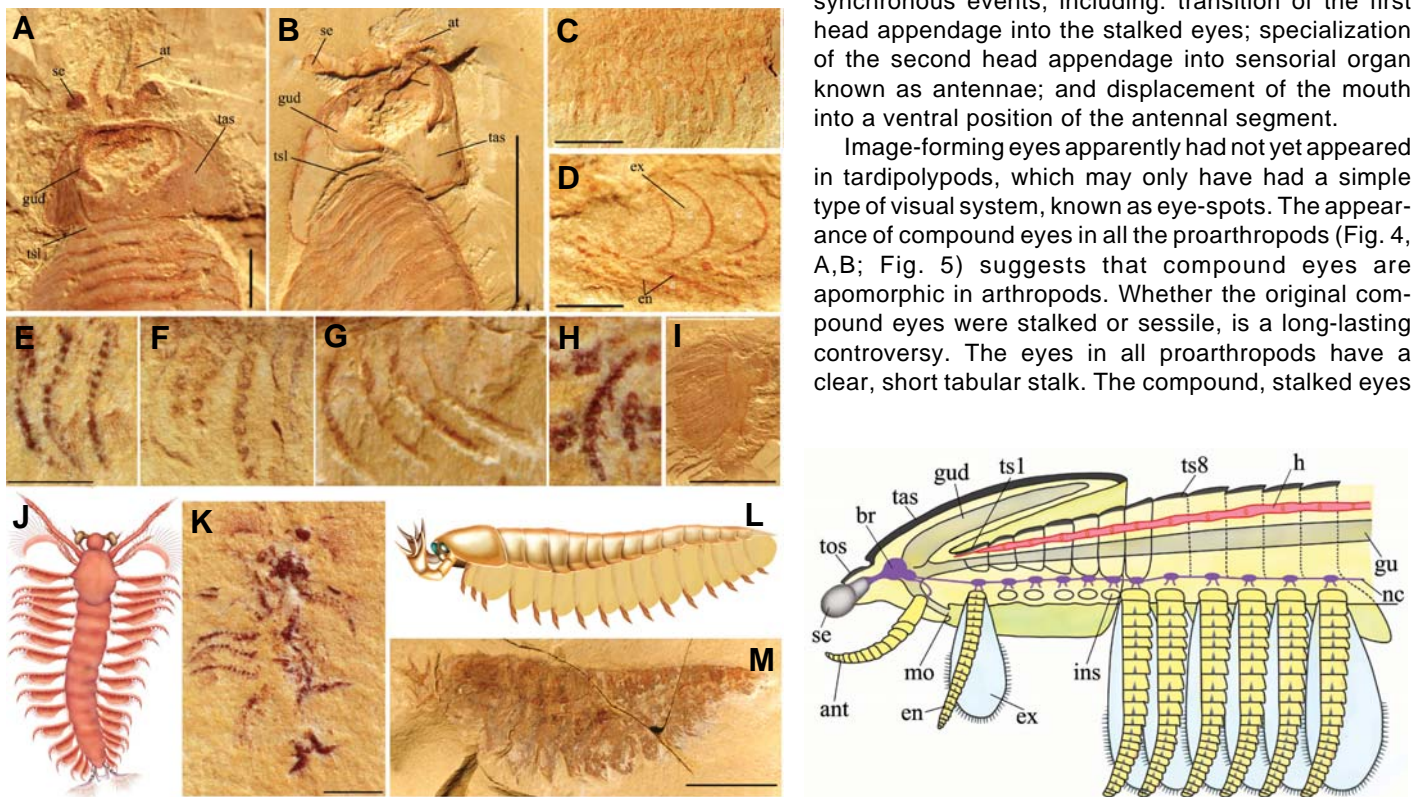


Fig. 4 (Left). Lower Cambrian arthropods. (A,B) Dorsal view of the anterior-displacement of the 2-segmental head and the anterior trunk portion of *Fuxianghuia*, showing the following features: stalked eyes, antennae, a large posterior-extended head shield and gut diverticula. (C,D) Biramous trunk appendages of *Shankouia*, showing lobopodian-like endopods and simple flap-like exopods. (E-H) Tubular-shaped, multi-segmented endopods among stem groups of the Euarthropoda. (I) Bipartite endopod with expanded and serrated basipod of a stem group of the Euarthropoda. (J,K) Restoration and photograph of the first Mandibulata, *Ercaia*. (K-L) Restoration and photograph of *Haikoucaris*, showing a pair of cheliceae-like structures consisting of 2 segmented peduncle and 3-fingered claw. Abbreviation: at, anetennae; en, endopod; ex, exopod; gud, gut diverticula; se, stalked eye; tas, tergite of antennal segment; ts, trunk segment. Scale bars: 2 mm in (A,G); 1 cm in (B,C,I,M); 0.2 mm in (D,E,H); 1 mm in (F,K).

Fig. 5 (Right). Schematic restoration of the tentative groundplan of the Arthropoda. Abbreviation: ant, antennae; br, brain; en, endopod; ex, exopod; gu, gut; gud, gut diverticulum; h, heart; ins, insertor; mo, mouth; nc, neural cord; se, stalked eyes; tas, tergite of antennal segments; tos, tergite of the ocular segment; ts, tergite of the trunk segment. This figure is adapted from Fig. 4 in Waloszek *et al.*, (2005).

arguably characterize the original body plan for the arthropod eyes. The tabular, possibly segmented eyestalk represents tabular extensions of the body wall in homology with tubular limbs, which argues for segmental origin for the anterior-most head part in arthropods and evolved from the anterior-most, limb-bearing head segment in worm-like ancestor resembling the *Hallucigenia-Cadiodyctyon* clade.

The flagelliform antennae have long been accepted as the original arthropodian version of the uniramous antennae (Fig. 4A). The long and multi-segmented flagelliform antennae widely appear among trilobites and extant arthropods with a mainly sensorial function. This flagelliform hypothesis however is refuted by the present study of the proarthropods, whose antennae are short and stout, consisting of a limited number of segments, about 15 (Fig. 4A). These short, limb-shaped antennae occurring both in all proarthropods and in some stem lineage euarthropods (*Canadaspis*, *Primicaris* and bradoriids, and *Ercaia*) represent the groundplan of the arthropod antennae (cf. Maas *et al.*, 2004).

The mouth in the groundplan of the Panarthropoda, as exemplified by tardipolypods, is situated at the terminal end of the body and in arthropods the mouth is located on ventral side of the antennal segment. The displacement of the mouth to a new position represents one of the major evolutionary changes in the origin of arthropods. The looped anterior digestive track in arthropods argues for this mouth displacement as the result of the back curving of the first head segment. *C. catenulum* (Fig. 3, D-F) and *H. fortis* (Fig. 3, A-C) have an expanded and ventrally-curving head and an anterior-ventrally situated mouth, representing an intermediate stage during mouth displacement.

The transformation of uniramous trunk limbs into biramous trunk limbs was one of the important changes during the origin of arthropods and it was marked by innovation of the flap-like exopods (Fig. 4, C,D; Fig.5). The exopods represent the extended pouch of body wall adjacent to the base of limb. They morphologically resemble insect wings, suggesting that the developmental process of the exopods is similar to that for wing development. It suggests that the coordinating genetic cassette for the insect wing-developing is very ancient, used by the first arthropods for exopod development. It was recruited later by insects for wing morphogenesis.

The groundplan of the arthropod trunk-limbs, as exemplified by *Shankouia zhenghei* and possibly *Chengjiangocaris longiformis*, is biramous, with the least differentiation among the different segments except for *Fuxianghuia protensa*, which had a trunk differentiated into a limb-bearing thorax and a limb-less abdomen. This limb-less abdomen is a secondarily derived feature, likely a result of the repressed expression of the *Distalless* gene. The groundplan of the arthropod endopods is a simple tubular form, consisting of multi-segments. It is not much different from the groundplan of the panarthropod limb except for the existence of pivot joints between two adjacent segments in the endopods of the arthropod groundplan. The presence of pivot joints suggests the presence of intrinsic muscles in the first arthropod endopod.

Groundplan of the Euarthropoda

Walossek has established Euarthropoda as representing a monophyletic group that includes all the extant arthropods and some extinct arthropod lineages, such as trilobites, naraoiids, and numerous others (Walossek 1999; Maas & Walossek 2001). The

monophyletic status of the Euarthropoda as proposed by the authors, is based on them having a head consisting of the ocular, antennal and three biramous limb-bearing segments: the post-antennal limbs composed of a rigid and antero-posteriorly compressed basipod and seven or fewer jointed endopods (Fig. 4I). Therefore it means that the transition of Proarthropods to Euarthropoda was marked by two separate events, e.g., the evolutionary origin of multiple, segmented syncephalons; and the formation of euarthropod limbs. The fossil evidence however shows that these two events were not synchronic and the origin of the syncephalon event predates formation of euarthropod limbs.

Whereas the head in extant arthropods is composed of either 6 segments in Mandibulata or 7 segments in Chelicerata, the head in Lower Cambrian arthropods, except for proarthropods, mostly contains 5 segments. The head in the euarthropod groundplan was five-segmented, composed of ocular, antennal, and three post-oral segments. The anterior two segments, referred to as the procephalon, constitute the anterior head part that is in a homology virtually to proarthropod head. The ocular segment in most species had a pair of stalk eyes but stalk-less sessile eyes were also very common among the stem groups of the euarthropods. These sessile eyes were either fixed dorsally on the head shield as seen in trilobites, or ventrally in trilobitiforms and *Misszouia*. These sessile compound eyes are secondarily derived; presumably the expression of the *Distalless* gene (organizer center for tabular structure) in these species had been selectively repressed by *otd/ems* in the ocular segment.

During the transformation of proarthropods to euarthropods, the antennae in the antennal segment either remain unchanged, or specified into the flagelliform sensorial organ or raptorial limb-like feeding organ. The origination of raptorial limb-like antennae was the first evolutionary event that led the evolutionary line forward to the Chelicerata.

The groundplan of the euarthropodan post-oral head, as exemplified by stem-lineage euarthropods from the Maotianshan Shale, is characterized by a three segmental mode, and each of the segments carried a pair of the least differentiated biramous appendages in homology with trunk appendages. Unlike to the other stem-lineage euarthropods, the post-oral head in *Ercaia minuscula* was comprised of 4-segments, whose first pair was in a specified form corresponding to the second pair antennae of the Crustacea (Chen *et al.* 2001). Presumably *Ercaia minuscula* is the fossil representative of the ancestral form of the Mandibulata, which was deeply rooted among the proarthropods or among the proarthropod-euarthropod transition.

The post-antennal limb design of the euarthropod groundplan has been defined as a bipartite mode, with an expanded proximal portion (called basipod) and a distal portion (consisting of 7 or less segments). This bipartite post-antennal limb is associated with feeding and locomotion function respectively (Walossek and Møller, 1990; Walossek, 1993). It has been interpreted to be monophyletic and the key novel event in the origin of Euarthropoda. This monophyletic hypothesis is however challenged by fossil evidence from the Lower Cambrian Maotianshan Shale. Instead of the predicted bipartite limb, the tabular and multi-segmented post-oral limb widely occurred among distally separated stem-groups of early euarthropods, including: the first Mandibulata (*Ercaia minuscula*) (Fig. 4, E,K) and the stem-groups of Chelicerata

(*Leachollia*, *Fortiforceps* and *Tanglangia*) and other extinct euarthropods (*Saperion*, *Canadaspis*, *Isoxus*, bradoriids, and *Primicaris*). The limb in some species is in a tubular shape, having a slight differentiation along its main axis, and consisting of about 14 segments (*Primicaris lavaformis*, *Ercaia minuscule*, *Canadaspis laevigata*) whereas in other species consists of fewer segments and displays a bipartite form with an expanded and segmented proximal portion (*Cindarella eucalla*, *Saperion glumaceum* and *Isoxus*) (Ramskold *et al.*, 1997; Chen, 2004). This bipartite, multi-segmented limb-design presumably represents an intermediate stage from least differentiated tubular form to bipartate mode. The limb design in *Saperion* comprises 9 segments and the 3 proximal segments are expanded with serrated inner edge (Ramskold *et al.*, 1997). I speculate that these 3 serrated segments are the precursor of the expanded basipod and the basipod may have evolved from these expanded proximal limb-segments through their fusion.

Possible groundplans of Mandibulata

The extant arthropods including Crustacea, Hexapoda, Myriapoda and Chelicerata can be grouped into two deeply-rooted clades, the Mandibulata and the Chelicerata. Extant Mandibulata is composed of the Hexapoda, Myriopoda and Crustacea. The head segmentation and related appendages in the Hexapoda and Myriapoda are homologous with those in Crustacea except for the first post-oral segment, which is known as intercalary segment in stead of 2nd antennae. The intercalary segment has long been regarded as an appendage-less structure until recently when the labrum was reinterpreted as fused paired appendages of intercalary segment (Finkelstein and Perrimon, 1991; Popadic *et al.* 1998; Boyan *et al.*, 2002) and the head was reinterpreted in the 6-segmental mode (Zacharias *et al.*, 1993; Minelli, 2001). The six segments in the insect head are arranged from anterior: ocular, antennal, intercalary, mandibular, maxillary and labial segment. The insect head segmentation and related structures show a striking homology with those in Crustacea, suggesting that insects (possibly including Myriopoda also) likely originated among the Crustacea or from a crustacean-like ancestor. The transition from 2nd antennae into a feeding organ known as labrum through anteriorly-displacement and the fusion of the paired 2nd antennae was one of major changes in the origin of the insect.

Ercaia minuscule from the Maotianshan Shale has a 6-segmented head and the first pair of the modified post-oral appendages corresponds to the 2nd antennae in extant Mandibulata. It was first described as an ancestral form of Crustacea (Chen *et al.* 2001) and here is interpreted as the fossil representative of the first Mandibulata.

The head in Mandibulata groundplan as exemplified by *E. minuscule* is composed of 6 segments, which are arranged from anterior: ocular, the 1st antennal, the 2nd antennal, and 3 least differentiated appendages (Chen *et al.*, 2001). This early Mandibulata was small, 2-4 mm long, and had an ocular segment identical basically to that of proarthropods. Like the ocular segment in proarthropods, it was small, and had an oval tergite jointed posteriorly with head shield, and a pair of stalked compound eyes attaching ventro-laterally to this segment. The antennae resemble those in proarthropods in general morphology but they bear setae, suggesting more advanced than the setae-lacking

antennae in proarthropods. The 2nd antennae are a newly derived character, composed of a relatively small 4-5-segmented endopod and a large crescent-shaped exopod with prominent setae on its margin. Posterior to the A2, there are likely three biramous head appendages, which resemble trunk appendages. The endopods in post-A2 appendages are in a tubular shape, consisting of 14 segments. The primitive nature of the homologous, post-A2 head appendages in *E. minuscule* suggest that Mandibulata was deeply rooted at very basal position of the arthropod evolutionary history. Recent discovery of an epipodite-bearing eucrustacean *Yicaris* from Yonshan, Yunnan (not far from the fossil site of *E. minuscule*) within stratum about coetaneous with the Lower Cambrian Maotianshan Shell (Zhang *et al.*, 2007). These finding indicates that the first appearance of Eucrustacea were much earlier than we thought.

Groundplan of the Chelicerata

Chelicerates are widely accepted as an unquestioned, basic monophyletic group of the Arthropoda. They share a distinct and conservative body plan, with features such as the prosomal - opisthosomal subdivision; and a cheliceral segment with a pair of morphologically unique chelicerae. Chelicera is named for the presence of a pair of modified head uniramous appendages called chelicerae. The chelicerae consist only of 3 articles, the proximal peduncle and distal two, which form of chela. The chelicerae were long been regarded as the modified second antennae and on basis of molecular developmental and neurobiological study now reinterpreted as the modified first antennae. The groundplan in chelicerate head consists of a two-segmental anterior head and 5-segmental posterior head (Dunlop 1999; Chen *et al.*, 2004b). In its anterior head the ocular segment bears dorsal sessile compound eyes and antennal segment bear a pair of anteriorly-pointing raptorial uniramous appendages, the chelicera. The posterior head as exemplified by Silurian *Offacolus kingi* bear 5 pairs of biramous appendages (Sutton *et al.* 2002).

The large modified antennae known as the "great appendages" have long been recorded from the Early to Middle Cambrian. These "great appendages" that comprise proximal peduncle and distal claw are similar to chelicerae in general morphology. Despite the similarity, no one had ever realized the possible evolutionary relationship of these peculiar Cambrian "great appendage" arthropods with Chelicerata until the fossil finding of *Haikoucaris ercaiensis* in 2004 (Chen *et al.*, 2004b) (Fig. 4, L-M). This animal recorded from the Lower Cambrian Maotianshan Shale has a pair of chelicerae-like structures, which fill the morphological gap between the Cambrian "great appendage" and the chelicerae of the crown-lineage Chelicerata. This chelicerae-like structure consists of the 2-segmental peduncle and 3-fingered claw. It resembles chelicerae but had one more segment both in its peduncle and claw, and similar to the "great appendage" but one segment less in claw of most Cambrian "great appendage" creatures. Because of the striking homology with chelicerae, I here call these "great appendage" as prochelicerae, which are interpreted as a precursor of the chelicerae.

The head ground plan in Chelicerata, as exemplified by prochelicera, is a five-segmented model. The five segments are arranged from anterior as follows: ocular segment, procheliceral segment, and 3 post-antennal segments. Each of the 3 post-

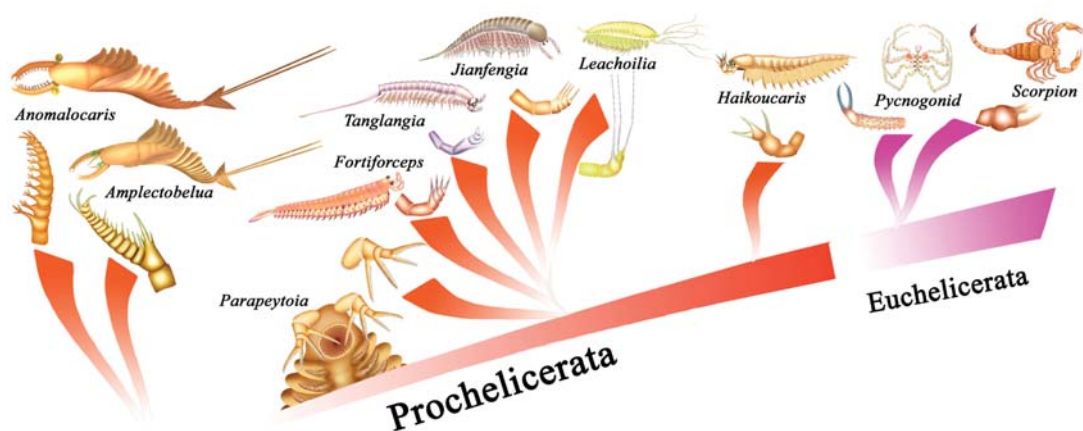


Fig. 6. Summary model of the major change in grasping head organ in antennal segment of Chelicerata.

antennal segments has a pair of biramous appendages identical to those in the trunk. Their endopod is mostly in simple, multi-segmented with least differentiation along the main axis (*Fortiforceps foliosa*, *Leachoilia illecebrosa*, and *Tanglangia longicaudata*), but *Haikoucaris ercaiensis* has only 7 segments, likely grouping into two function portions. The primitive nature of the limb design in most of Prochelicerata suggests that Chelicerata was deeply rooted among the stem-lineage arthropods. The transformation of the short, stout antennae into bipartite prochelicerae was the first key event that led to the line of the Chelicerata evolution. The transformation of Prochelicerata to Euchelicerata in head is marked by the two likely synchronous events: e.g., the reduction of the procheliceral segments and the recruitment of 2 more segments from trunk (Fig. 6).

Cristozoan groundplan

Neural crest cells contribute to the development of peripheral neural system, endocrine glands, pigment cells, most of the bones and cartilage of the skull, the bones and cartilage of the jaw and pharyngeal arches, and the teeth. Hall (2000) suggests vertebrates are quadroblastic organisms, with the neural crest constituting a fourth germ layer. The neural crest apparently was a watershed for dividing of the vertebrates from their invertebrate ancestor and Holland and Chen (2001) therefore coined a term Cristozoa (the crest animals) for representing craniates and their immediate precursors, although Wada (2001) suspected the neural crest cells as a novel vertebrate cell population.

Among the most significant fossil discoveries from Lower Cambrian Maotianshan shale are the fish-like early crest animals including *Haikouella* (Chen *et al.*, 1999; Chen and Li, 2000; Holland and Chen, 2001; Mallatt and Chen, 2003), *Yunnanozoon* (Hou *et al.*, 1991; Chen *et al.*, 1995a), *Haikoichthus* and *Myllokunmingia* (Shu *et al.*, 1999b and 2003a; Hou *et al.*, 2002), which represent different evolutionary stages in the line toward craniates. Among them, *Haikouella* is one of the most primitive crest animals, lying at basal position of the Cristozoa evolution (Fig. 7, C-J; Fig. 8). *Yunnanozoon* (Fig. 7, A,B) is a close relative with *Haikouella*. Because of incompleteness in the earlier discovered specimens, the phylogenetic affinities of *Yunnanozoon* have been a subject of controversy and this animal was first described as a chordate (Chen *et al.*, 1995a) and later reinter-

preted as a hemichordate (Shu *et al.*, 1996 and 2003b). The hemichordate hypothesis was heavily based on the misinterpretation of several key anatomical characters of this animal, and was refuted by Chen and Li (1997). *Yunnanozoon* not only has a mandibular arch but is also preserved with a pair of large lateral eyes in a complete specimen (Fig. 7, A,B), suggesting a more advanced phylogenetic position than that of *Haikouella* (Chen 2004, 2008). The presence of W-

shaped myosepta and fin rays suggests that *Haikoichthus* and *Myllokunmingia* are possible true craniates (Javier, 1999). They however lacked a mineralized skull and the reliable evidence for supporting the presence of telecephalon and otic capsules, casting the shadow on these animals as true craniates. Alternatively, *Haikoichthus* and *Myllokunmingia* may represent advanced forms of the pre-craniate crest animals.

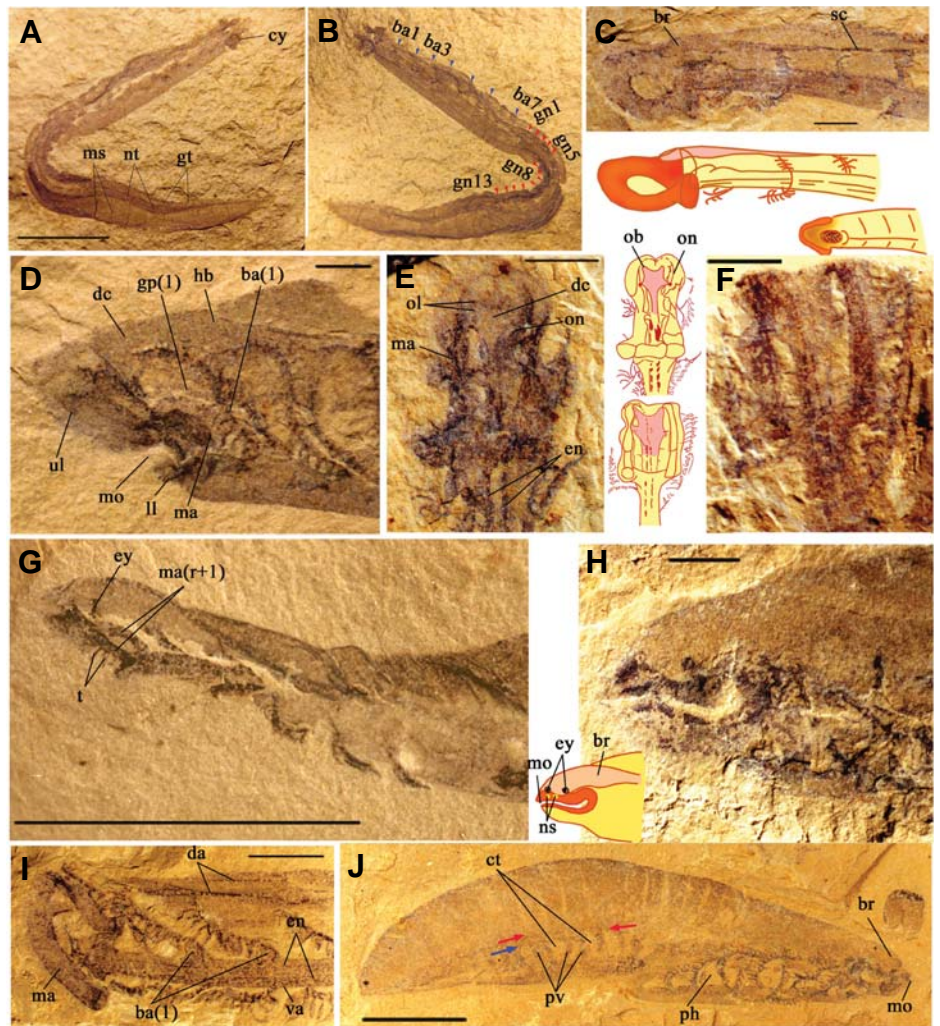
Presence of the visceral skeleton, branchial denticles and the evidence of muscular ventilation suggest that *Yunnanozoon* and *Haikouella* had definitive neural crest cells and were definitive crest animals (Holland and Chen, 2001; Mallatt and Chen, 2003). The presence of the neural crest allowed the crest animals to become one of the most successful animal groups. These two early crest animals display a number of characters typical of vertebrates, but they lack some vertebrate characters, such as a skull, ear, fin rays, and definitive telencephalic forebrain. These vertebrate features must have evolved at later stages in the evolutionary line leading to craniates (Mallatt and Chen, 2003), arguing for the existence of pre-craniate evolutionary history among the crest animals. The crest animals therefore embrace stem- and crown- groups.

Among the stem group of the crest animals, *Haikouella* is a most remarkable one. This animal has preserved with extraordinary details of key anatomical characters including brain, eyes, oral tentacles, muscle fabrics (Fig. 8) and other organs and at a very basal position in the evolution history of the crest animals, provides a unique key for novel understanding of the groundplan of the crest animals.

The groundplan of the crest animals as exemplified by *Haikouella* and *Yunnanozoon* has an elongated, lancelet-like body only a few centimeters long which was bilaterally symmetrical. A distinct head bears a pair of eyes on lateral sides, nostrils on front, and a ventral buccal cavity at its hind end. It also has large paped lips and a distinct lower lip (Fig. 8). Similar characters also exist both in agnathans and in sharks (Mallatt, 1996), representing apomorphic characters of the Cristozoa (Fig. 8). The oral cavity has a pre-pharynx position, with a skeletal ring for supporting oral tentacles. This tentacle-supporting ring is a sharing feature with cephalochordates, also present in larval lamprey, possibly representing plesiomorphic character of the Cristozoa.

The groundplan of the Cristozoa pharynx system as exemplified by *Haikouella* is characterized by having only 6 post-man-

Fig. 7. Fossil representatives of the early crest animals from Lower Cambrian. (A,B) Part and counterpart of a completely-preserved *Yunnanozoon* specimen from Chengjiang, Yunnan (southern China), showing: a pair of big eyes, pharynx with 7 pair of branchial arches; 13 pairs of gonads; gut and notochord. **(C)** A laterally compacted brain in anterior part of *Haikouella*. **(D)** Mouth, upper lip, lower lip, gill pouch, brain, spinal cord in a laterally compacted head of *Haikouella*. **(E,F)** Dorso-ventrally compacted, anterior portion of *Haikouella*, showing brain with a pair of olfactory lobe and optic nerves. **(G)** A laterally compacted specimen of *Haikouella*, showing eyes, oral cavity with tentacles and right and left branches of mandibular artery. **(H)** A head of *Haikouella*, with preservation of brain, a pair of eyes, and nostrils. **(I)** Pharynx of *Haikouella*, preserved with mandibular artery, branchial arches, dorsal and ventral aorta and endostyle. **(J)** Laterally compacted *Haikouella* showing: cartilage protovertebrae, connecting tissues and its dorsal margin, dorsal margin of notochord. Abbreviation; ba, branchial arch; br, brain; ct, connecting tissue; da, dorsal arteria; dc, diencephalic brain; en, endostyle; ey, eye; gn, gonad; gp, gill pouch; gt, gut; hb, hindbrain; ll, lower lip; ma, mandibular artery; ma(r+l), right and left limb of mandibular artery; mo, mouth; ms, myoseptum; ns, nostrils; nt, notochord; ol, olfactory lobe; on, ocular nerve; ph, pharynx; pv, protovertebrae; sc, spiral cord; t, tentacle; ul, upper lip; va, ventral arteria. Scale bars, 2 mm in A-B, D-F, and H-J; 1 cm in C,G.



dibular arches and lacking a mandibular arch (Fig. 7I; Fig. 8). The mandibular region is marked by the mandibular artery without branchial bar associated with this artery. *Yunnanozoon* had 7 branchial arches and the first pair corresponds to the mandibular bar. It suggests that the origination of the mandibular arch was not synchronous with that of the post-mandibular arches, but occurred in a later time among the pre-craniate history. These branchial arches are separated by wider branchial slits, and relatively thicker and jointed but least differentiated along their main axis. The widely spaced, thick skeletal branchial bars indicate that the muscular ventilation mechanism had evolved at very beginning of the cristozoan evolutionary history. The pharynx as evidenced by *Haikouella* and *Yunnanozoon* is protected by a pair of water chambers known as atria. Atria are a plesiomorphic character and are also present in proto-chordates including ascidians and cephalochordates but disappear in craniates. The atria are a plesiomorphic character of the crest animals and might function for protecting the gills from muddy and sandy substratum during swimming or hov-

ering close to the muddy sea bottom (Holland and Chen, 2001).

The brain in the Cristozoa groundplan as exemplified by *Haikouella* is relatively large and expanded at the pre-mandibular location (Fig. 7, C-F; Fig. 8). This pre-mandibular part of the brain interruptedly contains mainly the diencephalon and middle brain at its hind end. The hindbrain is elongated and narrows posteriorly by its end at the third (vagal) branchial bar (Fig.7, C). Dorso-ventrally compacted specimens confirm the absence of large cerebral hemispheric structures related to telencephalon

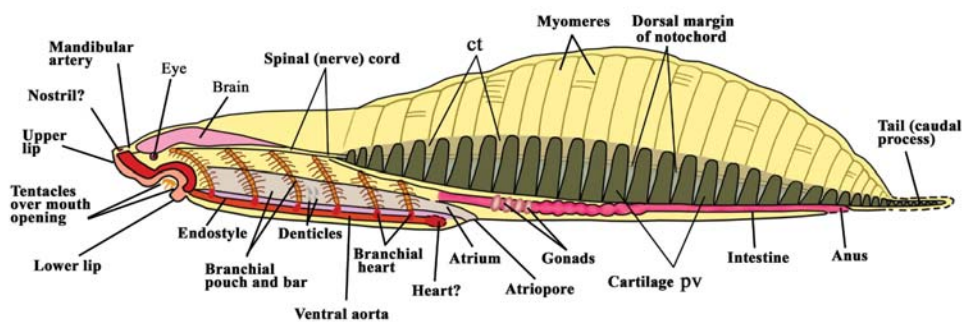


Fig. 8. Anatomy of the Early Cambrian crest animal, *Haikouella*.

brain, except for a pair of small bulges that are interpreted as olfactory lobes (Fig. 7, E-F).

Vertebrates get their name from their axial skeleton known as the vertebral column and it is a vertebrate-only character, but is not best developed until the mammalian evolutionary stage. The notochord is the axial hydrostatic skeleton, which is a chordate-only character. The notochord-only axial structure appears only in larval stage of urochordates but well-developed throughout the life in cephalochordates. Notochords exist in vertebrates throughout their life, but it tends to degenerate, and the mineralized vertebral column instead of notochord plays the role of axial skeleton. That origin and timing of vertebral column evolution remains a great puzzle.

The axial skeleton in the Crustozoa groundplan as exemplified by *Haikouella* and *Yunnanozoon* is characterized by having a large distinct notochord and cartilage protovertebrae (Fig. 7J; Fig. 8). The notochord presents throughout the life cycle, resembling that in cephalochordates that have an elongated form, narrowing anteriorly over the pharynx and posteriorly in the caudal process. It was enclosed in a thick sheath that consists of circular and oblique fibers, the same as in extant chordates (Fig. 7J; Fig. 8). This sheath is continuous with a layer of a connecting tissue; within this connecting tissue there develop irregular cartilaginous thickenings comparable to protovertebrae of the extant lamprey (Young, 1981; Chen and Li, 2000; Holland and Chen, 2001). This cartilage protovertebrae is the precursor of the vertebral column and represents an apomorphic character of the crest animals.

Muscle segments (myomeres) and the myosepta (or myocommata) between the segments are present in both extant cephalochordates and craniates. In these taxa, the myosepta have a complex V or W shape. In *Haikouella* and *Yunnanozoon*, there are about 2 dozen myomeres, which are separated by myosepta (Fig. 1., J; Fig. 8). Different from V or W shape, these myosepta however are simply straight. Because of their unusual simplicity and dorsal location, they were reinterpreted as a large, segmented fin (Shu *et al.*, 1996) and as cuticular rings (Conway Morris, 2000). Preservation of muscle fibers in some *Haikouella* and wrinkled overlying skin on the myomeres in some *Yunnanozoon* confirm that these segmental structures are truly myomeres (Chen and Li, 1997; Mallatt and Chen, 2003). The simply myosepta may represent a primitive character of the crest animals. The resemblance in the complex shape of the myosepta between cephalochordates and advanced crest animals presumably evolved through parallel evolution. Young larvae of lancelets and tunicates with the same simple straight, segmental muscles swim well (Holland and Chen, 2001).

Simple cone-shaped denticles exist on the pharyngeal floor in *Yunnanozoon* and *Haikouella*. It suggests that the teeth are deeply rooted, representing an apomorphic character of the Crustozoa, and they have emerged before the onset of mineralized dermal skeletons. The early teeth as exemplified by *Haikouella* and *Yunnanozoon* were simply cone-shaped, situated on the pharyngeal floor.

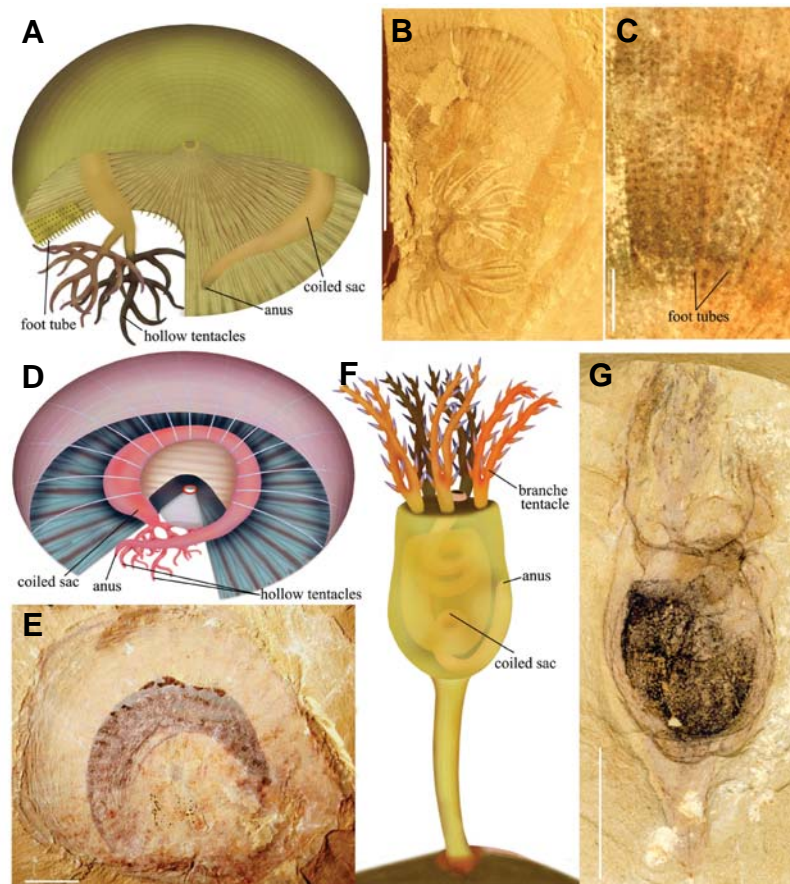


Fig. 9. Early Cambrian lophophore-bearing animals. (A-E) *The pelagic medusiform lophophore-bearing animals, Rotadiscus (A-C) and Eldonia (D-E). (F,G)* Solitary sessile lophophore-bearing animals, Phlogites (=Chenungkongella). Scale bars all are 1 cm.

Body plans of the lophophore-bearing animal groups

The diversity of Cambrian metazoan body plans includes some whose relationships to modern phyla have been controversial (Gould, 1989). Two major groups of Early Cambrian enigmatic animals both had a crown of hollow ciliated tentacles (also called a lophophore) encircling their mouth. Their anus lies outside the tentacle crown but nearby. One of the enigmatic groups is represented by *Phlogites* (*Chenungkongella*), which was a solitary sessile suspension feeder (Fig. 9, F,G). *Phlogites* was soft-bodied, with a goblet body, which consisted of a proximal stalk, a calyx and branched circumoral arms. The gut was coiled, U-shaped, with the anus on the side of body. This animal had been interpreted either as a tunicate (Shu *et al.* 2001a) or as an endoproct (Luo *et al.* 1999). The body plan, based examination of more completely preserved specimens, shows this animal was likely not a tunicate, or an endoproct, but likely a lophophorate animal (Chen *et al.*, 2003).

The medusiform animals *Eldonia* and *Rotadiscus* represent the other animal group which were dominant among the Cambrian pelagic community (Fig. 9, A-E) (Walcott, 1911b; Sun and Hou, 1987; Chen *et al.*, 1995c; Zhao and Zhu, 1994; Zhu *et al.*, 2002). The body plan of *Eldonia* and *Rotadiscus* is characterized by having a low bell-shaped body, with a convex upper surface

and a concave lower surface. The upper surface was soft-bodied, supported by fine tabular structures in *Eldonia* or a weakly mineralized hard skeleton in *Rotadiscus*. The internal anatomy is however asymmetric and complex, having a sub-horizontally clockwise (viewing from above) coiled sac. The alimentary canal situates dorsally in the coiled sac, ending with mouth and anus ventrally, both off the centre on one side of the disc and close to each other. A crown of hollow branched tentacles encircles the mouth. A hydraulic sac lies ventrally in the alimentary canal and it is divided into numerous segmental parts. These hydraulic compartments extend radially. Each of these radially-extended sacs bears a canal, which connected with a ring canal centrally and bifurcated in its peripheral part. The lower surface is soft-bodied, bearing radial rows of elongated structures, which correspond in distribution to the radial canals (Fig. 9, C). These structures are 0.2 mm wide and 0.35 mm long but the innermost one is much larger and comparable to foot tubes in echinoderms.

The phylogenetic affinity of the medusiform organisms, especially *Eldonia*, has evoked much controversy since 1911 when it was originally described as a holothurian (Walcott, 1911), or considered as an echinoderm (Clark, 1912; Croneis and McCormack, 1932). Contrary to the echinoderm hypothesis, it has proposed been as a Cnidaria, but being placed into different groups in Cnidarians, as a siphonophore (Madsen, 1956), a coelenterate medusa (Lemche, 1960), a scyphozoan (Sun and Hou, 1987), and as a sedentary (Dzik *et al.*, 1997) or pelagic lophophorate (Chen *et al.*, 1995). Conway Morris and Robison (1988) and Chen and Erdtmann (1989) interpreted it as a problematic. These medusiform organisms had a well-developed water vascular system that resembles that in echinoderms, suggesting that they were likely a basal deuterostome, closely related with echinoderms. Walcott original interpretation is correct.

Body plan of the vetulicolians: are they basal deuterostomes?

Among Cambrian organisms, vetulicolians (Shu *et al.*, 2001b; Chen and Zhou, 1997) are one of the big Cambrian puzzles whose affinities have been controversial, being considered as an extinct arthropodan Class called Vetululida (Chen and Zhou, 1997) or as a deuterostome group, either basal (Shu *et al.*, 2001b) or more derived, likely close to the tunicates (Gee, 2001; Lacalli, 2002; Chen *et al.*, 2003; Aldridge *et al.*, 2007). The recent issue of one of the vetulicolians, *Xidazoon* (junior synonym of *Pomatrum*; Luo *et al.*, 1997) as a vertebrate (Shu *et al.*, 1999a) is one of the most disparate views with arthropodan or arthropod-affinity hypothesis (Chen & Zhou, 1997; Bergström & Hou, 2003; Chen, 2004; Briggs *et al.*, 2005; Caron, 2006).

Vetulicolians were large Cambrian animals, sharing a body plan that has a body with super-

ficial resemblance to tadpole larvae, composed of a simple cone-shaped head (Fig. 10A), a larger expanded segmented thorax and a dorso-ventrally-flattened abdomen (Fig. 10, B-C). Their mouth and anus lay at either terminal end of the body.

The head is only well represented in a few specimens, in resemblance to the head of the tardipolypods. The head in both animal groups is non-segmental and cone-shaped, lacking eyes and other structures, with a small mouth situated at the terminal end.

The expanded thorax represents the anterior half of the trunk, being laterally compressed (*Vetulicola*, *Didazoon* and *Heteromorphus*), or rounded (*Pomatrum*=*Xidazoon* and *Banffia*). It is segmented, divided into various sub-equal segments, mostly six, but sometimes fewer, possibly five in *Heteromorphus* and uncertain in *Banffia* (possibly numerous). Each of the thoracic segments in most species had a pair of distinct gas exchange organ "gills" (Fig. 10, G-H), which were contained in the branchial

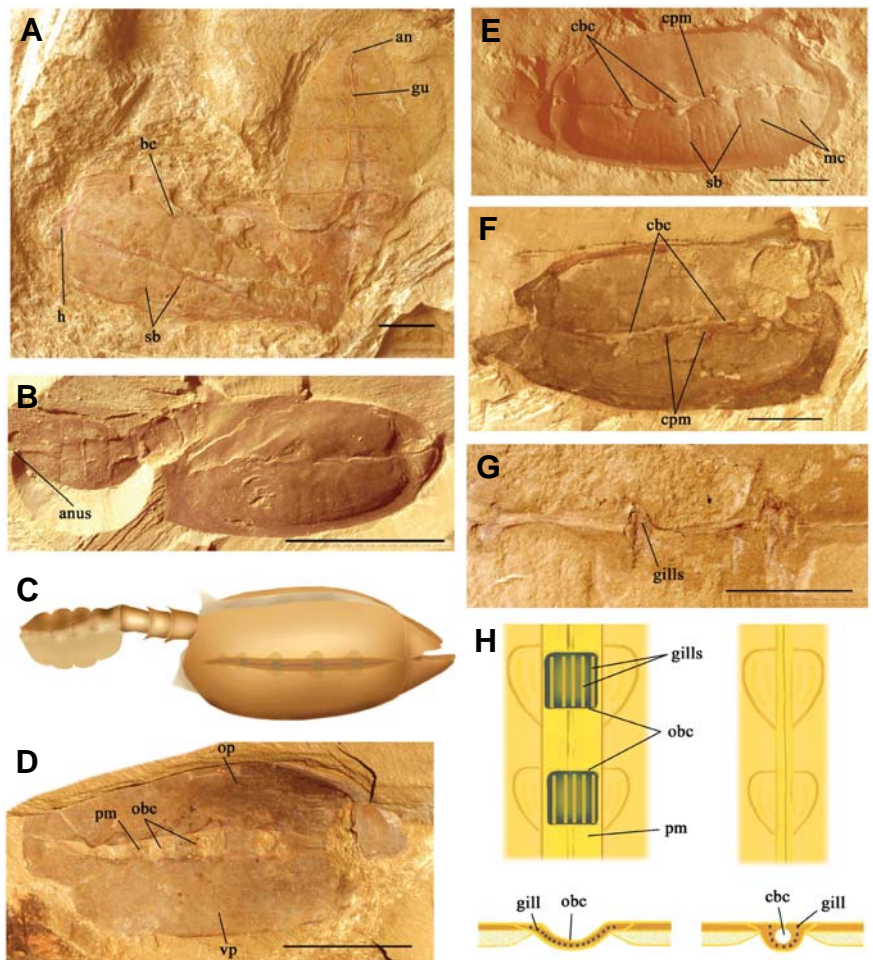


Fig. 10. Early Cambrian arthropod-like animals. *Vetulicolia*: (A) a complete, dorsally-compacted specimen of *Pomatrum* (=Xidazoon), preserved with a short-conic head, segmented thorax and abdomen. (B-H) Specimens (B, D-G), restoration (D) and schematically drawing of the branchial chambers in *Vetulicola*, showing: close (E, right column of H) and open state (C, D, left column of H) of the pleural area and related branchial chambers, and gills (G). Abbreviation: bc, brachial chamber; cbc, closed branchial chambers; cpm, closed pleural member; h, head; mc, microscopic canal; obc, open branchial chambers; pm, pleural member; sb, segmental boundary. Scale bars all are 1 cm.

chambers formed by subsidence of the body wall (Fig. 10, E-H). Similar structures are also present in many modern decapod crustaceans. The gills are contained in the branchial chamber that provides protection for the fragile gill filaments.

The exoskeleton design in the thoracic region is similar to arthropods because it is differentiated transversely into the following four parts, e.g., a dorsal and ventral hard plate, two lateral flexible pleural membranes between ventral and dorsal plates (Fig. 10, C,H). The dorsal and ventral plates were usually finned in the median plane. The exoskeleton of the dorsal and ventral plates is relatively thick, consisting of a thin, dark outermost layer and a thick, pitted inner layer. The outer layer presumably corresponds to the epicuticle of arthropods, bearing a smooth outer surface, whereas the inner layer corresponds to procuticle of extant arthropods, that bears microscopic canals (Brusca and Brusca 1990). Between the dorsal and ventral plates are lateral pleural membranes, which are the flexible part of the thorax, extending longitudinally through the entire length of the thorax. The pleural areas bear five or four pairs of pear-shaped brachial chambers concealed within the pleural membrane. These chambers had been interpreted as the homology of the gill pouches in chordates (Shu *et al.*, 2001b). In contrast, the body wall is continuously extended, representing subsidence of the body wall that conflicts with the “gill-pouches” hypothesis.

The abdomen was compacted dorso-ventrally in an oar-like form, lacking appendages. It is segmented and each segment is boxed by a dorsal plate, two narrow lateral pleural joints and a ventral plate (Brusca and Brusca, 1990). The segmented sclerites are jointed with an arthropodial membrane.

Deuterostome affinity of the vetulicolians is refuted by evidence of the segmented, jointed exoskeleton that is an arthropod-only character. The putative deuterostome gill slits are actually a subsidence of the body wall and the similar structure known as branchial chambers are also present in a number of modern decapod crustaceans. Whereas the segmented, jointed exoskeleton shows affinity with arthropods, the non-segmented head with the mouth at the terminal end in vetulicolians denies their arthropod identity. Their head however resembles that of some tardipolypods (*Microdictyon* and *Paucipodia*) with non-segmented heads. One may speculate that vetulicolians had evolved among tardipolypods but from a species different than that which led to the line of arthropod evolution.

Discussion

Evolution of head segmentation in Panarthropoda

The head in arthropods is composed of serial segments but the serial homology and segmental identity in the extant arthropod head have been obscured by fusion, modification and replacement in some taxa, especially insects. Because of this, the head segmentation in arthropods has been a long-lasting controversy regarding these questions: how many segments and which ones are nested in the head; how many head segments are in the ancestral groups of the arthropods; and is the anterior head segmental or non-segmental origin? The comparative studies of the fossil representatives in different evolutionary stages from the Lower Cambrian Maotianshan Shale contribute novel understanding to the answers of these questions.

The Cambrian limb-bearing worm-like animals known as

tardipolypods represent stem-lineage panarthropods. This animal group has a simple tagmosis of the body into an anterior head and a posterior trunk. Its head is scarcely distinguishable from the trunk and its appendages are similar to those in the trunk but are more dorsally situated. The tardipolypods contain three different types of head, e.g. non-segmented head, one segmental head and two segmental head. Among them, the two segmental head has a special interest for sharing an identical number of head segments with that in the stem-lineage arthropods known as proarthropods. The groundplan of the arthropodal head as represented by proarthropods was short, comprised of two segments, which corresponding respectively to the ocular and antennal segment of the anterior head region of euarthropod syncephalon (Fig. 5; Fig. 11B). This 2-segmental primitive head is known as procephalon. The ocular segment bears a pair of stalk eyes and the antennal one has a pair of short antennae.

The morphological gap between the two-segmented head in

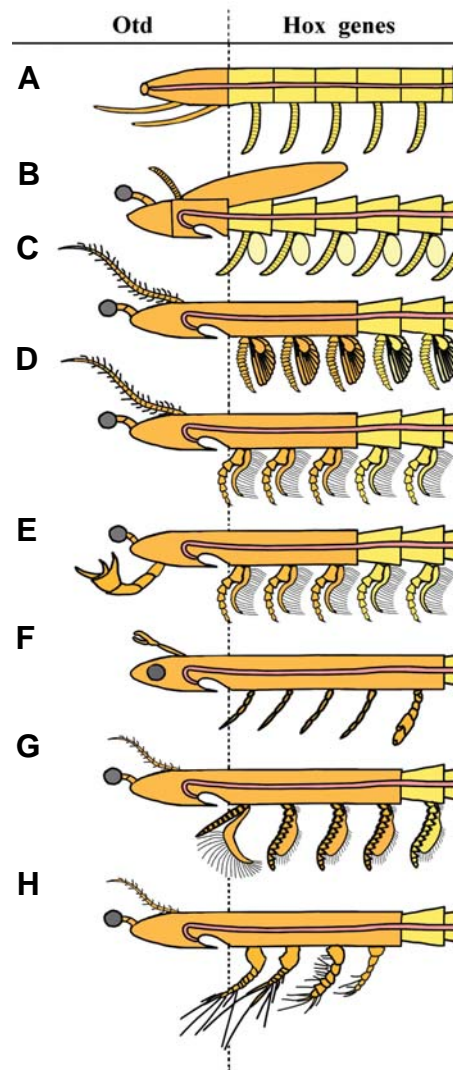


Fig. 11. Head groundplans of: *Pararthropoda* (A); *Proarthropoda* (B); *stem-lineage Euarthropoda* (C-D); *Chenlicarata* (E); *Euchelicerata* (F); *Mandibulata* (G); and *Eucrustacea* (H). Brown color representing head; yellow, trunk.

tardipolypods and arthropods is very great and the evolution of the tardipolypod head to the arthropod head occurred rapidly and remains poorly understood.

The evolution of arthropod head segmentation is marked by the recruitment of new segments from the trunk to form the new post-oral head region. The Maotianshan Shale yields diverse stem groups of the euarthropods, whose head had more segments, usually 5 (Fig. 11, C-E). The 5-segments are the principal characteristic of the groundplan in the arthropod head, whose anterior 2 segments are virtually identical to the head in proarthropods. The posterior 3 ones are a novel structure, which were presumably recruited from the anterior trunk. The question of why the first head expansion of exactly 3 segments from trunk remains a puzzle.

Stem lineages of Euarthropods are diverse in the Maotianshan Shale deposits. Unlike proarthropods, the head in the stem-lineage euarthropods is larger, composed of 5 segments. The anterior 2 segments are virtually identical to the head in proarthropods. The posterior 3 segments are additional structures, which were presumably recruited from the anterior trunk through the loss of related joints between the anterior trunk tergites. Each of the segments carries a pair of appendages identical to that in the trunk. Why the first head expansion that had recruited 3 but not in other different segments from trunk remains mysterious. The head in crown lineage arthropods however contains more than 5 segments, 6 in crown groups of Mandibulata and 7 in the crown groups of Chelicerata (Fig. 11). This suggests that the head segmentation in crown lineages of Euarthropoda has been achieved with two separated expansion events.

The presence of a stem-group crustacean (*Ercaia*) with a 6-segmental head from the Lower Cambrian Maotianshan Shale suggests that the second event of head expansion in the Mandibulata was deeply rooted among the proarthropod/euarthropod transition in the Early Cambrian (Chen *et al.*, 2001), and predated the second event of head expansion in the Euchelicerates. A 7-segmental mode characterizes the groundplan in the euchelicerate head and it suggests that the second expansion event occurred in the Silurian when the first known 7-segmental chelicerate head is reported from fossil the record (Sutton *et al.*, 2002).

Is the anterior-most part of the head a true segment or does it represent the non-segmental acron?

Because the head in insects has been highly modified and some of its structures have been displaced, the head segmentation and the identity of the head structures have long been an issue of controversy. The labrum (or upper lip), a plate-like process anterior of the mouth field, is the most unexpected structure. It led some authors to speculate that the anterior-most head part is of non-segmental origin. They coined the term "acron" for representing this non-segmental part. The acron includes the labrum only according to some authors (Snodgrass, 1935; Rempel, 1975; Scholtz, 2001) but it has been interpreted to represent a fused anterior head part including the labrum, eye and protocerebrum (Brusca and Brusca, 1990). Based on the above interpretation, the pre-oral head part is composed either of a non-segmental part and two segments, or of a non-segmental part and a segment. The appendicular origin of the labrum however has been well supported both by embryological (Eastham, 1930; Butt,

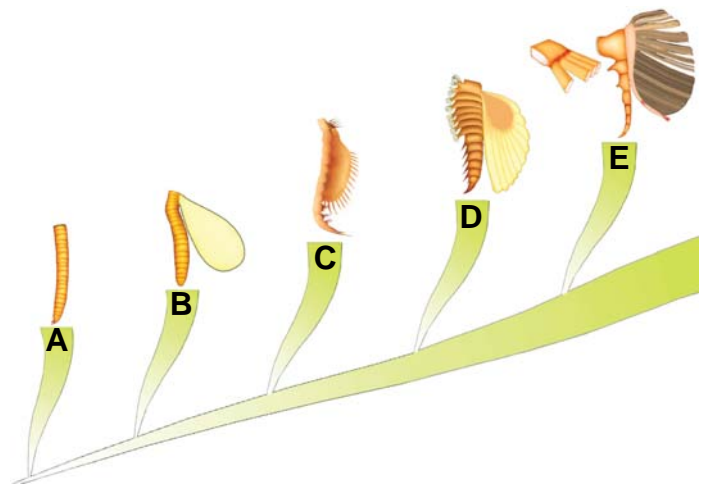


Fig. 12. Post-antennal limb designs of: (A) stem-groups of Panarthropoda, in uniramous, multi-annulated tubular form; (B) Proarthropoda, in biramous, with a flap-like exopod and a simple tubular, multi-segmented endopod; (C-D) stem groups of Euarthropoda, in biramous structure, with endopods, which are either in a simple, multi-segmented tubular form (C), or differentiated into a distal and an expanded multi-segmented proximal domain (D); (E) advanced form of stem groups in Euarthropoda, in a distinct bipartite structure consisting a distal limb and an expanded large basipod with serrated inner edge (D).

1960; Rempel, 1975) and molecular developmental studies (Schmidt and Technan, 1992). The questions of whether the labrum represents the anterior-most segment, or alternatively of the intercalary segment, and the insect head is 6 segmental or 7 segmental mode are still part of ongoing debate.

Fossil evidence reveals that the order of arthropod head segmentation is conservative and deeply rooted in the first arthropods. None of the structure has ever been observed from fossils at a position anterior to the ocular segment in early arthropods. The 6-segmental mode for insect head and the labrum as fused appendages of the intercalary segment are well grounded also by fossil observation.

Evolution in Panarthropod appendages

The diverse arthropod fauna together with their worm-like ancestors provide a unique window for establishing the evolutionary scenario of the arthropod appendages. It suggests that the arthropods are derived from a worm-like ancestor in close resemblance to the "*Hallucigenia+Cardiodictyon*" clade. This presumed worm-like ancestor had a simple elongated body divided into serial segments. Each segment had a pair of uniramous appendages that were soft-bodied and unjointed in a single tabular form. These appendages consisted of multiple annulations, and lacked intrinsic musculature. The preservation of a central cavity in some appendages suggests that these limbs were hydraulic. The appendages were paired and undifferentiated except for head appendages that are in a more dorsal location and slender.

The evolutionary origin of the Arthropoda is marked by the transformation of the head appendages into sensorial organs, and in the transformation of the uniramous trunk appendages into biramous appendages (Fig. 11, A-B). The post-antennal limbs in the first arthropods were tubular, slowly tapered and multi-

annulated, bearing a striking resemblance with those in worm-like ancestors. They were composed of 17-20 articles, which are regularly ring-shaped, and connected to each other by a pivot joint (Hou *et al.*, 1999; 2004). The presence of a pivot joint suggests the presence of intrinsic muscles and that these ring-shaped articles were true segments.

The PD (proximodistal) specification lays the core in the evolutionary change of the limb design. This change drove the limb in a progressively increasing in differentiation along main axis by stepwise from the simple, multi-segmented tubular limb (Fig. 12, B-C) to bipartite design (Fig. 12E), via of intermediate step (Fig. 12D). The limb design in intermediate step as exemplified by *Saperion* and *Canadaspis* displays a clear P-D domain differentiation, with an expanded multi-segmented proximal domain. The transformation of this intermediate form into limb with two functional domains is likely marked with fusion of multi-segmented proximal domain into a single-segmental proximal domain known as basipod.

Both limb and antennae in extant arthropods share the same PD-axis patterning genes including: *Distal-less* (*Dll*), *dachshund* (*dac*) and *homothorax* (*hth*) during their development (Dong *et al.*, 2001). These patterning genes are either co-existent each other, or in a mutually exclusive interaction. Among the two different states of interaction in these patterning genes, the mutually exclusive regulatory one has competence for developing sharp boundary between domains. Both the limb and antennae as exemplified by proarthropods are in a simple tubular shape with least PD differentiation in early arthropods. This mutually exclusive regulatory interaction for limb or antennae patterning had not yet appeared in the early arthropods. Presence of bipartite antennae known as "prochelicerae" in stem groups of Chelicerata and bipartite limb among the stem groups of Euarthropoda implies that this mutually exclusive regulatory interaction of the patterning genes had evolved for helping reaction of the sharp boundary between the domains both in antennae and in limbs.

The appendage differentiation between head and trunk implies that the two axial molecular domains, e.g. *Otd/ems* and *Hox* genes had been established at the very beginning of arthropod evolutionary history. The *Otd/ems* genes in the head region were selectively expressed for being responsible in the modification of the head simple tubular appendages into stalked compound eyes and antennae respectively. *Hox* genes were selectively expressed in the trunk region, responsible for biramous appendage development. The exopod is a novel structure in the arthropods and its flap-like form represents an extended pouch of the body wall, which resembles with insect wing in general morphology and presumably had a gene cassette similar to that in wing developing of the extant insects.

Euarthropoda has been defined as monophyletic both on a 5-segmental head and the post-antennal limb design (Walossek, 1999; Maas and Walossek, 2001). The authors proposed that the post-antennal limb in Euarthropoda has a rigid, anterior-posterior compressed proximal limb portion (the basipod) and a 7-segmented endopod (Fig. 4, I). This bipartite limb design along its main axis however does not always appear among the stem groups of the Euarthropoda. The post-antennal limbs in numerous species among the distally related stem-groups of the Euarthropoda (*Canadaspis*, *Isoxus*, *Ercaia*, *Primicaris*, *Tanglangia*, *Fortiforceps*, *Leachoilia*, *Saperion* and *bradoriids*) do not appear

in an expected bipartite design but in a simple, sub-tubular shape with slight differentiation along their main axis (Fig. 4, E-H). These limbs consist of 9 to 15 segments. It implies that this bipartite limb design is not monophyletic. The A-P compressed basipod with an expanded and serrated inner edge is likely associated with feeding activity for breaking large food items into pieces and for anterior transportation of food into mouth. The origination of the expanded basipod with a serrate inner edge therefore is a pivotal event for changing the feeding behavior from a microphagous into a macrophagous organism.

The speciation of the appendages in arthropods has evolved roughly in order of the anterior toward posterior along body axis during arthropod evolution. The first event of appendage speciation was marked by the transformation of the first appendage into a visual organ, the eye stalk. The following event was the modification of the short limb-like structure in the second segment into a whip-like sensorial organ or grasping organ separately at transitional stage from proarthropods to euarthropods.

The speciation of the post-antennal appendages in the head of the Mandibulata is represented by three separate events. The early event exemplified by *Ercaia* is marked by the transformation of the first post-oral appendage into the 2nd antennae (Fig. 4, J-K). The newly discovery of Early Cambrian eucrustaceans both from north-eastern Yunnan, China (Zhang *et al.*, 2007) and from Shropshire, England (Sivetter *et al.* 2001) suggests that the origination of gnathus appendages followed soon after the origination of the 2nd antenna. The gnathal appendages (called mandible, maxilla 1 and maxilla 2) are the feeding organs, representing modified structures of the second to fourth post-antennal appendages.

The intercalary segment represents the first post-antennal segment both in insects and myriopods and it has long been interpreting as an appendage-less segment. The molecular studies (Boyan *et al.* 2002; Popadic *et al.* 1998) and the presence of tritocerebrum-originated nerves (Boyan *et al.*, 2002) propose that the labrum is the fused paired appendages of the intercalary segment. The modification of the 2nd antennae into a feeding organ, the labrum, is one of the important evolutionary changes leading to the lines of insects and Myriopods.

The differentiation of the appendages also occurred in order from the caudal one to the anterior. The loss of appendages characterizes this anterior-directed differentiation, linked to the process of abdominalization (Boxshall, 2004). Abdominalization may have occurred repeatedly in the evolutionary history of the arthropods. *Fuxianhuia* is the fossil representative of the earliest abdominalization event at the very beginning of arthropod evolutionary history.

Molecular and morphological head/trunk boundary evolution in Panarthropoda

Molecular and genetic research has confirmed that all bilateral animals, including arthropods and humans, possess a common genetic mechanism of the *Hox* cluster genes for patterning the anterior/posterior (A/P) axis along the body main axis except for its anterior-most part, which is in outside of *Hox* gene expression domain. Recently it has become evident that the anterior margin of the *Hox* gene domain is conserved among different extant arthropod groups, which lie at the position closely related to the border between the anterior and

posterior developing head of the extant arthropods but not tied to the morphological boundary between head and trunk (Fig. 11). The anterior head outside of the Hox gene expression is comprised of the two segments, e.g. the anterior ocular and posterior antennal segment, which is innervated by protocerebrum and deutocerebrum respectively. The *otd/Otx* genes are involved both in the anterior head and the rostral brain patterning. The boundary of the above two developing body subdivisions, e.g. the anterior *otd/Otx* and posterior Hox-specified region are conserved not only across the different extant arthropod groups but throughout the different bilaterian animals also. That is why the fact that the molecular boundary does not match with the morphological head/trunk boundary remains difficult to understand. The fossils may provide the answer.

The morphological boundary of the head/trunk is not conservative. The trunk in proarthropods represents the post-antennal part of the body and the boundary between the head and trunk matches well with the molecular boundary between the *Hox* gene expression region anteriorly and the *ems/otd* gene expression region posteriorly. The arthropod head has a tendency of expansion with posterior displacement of the morphological head/trunk boundary and that is why the molecular boundary has a more anterior position than the morphological boundary (Fig. 11).

The head-segment-gain expansion characterizes the arthropod head evolution and it is achieved through the posterior shifting of the head rear border. The original position of the head/trunk boundary in arthropods as exemplified by proarthropods was situated at the rear margin of the antennal segment at a much more anterior position than that in extant arthropods. It implies that the molecular boundary of the anterior *otd/Otx* and the posterior Hox domain is very conserved and ancient, and its relationship with head segmentation has not changed since its first establishment in the earliest arthropods.

This ancient head/trunk boundary has morphologically vanished, however, leaving virtually no distinct morphological mark in all the extant arthropods except for few extant species. For instance, in developing *Drosophila*, a transient structure known as the cephalic furrow (CF) may represent the relic of this ancient head/trunk boundary. The CF lies at the juxtaposition of the patterning system that defines the procephalic and segmental germ (Costa *et al.*, 1993; Vincent *et al.*, 1997) and the cells in this transient structure all unfold back onto the surface of the embryo contributing to the ectoderm in later stages (Costa *et al.*, 1993), and is interpreted as an early organizer region analogous to the midbrain-hindbrain border in vertebrates (Bally-Cuif & Wassef, 1995; Joyner, 1996). The antennal segment forms a posterior part of the early arthropod head and it bears a very large and posteriorly-extended cephalic shield. This large and posterior-extended tergite of the antennal segment is a character of the first arthropod head and a similar structure also reappears in some extant Crustacea (Casanova *et al.*, 2002).

Is the tripartite brain deeply rooted?

The developmental regulatory genes of the *orthodenticle* (*otd/Otx*) gene family and the Hox gene family not only act in the specification of the embryonic, antero-posterior body axis but

also are involved in the brain development of both insects and mammals. The *otd/Otx* genes are involved in their rostral part of the brain development and the *Hox* genes in their caudal brain development. The molecular and genetic similarity between insects and mammals in their brain development has led some to draw the conclusion that a tripartite organization of the embryonic brain was already established in the last common bilaterian ancestor of protostomes and deuterostomes (Hirth *et al.*, 2003).

The present study however denies this early origin of the tripartite brain organization. The arthropods have readable head segments and their head segments and related appendages are closely related to brain regionalization. The relationship of the head segment (related structure) with brain regionalization is consistent across distally-related groups of the extant arthropods. It implies that this relationship is very conserved and ancient, and has been established since the first appearance of the arthropods. The visual system in the ocular segment is an important component of the protocerebrum and the olfactory centers of antennal segment is the major component of the deutocerebrum. As exemplified by Lower Cambrian proarthropods, all these systems and the related protocerebrum and deutocerebrum already presented in the first arthropods.

The caudal brain comprises the posterior subesophageal ganglion and three subesophageal neuromeres, which are connected to the second antennal and three gnathal segments of the posterior head, respectively. It is connected with the post-antennal head segments and apparently had not yet developed in the first arthropods, whose post-antennal head had not yet developed. The caudal brain is therefore a secondarily derived structure and likely originated from modification of the trunk ganglia. In extant insects, the caudal brain (tritocerebrum) is composed of the mandibular, maxillary and labial neuromeres connected with respectively with feeding organs including: labrum, mandibular, maxillary, and labial. The stem groups of Euarthropoda have only three post-oral segments and related unspecialized appendages. These post-oral head segments and related appendages are in a striking homology with those in the trunk. The caudal brain had unlikely evolved in early euarthropods.

Origin of the neural crest cells

The crest animals contain both craniates and the extinct stem-lineage craniates. Among the Early Cambrian crest animals, *Haikouella* and *Yunnanozoon* were the most-primitive. These animals are preserved with products of neural crest cells (cartilaginous branchial bars with gill rays, pharyngeal denticles) and show evidence of the presence of branchiomic ventilatory muscles. These muscles are produced essentially through neural crest cell signaling (Graham and Smith, 2001), suggesting the presence of such neural crest cells. *Haikouella* and *Yunnanozoon* were definite crest animals. In addition to the emergence of the neural crest cells, the animals also display some other craniate features including a large differentiated brain, protovertebrae, nostrils and a pair of lateral eyes, lower and upper lips. But they lacked some craniate features, such as a skull, ears, telencephalon, and fin rays. It seems obvious that the craniate traits had evolved through two separate evolutionary events. The emergence of the neural crest cells, and development of relatively large

brain, lateral eyes, nostrils, upper and lower lips, protovertebrae, pharyngeal denticles, and widely-spaced and jointed thick branchial arches characterize the first event, which are represented by the extinct stem group of the crest animals including *Haikouella* and *Yunnanozoon*. *Haikoichthus* and *Mylokunmingia* also were pre-craniate crest animals, but more advanced than *Haikouella* and *Yunnanozoon*. The following evolutionary event is characteristic of the development of other craniate characters including a skull, ears, telencephalon, and fin rays, and gave rise to craniates.

Neural crest cells derived from almost the entire length of the anterior-posterior axis of the side edges of the crest of the neural plates, except for anterior to the prospective diencephalon corresponding to telencephalon, where the neural crest cells are not formed. The cephalic neural crest cells derived from neural folds caudal to the epiphylis (corresponding to the region of the posterior diencephalon, mesencephalon and rhombencephalon) exerts a critical effect of the rostral protrusion of the eyes first and then the cerebral hemispheres beyond the anterior end of notochord (Etchevers *et al.*, 1999; Creuzet *et al.*, 2006). The anterior border of the neural crest presumably first established at the first emergency of the crest animals and it was situating at anterior margin of the head (corresponding to anterior margin of the diencephalon) in the first crest animals. The relationship of the anterior border of the neural crest with brain seems to be conserved and has no change since its first establishment. The telencephalon that lies anterior to the diencephalon is a novel structure and had originated in the precraniate/crabriate transition through the anterior expansion of the brain. It is why the neural crest cells are not formed in the anterior-most portion corresponding to the telencephalon.

The dorsal midline ectoderm in invertebrate chordates, including extant tunicates and amphioxus, expresses many genes (*BMP2*, *Pax3/7*, *Msk*, *Dll*, and *Snail*) identical to those in vertebrate neural crest cells (Holland and Holland, 2001; Wada, 2001). Many of the *cis*-regulatory elements for the positioning of the neural crest cells are also present in amphioxus (Manzanares *et al.*, 2000). The remarkable similarity of the expression patterns between the vertebrate neural crest cell and ascidian dorsal midline epidermis had led Wada (Wada, 2001) denying neural crest cells as a novel vertebrate cell. Intriguingly, the cells expressing these genes in tunicate and amphioxus do not migrate; none of them differentiate into a wide range of tissues (Holland and Holland, 2001). Why do these ectodermal cells in amphioxus not evolve into neural crest cells, and what are they waiting for?

It is now known that the two transcription factors AP2 and *Distal-less* are critical in the formation of neural crest cells. The *Distal-less* gene in amphioxus has only one copy per haploid genome while in vertebrates it has 5 or 6 closely related copies originating from a single ancestral gene resembling that in amphioxus (Neidert *et al.*, 2001). These distal-less homologues have established new functions and at least 3 of them function in the patterning of neural crest cells. All these suggest that the acquisition of the migratory and differentiation properties of the ectodermal cells in amphioxus is likely waiting for the duplication and divergence of the *Distal-less* genes (Holland *et al.*, 1996) and for the archivement of novel regulatory mechanism of Hox genes (Wada, 2001).

Amphioxus has only a single *Hox* gene cluster with 13 genes, which are arranged in the same order as the corresponding

mouse genes. Within the craniates, the Lampreys *Petromyzon marinus* and *Lampetra fluviatilis* may have between 2 and 4 *Hox* gene clusters and the rest of the craniates, including the "lower" craniate the Hagfish *Eptatretus stoutii*, have 4 or more *Hox* gene clusters. This difference suggests that craniates have evolved at least two runs of wholesale duplication of the genome. The origination of the neural crest cells in the first crest animals including *Haikouella* and *Yunnanozoon* suggests that the first run of genome duplication had occurred at the beginning of the evolutionary history of the crest animals in the Early Cambrian.

Cristozoa groundplan in number of the branchial system

Pharyngeal arches (or pouches) are a basic chordate feature. They are numerous (up to a few hundred) and closely spaced in adults both in invertebrate chordates (ascidians and cephalochordates) and in hemichordates. In contrast to invertebrate chordates and hemichordates, the crest animals have much fewer and widely spaced branchial arches (or pouches), mostly no more than seven, except for agnaths, which have more branchial arches (or pouches) both in the living hagfish *Eptatretus* and in fossil agnathans (Janvir, 2004). The number of the gill-compartments in Silurian-Devonian galeaspid displays up to 45 (Janvir, 2004).

The brachial arches in the groundplan of the crest animals, as exemplified by *Yunnanozoon* and *Haikouella*, is characterized by having only 6 post-mandibular branchial arches, and a mandibular arch, which developed during the evolutionary stage of the pre-craniate crest-animals but had not yet evolved at the very beginning of the pre-craniate crest animals such as *Haikouella*. The branchial arches in the pre-craniate crest animals were morphologically slightly differentiated along their D-V axis and among different branchial arches.

The development of the branchial arches among the invertebrate chordates, especially in amphioxus, yields intriguing insight into the evolutionary origin of the branchial system in the crest animals. The branchial slits in extant amphioxus larvae number only a few and they first developed in the mid-ventral line and moved to the right side after 14 gill slits had formed (Young, 1981). A set of additional 8 gill slits are added to the first row after this. The larvae become bilaterally symmetrical by removing a set of 8 gill slits from the first 14 right row slits to the left and the loss of the remaining 6 gill slits. Further slits in amphioxus are added in pairs during development. The development of 8 pairs of slits in larvae is critical and especially interesting for understanding the origin of cristozoan branchial arches because the number of slits is similar to that in the groundplan of the crest animals. The branchial system with just a few branchial arches in Cristozoa is likely a larval version of the pre-Cristozoa ancestor achieved through paedomorphic processes.

The anterior-most branchial arch is the mandibular one, which appeared among the pre-craniate crest animals. The groundplan of the cristozoan mandibular arch, as exemplified by *Yunnanozoon*, is with a slight differentiation along its dorso-ventral (D-V) axis. Extant lamprey also have no obvious D-V differentiation. The transformation of the mandibular brachial arch into the jaw is marked by D-V speciation of the mandibular branchial arch, representing one of the earliest evolutionary novelties in the gnathostome vertebrate history. The establishment of the *Hox* gene-coded anterior-posterior (AP) axis and the *Dlx*-gene coded

dorsal-ventral (DV) axis in the pharyngeal area presumably are responsible for this event (Shigetani *et al.*, 2005). No obvious D-V axial speciation of the mandibular arch and the slight differentiation between the branchial arches imply that this Cartesian grid-like specification developmental mechanism had not yet been established in both the pre-craniate crest animals and in the agnathus.

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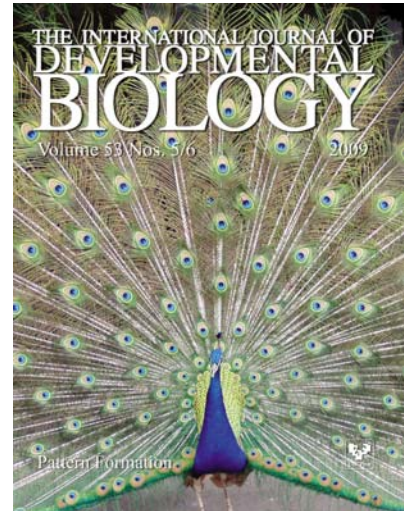
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