Unveiling an enigmatic fossil vertebrate from the Middle Devonian

The evolutionary history in deep time has long been the frontier of natural science. The fossil record provides the sole physical evidence for evolutionary processes and biodiversities in the past, but our understanding of fossil species is still far from sufficiency. It sometimes happens that an enigmatic fossil species cannot be compared with other known species owing to its very strange, incomprehensible body form. Such an enigma lasts for a long time until a breakthrough in paleontology.

Palaeospondylus gunni represents such an enigma. This species was first reported in 1890 [1] and is found in the Middle Devonian (about 390 million years ago) deep lacustrine deposits [2]. Thus far, thousands of specimens of *Palaeospondylus* have been collected, but this species remained mysterious, making its classification very difficult. This small (about 5 cm in body length) fishlike creature appears not to possess paired fins, unlike most extant fishes. Teeth are also lacking. In addition, the cranial dermal bones that cover the brain and sensory organs in most vertebrates are missing from *Palaeospondylus*. These features imply that this species possesses a "primitive" type of body and belongs to the lineage of cyclostomes, a jawless vertebrate group that diverged from the lineage of most vertebrates around 500 million years ago. On the other hand, *Palaeospondylus*, whose name stands for "ancient vertebrae", is furnished with well-developed vertebrae. Since distinct vertebrae are seen in evolutionarily new groups of vertebrates, such as teleost fishes and tetrapods, this feature contradicts a close relationship to cyclostomes.

The Devonian period was "the age of fishes", in which the early evolutions of, for example, sharks and bony fishes, ancestors of terrestrial vertebrates, proceeded to build the morphological diversity seen at the present time. Thus, if the enigmatic Middle Devonian *Palaeospondylus* belongs to any group, it will provide an important clue to understanding the early evolution of that group.

One reason for the lack of clarity regarding the phylogenetic position of *Palaeospondylus* is that, despite improvements in observation techniques, it has been unclear what parts the skull is divided into because the boundaries of the joints could not be clearly observed. We successfully solved this problem by strategically preparing the best specimens and ultrahigh-resolution, high-contrast conditions: we searched for extremely rare fossils where skulls were completely covered by the matrix rock, to observe the skeleton inside the rock by X-ray CT imaging. Normally, the rock is split up on the surface where the fossil is located, but in this case, we wanted to examine completely intact skulls, so we found two specimens with only the heads covered by rock (Fig. 1). We then performed synchrotron radiation X-ray micro-computed tomography (SRXμCT) at SPring-8 **BL20B2** [3].

The specimens were first scanned with conventional micro-CT to detect the positions of skulls within the rocks. Then, the specimens were trimmed stepwise to reduce their sizes to obtain higher resolutions at SRXμCT. As a result, we were able to observe complete skulls showing a detailed morphology inside the rock at resolutions of up to 1.46 μm per pixel (Fig. 2(a)). The valuable 3D morphological data obtained in this research has also been published in the database MorphoSource (https://www.morphosource.org) and made available to researchers around the world.

The key point of this research is that we were able to observe even the microstructures inside the skeletal elements in three dimensions. In particular, the spaces housing cells, or cell lacunae (Fig. 2(b)), were clearly recognizable. Detailed observations revealed that the histological features of the *Palaeospondylus* skull elements were comparable to those found in living bony fishes and tetrapods. By following the cell lacunae on the tomographic images, it was possible to determine the locations of boundaries between skeletal elements.

The morphology of each skeletal element of the *Palaeospondylus* skull can now be compared with that of other vertebrates. For example, a close examination of the occipital part of the skull revealed

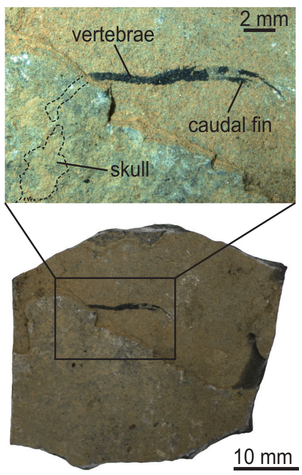

Fig. 1. Fossil specimen of *Palaeospondylus gunni*, NSMPV 24679, from the Middle Devonian of Scotland (now at the National Museum of Nature and Science, Tsukuba, Japan). Rare specimen with only the skull covered by rock.

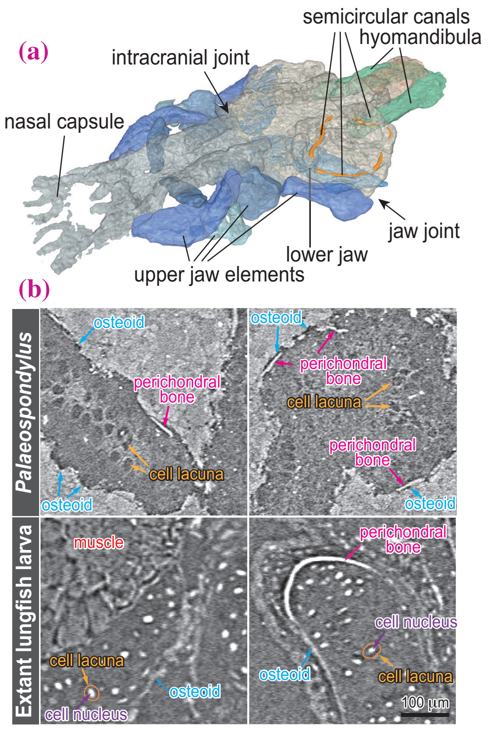

Fig. 2. (**a**) Skull of *Palaeospondylus*. (**b**) Skeletal histologies of *Palaeospondylus* and the extant sarcopterygian *Neoceratodus forsteri* (Australian lungfish).

three tubes where the three semicircular canals used to be housed (Fig. 2(a)), providing definitive evidence that *Palaeospondylus* is among the jawed, rather than jawless vertebrates. Among the many other morphological features revealed, one of the most surprising was the presence of an intracranial joint, which divided the skull into two (Fig. 2(a)). This intracranial joint is a feature shared with sarcopterygian vertebrates, the lineage consisting of coelacanths, lungfishes, and tetrapods. In addition, some other features of *Palaeospondylus* were shared with tetrapods among sarcopterygians.

These morphological characteristics enable the phylogenetic analyses of *Palaeospondylus* for the first time since its discovery in 1890, eventually placing this species within the lineage of tetrapods (Fig. 3). The Devonian period was a time when vertebrates began their forays onto land, and some tetrapods, such as *Eusthenopteron*, of this period possessed fins instead of limbs, despite the name of the group. The results of our phylogenetic analyses demonstrated that *Palaeospondylus* was likely closer to tetrapods possessing either limbs or limb-like fins, such as *Acanthostega*, *Elpistostege*, and *Tiktaalik*, than to tetrapods possessing fish fins, such as *Eusthenopteron*. The enigmatic *Palaeospondylus* was, to the best of our current knowledge, a member of the lineage of our ancestors expanding onto land. The absence of teeth, cranial dermal bones, and paired appendages in *Palaeospondylus* suggests that the morphotype of this species is comparable to that of the larvae of tetrapods. The combination of the larval-like body plan and the relatively short jaw resulted in the retracted mouth opening of *Palaeospondylus* (Fig. 3), the latter of which is seen in only limited species of tetrapods (among living tetrapods, some caecilians have a similar proportion), had probably been an obstacle to the classification of *Palaeospondylus*, as it rendered a very strange, incomprehensible impression.

Although the question of whether *Palaeospondylus* was a true larva or merely a larval-like form remains, our finding is expected to make a significant contribution to the overall picture of the evolutionary history of vertebrates over a period of 500 million years.

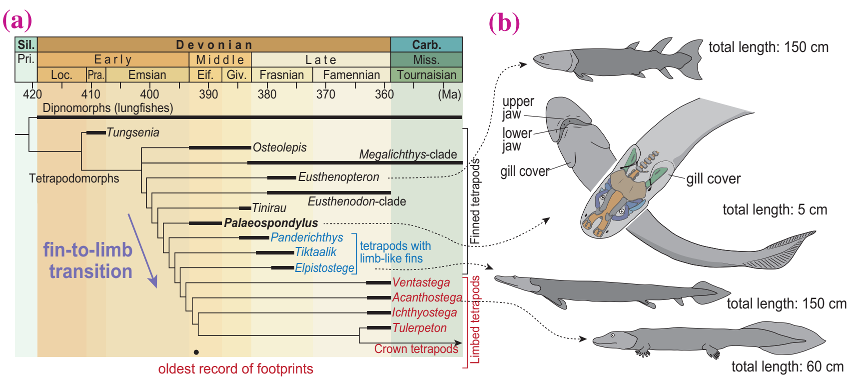

Fig. 3. (**a**) Phylogenetic position of *Palaeospondylus*. Bold lines show ages of fossils of corresponding taxa. Carb., Carboniferous; Eif., Eifelian; Giv., Givetian; Loc., Lochkovian; Miss., Mississippian; Pra., Pragian; Pri., Pridoli; Sil., Silurian. (**b**) Life restorations of Devonian tetrapods. From top to bottom, *Eusthenopteron*, *Palaeospondylus*, *Elpistostege*, and *Acanthostega*.

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