Palaeopathology in a Cretaceous terrestrial lizard from China.

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Abstract
The lizard genus *Yabeinosaurus* is a common and relatively well-known member of Chinese Lower Cretaceous Jehol Biota, found in both the Yixian and Jiufotang formations of north-eastern China. Previous research on *Yabeinosaurus* has revealed information on its morphology, phylogenetic position, coloration, diet, and viviparous reproductive strategy. Herein we describe a new specimen preserving the skull and postcranial skeleton. The skull shows features characteristic of *Yabeinosaurus robustus*, but reveals the morphology of the vomer for the first time. In the postcranial skeleton, the most significant feature is a malformation of the fibula resulting from a fracture that occurred several months before the animal died, possibly as the result of intraspecies aggression or a predation attempt.

Key words: Fossil lizard; *Yabeinosaurus*; pathology; China; Cretaceous

Introduction

The Early Cretaceous Jehol Biota is known from deposits of the Yixian and Jiufotang formations of western Liaoning, adjacent Inner Mongolia, and northern Hebei, and from the Huajiying Formation of northern Hebei. Temporally it ranges from the Barremian to Aptian, i.e., for at least 10 Ma (130–120 Ma) (Pan et al. 2013). The Jehol Biota includes a rich assemblage of vertebrates, invertebrates and plants, which together open an important window into the Early Cretaceous life of this region (Zhou 2014). Among the vertebrates from the Jehol Biota are several lizards, including some of the most complete lizard specimens of this age anywhere in the world (Evans et al. 2005; Evans and Wang 2012; Zhou 2014). The first lizard to be described from the Jehol Biota, *Yabeinosaurus tenuis* (Endo and Shikama 1942), was based on a single juvenile specimen from the Yixian Formation at Tsaotzushan (alternative transliteration Zaocishan), in what is now Liaoning Province. However, the holotype was lost in World War 2, leaving only a text description and poor quality images. Over the past two decades, the recovery of additional *Yabeinosaurus* specimens has clarified our understanding of this taxon. The new specimens include adult individuals (e.g. IVPP V 13285) with snout-pelvis lengths (SPL) ranging from 300- 350 mm, and also sub-adults (IVPP V 13284) and juveniles (IVPP V 12641). Ji et al. (2001) designated a neotype for *Y. tenuis*, but they chose another poorly ossified juvenile specimen. With the identification of a new species of *Yabeinosaurus* (*Y. bicuspidens*) from the type locality (Dong et al. 2017), the immaturity of Ji et al.'s neotype made it impossible to determine whether that specimen was a juvenile of the original *Y. tenuis* or of *Y. bicuspidens*. Dong et al. (2017) therefore recommended that *Y. tenuis* should be regarded as a nomen dubium, and that the well-preserved specimens more recently referred to *Y. tenuis* should be transferred to a new, diagnosable, species, *Y. robustus* for which IVPP V 13285 was designated as the holotype.

Yabeinosaurus robustus is one of the most completely known Early Cretaceous lizards. More than 30 specimens are recorded, and these include individuals that provide evidence of diet (fish: Wang and Evans 2011; Evans and Wang 2012; crayfish: Xing et al. 2019), skin coloration (transverse banding: Xing et al. 2019), and reproductive strategy (viviparity: Wang and Evans 2011). *Yabeinosaurus* appears to have been a primarily terrestrial lizard that foraged in and around the water. Here we describe a specimen of *Y. robustus* that shows a hind limb pathology.

Institutional abbreviations

YLSNHM, Yingliang Stone Nature History Museum, Nan'an, China

Material and methods

The specimen, YLSNHM01202, was recovered from the Jiufotang Formation at the Lamadong locality, Jianchang County, Liaoning, China and is housed in the Yingliang Stone Nature History Museum (YLSNHM), Nan'an, China. The Jiufotang Formation is composed primarily of lacustrine sandstones, shales, mudstones, and interbedded tuffs (Chang et al. 2009), and has been dated as Aptian, approximately 120 Ma (Smith et al. 1995; He et al. 2004, 2006). To date, the Lamadong locality has yielded further specimens of *Yabeinosaurus* (Xing et al. 2019) as well as confuciusornithid, enantiornithine, and ornithurine birds, pterosaurs, and mammals (Wang and Zhou 2003; Zhou et al. 2003).

A Canon digital camera (SD Mark III EF 100mm f/2.8 IS USM) and a Dino-Lite USB Digital Microscope (200x optical magnification) were used to image the specimen, followed by a focus stacking procedure using helicon Focus 5.1 and Adobe Photoshop software.

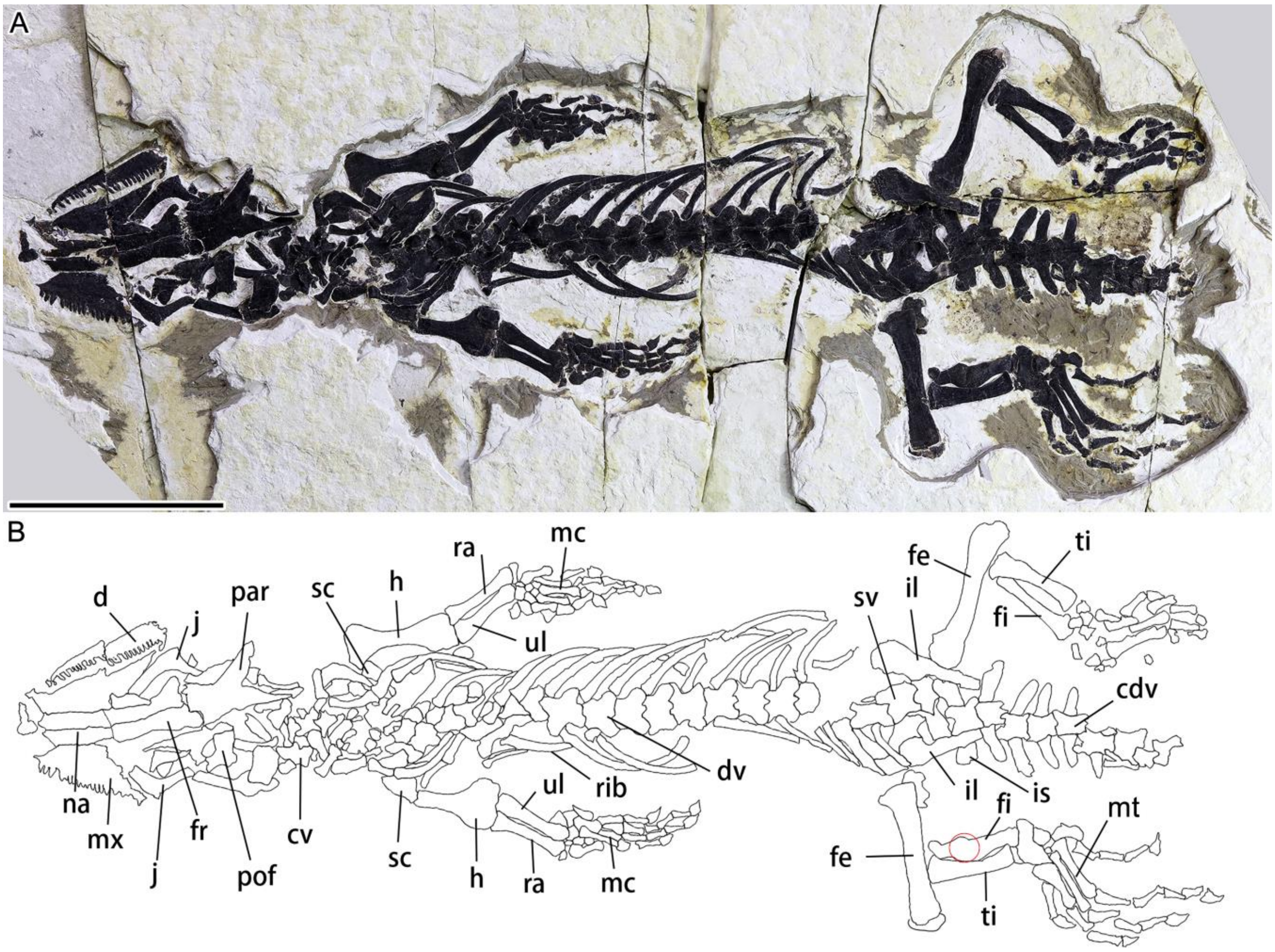

Fig. 1 The complete specimen of *Yabeinosaurus robustus* (YLSNHM01202). Scale bar = 50 mm. Abbreviations: cv, cervical vertebra; cdv, caudal vertebra; d, dentary; dv, dorsal vertebrae; fe, femur; fi, fibula; fr, frontal; h, humerus; il, ilium; j, jugal; mc, metacarpals; mt, metatarsals; mx, maxilla; na, nasal; par, parietal; pof, postorbitofrontal; ra, radius; sc, scapulocoracoid; sv, sacral vertebra; ti, tibia; ul, ulna.

Results

Morphology and attribution

YLSNHM01202 can be attributed to *Yabeinosaurus robustus* (Dong et al. 2017) based on the morphology of its skull (e.g. narrow paired nasals; paired sculptured frontals; interdigitated frontoparietal suture; elongate parietal with posteriorly directed supratemporal processes; L-shaped jugal with small posteroventral spur; large postorbitofrontal; deep maxilla) and dentition (unicuspid and slightly recurved).

The new specimen of *Y. robustus* has an SPL of approximately 225 mm, and an estimated total length of 600 mm. As such, it is larger than the holotype (IVPP V 13285, ~190 mm SPL) but smaller than the largest known specimen (IVPP V.13285) with an estimated SPL of 300 mm. YLSNHM01202 was probably sexually mature, but the disarticulation of the skull and the incompletely fused limb bone epiphyses demonstrate that it had not reached full adult size.

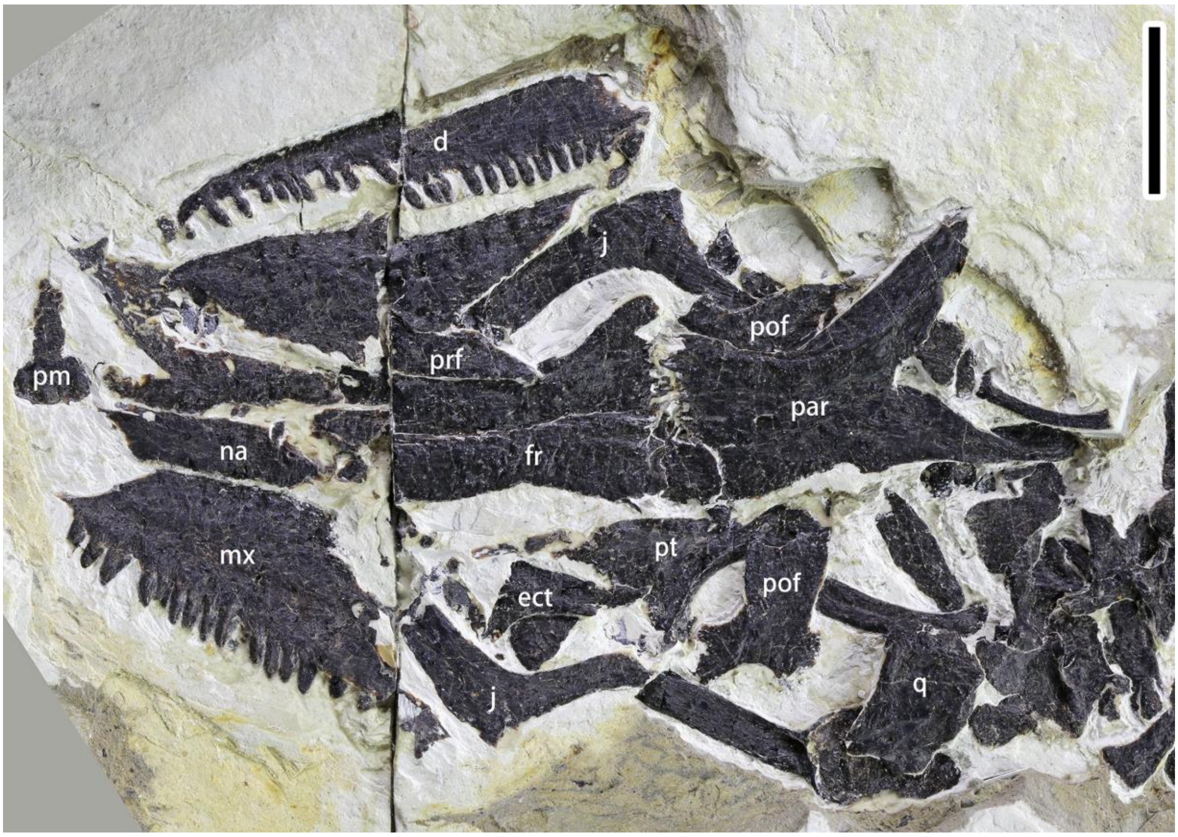

Fig. 2 Morphology of skull of *Yabeinosaurus robustus* (YLSNHM01202). Note that the break through the rostral part of the skull has resulted in a small misalignment of the affected elements. Abbreviations: d, dentary; ect, ectopterygoid; fr, frontal; j, jugal; mx, maxilla; na, nasal; par, parietal; pm, premaxilla; pof, postorbitofrontal; prf, prefrontal; pt, pterygoid; q, quadrate. Scale bar = 10 mm.

As in most *Y. robustus* specimens, the premaxilla of YLSNHM01202 is a single bone, with no obvious trace of a midline suture. This supports the conclusion that the apparent persistence of a suture line in the largest known skull of *Y. robustus* (IVPP V.13285, Evans et al. 2005) is atypical. However, YLSNHM01202 itself is unusual in that it lacks an obvious parietal foramen, a feature retained, albeit small, even in IVPP V13285. YLSNHM01202 does provide the first clear view of the vomer, the right bone lying anterior and medial to the tip of the right maxilla. It is narrow anteriorly, with a short premaxillary process, and widens posteriorly into a thin plate.

The postcranial skeleton also matches that of previous descriptions and emphasizes the robustness of the skeleton and the rather short, but powerful limbs. However, YLSNHM01202 is primarily of interest in the pathology of the left fibula.

Paleopathology

The right fibula illustrates the normal shape of this element –almost parallel-sided in the proximal 80% of the shaft, and expanded distally at the joint with the astragalocalcaneum. The bone is thinner than the tibia, but is still robust. By contrast, the left fibula is strongly misshapen. Both the internal (tibial side) and external margins of the proximal fibula component are expanded, in contrast to the distal fibula component in which a similar expansion occurs only on the internal margin.

Fig. 3 The palaeopathological details of the left hind limb of *Yabeinosaurus robustus* (YLSNHM01202). A, left tibia and fibula, B, detail of fracture callus on left fibula. Black arrows indicate infilled vascular channels described in the text. Abbreviations: a-c, astragalocalcaneum; fi, fibula; fr, fracture line; ti, tibia. Scale bars: A = 5 mm; B=1 mm

There does not appear to be any shortening of the overall bone length. A second alteration is in the proximal articulation of the affected bone. It is unclear whether the apparent tuberosity is a superimposed fragment, an epiphysis that has partially fused in a non-aligned manner, or a displaced cyamella (a sesamoid that lies between the femur and fibula, Rewcastle 1980).

The middle two thirds of the affected bone are roughened, compared to the surface of normal bone. The most prominent feature is the increase in transcortical channels, compared to the unaffected, adjacent tibia. The white spots (arrowed in Figure 3B) are infilled vascular channels. These are analogous to Grüneboom et al.'s (2019) description of trans-cortical capillaries in normal mouse bones. Whereas the latter were 10-20 µm in diameter, Herisson et al. (2008) described 21.6 +/- 0.9 µm diameter skull channels in mice, noting similar features in humans but with 5.3-fold greater diameter. The size of the channels in *Yabeinosaurus* is within that range.

The affected fibula lacks any of the filigree reaction associated with infection. The increased vasculature (channels) is compatible with a healing callus from a mid-shaft fracture.

Discussion

The peculiarity of this specimen is the abnormally enlarged and misshapen left fibula and the presence of a shallow groove in the midshaft indicative of a fracture experienced by this lizard when alive. A clean bone break followed by subsequent displacement of the two bone ends could leave a malformation of this kind, but judging from the degree of callus development, the injury probably occurred several months before the animal died. Pritchard and Ruzicka (1950) recorded that it took *Zootoca* (*Lacerta*) *vivipara* about 31 days to make a bony union in a fractured femur at 32-37°C, and Di Guiseppe et al. (2014) reported that a fractured chameleon humerus took six months to form a callus. In the *Yabeinosaurus* specimen, there are no obvious abnormalities in the associated tibia, the ankle, or the foot to suggest that these regions were being unusually strained. This suggests that, although presumably painful, the injury did not incapacitate the lizard, probably because the fibula is not weight bearing in this group (Rewcastle 1980) and it is bound to neighbouring bones by strong ligaments.

Most information on limb bone fracture occurrence comes from mammals, both domestic (e.g. Ben Ali 2013), where the injury is most likely to be due to vehicle impacts or occasional falls, and wild (e.g. small mammals: Forsman and Otto 2006, Bosch et al. 2016; carnivores: Argyros and Roth 2016; primates: Lovell 1990), although there is also some data on wild birds (e.g. Brandwood et al. 1986; Goodman and Glynn 1988). In each of these groups, fractures of the fibula are reasonably common (4% Grey squirrels, 18% of Virginia Opossums: Bosch et al. 2016). There is much less information on limb bone fractures in wild reptiles, and a majority of that stems from external observations of injury (e.g. missing toes or external scars, e.g. Vervust et al. 2009) rather than more detailed X-ray or CT examination. One exception is a radiographic study by Naldo et al. (2009) on a population of the agamid *Uromastyx* in Abu Dhabi which found five healed limb fractures in a sample of 90 lizards examined. However, with the exception of one illustrated femur, there was no record as to which bones were damaged. The palaeontological record of limb bone fractures is scattered through the primary literature. Most relates to dinosaurs (Rothschild and Martin 2006; Tanke and Rothschild 2014).

Limb injuries in wild reptiles are more likely to result from intraspecific fighting or predator action than from falls or impact injuries. Of 465 lizards (*Podarcis sicula*) on one island, Vervust et al. (2009) recorded 55.48% (=258) with at least one injured toe (more males than females). On a second island, the proportion was lower (12.87). Proportionally there were more injuries on the hind limbs than the forelimbs. They concluded that most of the injuries were due to intraspecific aggression, with a few (e.g. tail loss) resulting from predation attempts. *Yabeinosaurus* is a large lizard with strong teeth that seems to be relatively common in the Jehol community. Conspecific fighting seems the most plausible explanation for the injury, although we cannot rule out a predator attack.

The new specimen thus provides further palaeobiological information on what is, currently, one of the best known fossil lizards.

Conclusions

The specimen of *Yabeinosaurus robustus* described herein provides additional information on the skull (vomer) and displays a healing fracture of the left fibula. Judging by the development of the fracture callus, the injury occurred several months before the death of the lizard and is unlikely to have been the cause of death. The injury may have occurred as a result of intraspecific fighting or an unsuccessful predation attempt.

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Declaration of interest

The authors declare that they have no competing interests

References

Aygyros GC, Roth AJ. 2016. Prevalence of healed long-bone fractures in wild carnivores from the Northeastern United States. J Zoo Wildlife Med 47: 879–882.

Ben Ali, LM. 2013. Incidence, occurrence, classification and outcome of small animal fractures: a retrospective study (2005-2010). World Acad Sci Eng Tech Int J Anim Vet Sci 7: 191–196.

Bosch AM, Benson KJ, Mead AJ. 2016. Natural skeletal pathologies in a population of Gray Squirrels, *Sciurus carolinensis*, from Putnam County, Georgia. Georgia J Sci 74 (2), Article 6.

Brandwood A, Jayes AS, Mcn Alexander R. 1986. Incidence of healed fracture in the skeletons of birds, molluscs and primates. J Zool 208: 55–62.

Chang SC, Zhang HC, Renne PR, Fang Y. 2009. High-precision 40Ar/39Ar age for the Jehol Biota. Palaeogeogr Palaeoecol 280: 94–104.

Di Guiseppe M, Faraci L, Luparello M. 2014. Use of intramedullary pin for humeral fracture repair in a *Chamaeleo chamaeleon*. Natura rerum 3: 63–69

Dong LP, Wang Y, Evans SE. 2017. A new lizard (Reptilia: Squamata) from the Early Cretaceous Yixian Formation of China, with a taxonomic revision of *Yabeinosaurus*. Cretaceous Res 72: 161–171.

Endo R, Shikama T. 1942. Mesozoic reptilian fauna in the Jehol mountainland, Manchoukuo. Bull Cent Nat Mus Manchoukuo 3: 1–19.

Evans SE, Wang Y, Li C. 2005. The Early Cretaceous lizard *Yabeinosaurus* from China: resolving an enigma. J Syst Palaeont 3: 319–335.

Evans SE, Wang Y. 2012. New material of the Early Cretaceous lizard *Yabeinosaurus* from China. Cretaceous Res 34: 48–60.

Forsman ED, Otto IA. 2006. Healed fractures and other abnormalities in bones of small mammals. Northwest Nat 87: 143–146.

Goodman SM, Glynn C. 1988. Comparative rates of natural osteological disorders in a collection of Paraguayan birds. J Zool 214: 167–177.

Grüneboom A, Hawwari I, Weidner D, Culemann S, Müller S, Henneberg S, Brenzel A, Merz S, Bornemann L, Zed K, Wuelling M, Kling L, Hasenberg M, Voortmann S, Lang S, Baum W, Ohs A, Kraff O, Quick HH, Jäger M, Landgraeber S, Dudda M, Danuser R, Stein JV, Rohde M, Gelse K, Garbe AI, Adamczyk A, Westendorf AM, Hoffmann D, Christiansen S, Engel DR, Vortkamp A, Krönke G, Herrmann M, Kamradt T, Schett G, Hasenberg A, Gunzer M 2019. A network of trans-cortical capillaries as mainstay for blood circulation in long bones. Nature Metab 1: 236-250.

He HY, Wang XL, Zhou ZH, Wang F, Boven A, Shi GH, Zhu X. 2004. Timing of the Jiufotang Formation (Jehol Group) in Liaoning, northeastern China and its implications. Geophys Res Lett 31: 261–268.

He HY, Wang XY, Zhou ZH, Jin F, Wang F, Yang LK, Ding X, Boven A, Zhu RX. 2006. 40Ar/39Ar dating of Lujiatun Bed (Jehol Group) in Liaoning, northeastern China. Geophys Res Lett 33: 347–360.

Herisson F, Frodermann V, Courties G, Rohde D, Sun Y, Vandoorne K, Wojkiewicz GR, Masson GS, Vinegoni C, Kim J, Kim DE, Weissleder R, Swirski FK, Moskowitz MA, Nahrendorf M. 2018. Direct vascular channels connect skull bone marrow and brain surface enabling myeloid migration. Nature Neurosci 21: 1209-1217.

Ji SA, Lu LW, Bo HC. 2001. [New material of *Yabeinosaurus tenuis* (Lacertilia)] [Land and Resources] 2001, 41–43. Chinese.

Lovell NC. 1990. Patterns of injury and illness in Great Apes. Washington DC: Smithsonian Institution Press.

Naldo JL, Libanan NL, Samour JH. 2009. Health assessment of a Spiny-Tailed lizard (*Uromastyx* spp.) population in Abu Dhabi, United Arab Emirates. J Zoo Wildlife Med 40: 445–452.

Pan Y, Sha J, Zhou Z, Fürsich F. 2013. The Jehol Biota: definition and distribution of exceptionally preserved relicts of a continental Early Cretaceous ecosystem: Cretaceous Res 44: 30–38.

Pritchard JJ, Ruzicka AJ. 1950 Comparison of fracture repair in the frog, lizard and rat. J Anat 84: 236–261.

Rewcastle SC. 1980. Form and function in lacertilian knee and mesotarsal joints; a contribution to the analysis of sprawling locomotion. J Zool 191: 147–170.

Rothschild BM, Martin LM. 2006. Skeletal impact of disease. New Mex Mus Nat Hist Sci Bull 33. 226 pp.

Smith PE, Evensen NM, York D, Chang MM, Jin F, Li JL, Cumbaa S, Russell D. 1995. Dates and rates in ancient lakes: Ar- Ar evidence for an Early Cretaceous age for the Jehol Group, north-east China. Can J Earth Sci 32: 1426–1431.

Tanke DH, Rothschild BM. 2014. Paleopathologies in Late Cretaceous Hadrosauridae from Alberta, Canada. In: Eberth DA, Evans DC, editors. Hadrosaurs. Bloomington: Indiana University Press; p. 540–571.

Vervust B, Van Dongen S, Grbac I, Van Damme R. 2009. The mystery of the missing toes: extreme levels of natural mutilation in island lizard populations. Funct Ecol 23: 996–1003.

Wang XL, Zhou ZH. 2003. Mesozoic Pompei. In: Chang MM, Chen PJ, WangYQ, Wang Y, Miao DS, editors. The Jehol Fossils: The emergence of feathered dinosaurs, beaked birds and flowering plants. Shanghai: Shanghai Scientific and Technical Publishers; p. 19–35.

Wang Y, Evans SE. 2011. A gravid lizard from the Early Cretaceous of China: insights into the history of squamate viviparity. Naturwiss 98: 739–743.

Xing L, Taylor RS, Niu K, Evans SE. 2020. Integumentary remains and abdominal contents in the Early Cretaceous Chinese lizard, *Yabeinosaurus* (Squamata), demonstrate colour banding and a diet including crayfish. Cretaceous Res 108: https://doi.org/10.1016/j.cretres.2019.104320.

Zhou ZH. 2014. The Jehol Biota, an Early Cretaceous terrestrial Lagerstätte: new discoveries and implications. Natl Sci Rev 1: 543–559

Zhou ZH, Barrett PM, Hilton J. 2003. An exceptionally preserved Lower Cretaceous ecosystem. Nature 421: 807–814.