

Triassic marine reptiles gave birth to live young

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Sauropterygians form the largest and most diverse group of ancient marine reptiles that lived throughout nearly the entire Mesozoic era (from 250 to 65 million years ago)^{1,2}. Although thousands of specimens of this group have been collected around the world since the description of the first plesiosaur in 1821 (ref. 3), no direct evidence has been found to determine whether any sauropterygians came on shore to lay eggs (oviparity) like sea turtles, or gave birth in the water to live young (viviparity) as ichthyosaurs and mosasauroids (marine lizards) did⁴⁻⁶. Viviparity has been proposed for plesiosaur, pachypleurosaur and nothosaur sauropterygians⁷⁻¹⁰, but until now no concrete evidence has been advanced. Here we report two gravid specimens of *Keichousaurus hui* Young from the Middle Triassic of China. These exquisitely preserved specimens not only provide the first unequivocal evidence of reproductive mode and sexual dimorphism in sauropterygians, but also indicate that viviparity could have been expedited by the evolution of a movable pelvis in pachypleurosaurs. By extension, this has implications for the reproductive pattern of other sauropterygians and Mesozoic marine reptiles that possessed a movable pelvis.

Within the Sauropterygia, the two gravid specimens reported here are referred to the Pachypleurosauridae of the Pachypleurosauria on the basis of the presence of the following diagnostic features: the upper temporal opening smaller than the orbit, the presence of a distinct trough on the dorsal surface of the retro-articular process of the articular, no distal expansion of the sacral ribs, the pachyostotic pre- and postzygapophyses, and the reduced process-like dorsal iliac blade of the ilium². They can be further assigned to *Keichousaurus hui* Young, 1958 on the basis of the cervical region being longer than the trunk region, the short and blunt snout, the elongate upper temporal opening only slightly shorter than the orbit, the anterior position of the parietal opening, and the humerus longer and more robust than the femur⁹. Both specimens, housed in the National Museum of Natural Science (NMNS), Taichung, Taiwan, are nearly complete. One (NMNS-cyn2002-01) contains two embryos on each side, and the other (NMNS-VL191) at least three crushed embryos on each side. Both were collected from the Triassic limestone of Xingyi area, Guizhou province, southwestern China, probably from the late Middle Triassic Zhuganpo Member of the Falang Formation, as suggested by the fact that all other known specimens of this taxon have recently been confirmed to come from this member¹¹.

NMNS-cyn2002-01 is preserved in dorsal view. Its preserved length is about 296 mm, lacking the posterior portion of the tail after caudal 25 (Fig. 1a). The two embryos preserved on the right side are clearly more posterior in position than the two on the left, and at least the posterior one is directed posteriorly. The latter embryo, preserved in ventral view, reaches the second caudal rib posteriorly (cloacal or vent region) and the 11th dorsal (the 38th) vertebra anteriorly (Fig. 2). The anterior embryo, exposed in dorsal view, is not as well preserved, and is partly overlaid by the posterior embryo. The two left embryos are clearly separated from one another. The anterior one reaches anteriorly to the third dorsal

(the 30th) vertebra and the posterior one ends just anterior to the first sacral rib. The posterior one, exposed in ventral view, is clearly directed posteriorly. The anterior one is preserved in lateral view and seems to be directed anteriorly on the basis of the position of its scapula. The four embryos are mostly in articulation and their distribution on each side indicates that female *Keichousaurus hui* had a pair of oviducts as in ichthyosaurs¹² and many extant lizards¹³.

NMNS-VL191 has a total length of about 193 mm. It is preserved in dorsal view and compressed dorso-ventrally (Fig. 1b). It contains more embryos than NMNS-cyn2002-01 but they are poorly preserved. On the left side three embryos can be detected, all of which are posteriorly directed according to their rib orientation (Fig. 3). In contrast with NMNS-cyn2002-01, the left embryos of NMNS-VL191 are more posteriorly positioned than the right ones, posteriorly reaching the fourth caudal rib (although it may have been exaggerated by dorso-ventral compression *post mortem*), whereas the right embryos are more anteriorly placed, reaching the sixth dorsal (the 32nd) vertebra anteriorly. As in NMNS-cyn2002-01, the presence of embryos on both sides in NMNS-VL191 shows that female *Keichousaurus hui* must have had a pair of oviducts in life.

It has been well documented that embryos of ichthyosaurs are normally positioned head forwards as in modern cetaceans and that the gravid specimens with embryos head backwards represent an abnormal condition and might have caused the death of both mothers and embryos¹⁴. Most of the embryos in the two gravid specimens of *Keichousaurus hui* are head backwards and, similarly, they and their mothers might have been killed during birth because of abnormal carriage.

Although sexual dimorphism is common in the Pachypleurosauria^{8,9,15}, actual sex of each of the two morphotypes has never been demonstrated. In *Keichousaurus hui*, two sexes can be distinguished by the length ratio between the humerus and femur, and the structural complexity of the former. In one morph (sex X) the humerus is nearly as long as the femur and structurally simple, whereas in the other (sex Y) it is much longer than the femur and structurally massive⁹. In the two gravid specimens of *Keichousaurus hui*, the humerus lacks complicated structures and its length ratio to that of the femur is very similar to that of the specimens of sex X⁹. Therefore, in *Keichousaurus hui* sex X represents female and sex Y represents male. Because dimorphism in small European pachypleurosaurs such as *Neusticosaurus* and *Serpianosaurus*^{8,9,15} is closely

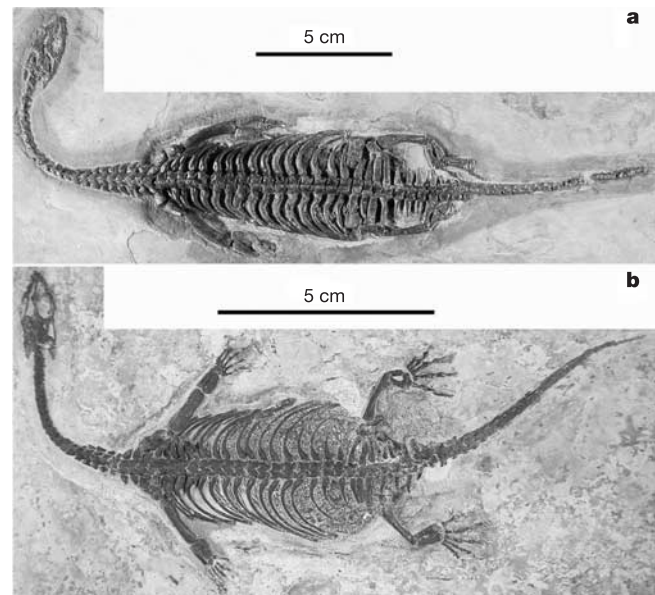


Figure 1 Two gravid specimens of *Keichousaurus hui* in dorsal view. a, NMNS-cyn2002-01; b, NMNS-VL191.

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comparable to that seen in *Keichousaurus hui*, it is now possible to sex the two morphs in those taxa as well.

Primitive sauropterygians, such as pachypleurosaurians and nothosaurians, lived in near-shore environments, intraplatform basins and shallow epicontinental seas², and were capable of leaving the water to brood or bask¹⁶, indicating that either oviparity or viviparity could have been practical strategies for these marine reptiles. Therefore, the achievement of viviparity in these primitive sauropterygians could be confirmed only by direct fossil evidence such as the two current specimens. In contrast with those of extant egg-laying sea turtles and other marine reptiles, the sacral ribs of *Keichousaurus hui* are rod-like and never fused to the sacral vertebrae proximally, and their middle element form a unique, peg-and-socket-like joint with the reduced dorsal blade of the ilium

distally. This indicates that a chain-like connection is present between the pelvis and sacrum (Fig. 4a). As in other marine reptiles such as ichthyosaurs and mosasaurs, the loss of a solid connection between the pelvic girdle and sacrum is correlated with aquatic habits. It has been suggested that the absence of a firm sacro-iliac joint in *Keichousaurus hui* would allow relative movement, possibly to accommodate stress generated during a sudden stop or sharp turn as the animal swam⁹. Once evolved, this joint would also allow the pelvic girdle to change its shape, maximizing the space of the birth canal. A chain-like sacro-iliac joint would certainly enhance labour, allowing the live young to pass through the birth canal and emerge as quickly as possible in a vulnerable marine environment.

As in *Keichousaurus hui*, the sacral ribs did not fuse proximally with their respective vertebrae nor tightly articulate distally with

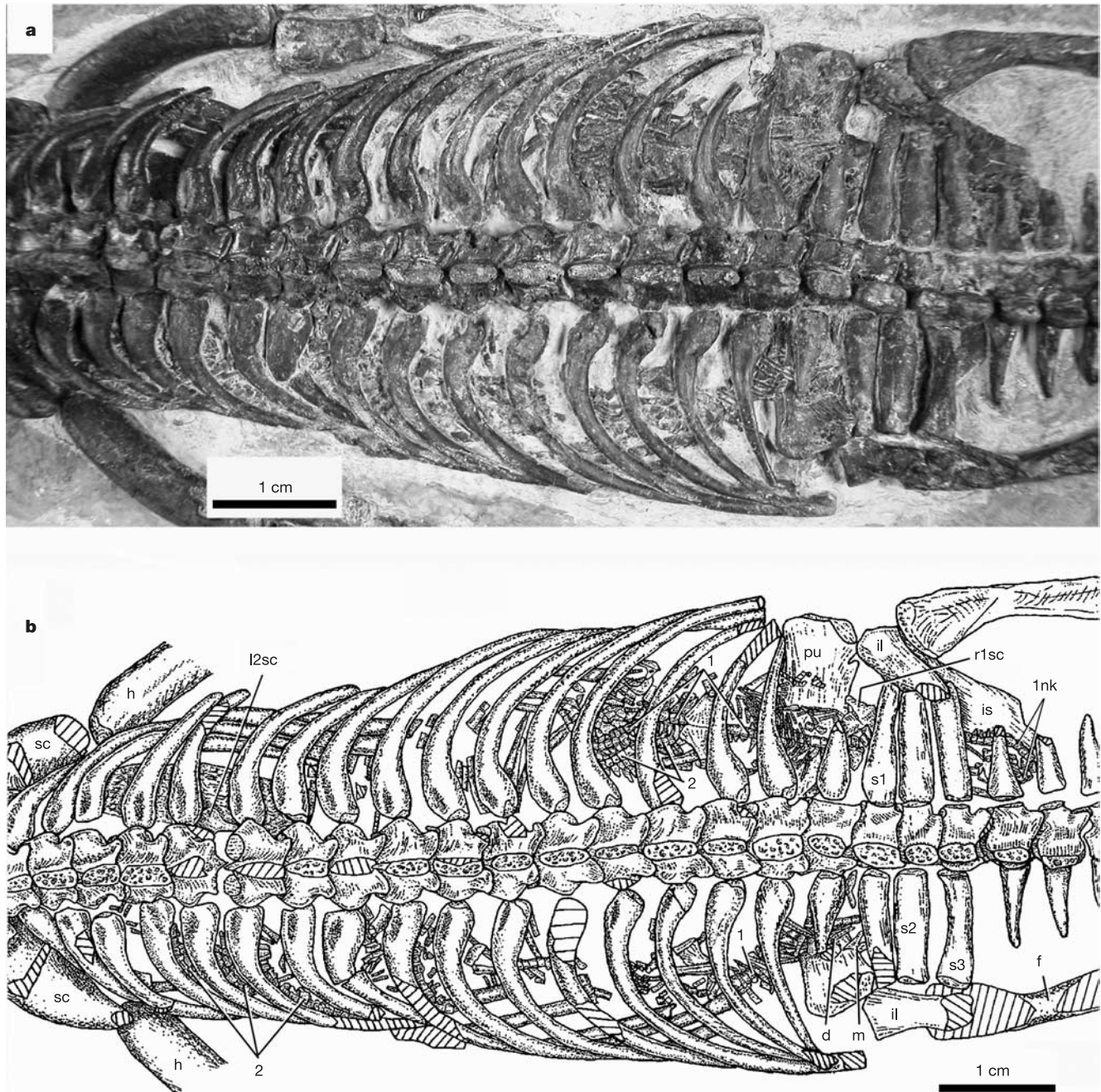


Figure 2 The trunk region of NMNS-cyn2002-01 in dorsal view. **a**, Actual specimen; **b**, drawing of **a**. Abbreviations: d, dentary; f, femur; h, humerus; is, ischium; l2sc, scapula of left anterior embryo; m, maxilla; pu, pubis of mother specimen; r1sc, scapula of right

posterior embryo; sc, scapula; s1–s3, sacral ribs 1–3; 1, posterior embryo of each side; 1nk, neck region of right posterior embryo; 2, anterior embryo of each side.

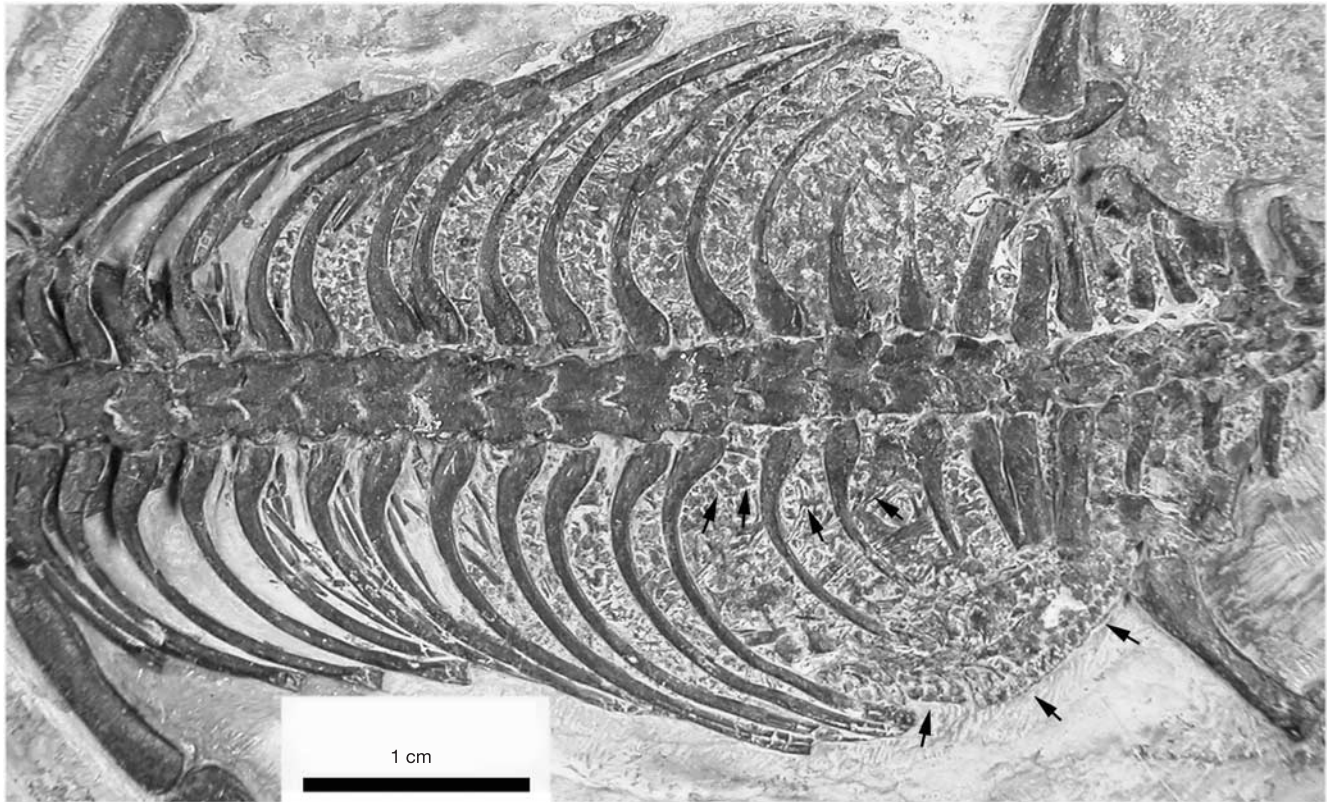


Figure 3 The trunk region of NMNS-VL191, showing three embryos (as indicated by arrows on left side).

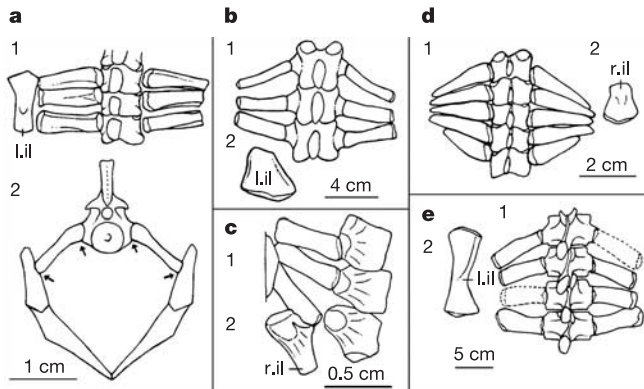


Figure 4 Sacral vertebrae and pelvis of selected sauropterygians.

a–c, Pachypleurosaur: **a**, *Keichousaurus hui* (1, the sacrum in dorsal view and left ilium in medial view, with its dorsal end directing downwards; 2, the reconstruction of 1 plus ventral part of the pelvic girdle, in posterior view; drawn based on NMNS-cyn2002-01); **b**, *Neusticosaurus* (= *Pachypleurosaurus*) *edwardsi* (1, the sacrum in dorsal view; 2, the left ilium in lateral view; redrawn from Fig. 19b in Carroll and Gaskill¹⁶); **c**, *Dactylosaurus gracilis* (= *s Schroederi*) (1, the sacrum in ventral and lateral view; 2, the right ilium in ventral view; redrawn from Fig. 1B in Sues and Carroll¹⁷). **d**, A nothosaur (*Lariosaurus valceresi*) (1, the sacrum in dorsal view; 2, the right ilium in medial view; drawn from Fig. 12B in Rieppel¹⁸). **e**, Plesiosaurs (1, the sacrum of *Cryptoclidus eurymerus* in dorsal view, redrawn from Fig. 14 in Brown²⁰; 2, the left ilium of *Bishanopliosaurus youngi* in medial view, redrawn from Fig. 6A in Sato *et al.*²⁵). Abbreviations: l.il, left ilium; r.il, right ilium; arrows indicate the movable (a chain-like) connection of the sacral ribs relative to the sacral vertebrae and to the pelvis, respectively.

the ilium in most other pachypleurosaur and nothosaur^{8,15–19}. This is generally interpreted as a phenomenon of pedomorphosis (juvenile condition)². In addition, the sacral ribs and the relevant elements of the pelvis such as the ilium were greatly simplified in

morphology, the former being bar-shaped and distally unexpanded or even tapering, whereas the dorsal blade of the latter became small and knob-like. These simplifications are comparable to those seen in *Keichousaurus hui*, suggesting that a similar mobility seen in the sacro-iliac joint of the former might have been present in those marine reptiles (Fig. 4b–d). If this is so, there was increased potential for the development of viviparity in these taxa as well. Furthermore, the pelvic girdle and sacrum also show a similar condition in advanced (derived) sauropterygians, such as plesiosaurs. To our knowledge, the sacral ribs are very simple in morphology and formed a weak connection with the bar-shaped ilium distally in adult plesiosaurs^{20–22} (Fig. 4e) although the fusion between the ribs and sacral vertebrae might occasionally occur in certain taxa (possibly in very old specimens)²³. Plesiosaurs, unlike pachypleurosaur and nothosaurs, were considered to be the inhabitants of open seas²⁴. Their strong, well-developed forelimbs and hindlimbs are believed to be important in swimming, suggesting that a firm sacro-iliac joint would have been more appropriate. Yet the morphology of the sacral ribs and ilium indicates that the mobility might have been retained in articulations between the pelvis and sacrum. This indicates that plesiosaurs, like the aforementioned sauropterygians, might have been committed to viviparity. □

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The evolution of alternative parasitic life histories in large blue butterflies

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Large blue (*Maculinea*) butterflies are highly endangered throughout the Palaearctic region, and have been the focus of intense conservation research^{1–3}. In addition, their extraordinary parasitic lifestyles make them ideal for studies of life history evolution. Early instars consume flower buds of specific host

plants, but later instars live in ant nests where they either devour the brood (predators), or are fed mouth-to-mouth by the adult ants (cuckoos). Here we present the phylogeny for the group, which shows that it is a monophyletic clade nested within *Phengaris*, a rare Oriental genus whose species have similar life histories^{4,5}. Cuckoo species are likely to have evolved from predatory ancestors. As early as five million years ago, two *Maculinea* clades diverged, leading to the different parasitic strategies seen in the genus today. Contrary to current belief, the two recognized cuckoo species show little genetic divergence and are probably a single ecologically differentiated species^{6–10}. On the other hand, some of the predatory morphospecies exhibit considerable genetic divergence and may contain cryptic species. These findings have important implications for conservation and reintroduction efforts.

Maculinea species have become the flagship butterflies for conservation in the UK and Europe^{2,11}. The severe decline of *Maculinea* populations during the twentieth century has been well documented, and all species have been included in the red data lists of most European countries³. Many management and reintroduction projects have been attempted, with variable results¹. The extinctions of the large blue (*Maculinea arion*) in the UK, the Netherlands and Belgium, the scarce large blue (*Maculinea teleius*) in the last two countries and the dusky large blue (*Maculinea nausithous*) in the Netherlands^{2,12}, have spurred increased conservation efforts, with large blue butterfly populations sometimes being used as bioindicators of habitat quality¹³.

Maculinea species are also the best-known examples of parasitic butterflies. Initially they feed on the flowers of specific Lamiaceae, Gentianaceae or Rosaceae host plants. When they reach the fourth instar, they drop to the ground and are picked up by *Myrmica*¹⁴ (or in a few cases *Aphaenogaster*¹⁵) ants and carried into the nest where they feed as parasites. Most currently recognized species, including the widely distributed species *M. arion*, *M. teleius* and *M. nausithous* and the east Asian *Maculinea arionides* prey on ant brood^{15–18}. In contrast, *Maculinea alcon* and *Maculinea rebeli* are ‘cuckoos’, whose larvae are fed primarily on regurgitations from ant workers, trophic eggs and prey items^{19,20}. These species have more elaborate adaptations of behavioural and chemical mimicry, and have thus been proposed to be derived relative to species that are strictly predatory²⁰. Several additional taxa occurring in the eastern Palaearctic have been proposed as species, including *Maculinea kurentzovi* and *Maculinea cyanecula*, but their status is still unclear and their life histories have not been described (see Supplementary Information)¹⁸.

More than 99% of the estimated 18,000 species of butterflies are herbivorous, but aphytophagy (carnivory and parasitism) has been fully documented in only about 80 species²¹. These are found primarily in the family Lycaenidae, to which *Maculinea* belongs, and are likely to be the result of the close relationship that the caterpillars of this family have with ants. Up to 75% of the approximately 5,000 species of Lycaenidae (*sensu stricto*) associate to some degree with ants, and whereas most of these relationships appear to be mutualistic, as many as 200 (4%) are known or suspected to be parasitic on ants²².

Maculinea belongs to the *Glaucopsyche* section of the Polyommataini, and *Sinia*, *Iolana*, *Caerulea* and *Phengaris* have been considered its closest relatives²³. In particular, the Oriental genus *Phengaris* has been proposed as the most likely sister group of *Maculinea* due to its similar morphology and the occurrence in the genus of both predatory and cuckoo parasitism on *Myrmica* ants^{4,5,24,25}. The present study reconstructs the evolution of ant parasitism, host plant association and speciation in *Maculinea*, and investigates whether the presently recognized species are likely to represent evolutionarily significant units for conservation.

Our molecular phylogeny includes 32 *Maculinea* specimens representing 31 geographically distinct populations of seven species