A New Mammaliaform from the Early Jurassic and Evolution of Mammalian Characteristics

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A fossil from the Early Jurassic (Sinemurian, ~195 million years ago) represents a new lineage of mammaliaforms, the extinct groups more closely related to the living mammals than to nonmammalian mammaliaforms. It has an enlarged cranial cavity, but no postdentary trough on the mandible, indicating separation of the middle ear bones from the mandible. This extends the earliest record of these crucial mammalian features by some 45 million years and suggests that separation of the middle ear bones from the mandible and the expanded brain vault could be correlated. It shows that several key mammalian evolutionary innovations in the ear region, the temporomandibular joint, and the brain vault evolved incrementally through mammaliaform evolution and long before the differentiation of the living mammal groups. With an estimated body weight of only 2 grams, its coexistence with other larger mammaliaforms with similar “triconodont-like” teeth for insectivory within the same fauna suggests a great trophic diversity within the mammaliaform insectivore feeding guild, as inferred from the range of body sizes.

_Hadrocodium wui_ (1) from the Lower Lufeng Formation of Yunnan, China, is distinguishable from all other nonmammalian mammaliaforms (2–14) and mammals from the Late Triassic and Jurassic in a long list of dental (15) and derived skull characteristics (Figs. 1, 2, and 3). The holotype specimen of _Hadrocodium_ displays several features typical of adults or subadults of late growth stages of other mammaliaforms and living mammals. The first adult feature is its large postcanine diastema, the gap between the functional canine and the first premolar (Fig. 1). A prominent postcanine diastema is characteristic of older individuals of _Sinoconodon_ (14), _Morganucodon_ (9–12), and _Kuehneotherium_ (8, 12), in which the anteriormost premolars are present in the smaller and younger individuals but lost without being replaced in the larger and adult individuals. _Hadrocodium_’s postcanine diastema is very large relative to the postcanine row, similar to adults of the late stages, but very different from the young individuals of _Sinoconodon_ (14) and _Morganucodon_ (12). The second adult feature of _Hadrocodium_ is the presence of wear facets on the molars (Fig. 1, D and E), showing that it had grown to a later stage of independent feeding. The third adult feature is a fully functioning temporomandibular joint (TMJ), which only appears beyond the suckling stage of early growth in extant monotremes (16–18) and therians (19–21).

The fourth feature is the absence of the meckelian sulcus in the mandible of _Hadrocodium_ (Fig. 3D). In living mammals, this sulcus is lost in the adult after the reabsorption of the anterior part of the Meckel’s cartilage, which would be associated with the meckelian sulcus on the dentary during embryonic stages (16–21). These adult features indicate that the type specimen of _Hadrocodium_ had undoubtedly developed beyond the early juvenile stages of living mammals. Its extremely small size is unlikely to be due to accidental sampling of an early ontogenetic stage. Its distinctive features are of phylogenetic importance.

_Hadrocodium_ is the sister taxon to the clade of triconodontids and extant Mammalia (Fig. 4), based on the parsimony analysis of 90 cranial and dental characters that can be recognized on its type specimen (22). Among 15 comparative taxa, it is more closely related to living mammals than are _Adelobasileus, Sinoconodon, morganucodontids_, and _Haldanodon_. The sister taxon relationship of _Hadrocodium_ to the clade of triconodontids and living mammals is supported by a large number of shared derived characters (Fig. 4B, node 6).

The first suite of apomorphies is on the medial side of the mandible of _Hadrocodium_, which has a smooth peristeal surface but lacks the postdentary trough and its medial ridge, and lacks the medial concavity of mandibular angle (Fig. 3). By contrast, more primitive morganucodontids (9–14), kuehneotheriids (8), Haramiyavus (6), and _Haldanodon_ (22) have a very prominent postdentary trough with a shelflike dorsal medial ridge, and all other nonmammalian mammaliaforms have a medial concavity on the mandibular angle (8–14, 23), as in nonmammalian cynodonts (9, 14, 24–27). The postdentary trough and the medial concavity on the mandibular angle respectively accommodated the prearticular/surangular and the reflected lamina of the angular (9, 25–27) that are the homologs to the mammalian middle ear bones (9, 14, 16–21, 23, 26). The absence of these structures indicates that the postdentary bones (“middle ear ossicles”) must have been separated from the mandible (Fig. 3).

_Hadrocodium_ lacks the primitive meckelian sulcus of the mandible typical of all nonmammalian cynodonts (24–27), stem groups of mammaliaforms (8, 9, 14, 23, 26, 27), triconodontids (28, 29), and nontribosphenic therian mammals (30). _Hadrocodium_ differs from all other stem mammaliaforms and most mammals in having a slightly inflected dentary angle, which is an autapomorphy (Fig. 1B).

The second suite of derived features is related to the enlargement of the brain in _Hadrocodium_ (Fig. 3). Its cranial vault is wider and more expanded in the alisphenoid and parietal region than those of all other nonmammalian mammaliaforms (7, 10, 14, 23) and all other Jurassic mammals (31–34) known to this date. The brain vault in the parietal region in _Hadrocodium_ is comparable to those of the mammalian crown group (31–34), but wider than in nonmammaliaform cynodonts (24, 25), _Sinoconodon_ (14), _Morganucodon_ (10, 33), and _Haldanodon_ (23) (Fig. 3). On the basis of the allometric scaling of a large sample of living and fossil mammals, the brain vault of _Hadrocodium_ is larger than expected for the mammals of its comparable skull width (Fig. 5A) and far wider than in any other Triassic-Jurassic mammaliaforms. Our scaling analysis shows that the small size of _Hadrocodium_, in and by itself, is not sufficient to explain its large brain (Fig. 5A). Related to the expansion of the brain vault, the cerebellar portion of the brain cavity is expanded more posteriorly than the level of TMJ (Figs. 1A and 3D). The occipital (posterior) wall of the brain cavity is convex posteriorly beyond the lambdoidal crest (Fig. 1A), instead of concave or flat as in cynodonts, other mammaliaforms, and all Jurassic mammals known to this date (7, 10, 14, 23–27). The third suite of derived features of _Hadrocodium_ is in the TMJ. All other nonmammalian mammaliaform crania have a TMJ positioned in about the same transverse level as the femur, whereas the occipital condyles (10, 14, 23, 27). By contrast, in _Hadrocodium_, the zygoma swings anteriorly from the cranial moiety of the squamosal, and the TMJ is positioned anterior to the level of

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the fenestra vestibuli and to occipital condyles (Fig. 2), in correlation with the expanded brain vault. A postglenoid depression is present on the lateral aspect of the squamosal between the zygoma and the cranial moiety. These are derived characteristics of some eutriconodonts (29), many multituberculates (35, 36), the monotremes *Obdurodon* (37) and *Tachyglossus* (16), and the majority of therian mammals (19–21). *Hadrocodium* has a well-developed postglenoid region behind the TMJ (Fig. 2), in correlation with the posterior displacement of the basioccipital and brain vault behind the TMJ. A similar pattern appears to be correlated with the detachment of the middle ear from the mandible in *Monodelphis* (33).

The fourth suite of derived features of *Hadrocodium* is in the petrosal (Fig. 2). The petrosal has a prominent promontorium (bony housing of the inner ear cochleae), more inflated than those of other mammaliaforms (7, 10, 14, 23, 38), triconodontids (39, 40), most multituberculates (35, 36, 40), and nontribosphenic therians (30). The large promontorium may be inversely correlated to the small size of the skull, as the inner ear may have negative allometry with the skull size. A shallow epitympanic recess, the location for attachment of the incus, is on the lateral side of the well-developed crista parotica, more derived than the condition of *Morganucodon* (41), but similar to the conditions of *Tachyglossus* and multituberculates. The recess lacks the distinctive incus fossa of *Ornithorhynchus* (18, 40, 41) and some triconodontids (39, 40); it is more posteriorly positioned relative to the TMJ than in these taxa. It is likely that *Hadrocodium* already developed a mobile articulation of the quadrate (incus) to the cranium, as in derived cynodonts (26), other mammaliaforms (41), and living mammals. The pterygoparoccipital foramen for the superior ramus of the stapedia muscle in *Hadrocodium* is completely enclosed by the petrosal, different from most mammaliaforms (38–41) (Fig. 2) but similar to those in monotremes and multituberculates. The paraparotic process of the petrosal lacks the bifurcating pattern common to *Sinoconodon*, *Morganucodon*, triconodontids, and some advanced cynodonts (38, 42). It lacks the ventrally projecting posterior paraparotic process seen in *Morganucodon*, triconodontids, multituberculates, and *Ornithorhynchus*.

The bony roof of the oropharyngeal passage is broad, flat, and almost featureless. There are no constrictions between the pterygoid and the basisphenoid, no pterygopalatine ridges, and no median ridge of the basisphenoid (Figs. 1B and 2C), all of which are primitive characters of nonmammalian cynodonts (24, 25), *Sinoconodon*, *Morganucodon*, and *Megazostrodon* and are present, although less developed, in *Adelobasileus* (7) and multituberculates (35, 36). The small hamulus of the pterygoid is similar to the condition in *Haldanodon*, *Ornithorhynchus*, and multituberculates but more reduced than the homologous transverse flange of cynodonts (24, 25), *Sinoconodon*, and *Morganucodon* (14). The posterior edge of the secondary bony palate (partially broken) lies posterior to the tooth row, more derived than in *Sinoconodon*, *Morganucodon*, and multituberculates but less than in *Haldanodon* (23), eutriconodontids, and the mammalian crown group taxa.

Our phylogeny shows that all cranio-dental diagnostic characters for the extant Mammalia evolved stepwise (27) and before the diversification of the extant mammalian clades (node 8 in Fig. 4B). The transformation from a more complex “double jaw hinge” (of the articular-quadrato and dentary-squamosal in *Sinoconodon*, *Morganucodon*, and *Haldanodon*) to the “single jaw hinge” joint (TMJ) (formed exclusively of the dentary-squamosal in *Hadrocodium*, triconodontids, and extant mammals) be-

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**Fig. 1. Hadrocodium wui gen. et sp. nov. (IVPP 8275).** (A) Lateral and (B) ventral views of restored skull. (C) Dentition (lateral view restoration). (D) Occlusion [based on scanning electron microscope (SEM) photos]. (E) Wear of molars (shaded areas are wear facets). The main cusp A of the upper molar occludes in the embrasure between the opposite lower molars. Abbreviations: an, angular process (dentary); bo, basioccipital; bs, basi-phenoid; c, canine; ce, cavum epipetricum; co, coronoid process (of dentary); dc, dentary condyle; er, epitympanic recess; f, frontal; fc, foramen cochleare (“perilymphatic foramen”); fst, fossa for stapelid muscle; f, fenestra vestibuli; hp, hamulus (of pterygoid); ii, upper and lower incisors; i, internal nares; iof, infraorbital foramen; j, jugal; jf, jugular foramen; lc, lacrimal; lt, lateral trough; M, molar; mx, maxillary; n, nasal; oc, occipital condyle; P, premolar; pa, parietal; pcd, postcanine diastema; pgd, postglenoid depression; pr, promontorium (petrosal); ptc, posttemporal canal (between petrosal and squamosal); px, premaxillary; sm, septomaxillary; so, supraoccipital; sof, sphenoorbital fissure; sq, squamosal; tmj, temporomandibular joint (dentary/squamosal jaw hinge); v3, foramen for the mandibular branch of the trigeminal nerve (v); xi, hypoglossal nerve (xii). Molar cusps following (17): A, B, and C, main cusps of upper molars; a, b, c, d, and e, cusps of the lowers.
The acquisition of "mammalian characteristics" shows an additive pattern in our mammaliaform phylogeny (Fig. 4C). The transition from the nonmammaliaform cynodonts to living mammals has a stepwise and incremental acquisition of the mammalian characteristics (24–27, 43), and there was no single episode of rapid evolution of a large number of derived characters. The node of crown-group Mammalia [following (42)] has four unambiguous synapomorphies, within the range of three to ten unambiguous synapomorphies for each of the intermodal segments on the backbone of the cladogram. This is consistent with a macroevolutionary pattern that prevailed in much of synapsid evolution (24, 25, 43).

Hadrocodium sheds light on evolution of the mammalian middle ear. It is the earliest known taxon that lacks the primitive attachment of the middle ear bones to the mandible but has an enlarged brain vault (suggestive of a large brain) (Fig. 5A). This extends the first appearance of these modern mammalian features back to the Early Jurassic, some 45 million years earlier than the next oldest mammals that have preserved such derived features, such as Triconodon from the Late Jurassic (31–34).

All other nonmammalian mammaliaforms with small brain vaults (Fig. 3, A to C) have retained the mandibular attachment of the middle ear bones, whereas Hadrocodium and living mammals (Fig. 3, D to I) with larger brain vaults have lost the mandibular attachment to the middle ear.

During the ontogeny of the marsupial Monodelphis, the chain of middle ear ossicles and the ectotympanic ring ossify and terminate their growth in size earlier than does the brain (33). In subsequent development, the ossicles and the ectotympanic ring, with their size fixed upon ossification, show negative allometry relative to the increasing size of the brain, the basicranium, and the entire skull. Negative allometry of the middle ear bones during development is known for monotremes (17, 44) and some placental (21). Negative allometry of adult middle ear bones occurs in diverse placental (45) and some marsupial (19) taxa of a wide range of body sizes.

Given this negative allometry of the middle ear ossicles and that the ossicles are connected to the basicranium through the fenestra vestibuli (33) and bound by connective tissues to the crista parotica (19, 21, 41), the peramorphic growth of the brain and the basicranium would cause the ectotympanic ring to move away from the mandibular angle, as the distance increases between the mandible and the crista parotica and fenestra vestibuli. The detachment of the ectotympanic ring from the mandible is in part correlated with the peramorphic growth of the brain (33) and the posterior displacement of the basicranium (Fig. 3). This may provide a mechanism that could have separated the middle ear (33), a crucial step in transforming the mandibular elements for feeding to the middle ear structures specialized for hearing (16–21, 26). The concurrence of the expanded brain vault and the separation of the middle ear from the mandible in Hadrocodium (Fig. 3) is consistent with the observed correlation of the peramorphic growth of brain and basicranium to the separation of the middle ear bones from the mandible during development (33).

Correlation of negative allometry of the middle ear elements to the peramorphic growth of the brain should be tested by further comparative studies of developmental rates that are
Fig. 3. Correlation of the expanded brain vault and the loss of the postdental trough and medial concavity of mandibular angle in Hadrocodium and more derived mammals. (I, Left) Internal view of dentaries (standardized to one jaw length, scales differ among taxa); the postdental trough, the medial concavity, and the meckelian sulcus on the mandibular angle are colored in blue. Abbreviations: cmr, medial concavity of the mandibular angle; pdt, postdental trough. (II, Middle) Dorsal view of the cranium (crania of different sizes are standardized to the same width between the left and right temporomandibular joints; scales differ among taxa); the areas in red represent the approximate extent of the brain endocasts. (III, Right) Measurement of the brain vault size (cranial width at the squamosal-parietal suture) relative to the width between the two TMJs; value on bar represents the width of brain vault in percentage of total skull width at the TMJs. Hadrocodium (85%) and mammalian crown groups (60% to 87%) with larger brain vaults show the separation of the middle ear ossicles from the mandible. Hadrocodium has a larger brain vault than expected for living mammals of its skull size (see allometry regression in Fig. 5B) and is similar to living mammals but different from other contemporaneous mammaliaforms. All primitive mammaliaforms (A) to (C) in the basal part of the tree have the postdental trough and medial concavity of mandibular angle (for postdental "ear" elements), as well as small brain vault (43 to 58%). The 58% value for Morganucodon, although larger than Haldanodon and Sinconodon, is far below the ~75% expected for extant mammals of similar skull size (Fig. 5A). (A) Sinconodon. (B) Morganucodon. (C) Haldanodon [after (25)]. (D) Hadrocodium [brain endocast outline based on the exposed borders on the right side]. (E) Monotreme Ornithorhynchus. (F) Monotreme Tachyglossus. (G) Multituberculate Chulsanbaatar [after (36)]. (H) Marsupial Didelphis [after (33)]. (I) Placental Asioryctes [after (31)].

The body mass of Hadrocodium is estimated to be 2 g from a skull length at 12 mm (Fig. 5B), on the basis of the well-established scaling relationship of body mass to skull size in 64 species of living lipotyphlan insectivore mammals (50). This taxon ranks among the smallest mammals (51) and is certainly the smallest mammal yet discovered in the Mesozoic. The smallest living insectivoran placentals has an adult weight of about 2.5 g. The smallest bat has an adult weight of about 2.0 g (51). The smallest Cenozoic fossil insectivore mammal has an estimated body weight of 1.3 to 2.04 g (51). The body masses of all other fossil mammals of the Cretaceous and Cenozoic are near or above 3 g (52). The diminutive body size of Hadrocodium greatly expands the range of body size for the early Jurassic mammaliaform insectivores (Fig. 5B). The morphological disparity (53, 54) within the earliest mammaliaform insectivore guild, as indicated by skull sizes (Fig. 5B), is almost equal to the range of disparity of living lipotyphlan insectivore mammals with diverse trophic adaptations (50, 51). The wide range of body size within the Lufeng mammaliaform fauna suggests a trophic differentiation within the insectivorous mammaliaform feeding guild (Fig. 5B), which is an important paleoecological feature for the early diversification of mammals, in addition to the splitting of phylogenetic clades, as documented elsewhere for the early diversification in some invertebrate groups (53) and for later mammals (52–54).

References and Notes

1. Etymology. Hadro, fulness (Greek); codium, head (Greek); for its very large brain capacity relative to the skull; Wui, after Dr. X.-C. Wu who, in 1985, discovered the holotype IVPP 8275 (Institute of Vertebrate Paleontology and Paleoanthropology, Beijing), a nearly complete skull, 12 mm in rostro-occipital length and 8 mm in width at the level of temporomandibular joint. The unprepared specimen was mentioned as a juvenile morganucodon-tid (14). Now after full preparation, the specimen shows many taxonomic differences from any previous known mammaliaforms and from mammals [see diagnosis (15)].


Fig. 5. (A) Scaling of brain vault size (width measured at the level of anterior squamosal/pa-rietal suture) relative to skull size (measured at the distance between the left versus right temporomandibular joints). This shows that allometry of small size of Hadrocodium, by itself, is not sufficient to account for its very large brain-case. Hadrocodium’s brain vault is larger (wider) than expected for the crown-group mammals with similar skull width from the allometrical regression. By contrast, all contemporaneous mammaliaforms (triangles: Sinoconodon, Morganucodon, and Hal-danodon) with the postdental trough and meckelian groove have smaller (narrower) brain vaults than those living mammal taxa (and (narrower) brain vaults than those living mammal taxa (and danodon) with the postdentary trough and meckelian groove have smaller (narrower) brain vaults than those living mammal taxa (and danodon) with the postdentary trough and meckelian groove have smaller (narrower) brain vaults than those living mammal taxa (and danodon) with the postdentary trough and meckelian groove have smaller (narrower) brain vaults than those living mammal taxa (and danodon) with the postdentary trough and meckelian groove have smaller (narrower) brain vaults than those living mammal taxa (and danodon) with the postdentary trough and meckelian groove have smaller (narrower) brain vaults than those living mammal taxa (danodon) with the postdentary trough and meckelian groove have smaller 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22. Supplementary data are available on Science Online at www.sciencemag.org/cgi/content/full/292/5521/1535/DC1.
47. Differentiation of the central nervous system in eutheri-ans (21, 46) occurs in advance of the ossification of TMJ, which, in turn, occurs before the ossification of middle ear ossicle (27, 46). The sequence of these events is the same as that of marsupials, indicating an unambiguous case of heterochrony among living therians (46), and references cited therein. The dentary/squamosal joint is also established earlier than ossification of the middle ear ossicles in monotremes (17, 18), similar to placental but different from marsupials (19, 20) and references cited therein. The Meckel’s cartilage and its derivative elements (the malleus and incus) are positioned on the basioccipital and well separated from the dentary in early ontogenesis in eutheri-ans (21). This is different from marsupials, in which the Meckel’s cartilage and its derivative elements are closely attached to the dentary until the final separation from the latter in a later postnatal stage (19, 20).
49. Teratological evidence shows that masticatory muscle attachment to the Meckel’s cartilage in abnormal human development can delay its reabsorption (48). Thus, reabsorption of the Meckel’s cartilage may be associated with the loss of mechanical stress and loading as the masticatory muscles shifted their attachment from Meckel’s cartilage to the dentary during normal development (48). Maier (19, 20) also proposed that movement of the dentary during suckling would result in disruption of the Meckel’s cartilage.
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Chromatin Docking and Exchange Activity Enhancement of RCC1 by Histones H2A and H2B

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The Ran guanosine triphosphatase (GTPase) controls nucleocytoplasmic transport, mitotic spindle formation, and nuclear envelope assembly. These functions rely on the association of the Ran-specific exchange factor, RCC1 (regulator of chromosome condensation 1), with chromatin. We find that RCC1 binds directly to mononucleosomes and to histones H2A and H2B. RCC1 utilizes these histones to bind Xenopus sperm chromatin, and the binding of RCC1 to nucleosomes or histones stimulates the catalytic activity of RCC1. We propose that the docking of RCC1 to H2A/H2B establishes the polarity of the Ran-GTP gradient that drives nuclear envelope assembly, nuclear transport, and other nuclear events.

RCC1 can be considered as a chromatin marker. Catalysis of guanine nucleotide exchange on Ran by RCC1 to produce Ran-GTP is essential for mitotic spindle assembly and nuclear envelope formation (7–4). Once enclosed by the envelope, chromatin-bound RCC1 generates a Ran-GTP gradient across nuclear pores that permits vential nucleocytoplasmic transport (+). The docking mechanism for RCC1 onto chromatin is unknown. RCC1 may bind DNA in vitro, but removal of the NH2-terminal domain of RCC1 by protease prevents this activity. The chromatin docking and exchange activity of RCC1 were not observed in the absence of histones H2A and H2B.

A

B

Fig. 1. RCC1 binds mononucleosomes. (A) HeLa nuclei were digested with micrococcal nuclease and centrifuged through a linear 8 to 20% sucrose gradient. Samples of individual fractions were electrophoresed through a tris-borate EDTA-agarose gel and visualized by ethidium bromide staining (top) or precipitated with trichloroacetic acid, subjected to SDS-PAGE, and immunoblotted (N-19, Santa Cruz) for endogenous RCC1 (bottom). (B) Immobilized GST, GST-RCC1, or GST-RCC1(23–421) was incubated with intact or trypsinized H1-depleted mononucleosomes. After washing, proteins were eluted, subjected to SDS-PAGE, and stained with Coomassie.