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Primitive dinosaur skeleton from Argentina and the early evolution of Dinosauria

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WE report here the discovery of a primitive dinosaur skeleton from Upper Triassic strata in northwestern Argentina. The 1-m-long skeleton represents a new taxon, *Eoraptor lunensis* gen. et sp. nov., which is close to the predicted structure and size of the common dinosaurian ancestor¹⁻⁵. The skull, which has a unique heterodont dentition, does not exhibit any of the specializations of the major dinosaurian clades (Ornithischia, Sauropodomorpha, Theropoda). The forelimbs are less than half the length of the hind limbs, which suggests an obligatory bipedal posture. Although close in overall form to the common dinosaurian ancestor, the functionally tridactyl, grasping/raking hand and other features show that *Eoraptor* is allied phylogenetically with theropods. The discovery of *Eoraptor* supports the hypothesis that dinosaurs diverged rapidly at small body size from a common ancestor, with the

principal herbivorous and carnivorous lineages present by the middle Carnian.

Skeletal remains of the earliest (middle Carnian) dinosaurs are rare and have been unearthed mostly from the fossiliferous Upper Triassic Ischigualasto Formation in northwestern Argentina. These dinosaurs include the ornithischian *Pisanosaurus*⁶ and the theropod *Herrerasaurus*^{1,7-9}. The new skeleton, a contemporary of *Herrerasaurus* in the lower third of the formation (R.R.R., C. C. Swisher, P.C.S., C.A.F. and A.M.M., manuscript in preparation), represents a third species. Despite its small size, closure of sutures in the vertebral column and partial fusion of the scapulocoracoid suggest that the skeleton had reached adult size.

Order Saurischia

Suborder Theropoda

Eoraptor lunensis gen. et sp. nov.

Etymology. *Eos*, dawn (Greek) in reference to its primitive structure and early temporal occurrence; *raptor*, plunderer (Latin) in reference to its carnivorous habits and grasping hand; *luna*, moon (Latin) and *-ensis*, place (Latin), in reference to the type locality.

Holotype. PVSJ 512 (Museo de Ciencias Naturales, Universidad Nacional de San Juan, San Juan, Argentina), an articulated skeleton lacking only the distal caudal vertebrae (Figs 1 and 2). The skeleton was preserved in a muddy siltstone in close association with many of the common vertebrates in the Ischigualasto fauna (*Herrerasaurus*, *Saurosuchus*, *Aetosauroides*, *Ischigualastia*, *Exaeretodon*, and an undescribed small carnivorous cynodont).

Horizon and locality. Ischigualasto Formation (Upper Triassic: middle Carnian); Valley of the Moon (Ischigualasto Provincial Park, Ischigualasto-Villa Unión Basin, northwestern Argentina) (R.R.R. *et al.*, manuscript in preparation).

Diagnosis. Small 1-m-long theropod with external naris slightly enlarged, premaxilla with slender posterolateral process, and leaf-shaped premaxillary and anterior maxillary crowns.

The skull follows a primitive saurischian design, with transversely narrow proportions, a relatively large antorbital opening, a small subnarial foramen beneath the external naris, and a forked posterior process on the jugal (Fig. 1a, b). Derived cranial characters that distinguish ornithischians, sauropodomorphs, and theropods, however, are absent. For example, there is no

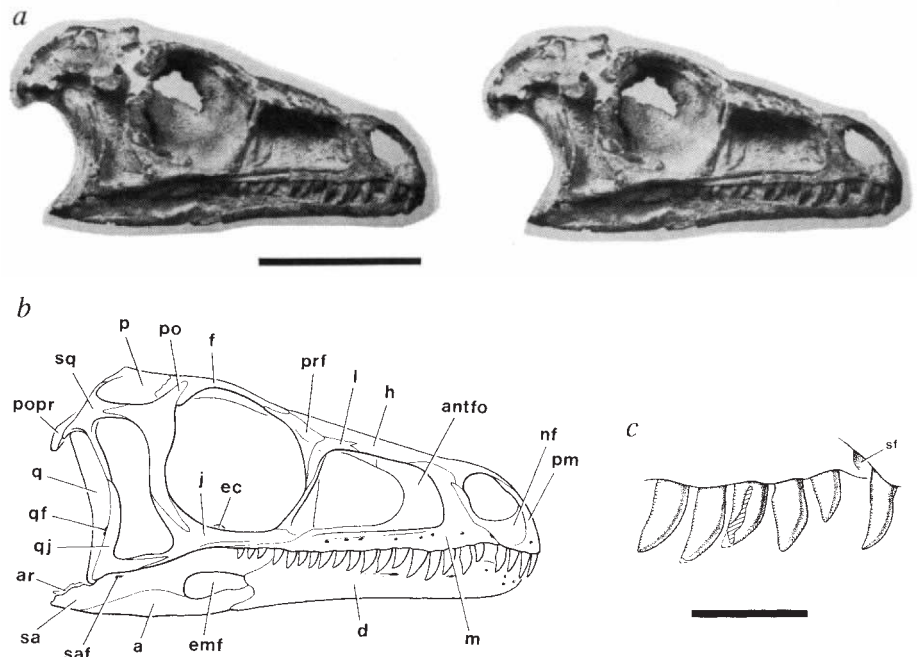


FIG. 1 PVSJ 512, *Eoraptor lunensis*, new species. *a*, Stereopair and *b*, reconstruction of the skull in right lateral view. *c*, Right posterior premaxillary and anterior maxillary teeth in lateral view.

Abbreviations: a, angular; antfo, antorbital fossa; ar, articular; d, dentary; emf, external mandibular fenestra; ec, ectopterygoid; f, frontal; j, jugal; l, lacrimal; m, maxilla; n, nasal; nf, narial fossa; p, parietal; pm, premaxilla; po, postorbital; popr, paroccipital process; prf, prefrontal; q, quadrate; qf, quadrate foramen; qj, quadratojugal; sa, surangular; saf, surangular foramen; sf, subnarial foramen; sq, squamosal. Scale bar, *a*, 5 cm; *c*, 1 cm.

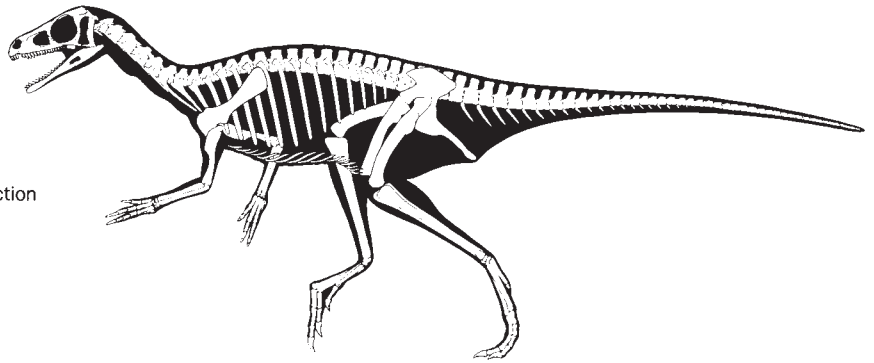


FIG. 2 *Eoraptor lunensis*, new species. Skeletal reconstruction based on PVSJ 512 (length 1 m).

development of an intra-mandibular joint, as occurs in *Herrerasaurus* and other theropods⁹, and the external naris and the narial fossa are not greatly enlarged as in sauropodomorphs¹¹. The dentition is heterodont (Fig. 1b, c). The posterior half of the upper tooth row has serrated, recurved crowns typical of theropods. The crowns in the anterior half of the tooth row, in contrast, are leaf-shaped with a basal constriction that most closely resembles the crown shape in basal sauropodomorphs^{11,12}.

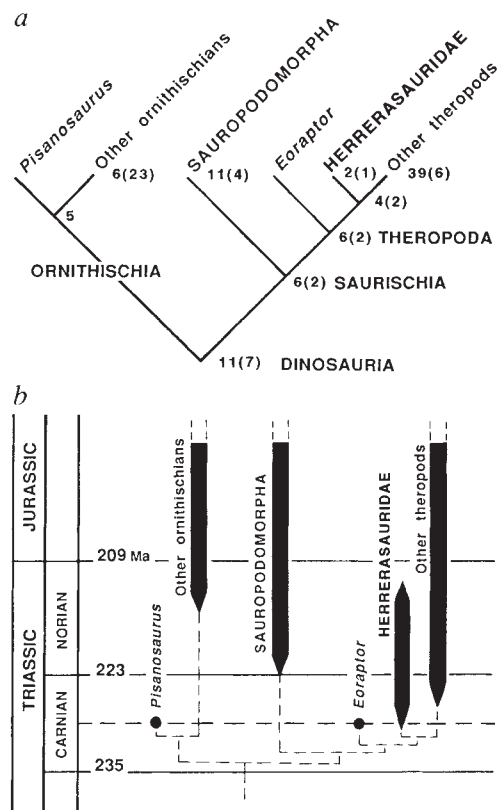
The skeleton exhibits several dinosaurian hallmarks, such as a long deltopectoral crest on the humerus, reduction of the outer digits of the hand (Fig. 2), and an ascending process on the astragalus¹. Similar to the skull, the skeleton lacks many derived characters that distinguish later dinosaurs. There are only three sacral vertebrae that support the pelvis, the lowest number that occurs among dinosaurs, and the hand retains a fifth digit, albeit reduced to a short metacarpal. There are no pneumatic cavities in the presacral vertebrae, as often occurs among saurischians,

but all of the long bones of the skeleton are strongly hollowed as in theropods.

A detailed cladistic analysis of the early branching history of Dinosauria supports a basal split into ornithischians and saurischians, the latter including herrerasaurids (Fig. 3a). Although *Eoraptor* is structurally close to the common dinosaurian ancestor, several advanced features place it among the saurischians as the most primitive known theropod (Fig. 3). Key saurischian synapomorphies (derived characters) present in *Eoraptor* include a subnarial foramen in the skull (Fig. 1c, sf) and modifications that increase the flexibility of the neck (epiphyseal processes and slender, interconnecting ribs). Features that link *Eoraptor* with *Herrerasaurus* and other theropods include a predatory manus with trenchant unguals and enhanced grasping and raking capabilities (elongate distal phalanges, metacarpal pits for phalangeal hyperextension)^{1,2}.

The discovery of *Eoraptor* has opened a window into the early evolution of theropods. The contemporaneous occurrence of a

FIG. 3 a, Hypothesis of early dinosaurian phylogeny based on numerical cladistic analysis of 132 anatomical characters in 12 terminal taxa (consistency index equals 0.85; P.C.S., unpublished data). Numbers at nodes indicate total number of synapomorphies. Synapomorphies that may occupy more than a single node (primarily due to missing data in rare species) are shown in parentheses under delayed transformation¹². Synapomorphies supporting dinosaurian monophyly and internal nodes include: **Dinosauria**, postfrontal absent^{1,2,9}, frontal participation in supratemporal fossa^{1,9}, ectopterygoid overlapping pterygoid⁹, quadrate head exposed laterally^{1,9}, post-temporal opening reduced to a foramen^{1,9}, dorsal vertebra added to sacrum^{1,5,6}, deltopectoral crest 35% or more of humeral length^{1,3-5,13}, manual digit IV narrower than digits II and III and lacking an ungual^{1-3,5,13,14}, acetabular antitrochanter partially divided by notch¹, brevis fossa^{1,14}, femoral head subrectangular^{1-3,5,6,8,15} with angular greater trochanter, femoral medial tuberosity rudimentary, cnemial crest on tibia^{1,2,14}, ascending process on astragalus^{1-6,14,15}, astragalus with laterally facing calcaneal facet, calcaneal medial process rudimentary¹, distal tarsal 4 heel shallow¹, metatarsal 4 shaft sigmoid. **Ornithischia**, subtriangular maxillary/dentary crowns^{2,16}, largest maxillary/dentary tooth in centre of tooth row¹⁶, dentary coronoid process¹⁶, coronoid region half or more as deep as dentary ramus, external mandibular fenestra reduced; **Saurischia**, subnarial foramen, jugal overlaps lacrimal, jugal posterior process forked, epiphyses on mid- and posterior cervical vertebrae², hyposphene-hypantrum articulation in dorsal vertebrae^{2,3}, mid-cervical ribs long and parallel to cervical column, distal carpal 5 absent, phalanx 1 of manual digit I equal to or longer than metacarpal 1^{2,3}, astragalus ascending process wedge-shaped, metatarsals 2-4 with overlapping proximal ends. **Theropoda**, cervical epiphyses prong-shaped, extreme hollowing of centra and long bones², manual digits IV and V vestigial^{2,13}, metacarpals 1-3 with extensor depressions for phalangeal hyperextension, manus more than 50% length of humerus+radius. **Herrerasauridae + other theropods**, intramandibular joint^{1,2,9}, distal caudal prezygapophyses elongate^{2,8}, scapular blade strap-shaped, manual digits I-III with long penultimate phalanges and trenchant unguals², pubic blade at least six times as long as broad, pubic foot¹, proximal end of fibula 75% or more of proximal width of tibia. b, Corresponding phylogram showing the recorded temporal range for each taxon, with dashed line indicating the approximate age (230 Myr; R.R.R. *et al.*, manuscript in preparation) of the dinosaurs from the Ischigualasto Formation (*Pisanosaurus*, *Eoraptor*, *Herrerasaurus*). Solid bars show the earliest record of ornithischians that



are more advanced than *Pisanosaurus*^{17,18}, prosauropod sauropodomorphs^{19,20}, and theropods that are more advanced than *Eoraptor* and *Herrerasaurus*²¹. Herrerasaurids appear first during the Carnian in South America^{1,7,22} and in North America^{23,24}, where they persist into the Norian.

dinosaur as primitive as *Eoraptor* with the ornithischian *Pisanosaurus* and more advanced theropod *Herrerasaurus* supports the hypothesis that dinosaurs diverged rapidly at small body size from a common ancestor during the early Carnian (Fig. 3b). By the middle Carnian, the principal herbivorous and carnivorous lineages of dinosaurs were established. □

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Molecular identification of microorganisms associated with parthenogenesis

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CYTOPLASMICALLY inherited microorganisms are widespread in insects and have been implicated as causes of female parthenogenesis (females developing from unfertilized eggs) and cytoplasmic incompatibility^{1–15}. Normal sexual reproduction can be restored by treatment with antibiotics^{1–4}. Sequence analysis of the DNA encoding 16S ribosomal RNA has shown that cytoplasmic incompatibility bacteria from diverse insect taxa are closely related (they share >95% sequence similarity) and belong to the alpha subdivision of Proteobacteria^{5–7}. Here we show that parthenogenesis-associated bacteria from parasitoid Hymenoptera also fall into this bacterial group, having up to 99% sequence similarity to some incompatibility microorganisms. Both incompatibility and parthenogenesis microorganisms alter host chromosome behaviour during early mitotic divisions of the egg^{13–17}. Incompatibility bacteria act by interfering with paternal chromosome incorporation in fertilized eggs, whereas parthenogenesis bacteria prevent segregation of chromosomes in unfertilized eggs. These traits are

adaptive for the microorganisms. On the basis of their sequence similarities, we conclude that parthenogenesis bacteria and cytoplasmic incompatibility bacteria form a monophyletic group of microorganisms that 'specialize' in manipulating chromosome behaviour and reproduction of insects.

Identification of insect bacterial symbionts has long been hampered by the inability to culture these fastidious prokaryotes. But the development of the polymerase chain reaction (PCR) and use of DNA sequence encoding 16S RNA (16S sequences) in microbial phylogeny¹⁸ has made it possible to determine their phylogenetic position. We amplified and sequenced the bacterial 16S ribosomal DNA from six parthenogenetic strains of *Trichogramma* wasps (three different species) and one parthenogenetic strain of *Muscidifurax uniraptor*. *Trichogramma* wasps are minute (around 0.5 mm) parasites of insect eggs, primarily those of Lepidoptera. *Muscidifurax uniraptor* is a Pteromalid pupal parasitoid of houseflies. Each of these strains harbours microorganisms associated with parthenogenesis. To check for PCR amplification of bacteria not associated with parthenogenesis, sexual strains of *Trichogramma* were used as a control. Some forms of parthenogenesis in *Trichogramma* are genetically based and are not associated with microorganisms^{1,3}. As a second control, three strains of *Trichogramma* with genetic parthenogenesis were also examined for cytoplasmic

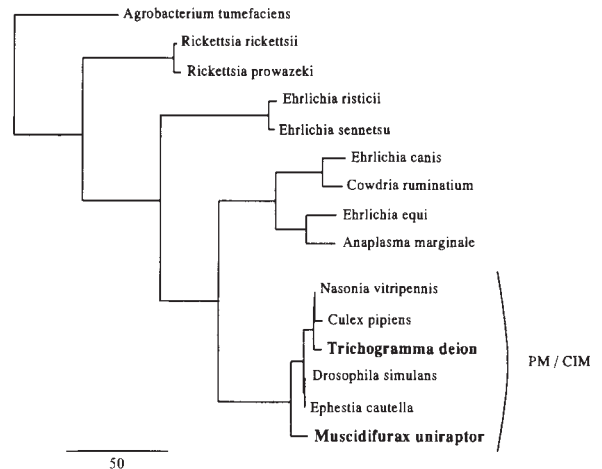


FIG. 1 Most parsimonious phylogenetic tree of parthenogenesis microorganisms (PM) of *Trichogramma deion* (Bautista strain) and *Muscidifurax uniraptor*, several cytoplasmic incompatibility microorganisms (CIM)^{5,6} and other representatives of the alpha subdivision of the Proteobacteria^{21–23}. The 16S rDNA sequences of PM (bold) and CIM are identified by the host species from which they were isolated. *Escherichia coli* (gamma subdivision) was used as an outgroup. Sequences were manually aligned using regions of the 16S gene that are conserved in eubacteria²¹. The aligned sequences of parthenogenesis microorganisms were 1,512 bases in length, including gaps. Gaps were treated as a 'fifth' base. The aligned sequence dataset was analysed with PAUP 3.0 (ref. 20) using the Branch and Bound algorithm to find the shortest tree(s). Two parsimonious trees (length 1,195 bases) were generated. They only differed in the positioning of the *Drosophila simulans* and *Ephestia cautella* microorganisms within the PM/CIM group. This is a result of the fact that only a partial 16S sequence is available for these two cytoplasmic incompatibility microorganisms. Sequences of 16S ribosomal genes of the *M. uniraptor* and *Trichogramma* symbionts were determined using the following procedure. Total DNA was extracted from white pupae (100 of *Trichogramma* spp. and 5 of *Muscidifurax*) after these were surface-sterilized with 70% ethanol, thoroughly washed with sterile water and homogenized in a Mini-Bead beater (Biospec). After precipitation with ethanol, bacterial 16S rDNA was PCR-amplified using conserved 16S rDNA primers (fD2 and rP2; ref. 21). PCR product was then directly cloned into T-tailed M13mp18 vector and sequenced by the Sanger method using Sequenase vr. 2.0 kit (US Biochemical; for sequence primers, see refs 5, 27). The nucleotide sequence data of parthenogenesis bacteria will appear in Genbank under the following accession numbers; *M. uniraptor*, L02882; *T. cordubensis*, L02883; *T. deion* TX, L02884; *T. pretiosum*, L02885; *T. deion* Mo, L02886; *T. deion* Ba, L02887; *T. Deion* SD, L02888.