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Graciela Piñeiro, Pablo Núñez Demarco, Melitta Meneghel

The hypotheses about the origin of the primitive amniotic tarsus are very speculative. Early studies argued that ~~the origin of~~ the astragalus, one of the largest proximal bones in the tarsus of basal amniotes ~~was produced~~ by the fusion of two or three of the amphibian tarsal bones, the intermedium, the tibiale and the proximal central (c4). Others however, considered that just the ~~former~~ transforms into the primitive astragalus. More recent work~~s~~ ~~have~~ ~~suggested~~ the fusion of the amphibian intermedium, the proximal centrale (c4) and eventually the c3 based ~~in~~ the presence of a ~~purposed tripartite or~~ multipartite structure of the ~~*Captorhinus*'s~~ astragalus. However, this statement ~~becomes~~ controversial ~~when~~ the absence of these bones in the ontogenetic stages of either fossil or extant reptiles ~~cannot be explained~~; thus an alternative hypothesis arises proposing the origin of the amniotic astragalus as a neomorph. Described tarsi exhibiting a putative tripartite structure of possible juvenile diadectids as well as disarticulated tarsal elements of the earliest known amniote *Hylonomys lyelli*, do not demonstrate the veracity of these evidence. Very well preserved tarsi of the Early Permian aquatic *Mesosaurus tenuidens*, representing the most complete ontogenetic succession known for a basal amniote (the other exceptional one is provided by the Late Permian eosuchian *Hovasaurus boulei* Piveteau, 1926) suggest that there is more than one ossification center for the astragalus which fuse during late embryonic stages or early after born. A *Mesosaurus* fetus in an advanced stage of development shows ~~that the tarsus is represented by a single bone, most probably~~ the astragalus~~,~~ clearly formed by the fusion of two or three bones. The tarsus in adult mesosaurids possesses eight to nine bones, the typical structure found in basalmost amniotes~~,~~ ~~and~~ two centralia (possibly c1and c2) are always present distal to the astragalus, being partially or totally fused to it in mature individuals. A primitive, amniote-like tarsal structure is also observed in Carboniferous very basal forms as the embolomeres *Proterogyrinus*, *Gephyrostegus*, *Westlothiana*, the captorhinomorph *Labidosaurus* and even in microsaurs like *Tuditanus* and *Pantylus*, taxa that were considered true amniotes in their former descriptions. Therefore, the structure of the amniotic tarsus, including the configuration of the proximal series formed by the astragalus and the calcaneum, a typical pair of enlarged bones, could have been

established well before than the first recognized amniote walked on Earth. Thus, it could have appeared convergently in not related, primitive groups, or it ~~is~~ part of a transformation series that involves taxa more closely related to the early amniotes than currently accepted.

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tarsus in adult mesosaurids possesses eight to nine bones, the typical structure found in basalmost amniotes, and two centralia (possibly c1and c2) are always present distal to the astragalus, being partially or totally fused to it in mature individuals. A primitive, amniote-like tarsal structure is also observed in Carboniferous very basal forms as the embolomeres *Proterogyrinus*, *Gephyrostegus*, *Westlothiana*, the captorhinomorph *Labidosaurus* and even in microsaurs like *Tuditanus* and *Pantylus*, taxa that were considered true amniotes in their former descriptions. Therefore, the structure of the amniotic tarsus, including the configuration of the proximal series formed by the astragalus and the calcaneum, a typical pair of enlarged bones, could have been established well before than the first recognized amniote walked on Earth. Thus, it could have appeared convergently in not related, primitive groups, or it is part of a transformation series that involves taxa more closely related to the early amniotes than currently accepted.

The ontogenetic transformation of the mesosaurid tarsus: a contribution to the origin of the amniotic astragalus

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Introduction

The origin of the astragalus and the calcaneum in the ankle of basal amniotes has been considered as an adaptation to terrestrial locomotion and a key innovation in the amphibious to reptilian (and synapsid) grade transition (Romer, 1956). Considering the elements present in the most basic amphibian tarsus, it is clear that there was a strong reduction of bones that form the primitive amniotic tarsus. ~~That~~ can be explained by fusion or ~~losing~~ of some tarsal bones in the ancestral amniotes and several poorly known transitional forms, in which the homology of the elements cannot be well established (O'Keefe et al., 2006). According to previous contributions, the origin of the astragalus as well as the identification of the ancestral bones that give origin to it ~~is~~ ~~a~~ contentious event (Peabody, 1951). ~~However~~, it is widely acknowledged that the calcaneum is derived from the fibulare, meaning from only one of the components of the amphibian tarsus. Historically, most authors supported the classic hypothesis of a unitary origin for the astragalus, from the intermedium or from the fusion of this bone with the tibiale (Peabody, 1951). However, ~~this last~~ author, following Holmgren (1933), suggested that ~~the origin of~~ the astragalus is produced by the fusion of two or three bones; mainly the intermedium, one of the proximal centralia (c4) and eventually, the tibiale (Peabody, 1951, figure2). A modification of this proposal, although supporting the composite origin for the astragalus, was suggested by O'Keefe et al. (2006) by including also the third central as a component of the fused element. Indeed, there is evidence of a fusion between the tibiale and the proximal centrale (c4) in the embolomere *Gephyrostegus* (Schaeffer, 1941; Holmes, 1984) which possesses an amniot~~ic~~-like tarsus, thus, this fusion may have occurred early in the primitive evolution of the amniotic tarsus. Embryologic studies show just two cartilaginous condensations close to the distal end of the fibula, one for the astragalus and the other for the calcaneum (Schaeffer, 1941; Rieppel, 1993), remaining ~~contention~~ about the presence of additional anlagen for the tibiale. Mainly ~~according~~ to this evidence, the extended view about the origin of the astragalus before Peabody´s (1951) contribution was in favor of a slightly transformed intermedium as the precursor. Other characteristic of the primitive amniotic tarsus is the articulation of proximal tarsal elements (astragalus and calcaneum) with centralia 1 and 2, which are placed distally and often can be fused to each other (Peabody, 1951). ~~That~~ fused element, commonly named as ~~the~~ centrale or lateral centrale has been suggested to form the navicular bone, characteristically present in therapsid-grade synapsids and mammals (Broom, 1915; 1924). Moreover, five distal tarsals are present, being the first and the fourth commonly the largest.

Peabody´s (1951) hypothesis was posteriorly refused by Rieppel (1993) who based on embryological evidence from extant reptiles stated that the reptilian~~,~~ as well as the synapsid astragalus~~, is a~~ neomorph. But Rieppel’s (1993) suggestion ~~did not convince at all~~ and the hypothesis on the ~~tripartite~~ structure of the reptilian astragalus remains. Recent reports ~~of~~ well-preserved tarsi from apparently young individuals, which will be discussed ~~later~~ (Kissel et al., 2002; Berman and Henrici, 2003; O’Keefe at al., 2006; Meyer and Anderson, 2013), prove that the matter is still ~~open~~.

Here we investigate the origin and evolution of the amniotic astragalus by a thorough study of several almost complete and also incomplete mesosaurid skeletons and natural external molds and casts, including well-preserved feet. Moreover, well preserved, isolated astragali and calcanei of individuals in different ontogenetic stages, including the tarsus of one *Mesosaurus* fetus and newborn individuals were also analyzed ~~for~~ completing an ontogenetic sequence previously unknown for any other basal amniote. This amazing record provides useful data for characterizing early and late juvenile stages ~~based on the tarsal structure~~ as well as help us to ~~know~~ the ~~transition~~ to the acquisition of the adult tarsal morphology. We present a synoptic view ~~about~~ the evidence ~~we~~ found for ~~determining the homology of~~ the primitive amniotic astragalus to the amphibian intermedium plus possible the tibiale and proximal centralia and propose that the fusion of these elements occurs during ~~the~~ embryonic stage ~~to produce~~ a very specialized single bone in the newborns. We also report the invariable presence of a navicular-like bone (fusion of c1+c2?) in *Mesosaurus tenuidens* (*contra* Modesto, 1996a-b; 1999) and discuss the possibility that ~~this character~~ can be polymorphic for mesosaurs as observed in basal synapsids (Romer and Price, 1940).

Institutional Abbreviations: FC-DP: Fossil Vertebrates of Facultad de Ciencias, Montevideo, Uruguay; GP/2E: Instituto de Geociencias (section Palaeontology), São Paulo University, São Paulo, Brazil; SMF-R: Senckenberg Institut, Frankfurt, Germany, MN: Museu Nacional de Rio de Janeiro, Brazil; AMNH: American Museum of Natural History, New York, USA.

Materials and methods

The specimens used in this study are part of several palaeontological collections and consist ~~in~~ almost complete and well preserved *Mesosaurus tenuidens* individuals and partially preserved skeletons that include the hind limbs, which are the subject of our study. They allow us to address the structure of the mesosaur tarsus and its component bones at different stages of development. All these materials plus isolated complete astragali and calcanei from juvenile and mature individuals were analyzed by using a binocular microscope and different techniques of photography, as well as digital drawings. Specimens from FC-DPV, GP/2E, MN and SMF-R were personally analyzed by the senior author (GP), while the specimens from AMNH were ~~just~~ studied from ~~pictures~~ kindly provided by personnel of that institution.

SYSTEMATIC PALAEONTOLOGY

Amniota Haeckel, 1866

Proganosauria, Baur, 1889

Mesosauridae Baur, 1889

Mesosaurus tenuidens Gervais, 1864-1865

Figures 1-2, 4-5

Performed study remarks. In order to know the structure and ontogenetic variation of the mesosaurid tarsus particularly that of the astragalus, we carried out an anatomical study of 50 mesosaurid specimens assigned to the species *Mesosaurus tenuidens*. We selected 18 individuals with well-preserved tarsi, including a foetus in late stage of development~~, for~~ represent an idealized ontogenetic transition (Figs. 1-2). ~~In a general view, the~~ mesosaurid tarsus displays a primitive construction regarding the structure observed in other basal amniotes as *Hylonomus lyelli* (Carroll, 1964; Meyer and Anderson, 2013), *Paleothyris acadiana* (Carroll, 1969) and *Petrolacosaurus kansensis* (Peabody, 1952) (Figs. 3-4). It is essentially equivalent to the tarsus of basal synapsids (Romer and Price, 1940; Romer, 1956) and it even mirrors the structure described for microsaurs (particularly *Tuditanus*, Carroll and Baird, 1968) and for the embolomeres *Gephyrostegus* (Carroll, 1970), *Westlothiana* (Smithson et al., 1993) and *Proterogyrinus* (Holmes, 1984).

The astragalus of immature mesosaurids is a delicate, roughly rounded or ~~maybe~~ subquadrangular bone bearing an evident dorso-medial thick border which will ~~be~~ develop~~ed~~ in very well defined articulating areas for the fibula and the tibia during growth ~~and producing respectively,~~ an slightly excavated central area in the dorsal margin and a broad, almost plane and sub-triangular surface medially placed. Th~~o~~se thickened margins can be seen even in ~~very~~ small newborn individuals (see Fig. 2D-G). ~~But~~ the ~~astragalus'~~ morphology changes dramatically during ontogeny; mature individuals bear a stout roughly squared bone with broad articulating facets for the crus (Fig. 4 A and C). It also possesses a wide, shelf-like latero-distal facet for receiving the centrale or navicular (Fig. 3), which can be totally separated from the astragalus, or partially fused in a way that the free, unfused part of the bone can only be seen ~~on the ventral surface~~ (Fig. 4).

Despite the mesosaur astragalus does not show the tripartite structure seen in *Captorhinus* (Peabody, 1951; Fox and Bowman, 1966; Kissel et al., 2002 and references herein) it seems to have been derived from the junction of three bones as we can deduce from the tarsus of the foetus (Fig. 5) where we interpret although with doubts, that the ~~bone forming the tarsus is an~~ astragalus formed by the suturing of the intermedium, the fibular and maybe the proximal centrale (c4). Indeed, some of the original sutures remain~~ed~~ in some specimens, but they show a different pattern than that described by Peabody (1951) (see figure 4A). In his 1993's study, Rieppl~~e~~ stated that the mesosaurid astragalus does not show any evidence of being a fusion of the amphibians tarsal elements; to him all the accidents (e.g. delicate grooves or thickness) seen in the ventral surface correspond to attachments of muscles and tendons and the medial groove delimitates the passage of the perforating artery.

In sum, the mesosaur tarsus consists in two proximal bones identified as the astragalus and the calcaneum plus a single navicular-like element and five elements in the distal tarsal series (Fig. 3), totalizing 8 ~~or 9~~ tarsal bones. The bones that form the "navicular" may be the centralia 1 and 2 regarding that c4 and c3 ossify very early in the ontogeny of other fossil and extant reptiles, while the former~~s~~ are the last ~~in~~ become visible (Caldwell, 1994). C1 and c2 fusion is often identified as the centrale (e.g. Currie, 1981), or as distal centralia (e.g. Carroll, 1970) or lateral centrale (e.g. Modesto, 1999) despite they are bones always placed medially in the tarsus. Similarly, the c4 is called as the proximal central (e.g. Kissel et al., 2002, Berman and Henrici, 2003, this paper), or posterior centrale or even distal central, but there is no stable denominations to the c3. This lack of consensus in the literature when alluding to a determinate central~~ia~~ increase the confusion about the establishment of evolutive patterns for the early amniotic tarsus. So, we decided to refer the bone (or bones) placed distally to the astragalus in the mesosaur tarsus as the “navicular”. It firstly appears as two sutured (but not fused) bones (Figs. 1 and 2 K-O) and there seems to be a reduction of c1size, which becomes a pointed medial tip which is not preserved in most individuals because the fragility of its suture to c2 (see Figs. 1 and 2O and 4B-C). In *Mesosaurus* the “navicular” strongly abuts to the wide platform-like facet on the distal margin of the astragalus.

The presence of the “navicular” in mesosaurs is a novel characteristic, as all but one (Modesto, 1996a,b; 1999) previous workers did not mention ~~their~~ presence ~~in descriptions of the mesosaurid tarsus~~. Indeed, Modesto (1996a,b) described the presence of a lateral central ~~just~~ in *Stereosternum* and stated that this bone is never present in *Mesosaurus*. However we have enough evidence to confirm that a transversely elongated bone is invariably present distal to the astragalus in all the analyzed specimens—most frequently representing two sutured bones— identified as the centralia c1 and c2 present in “pelycosaurs” and other basal amniotes. As this bone abuts the astragalus in very mature individuals, as also seems to occur in *Captorhinus aguti* (Peabody, 1951), it becomes difficult to identify its presence in the *Mesosaurus* tarsus (see Fig. 4).

Description. All specimens from Uruguay were collected either in bituminous or non-bituminous shale of the Early Permian (Artinskian) Mangrullo Formation~~, as well as all the materials coming from Brazil were collected~~ in the correlative Iratí Formation (Santos et al., 2006). Each of the constituent tarsal elements will be described ~~for the specimens representing the transition regarding their ontogenetic stage and the morphological changes detected~~:

1- FC-DPV 2504 (Figs. 1A and 2A). An almost complete and well preserved *Mesosaurus tenuidens* foetus from Uruguay, which is curled as within an egg (Piñeiro et al., 2012a). It consists ~~in~~ an external mould of a small, still poorly ossified skeleton that suffered a strong dorsoventral compression during diagenesis. This is evidenced by the disposition of the ribs and feet which are overlapping each other~~,~~ as well as for the reduced three-dimensionality (suggesting strong compression) of the delicate skeleton~~, which represents the smallest mesosaur ever known~~. ~~While~~ some of the constituent bones of the feet may be not completely ossified ~~(considering the small size and the poor preservation of the manus)~~ the extraordinary preservation of the specimen allowed us to reconstruct the structure of the tarsus ~~and to describe the bones that seem to be present~~ (Fig. 5). ~~Just~~ one composite element can be seen, or three agglomerated bones with clear joints between them can still be observed. We ~~can~~ interpret this assembly as the embryonic astragalus (taking into account the composite hypothesis) but we do not dismiss the hypothesis that what we are seeing are in fact the astragalus precursors (see Fig. 6). The bones represented may be the intermedium, the tibiale and a proximal centrale, probably c4 which has proved to ossify early in aquatic reptiles ~~(~~c1 and c2="navicular" ~~may~~ ossify very late in mesosaurs~~,~~ Figs. 1-2). Other possibility is that the tibiale already fused to c4 and both to the intermedium, and we are observing two bones. The fibulare (the calcaneum precursor) ossify ~~very~~ after the c4 does (Caldwell, 1994), thus it may be possible that it is already present in the ~~foetus~~ tarsus. Considering the presence of only two ossified bones in juvenile individuals, it is possible that the intermedium and the tibiale fuse early in the ontogeny as some previous workers suggested (e.g. Gegenbaur and Williston, in Schaeffer, 1941). Indeed, the tibiale fuses to c4 in *Proterogyrinus* and there was proposed that both fuse also to the intermedium to produce the amniotic astragalus (Holmes, 1984). Distal tarsals could be not yet formed, but according to the presence of at least metatarsals II and III possibly total or partially ossified and the apparent~~ly~~ absence of metatarsal V, we are inclined to suggest that they are masked by the pedal aponeurosis or were displaced between the overlapped metatarsals (see Sheil and Portic, 2008 as a reference).

2-AMNH 23799 (Figs. 1B and 2B). This is one of the specimens referred by Rieppel (1993) and it is also a very small individual from Brazil. Despite its small size, the difference with the smaller FC-DPV 2504 is notable. The tarsus is obscured by the distal part of the tibia in the right limb but at the left, there can be seen the small, sub-~~squarer~~ astragalus showing clearly the typical roughly rounded articular facet for the tibia. It is slightly displaced from its original anatomical position and was preserved in an oblique arrangement respecting to the horizontal plane. The calcaneum is not preserved unless it is a very tiny element that was displaced beyond the astragalus, over the phalanges (Fig. 1B).

3-GP-2E 272 (Figs. 1C and 2C). This specimen is a well preserved very young individual from Brazil. Ribs are not as pachyostotic as ~~can be observed~~ in other immature specimens, but aside that condition, the specimen does not show relevant anatomical differences regarding *M. tenuidens*. The silhouette of part of the body can be reconstructed due to the preservation of the skin. The membrane that unites the toes to the claws can be delimited as well as the robustness of the leg musculature in even such a young individual. What could have been the plantar aponeurosis covers most part of the tarsal bones. However, two elements (maybe ossified cartilages) placed very close to the fibula are interpreted here as a possible astragalus (the largest bone) and an incipient, smaller calcaneum. It is also possible to see shadow-like structures that can be interpreted as some of the distal tarsals (e.g. d4), which starts ossification at very early ontogenetic stages in extant reptiles (Caldwell, 1994; Sheil and Portik, 2008). What appear to be scratch marks (according to Sedor and Costa Da-Silva, 2004) are observed close to the left foot, maybe produced by the individual before its sudden and perhaps unexpected death. But these structures can rather be part of the muscle and skin ~~that form~~ the base of the tail, exquisitely preserved. These taphonomic features support the hypothesis that the tarsal elements, even if still cartilaginous could have been perfectly preserved, but covered by the plantar aponeurosis, which is not frequently observed in fossil tetrapods.

4-SMF-R 4496 (Figs. 1D and 2D). This specimen constitutes an external mould of a partially preserved posterior trunk and tail, with associated pelvic girdle and limbs from the Iratí Formation. This is the specimen that ~~better~~ shows the structure of the tarsus in immature, juvenile mesosaurids; the preserved bones might be partially ossified. The specimen is comparatively larger than the two anteriorly described and the tarsus is formed by two roughly rounded small bones, which can be homologated with the astragalus (the largest) and the calcaneum (the smaller), which do not ~~meet together~~ but they are one in front to the other and positioned as in mature individuals. Despite its apparent general subcircular outline, the astragalus indeed shows a structure similar to that preserved in adults or sub-adult individuals bearing thickened articulating areas and some lines of sutures, although it is difficult to say how many bones may be involved.

5- AMNH 23795 (Figs. 1E and 2E) is an articulated, very complete skeleton of a young mesosaur, which bears a tarsus showing the same structure seen in SMF-R 4496 (probably because they are individuals of equivalent age). Both the astragalus and calcaneum can be clearly seen close to each other. Again, the astragalus shows the same structure as in previously analysed specimens and sutures between component bones are perfectly visible.

6- The specimens MN 4741 and SMF-R 4934 (Figs. 1F-G and 2F-G respectively) and GP-2E 664 (Figs. 1H and 2H) from Brazil are a little larger than the previously described materials and we can see for the first time the morphological differences between ~~both~~ the proximal tarsal bones in the ontogenetic ~~stage~~ series, being the astragalus ~~transformed into~~ a more stylized and easier ~~recognizable~~ element although the size of the specimens is still small. Distal tarsals appear to be more or less ossified at these stages (e.g. SMF-R 4513 in Figs. 1I and 2I). Astragalus and calcaneum are preserved close to their normal anatomical arrangement and the foramen for the perforating artery ~~starts developing~~ between these bones (see GP-2E 664 and SMF-R 4513, Figs. 1H-1I and 2H-2I).

7- SMF-R 4513 (Figs. 1I and 2I) is probably a young adult individual. ~~There are three bones present; two proximal tarsal elements visible,~~ the largest ~~one~~ is the astragalus which features a very similar morphology ~~like~~ to the one observed in more mature individuals. It is a stout bone ~~tending to reach the~~ L-shaped outline characteristic of ~~the~~ basalmost amniotes and some tetrapod taxa~~, probably ancestral groups~~ (see Fig. 6). The foramen for the perforating artery is placed at ~~the middle length~~ of the lateral margin, and an intimate area ~~of contact is being generated~~ between astragalus and calcaneum ~~at this point. However,~~ at later stages these bones develop a long contact through most of the entire lateral margin of the astragalus and the medial margin of the calcaneum (Figs. 1 and 2 J to Q). A small bone can be seen below the astragalus-calcaneum contact in SMF-R 4513~~, which is located over the distal tarsal elements~~. It could be the distal tarsal four or the “navicular” starting to ossify, which ~~will be well developed later,~~ in mature *Mesosaurus* specimens.

8- The remaining analyzed specimens (FC-DPV 2497, GP-2E 114, GP-2E 5610, SMF-R 4710, SMF-R 44 70, GP-2E 5816, GP-2E 6576, GP-2E 5740 and FC-DPV 2058 , (see figures 1J-R and 2J-R) represent adult individuals and most of them possess the complete series of tarsal elements: astragalus, calcaneum and “navicular”, as well as five distal tarsals, where the first and the fourth are the largest (Fig. 3). In *Mesosaurus* a significant delay in mesopodial ossification is noted, differing from ~~the~~ observed in most terrestrial tetrapods (Caldwell, 2002, 1994, 1992a, 1992b), ~~where~~ propodials, epipodials and metapodials are ossified ~~prior while~~ the mesopodials are still formed of cartilage. However, unlike other aquatic species as *Hovasaurus boulei* or living lepidosaurs (Caldwell, 2002, and references therein) mesosaurids present an early ossification of astragalus and calcaneum. Thus mesosaurid tarsal ossification proceeds following the sequence: intermedium, tibiale+central four, calcaneum and for the last, distal tarsal four, the “navicular” and the remaining bones (distal tarsals 1-3 and 5). The sequence of ossification of this lasts bones is not clear, however. This pattern of ossification is mostly according to recent discoveries in the fields of paleontology and developmental genetics looking for patterns and processes of vertebrate limb evolution (Caldwell, 1994; 2002 and references therein). Moreover, it highlights the potential conservatism of the underlying genetic controls on/of limb development patterns.

The astragalus is the largest bone in the mesosaurid tarsus and as mentioned above, it features an L-shaped outline in dorsal view. The proximal border is deep and bears an extended rectangular facet for the fibula, making an almost immobile articulation between these bones, as in basal synapsids (Romer and Price, 1940). The foramen for the perforating artery is poorly developed in young individuals. It is just marked by a simple shallow notch that appears at the middle of the astragalar lateral border and it does not correspond to a similar structure in the calcaneum (see Figs. 1 and 2I to L). However, it is well developed in larger (more mature) individuals where the notches in both bones approach each other to form a notable true foramen (see Figs. 1 and 2 M to Q). The groove for the passage of the perforating artery crosses the bone medially and proximally, where a rugose area is visible (Fig. 4). Probably it marks the line of suture of both of the larger bones seen in the fetus’ astragalus, implicating the intermedium and the c4+tibiale complex. Considering this hypothesis as the most probable, another line of suture located at the medial corner of the astragalus of adult individuals may correspond to the delimitation of the remains of the tibiale bone and includes the articular facet for the tibia at the medial margin (Fig. 4). This line of suture is also seen to be continuing at the medial margin, where it runs just above the articular facet for the tibia. This facet is wide and like a teardrop in shape which allows for a broad (motile) articulation with the tibia (Fig. 4 A and C). It is interesting to note that the same type of articulations (and very similarly shaped facets) for the fibula and the tibia were described for the pelycosaur tarsus, as well as the presence of a medio-ventral extension interpreted as a cartilaginous remnant of the tibiale (Romer and Price, 1940).

The “navicular” can be preserved as a separate element or it could be partially fused to the astragalus in some individuals (see Figs. 1J and 2J as a reference of the condition and Fig. 4). This polymorphic condition concerning the fusion of the centralia 1 and 2 recalls that observed in pelycosaurs in which some species show the centrale 1 and 2 as separate bones (e.g. *Ophiacodon*), while others show them fused (e.g. *Haptodus*) (Romer and Price, 1940) (Fig. 6). Moreover, the morphology of the c1in mesosaurids is very similar to that of the medial centrale of *Sphenacodon ferox* (according to Henrici et al., 2005). Perhaps the repositioning of that bone allied to the distal tarsal one by Henrici et al. (2005) should be changed and reconstructed as the fractured medial pointed part of the navicular in *Sphenacodon*.

Discussion

Evolution of the astragalus structure

Despite most previous workers (e.g. Carroll, 1964; Berman and Henrici, 2003; O´Keefe et al., 2006; Meyer and Anderson, 2013, and reference herein) have acknowledged the composite, tripartite origin of the astragalus after the persuasive contribution of Peabody in 1951, the reappraisal of that condition and their significance performed by Rieppel in 1993, introduced controversy. This ~~last~~ author refused the tripartite origin of the astragalus arguing ~~for~~ the lack of unequivocal ontogenetic evidence ~~that proves~~ that the bones which will form the composite astragalus are present in at least some stages of the ontogenetic development. He refuses the proposed composite origin of the astragalus by Peabody (1951) mainly based in the fact that this bone derives from a single ossification center in extant reptiles and according to Sewertzoff (1908) lizards have just a single block of cartilage close to the distal end of fibula and tibia where the astragalus later ossificates. It is also known that in *Sphenodon punctatus* the astragalus originates by the condensation of more than one condrogenic element but they fuse during the ontogenetic embryological stage (Rieppel, 1993).

Indeed, there are several known examples of ?stem amniotes or ?proto-reptiles that allow us to deduct the steps of fusion of the tarsal bones until reaching the amniote condition (Fig. 6). Thus, it is possible that, as the embryology of extant lizards suggests, the fusion of these elements ~~in~~ the development of the amniot~~a~~ ankle ~~is produced~~ in the embryonic stage and so it is not possible to address their original ossification centers anymore (Gauthier et al., 1988). Rieppel (1993) observed that associations of tarsal bones are common in amphibians and that while centralia 1 and 2 can be fused or separated, c3 and c4 may be fused, or rather, one of them can be lost. Thus, according to Rieppel (1993) the association between the tibiale and c4 may be casual and do not represent a condition of phylogenetic relevance. However, we can see a real transition from closely related, supposedly non amniote taxa (?proto-reptiles, ?stem amniotes), to the acquisition of the primitive amniotic tarsal configuration (see figure 6). Thus, if we consider the association of the tibiale and c4 observed in some *Proterogyrinus* specimens (Holmes, 1984) as the first step to the development of the amniotic tarsus (Holmes, 1984), we can reconstruct the succession including *Gephyrostegus* (Carroll, 1970) where the tibiale+c4 (and c3?, see O'Keefe et al., 2006) complex is associated to the intermedium to form the composite amniotic astragalus. Moreover, in that transformation the fibulare becomes the calcaneum and c1 and c2 remain as the only centralia present in early taxa, either as separated bones or fused to form a single element. The microsaurs (e.g. *Tuditanus punctulatus*, Carroll and Baird, 1968, but see also Carroll and Gaskill, 1978) could have been the last phylogenetic intermediaries (thus supporting the Laurin and Reisz, 1997 hypothesis of close relationships between lepospondyls and amniotes) or, they could have developed an amniotic-like tarsus convergently.

Taking into account the previous evolutive transition in favor to a composite (tripartite or even multipartite) origin of the amniotic astragalus, ~~which of course, may also include other taxa,~~ the interpretation of Peabody (1951) and posterior workers about the presence of more than one ossification center in the astragalus of *Captorhinus* and other basal amniotes seems sensate. But, other extensive descriptions of *Captorhinus* (e.g. Fox and Bowman, 1966) do not provide more conclusive evidence about the structure of the tarsus and, as Rieppel (1993) claimed, it is necessary to provide ontogenetic evidence to prove the homology of the bones composing the tripartite astragalus by their presence in the earliest stages of development of *Captorhinus*, *Hylonomus*, or diadectids (Romer, 1944; Romer and Byrne, 1931). The required evidence came partially from discoveries of possible juvenile diadectomorph tarsi including a putative composite astragalus formed by the intermedium, the fibulare and the proximal centrale (c4, as it was identified) (Berman and Henrici, 2003). Posteriorly, these materials were assigned to the species *Orobates pabsti*, a basal diadectomorph (Berman et al., 2004). Recently, the holotype specimen of *Orobates* described by Berman and Henrici (2003) (MNG 10181) as having a tripartite astragalus was subject of a deep study using micro-focus computed tomography scans (Nyakatura et al., 2015), which allowed for a thoughtful anatomical understanding of the specimen. The scanned image and digital reconstruction shows that there are six separated bones in the tarsus of *Orobates*, which morphology suggests that they could be homologated with immature astragalus and calcaneum plus four distal tarsals. Indeed, despite the very good preservation of the individual, it apparently was subjected to severe diagenetic distortion and the bones were embedded in a crystalline calcite matrix and there was a significant chemical substitution around their margins (cf. Nyakatura et al., 2015). That taphonomic feature could have produced a configuration that under direct examination, lead to the interpretation of Berman and Henrici (2003) about the presence of a composite astragalus in *Orobates*.

Berman and Henrici (2003) also described two associate (maybe sutured) tarsal bones which they recognized as the intermedium and the fibulare of a juvenile *Diadectes*. However, the shape of the bones, mostly subcircular, and their relative size and proportions remind the astragalus and calcaneum of a very young individual, taking into account the ontogenetic stages described here for the very basal amniote *Mesosaurus tenuidens*.

Concerning captorhinids, most of the isolated astragali figured by Peabody (1951) and assigned to *Captorhinus* clearly belong to mature animals, according to their size and structure (see Fox and Bowman, 1966 for comparison) and the smallest one already shows the same structure and morphology seen in the larger ones. If the astragali shown by Peabody (1951) partially represent an ontogenetic transformation series, ~~they~~ cannot confidently prove that the apparent tripartite structure is derived from the fusion of three or four of the amphibian tarsal bones. A feature that can be interpreted as a weakness of the tripartite structure is that the sutural lines and groove patterns present in *Captorhinus* as described by Peabody (1951), are only visible on the ventral surface of the bone, suggesting that the fusion started on the dorsal surface and was not completed in adult individuals. The same condition can be observed in the ~~big~~ captorhinid *Captorhinus magnus* (Kissel et al., 2002).

Regarding the condition in the purposed pes of a juvenile individual of the giant, largest known captorhinid *Moradisaurus grandis* figured and described by O'Keefe et al. (2006) it is difficult to make a commentary. The material was found isolated and we could not find any character supporting the assignation to *Moradisaurus* more than to any other basal tetrapod of the same size. Besides, it is difficult to include that material in an ontogenetic transformation series having just one isolated, putative juvenile pes that is purposely related to

Moradisaurus. Consequently, the anatomical comparison to the *Moradisaurus* pes figured in O'Keefe et al. (2005) is much too speculative.

Even considering the inconsistencies previously mentioned, the captorhinid inquiry regarding the evolution of the tripartite astragalar bone should be seriously taken into account in the light of the new evidence provided by mesosaurs. The pes of *Captorhinus aguti* described by Holmes (2003) is very interesting as the astragalus shows a very similar structure to that described here for *Mesosaurus tenuidens*. There is a well-defined suture for the tibiale which can be seen from the medial view, where a wide facet for articulation with the tibia is evident. Besides, the distal margin of the figured isolated astragalus is concave as in *Mesosaurus*, at the ontogenetic stage when the "navicular" is not yet abutted against it. Interestingly, the reconstruction of the pes provided by Holmes (2003) includes a suture representing the suture of what appears to be the c4+c3 complex, plus the "navicular", showing both pairs the same morphology. The suture uniting the intermedium+tibiale complex to the putative c4+c3 complex is not compatible as it runs across the tibiale (see figure 6 of Holmes, 2003). Besides, the putative existence of that junction would avoid the astragalus laterodistal projection to reach the distal tarsal 4. That projection is already present in the intermedium+tibiale complex. Thus, it is possible that in captorhinids, as in mesosaurs, the bones that fuse to form the astragalus (intermedium+c4+tibiale complex) are indeed those that form the lateral central (most possibly c1+c2). Hopefully, if very early juvenile skeletons of *Captorhinus* preserving the tarsus could be found, they will be in harmony to that shown by mesosaurs.

Revising the evidence from other basal amniotes as *Hylonomus lyielli* (Carroll, 1964; Meyer and Anderson, 2013) we found some inconsistencies related to the identification of the bones figured, perhaps as a result of an exacerbation of Peabody's (1951) tripartite origin of the astragalus. Thus, Meyer and Anderson (2013), following Carroll (1964, fig. 1) considered the calcaneum of *Hylonomus* as two times larger than the astragalus. According to the information found in Carroll (1964, p. 72, fig. 8) and based on the ontogenetic succession that we described here for mesosaurs, the calcaneum can sometimes ~~to~~ be equal to the astragalus or even a little larger, but never larger (that larger). Thus, we can deduce that bones from individuals of different ~~ages~~ were contrasted, or the bone identified as the calcaneum is indeed the astragalus. The bone consider to be the calcaneum by Meyer and Anderson (2013) possesses a consistent morphology with immature astragali, being a sub-spherical to roughly quadrangular element developing thick and prominent margins for the fibular and tibial articulations dorsally and medially. It even articulates ventrally with what appears to be the fused centralia 1 and 2 (a widespread condition among basal amniotes) (see figure 3 in Meyer and Anderson, 2013 and Fig. 5). Intriguingly, ~~both~~ of the astragali figured by Meyer and Anderson (2013) as belonging to *Hylonomus* show~~s no~~ signs of lines or grooves that suggest a composite origin.

The ontogenetic tarsal transformation in mesosaurs

Following the above ~~paragraphs~~ we ~~tend to~~ conclude that the referred putative ontogenetic proofs about the tripartite astragalus are not conclusive and might be inclined to follow the reasoning of Rieppel (1993) who based his hypothesis on the valuable evidence provided by mesosaurs. However, the morphological transformations observed in the skeleton of the mesosaurid *Mesosaurus tenuidens* during the ontogenetic development ~~include~~ significant changes in the evolution of the tarsus. From a *Mesosaurus tenuidens* foetus found in the Early Permian of Uruguay (see Piñeiro et al., 2012a) we knew that the cartilaginous precursors of the typical amniotic astragalus, join each other before of the birth, but sutures remain (Fig. 5). The following early stages are characterized by the presence of sub-spherical to roughly square small ~~bones, mainly representing the~~ astragalus ~~(as the mostly preserved)~~,

~~while~~ the morphology of the adult astragalus can be deduced through not always well preserved accidents (sutures, rugose surfaces and thickened margins). In the early stages both astragalus and calcaneus seem to have been separated as there is no evidence of contact between them. The foramen for the perforating artery is not visible at these early stages that we consider as juvenile mesosaurids. At the following stage, the astragalus becomes more quadrangular in shape, approaching to the calcaneum and an incipient foramen for the perforating artery starts to develop. At this stage, mesosaurids appear to be young but mature individuals. The remaining transformations are crucial for the growing of the individuals and improvement of their capabilities of prey capture and reproductive traits (see Ramos, 2015; Villamil et al., 2015; Piñeiro et al., 2012a). Thus, the tarsal bones ~~will~~ change in the path of increasing robustness and extending of surfaces for fusion to near tarsal elements and to favor strong tendon and muscle insertions. The tibial-astragalus articulation is the weakest in the mesosaur tarsus, as occurs in pelycosaurs (cf. Romer and Price, 1940). The “navicular” is a bone present in both synapsid and sauropsid amniotes, in the later, it is observed at least in their basalmost representatives. Therefore, a “navicular” is found in Captorhinomorpha, basal diapsids, some Parareptilia and Mesosauridae and in all pelycosaurs (Fig. 6). Later, it becomes a bone that is characteristic of just the proto-~~mammalians~~ and proper ~~mammalians.~~ It ossifies at a late stage (at the same time as the foramen for the perforating artery starts to be noted) and is separated from the astragalus in most individuals or abutting against the distal margin of this bone, even fusing partially with it in mature individuals (Fig. 4). That means that the presence of the “navicular in mesosaurs is indicative of maturity.

The morphological ontogenetic transformation presented here for *Mesosaurus tenuidens* is the most complete ever known for a basal amniote (cf. Laurin and Reisz, 1995) and as such, it constitutes a relevant database for studies of different nature. The information provided for this data base on the origin of the amniotic tarsus suggests that as Peabody

(1951) and previous authors (e.g. Holmgren, 1933) have stated, the astragalus is originated from at least three ossification centers near the tibial and fibular distal margin. However, we found evidence that the fusion of the amphibian tarsal' should occur at some point in very early stages of the development. According to our observations of the *Mesosaurus tenuidens* foetus which possesses an astragalus formed by at most three bones we can say that the mesosaurid astragalus is not a neomorph as Rieppel (1993) has suggested. The evidence taken from some ?proto-reptiles taxa as the embolomere *Proterogyrinus scheelei* (Holmes, 1984) can even support the tripartite hypothesis and the identification of the bones provided in the present work.

It is noteworthy that some taxa which are not classified as amniotes have an amiote-like tarsus or at least developed the large proximal tarsal bones that characterize the amniotic tarsus, the astragalus and the calcaneum (Fig. 6). Notable examples of this feature are the diadectids (Romer and Byrne, 1931; Romer, 1944) earlier analyzed, and the lepospondyl microsaurs with *Pantylus* (Carroll, 1968) and *Tuditanus punctulatus* as having the most intriguing amniote-like tarsus (Carroll and Baird, 1978). Moreover, the embolomeres *Proterogyrinus scheelei* (Holmes, 1984), *Westlothiana lizziae* (Smithson et al., 1993) and *Gephyrostegus bohemicus* (Carroll, 1970) also have a very amniote-like tarsus. Because mesosaurids are very basal amniotes (Laurin and Reisz, 1995; Modesto, 1996 a-b; Modesto, 1999; Piñeiro, 2004; Piñeiro et al., 2012b) ~~we explored within these last taxa in order to find homologies between supposed amphibian tarsi and their corresponding structure in mesosaurids according to the different ontogenetic stages described for the group. We made several interesting observations that support the already established homologies and possible evolutive paths on the origin of the primitive amniotic astragalus.~~ Particularly in *Proterogyrinus* the intermedium has a very similar structure than that of the astragalus of young mature mesosaurs and the tibiale is clearly sutured against the medial corner of the intermedium. The fibulare is also very similar to the calcaneum of the same stage (see Figs. 1 and 2) which is logic to presume that these bones are homologous, as already stated. The main question is what happens to the remaining bones to obtain the mesosaurid (=basal amniote) tarsus consisting in two large proximal elements plus one or two centralia and five distal tarsals. We do not find any evidence about the destiny of c3 and c4 in the described mesosaur ontogenetic transformation. However, based on the structure shown by *Proterogyrinus* (Holmes, 1984) where apparently the tibiale fuses to the c4 and taking into account that shown by *Labidosaurus* (Williston, 1917) where the intermedium and the tibiale also fused to c4, we hypothetized three possibilities or combinations: A, the astragalus is just formed by the intermedium+tibiale and c4 and c3 undergo a reduction in size until they finally disappear; B, it is formed by intermedium+tibiale+c4 and c3is reduced to be lost and C, astragalus results from the fusion of all the bones, i+te+c4+c3 (Fig. 7A). The last possibility (C), which is similar to that suggested by Peabody (1951) and posteriorly followed by other workers (particularly O'Keefe et al., 2006), has the particularity that does not imply the loss of bones but a repatterning to produce the amniotic tarsus. Moreover, there are also two possibilities for the formation of the "navicular": 1, results from fusion of c1 and c2 and 2, it is formed by just the c2 after the reduction and lose of c1 (see Fig. 7B). We found ~~probably~~ evidences of some of these fusions (the tibiale+?c4+ intermedium) in early stages of *Mesosaurus tenuidens'* ontogenetic development, but not of all. Maybe some could have produced in primitive taxa that maybe can be related to the origin of the amniotes.

If the hypotheses of the astragalus and the "navicular" formation are combined we can have the following six possibilities: A-1; A-2; B-1; B-2; C-1; C-2, from which we found some evidence just for the first four in mesosaurs.

The possible "implicit" relationship between mesosaurids and basal synapsids

In 1941, Friedrich von Huene proposed for the first time a phylogenetic relation between *Mesosaurus* and some basal pelycosaurs. That suggestion was not generally acknowledged by later authors who developed the currently accepted theory that mesosaurids are the basalmost sauropsids (Laurin and Reisz, 1995) or the basalmost parareptiles (Modesto, 1999). More recently, Piñeiro (2004) found some evidences that she understood gave support to von Huene's hypothesis (1941) but acknowledged that they should be tested in a phylogenetic context. Moreover, the nature of the mesosaurid skull, long discussed during more than a hundred years has been recently reassessed to denote the presence of a synapsid-like lower temporal fenestra in *Mesosaurus tenuidens* (Piñeiro et al., 2012b). This contribution gave credit to the observations made by von Huene (1941) several years ago about the morphology of the mesosaur skull. Similarly, the tarsus of mesosaurs has been studied by several authors and here we demonstrated that its structure is almost identical to that described for basal synapsids. Indeed, the tarsus is both structural and morphologically equivalent in the two groups, except because in pelycosaurs there is no evidence for the tripartite formation of the astragalus, thus generating doubts about the homology of this bones in synapsid and sauropsid amniotes (Riepple, 1993). Besides, there is a greater development of the calcaneum (Romer and Price, 1940), which in some taxa roughly acquires the size of the astragalus. On the contrary, the calcaneum of *Mesosaurus* is smaller than the astragalus and develops a lateral expansion in the area of the heel, possibly for insertion of flexor tendons including the Achilles tendon (Fig. 3).

Current morphological and comparative studies on the mesosaurid skeleton are suggesting other interesting similarities between mesosaurids and basal synapsids that will be properly described in a forthcoming paper, but these features are also shared with other basal taxa that are not even amniotes. For instance, mesosaurs share characters with taxa previously enclosed in Reptiliomorpha (Panchen and Smithson, 1988 but see also Smithson et al., 1993) and now considered as stem amniotes or with not well defined affinities. This commonly shared morphology among apparently not related but very basal taxa reflects the primitive nature of mesosaurids, already noted by Huene (1941) and other paleontologists. The example of the similar tarsal structure observed in mesosaurids, microsaurs, basal synapsids and non-amniote tetrapods suggests that the evolution of the astragalus and calcaneum as the most typical bones in the amniotic tarsus could ~~be an acquisition obtained much earlier than the first recognized amniote appeared and walked on the planet~~.

Conclusions. The changes produced in the mesosaur tarsus structure during ontogeny were established based on the study of several specimens preserved in different stages of development. This transformation series is the most complete known for a basal amniote as it includes even embryological information. Our results allow determining that the mesosaur tarsus includes 8 or 9 bones: astragalus and calcaneum plus centralia 1 and 2 (fused to form the mesosaur "navicular") and five distal tarsals. The "navicular" is proved to be present in all mesosaurids, even in *Mesosaurus* where it fuses to the astragalus in mature individuals. The early amniote astragalus is a composite bone as can be evidenced by the presence of at most three sutured bones in the tarsus of a mesosaur foetus in advanced stage of development. This tripartite structure is different to that ~~purposed~~ by previous authors mainly based on the tarsus of *Captorhinus aguti*, but can be seen even in the astragalus of very young individuals. Thus, our study rejects the hypothesis that the amniotic astragalus and calcaneous are neomorphs. Regarding the analyzed ontogenetic series we could determine that attainment of maturity in mesosaurs can be related to a determinate tarsus structure, which can be a good age indicator to extrapolate to other groups of basal amniotes. The evolution of the amniotic tarsus can be followed ~~throw~~ a series of transformations, including fusion of bones, ~~produced~~ in non-amniot~~ic~~ groups which were originally described as ~~truly~~ amniotes. ~~While~~ the taxa currently recognized as the most closely related groups to the origin of ~~the~~ Amniota retain~~s~~ an amphibian-like rather amniot~~ic~~-like tarsus, other~~s~~ apparently not related intriguingly achieved an equivalent amniotic structure. This fact leads us to suggest that very probably the amniot~~ic~~ tarsus ~~arises~~ much earlier than the earliest recognized amniote had appeared. Thus, the knowledge of the tarsal ossification sequences in the basal amniote *Mesosaurus tenuidens* may contribute to the establishment of homologies that ~~would~~ shed light ~~to~~ problems concerning phylogenetic relationships~~,~~ and ~~evolutionary trends~~ that have constrained the appearance of an amniotic-like tarsus in currently considered anamniote groups.

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Figure captions

Figure 1. *Mesosaurus tenuidens* ontogenetic transformation transition in the tarsus formation. Photographs of the 18 selected specimens preserving epipodial, mesopodial and metapodial elements, from a list of 50 that were revised during the present study. See text for further descriptions of each the included specimens. Scale bar: 5 mm.

Figure 2. *Mesosaurus tenuidens* ontogenetic transformation transition in the tarsus formation. Schematic outlines of the 18 selected specimens preserving epipodial, mesopodial and metapodial elements, from a list of 50 that were revised during the present study. See text for further descriptions of each the included specimens. Scale bar: 5 mm.

Figure 3. Anatomical reconstruction of the crus in an adult *Mesosaurus tenuidens*. Colors indicate the different elements that form the tarsus and the crus. Scale bar: 10 mm.

Figure 4. Ontogenetic transition of the “navicular” in *Mesosaurus tenuidens*. A, FC-DPV 1502, from left to right, photographs and interpretive drawings of isolated astragalus from a young individual, in dorsal, ventral and medial views respectively. The bone shows the typical square outline of immature individuals and the remains of sutures between the original anlagen more visible on its ventral surface. Note that there is no traces of the “navicular” preserved along the distal surface of the astragalus. B, GP-2E 5203, photograph and interpretive drawing of astragalus, calcaneum and incipient “navicular” of a young individual in dorsal view. Recall on that the “navicular” is already united to the astragalus by the c2 is formed by c1 and c2 and the suture

between them is still well visible. C, FC-DPV 1479, photographs and interpretive drawings of an isolated astragalus from an adult individual in dorsal, ventral and medial view. Observe that the “navicular” is now a single bone almost completely fused to the astragalus to produce the adult outline. C1 has transformed into a tip-like bone and remains separated from the astragalus, but it just can be seen from the ventral view. The wide and triangular facet for articulation with the tibia can be seen from the medial view. Anatomical abbreviations: a, astragalus; ca, calcaneum; c1, centrale one; c2, centrale two; c4, centrale four; ft, facet for the tíbia; i, intermedium; te, tibiale. Scale bar: 5 mm.

Figure 5. Preserved tarsus in a *Mesosaurus tenuidens* terminal foetus. A, SEM image of the pes which were preserved overlapped each other. B, Interpretive drawing of the tarsus including a possible identification of the preserved bones by regarding previous hypotheses about the origin of the amniotic astragalus. Anatomical abbreviations: ?i, possible intermedium; ?c4, possible central four; ?te, possible tibiale. Scale bar: 1mm.

Figure 6. Tarsus structure in basal tetrapods, including amniote and non amniote taxa. Schematic diagram for comparing the tarsus structure in the basal tetrapods *Acheloma* and *Greererpeton* (amphibian-like tarsus) respect to that of embolomeres and microsaurs (amniote-like tarsus) and truly and primitive amniotes. Note the similar structure and construction of the microsaur tarsus respect to the early amniote *Hylonomus*. See text for more details of the evolutive significance of the selected taxa. Abbreviations: as, astragalus; i, intermedium; te, tibiale; 1,2,3,4, centralia; i,ii,iii,iv,v, distal tarsal.

Figure 7. Hypotheses about the astragalus and the navicular formation. The schematic diagram shows the steps that lead to the formation of the amniotic tarsus, remarked the

series of possible transformations that could have produced the primitive astragalus (A) as well as those that prevailed into the evolution of the “navicular” bone.

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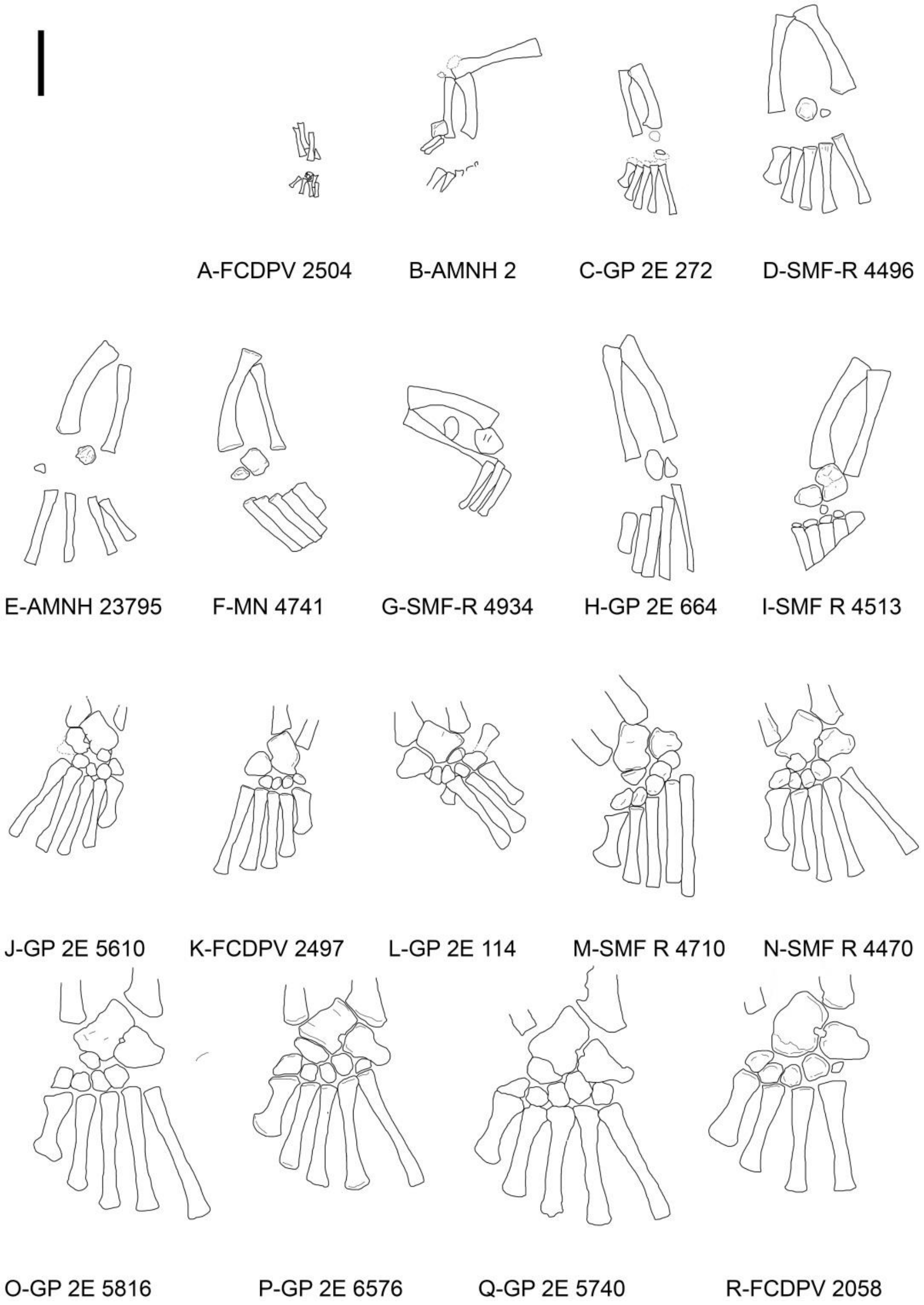

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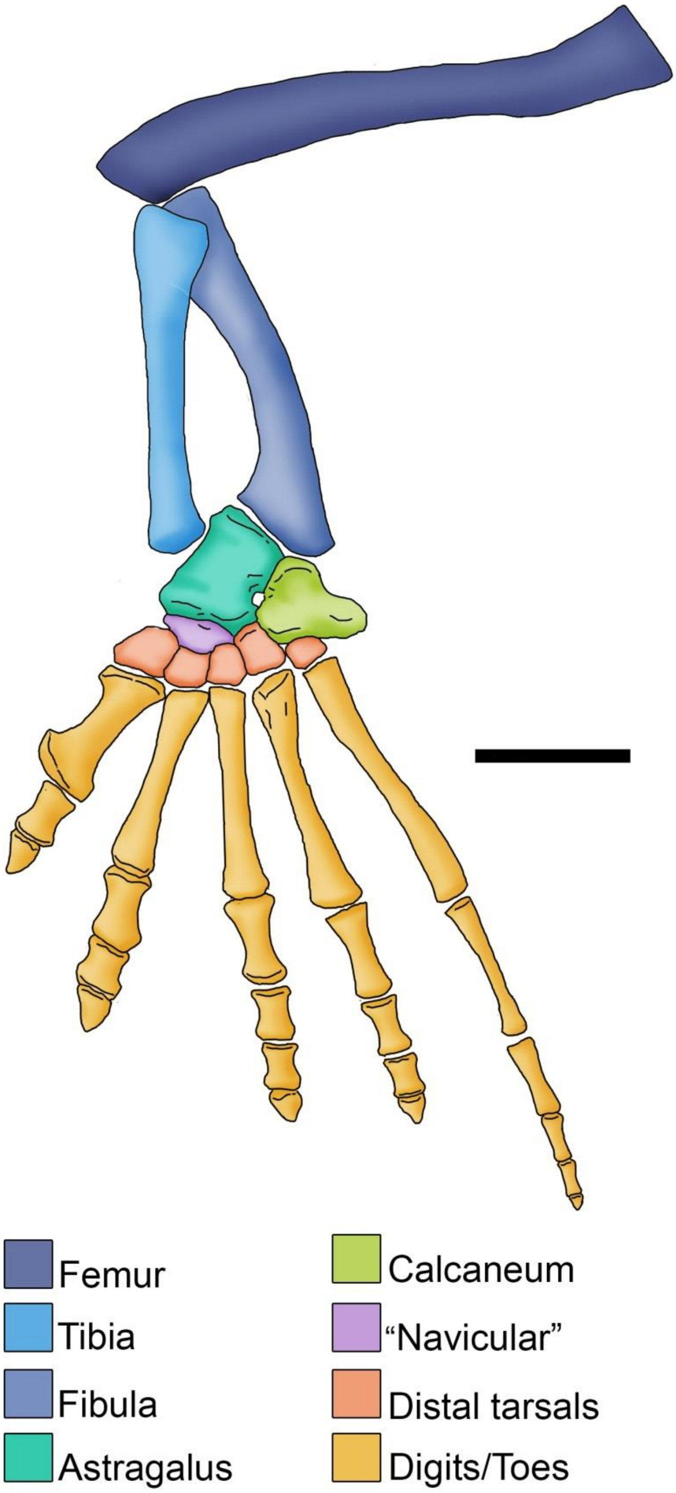

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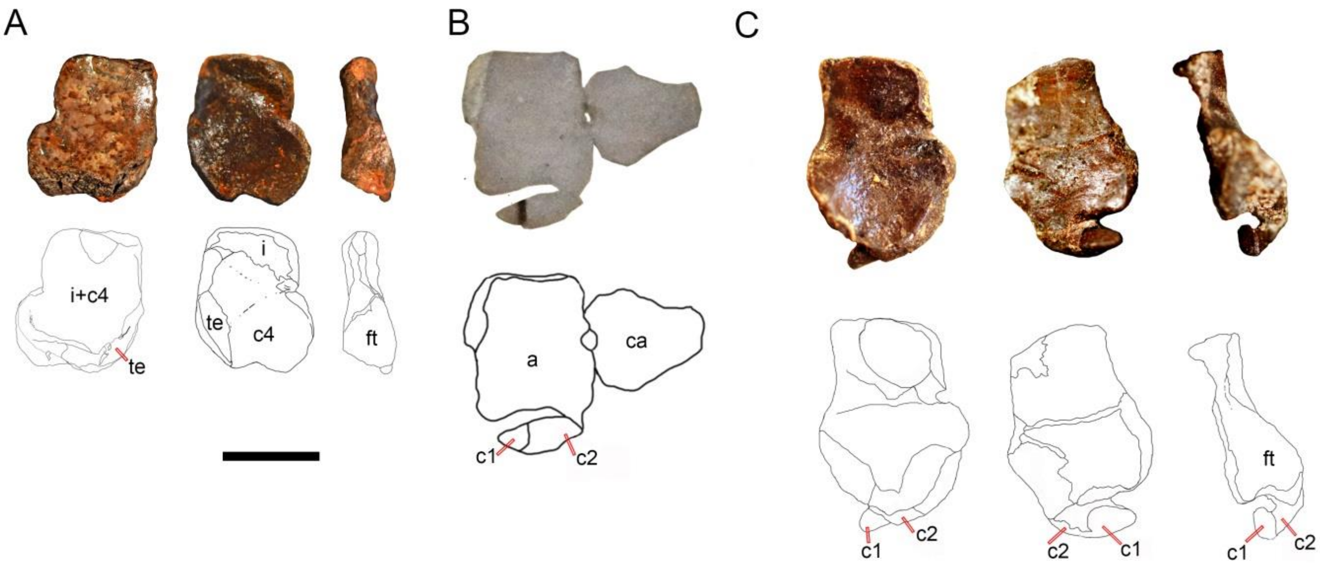

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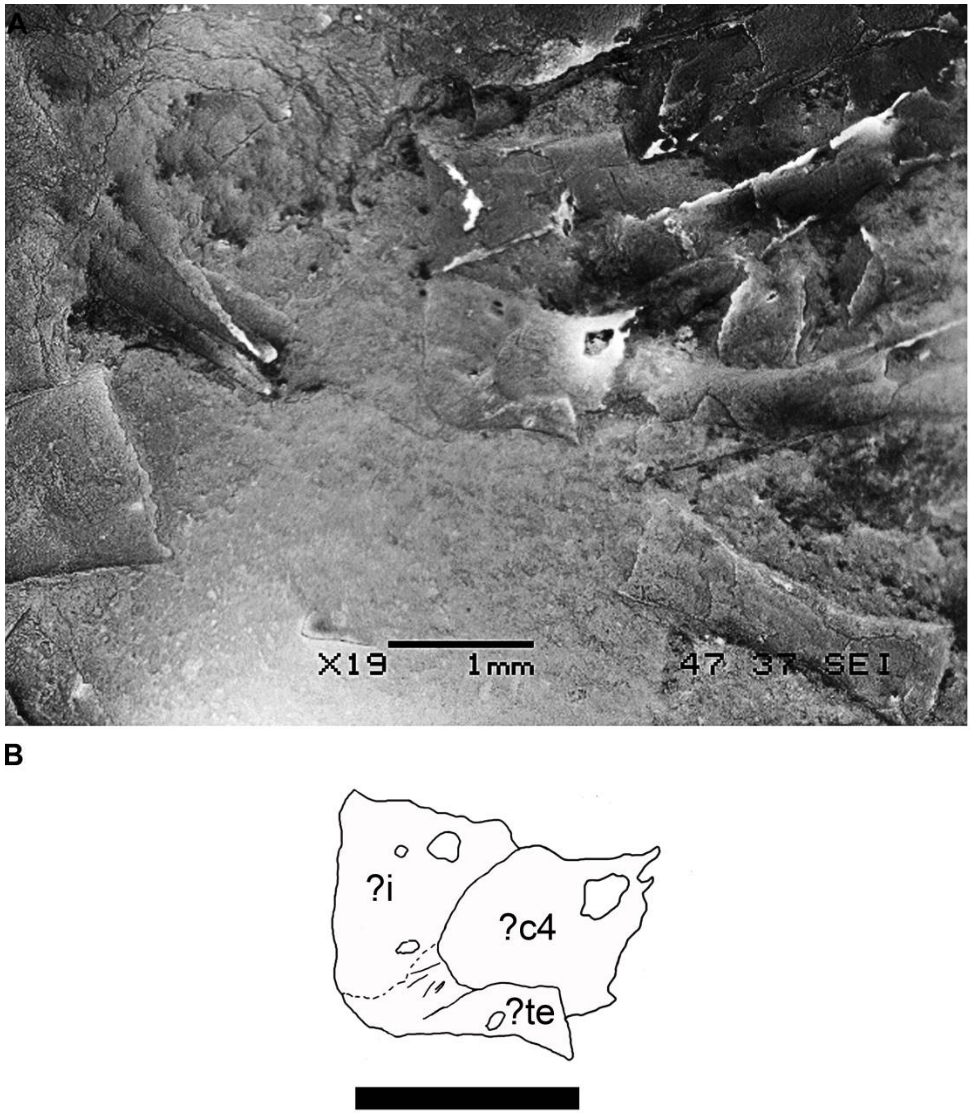

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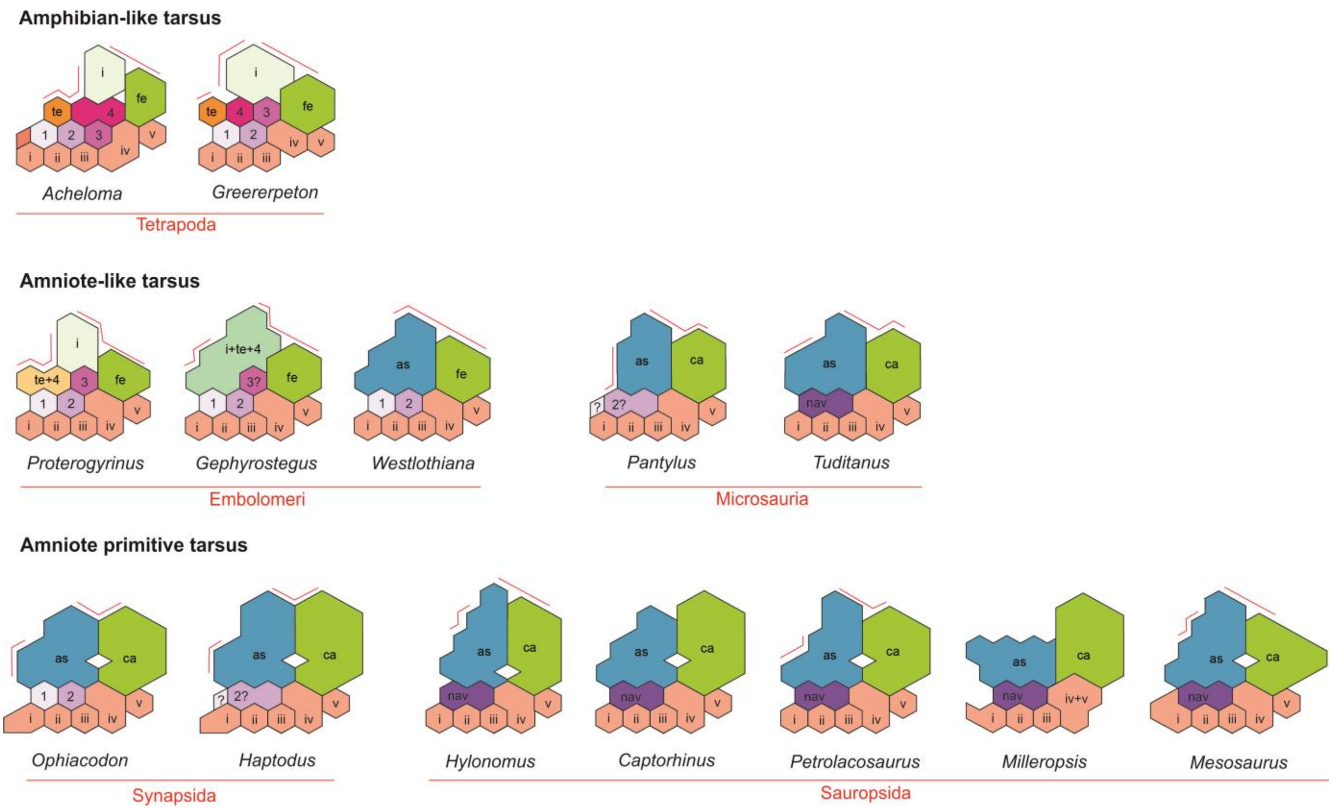

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