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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 origin of Amniota (Romer, 1956). Taking into account the elements present in the tarsus of basal tetrapods, it is clear that there was a strong reduction in the number of bones that form the primitive amniotic tarsus. This reduction can be explained by the fusion or loss of some tarsal bones in the ancestral amniotes despite the homology of these elements not always is well established. According to previous contributions, it is widely acknowledged that the calcaneum is derived from the fibulare, ie. from only one of the precursor bones present in the tarsus of non-amniote tetrapods. However, the origin of the astragalus, as well as the identification of the ancestral bones that give origin to it, are contentious (Peabody, 1951; Rieppel, 1993; Kissel, Dilkes & Reisz, 2002; Berman & Henrici, 2003; O'Keefe et al., 2006; Meyer & Anderson, 2013). Some authors supported the classic hypothesis of a unitary origin for the astragalus, from the intermedium (see Romer, 1956) or perhaps from the fusion of this bone to the tibiale (e.g. Holmgren, 1933; Gegenbaur, 1864 in Schaeffer, 1941). However, Peabody, 1951, following Holmgren (1933), suggested that the origin of the astragalus is produced by the fusion of three bones; mainly the intermedium, one of the proximal centralia (c4) and perhaps, the tibiale (Peabody, 1951, figure 2). 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 centrale as a component of the fused element (four-center hypothesis). Indeed, there is evidence of a fusion between the tibiale and the proximal centrale (c4) in *Gephyrostegus* (Schaeffer, 1941; Holmes, 1984) which possesses an amniote-like tarsus (Carroll, 1970), thus, this fusion may have occurred early in the evolution of the amniotic tarsus. Peabody's (1951) hypothesis was subsequently refuted by Rieppel (1993) who stated, based on embryological evidence from extant reptiles, that the reptilian astragalus is a neomorph. But Rieppel's (1993) suggestion was not widely accepted and the hypothesis on the multipartite structure of the reptilian astragalus remains plausible. Recent reports of well-preserved tarsi from apparently young individuals of several captorhinid species (Kissel, Dilkes & Reisz, 2002; Berman & Henrici, 2003; O'Keefe et al., 2005, 2006), which will be discussed later, demonstrate that the matter is still open.

Embryological studies show only two cartilaginous condensations close to the distal end of the fibula in most extant reptiles, one for the astragalus and the other for the calcaneum (Schaeffer, 1941; Rieppel, 1993), but the presence of additional anlagen for the tibiale, remains contentious. Mainly due to this evidence, the widespread view about the origin of the astragalus before Peabody's (1951) contribution was in favor of a slightly transformed intermedium as the astragalus precursor.

Another characteristic of the primitive amniotic tarsus is the articulation of the proximal tarsal elements (astragalus and calcaneum) with centralia 1 and 2, which are placed distally and often fuse to each other (Peabody, 1951). The fused element (c1+c2), commonly named the centrale or lateral centrale, has been suggested to form the navicular bone, characteristically present in therapsid-grade synapsids and mammals (Broom, 1915; 1924, Jenkins, 1971). Moreover, five distal tarsals are present, the first and the fourth commonly being the largest.

Here we investigate the origin and evolution of the amniotic astragalus by a thorough study of several almost complete and some incomplete mesosaur skeletons and natural external molds and casts, including well-preserved feet. Moreover, well preserved, isolated astragali and calcanea of individuals in different ontogenetic stages, including the tarsus of one non-hatched *Mesosaurus tenuidens* and hatchling individuals, were also analyzed for completing an ontogenetic sequence previously unknown for any other Early Permian amniote. This amazing record provides useful data for characterizing the tarsal structure in early and late juvenile stages, and helps us to understand the transition towards the acquisition of the adult tarsal morphology. We present a synoptic view of the evidence we found for homologizing the primitive amniotic astragalus to the intermedium plus possibly the tibiale and proximal centralia, and propose that the suturing of these elements occurred during the embryonic stage, producing a very specialized single bone in the hatchlings. 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 if this character is polymorphic for mesosaurs as observed in basal synapsids (Romer & Price, 1940).

Institutional Abbreviations: FC-DP: Fossil Vertebrates of Facultad de Ciencias, Montevideo, Uruguay; GP/2E: Instituto de Geociências (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 of 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 calcanea from juvenile and mature individuals were analyzed by using a binocular microscope and different techniques of photography, as well as by digital drawings. Specimens from FC-DPV, GP/2E, MN and SMF-R were personally analyzed by the senior author (GP), while the specimens from the AMNH were studied from photographs kindly provided by personnel of that institution.

Methods

In order to evaluate 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 non-hatched individual in a late stage of development, to represent an idealized ontogenetic transition (Figs. 1-6).

Distinction of juvenile from adult mesosaurs

The recognition of young, immature individuals from adult, mature ones was not easy to determine in mesosaurs. Modesto (1996a, 1999, 2006, 2010) made a detailed study of the characters that can be used to recognize the three monospecific genera that compose the Family Mesosauridae. He concluded that the main characters (e.g. tooth morphology, head-to-neck ratios, presacral vertebral counts, presence/absence of pachyostotic ribs and hemal arches) used

for taxonomic purposes are valid to separate three monospecific mesosaurid taxa. Nevetheless, Piñeiro (2002, 2004, 2008) revised some of the characters that have been previously used as taxonomically diagnostic and found that they could instead be ontogenetic conditions distinguishing alternatively immature and mature specimens or could even represent sexual dimorphism. Reliable characters that can be useful to differentiate juvenile (immature) from adult (mature) mesosaurid individuals can be derived from changes in the morphology and structure of the coracoid and the scapula in the shoulder girdle and the pubis in the pelvic girdle (Piñeiro, 2004). These bones are simple rounded plate-like structures in very young individuals, only acquiring the suchlike shape in adults; the coracoid develops into a roughly rectangular bone with anterior and medial convex margins (Modesto, 1996; Piñeiro, 2004). The coracoid notch pierces the bone medially but is very poorly developed in young individuals. It becomes a true coracoid foramen in adults, when both bones suture and eventually fuse to form the scapulo-coracoid. These bones can fuse leaving no trace of any suture between them, even in apparently young adults, or the suture may remain visible even in large, adult individuals (Piñeiro, 2002), evidencing perhaps intraspecific or sexual variability (Piñeiro, 2004). Similar morphological changes are seen in the pubis, from being a small, plate-like rounded bone to a more kidney-shaped element that develops a pubic notch or a true obturator foramen totally enclosed by bone. Other aspects of the skeleton morphology will be part of a forthcoming paper, and will not, therefore, be discussed here. Even though the characters reviewed above are useful as complementary data to help identify the development stage in mesosaurs, the presence of well ossified carpal and tarsal bones was the most useful feature for considering maturity in mesosaurs. We consider here that an individual is mature when in the tarsus, the astragalus and

the calcaneum approach each other and the foramen for the perforating artery appears between them.

Centralia and Navicular Nomenclature

The c1 is often named as the lateral centrale and the c2 as the medial centrale. But, when only one distal tarsal is seen (it could result from the fusion of c1+ c2 or it could be just the c2), it is often identified as the centrale (e.g. Schaeffer, 1941, Currie, 1981, Lewis, 1964, Reisz & Fröbisch, 2014), or as the distal centrale (e.g. Carroll, 1970) or as the lateral centrale (e.g. Peabody, 1952, Modesto, 1999, Reiz & Dilkes, 2003), even though these bones are always placed medially in the tarsus, or even as the navicular (Schaeffer, 1941). Similarly, the c4 is called the proximal centrale (e.g. Kissel, Dilkes & Reisz, 2002; Berman & Henrici, 2003) or posterior centrale (Olson 1964). On the other hand, there is no stable designation for the c3 and it can be mistaken for the c4 when it is called the proximal centrale (Carroll, 1970; Holmgren, 1933) or even considered a distal centrale (Fröbisch, 2008; Hall, 2007). This lack of consensus in the literature on how to refer to specific centralia increases the confusion about the establishment of evolutionary patterns for the early amniotic tarsus. Therefore, we decided to use the following naming criterion: we refer to the bone (or fused bones) placed distally to the astragalus in the mesosaur tarsus as the ‘navicular’, and we use the name "proximal centrale" only when it cannot be determined if it is the c4 or c3.

SYSTEMATIC PALAEONTOLOGY

Amniota Haeckel, 1866

Proganosauria Baur, 1889

Mesosauridae Baur, 1889

Mesosaurus tenuidens Gervais, 1864—1865

Figures 1—9

The mesosaurid tarsus (Figs. 1-9) displays a plesiomorphic construction regarding the structures observed in other basal amniotes as *Hylonomus lyelli*, *Paleothyris acadiana* and *Petrolacosaurus kansensis* (Carroll, 1964, 1969; Peabody, 1952; Reisz, 1981). It is also essentially equivalent to the tarsus of basal synapsids (Romer & Price, 1940; Romer, 1956) and it even mirrors the structure described for some microsaurs, particularly *Tuditanus*, and *Pantylus*, the embolomere *Proterogyrinus*, *Westlothiana* and *Gephyrostegus* (Carroll, 1968; 1970; Carroll & Baird, 1968; Holmes, 1984; Smithson, 1989, although see also Smithson et al., 1994) (Fig. 10).

Description. All specimens from Uruguay were collected either in bituminous or non-bituminous shale of the Early Permian (Artinskian) Mangrullo Formation (Piñeiro, 2004; Piñeiro et al., 2012a, b); all the material coming from Brazil was 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. 1-2A, 9). An almost complete and well preserved non-hatched *Mesosaurus tenuidens* from Uruguay, which is curled as if within an egg (Piñeiro et al., 2012b). It consists of an external mould of a small, still poorly ossified skeleton that suffered strong dorsoventral compression during diagenesis. This is evidenced by the displacement of the ribs and feet which are overlapping each other, as well as by the reduced three-dimensionality (suggesting strong compression) of the delicate skeleton, which represents the smallest mesosaur

225 yet found (see Figs. 1-2 to better appreciate the small size of the specimen). While some of the
226 constituent bones of the feet may not be completely ossified (considering the small size and the
227 poor preservation of the manus), the extraordinary preservation of the specimen allowed us to
228 reconstruct the structure of the tarsus and to describe the bones that seem to be present (Fig. 9).
229 Both astragali are preserved, but only one of them shows the precursor bones articulated (see
230 Fig. 9); the other was probably affected by the lateral compression that the specimen suffered
231 during the early stages of fossilization, producing the separation of the bones. Neither one is
232 preserved in its original anatomical position, but they were not too much displaced. Most
233 probably, considering the curled disposition of the skeleton, the astragali dropped from their
234 original position close to the zeugopodium to near the metatarsals when the soft tissues were
235 decomposed. A similar displacement is observed in very young specimens of *Hovasaurus boulei*
236 as figured by Caldwell (1994). The composite astragalus is shown as if it has turned itself over
237 before reaching its final position. This was obviously favored by the presence of the enclosing
238 egg membrane that prevented long transportation and loss of such tiny bones. Considering this
239 taphonomic explanation, and following the anatomical disposition of the bones we interpreted
240 the sutured bones, to be the intermedium, the tibiale (which possibly has fused to c4) and
241 possibly the c3, confirming Peabody's (1951) and O'Keefe et al. (2006) theory about the
242 presence of a composite astragalus in the tarsus of early amniotes. The c4 (and maybe also c3)
243 ossifies early in aquatic and terrestrial reptiles (Shubin & Alberch 1986; Rieppel, 1992a,b, 1993;
244 Caldwell, 1994, among others), and the former fuses to the tibiale in *Proterogyrinus scheelei*
245 (Holmes, 1984). On the other hand, c1 and c2 (='navicular') may ossify very late in mesosaurs,
246 (Figs. 4-6, 8). Thus, taking into account the tarsal structure shown by early amniotes, and

247 considering that mesosaurids are a very basal group, our suggested tarsal arrangement for the
248 non-hatched mesosaurid tarsus is plausible.

249 The distal tarsals are no visible in the specimen. They could be still unossified judging
250 from the fact that distal tarsals ossify later than metatarsals in amniotes and at least metatarsals
251 II, III, IV and V were partially, or possibly completely ossified in FC-DPV 2504, but no
252 metatarsal I, which is apparently absent (see Sheil & Portik, 2008 and references therein).
253 Otherwise (but very improbably) due to their very small size, they would not be visible if they
254 were displaced between the overlapping metatarsals.

255 2) GP-2E 272 (Figs. 1-3B). This specimen is a well preserved very young individual from Brazil.
256 The ribs are not as pachyostotic as can be observed in other immature specimens, but apart from
257 that condition, the specimen does not show relevant anatomical differences to *M. tenuidens*. The
258 silhouette of part of the body can be reconstructed due to the preservation of the skin. The
259 interdigital membrane that unites the toes to the claws can be delimited as well as the robustness
260 of the leg musculature, even in such a young individual. What could have been the plantar
261 aponeurosis covers most of the tarsal bones (Fig. 3B). However, two elements (maybe
262 mineralized cartilages) placed very close to the fibula are interpreted here as a possible astragalus
263 (the largest bone) and an incipient, smaller calcaneum, which was distally displaced. It is
264 difficult to believe that, covered by the, highly resistant plantar membrane, this tarsal bone can
265 appear as displaced from its original anatomical position. But considering that in very early
266 stages of development the astragalus and the calcaneum are the only bones ossified, we
267 hypothesize that the small size of the bone and gravity combined to move it distally after the
268 decay of flesh tissues started, particularly damaging the skin and muscle insertions. Otherwise,
269 the calcaneum is covered by the aponeurosis and it is not visible or it is a very small fragmentary

bone that is observed medially to the fibula (see Fig. 3B). It is also possible to see shadow-like structures that can be interpreted as some of the distal tarsals (e.g. dt4), which begin to ossify at very early ontogenetic stages in extant reptiles (Caldwell, 1994; Sheil & Portik, 2008). What appear to be scratch marks (according to Sedor & Costa Da-Silva, 2004) are observed close to the left foot, possibly produced by the individual before its sudden death. But these structures more likely are 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.

3) SMF-R 4496 (Figs. 1-3C). 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 best 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 described above; its tarsus is formed by two small roughly rounded bones, which can be homologized with the astragalus (the larger one) and the calcaneum (the smaller one), which do not meet, but lie one in front of the other and are positioned as in adult 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 suture lines. Although it is difficult to establish with confidence which of the original bones are involved, it is possible to suggest a putative arrangement based on the astragalus of the non-hatched mesosaurid (see Fig. 3C).

4) AMNH 23795 (Figs. 1-3D) 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 the calcaneum can be seen close to each other. Again, the astragalus shows the same structure as in the small, previously analysed specimens, and what appear to be sutures between component bones can be seen on the dorsal surface (see Fig. 3D).

5) MN 4741 and SMF-R 4934 (Figs. 1-3E-F respectively) and SMF-R 4513 (Figs. 1-3G) from Brazil are a little larger than the specimens previously described. Even though their similar still small size, SMF-R 4513 is probably ontogenetically older judging for the tarsal features. We can see for the first time the morphological differences between both the proximal tarsal bones in the ontogenetic series, the astragalus being transformed into a more stylized and more easily recognizable element (see for instance Fig. 3G). Astragalus and calcaneum are preserved close to each other, and the foramen for the perforating artery is incipient but visible at approximately the midpoint length between these bones (see SMF-R 4513, Figs. 1-3G). SMF-R 4513 (Figs. 1-3G) is probably an adult or a subadult individual. There are three bones present; two proximal tarsal elements are visible, the larger one being the astragalus which features a morphology which is similar to those observed in more mature individuals (Fig.3). It is a stout bone tending to reach the L-shaped outline characteristic of the basalmost amniotes and some other tetrapods (see the distribution and schematic morphology of the tarsal bones in Fig. 10). The foramen for the perforating artery is placed at the midlength of the lateral margin, and an intimate area of contact is being generated between astragalus and calcaneum at this point (Fig 3G). A small bone can be seen distal to the astragalus-calcaneum contact in SMF-R 4513, which is located proximal to the distal tarsal elements, including probably the dt4. It could be the 'navicular' starting to ossify, which will be well developed later, in mature *Mesosaurus* specimens.

6) At later stages, these bones develop a short contact through the lateral margin of the astragalus and the medial margin of the calcaneum (Figs. 4-6H to P), so, the remaining analysed 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 4-6H-P) represent adult individuals. 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 often the largest, although this can be very variable (Fig. 6).

In summary, the mesosaur tarsus consists of 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. 7), resulting in 8 or 9 tarsal bones. The bones that form the 'navicular' may be the centralia 1 and 2 considering that c4 and c3 ossify very early in the ontogeny of other fossil and extant sauropsids, while the former are the last to become visible (Caldwell, 1994).

RESULTS AND DISCUSSION

Following the evidence provided by the studied specimens, which notably includes the partially preserved tarsus of a non-hatched mesosaurid in an advanced stage of development, we can see the significant morphological transformation that the mesosaur astragalus experienced during ontogeny. The non-hatched *Mesosaurus tenuidens* found in the Early Permian of Uruguay (see Piñeiro et al., 2012a, b) is so exquisitely preserved that it allows us to describe the morphology of what we interpret to be a composite astragalus that is one millimeter in length! It possibly shows the precursors of the typical amniotic astragalus united by weak sutures (Fig. 9). The following postnatal, early stages of mesosaur ontogeny are characterized by the presence of

338 sub-circular to roughly square small bones, mainly representing the astragalus as a single bone
339 (and the more frequently preserved), although some young specimens still show the tripartite
340 structure (Figs. 1-3 C-E) which is not easy to observe directly from photographs because of the
341 very small size of the specimens. The sutures between the precursor bones in the astragalus of
342 larger, adult individuals can often be deduced from not always well preserved features (e.g.
343 sutures, rugose surfaces and thickened margins) (Figs. 6 and 8C).

344 In the early stages of development, astragalus and calcaneum seem to have been
345 separated, as there is no evidence of contact between them. The foramen for the perforating
346 artery is not visible; we consider both these features as useful in identifying juvenile, immature
347 mesosaurids. At the following stage, the astragalus becomes more quadrangular in shape,
348 approaches the calcaneum, and an incipient foramen for the perforating artery develops. At this
349 stage, mesosaurids appear to be young adults and possibly, mature individuals, judging by the
350 further ossification of the overall skeleton. The remaining transformations are crucial for the
351 growth of the individuals for improving their capabilities for capturing prey and for their
352 reproductive traits (see Ramos, 2015; Villamil et al., 2015; Piñeiro et al., 2012a). The proximal
353 border of the astragalus in adult individuals is deep and bears an extended rectangular facet for
354 the fibula, making an almost immobile articulation between these bones, as in basal synapsids
355 (Romer & Price, 1940). The foramen for the perforating artery is well developed in large
356 (mature) individuals where the notches in both bones approach each other to form a conspicuous
357 true foramen (see Figs. 4-6 H to P). The groove for the passage of the perforating artery crosses
358 the bone medially and proximally, where a rugose area is visible (Figs. 4 and 6). Probably it
359 marks the line of suture of both of the larger bones seen in the astragalus of the non-hatched
360 mesosaurid, implicating the intermedium and the c4+tibiale complex. Considering this

361 hypothesis as the most probable, another line of suture located at the medial corner of the
362 astragalus of adult individuals may correspond to the delimitation of the tibiale and includes the
363 articular facet for the tibia at the medial margin (Figs. 6, and 8). This suture line is also seen to
364 be continue at the medial margin, where it runs just above the articular facet for the tibia. This
365 facet is wide and teardrop-shaped, which allows for a broad (comparatively motile) articulation
366 with the tibia (Fig. 8 A and C), considering the oblique angle and the short surface at which the
367 contact is produced. It is interesting to note that the same type of articulations (and very similarly
368 shaped facets) for the fibula and the tibia were described for the 'pelycosaur' tarsus, as well as
369 the presence of a medio-ventral extension interpreted as a cartilaginous remnant of the tibiale
370 (Romer & Price, 1940).
371
372 **Limb ossification patterns**
373
374 In *Mesosaurus* a significant delay in mesopodial ossification is noted, following the pattern
375 observed in most aquatic tetrapods (Rieppel, 1992 a-b; Caldwell, 1994) such as *Hovasaurus*
376 *boulei* Currie, 1981, from which we also know an almost complete ontogenetic succession in the
377 development of the tarsus (Caldwell, 1994). Thus, long bones (propodials, epipodials and
378 metapodials) become ossified while the mesopodials are still formed of cartilage. However,
379 unlike in *Hovasaurus*, where the astragalus and the calcaneum of very young specimens are of
380 nearly the same size, in *Mesosaurus* the first is clearly larger than the latter, thus supporting the
381 hypothesis that the astragalus is the first bone to ossify in the mesosaur tarsus, arising from the
382 suturing and later fusion of at least three bones that are present in the non-hatched mesosaurid.
383 Taking into account this information, along with the evidence from Carboniferous tetrapods and

the evidence provided by the non-hatched specimen, the mesosaurid tarsal ossification proceeds in the following sequence: intermedium, tibiale+centrale 4 (and c3?, see Fig. 9 and O'Keefe et al. 2006), calcaneum, distal tarsal four, the 'navicular' and the remaining bones (distal tarsals 3—1 and 5). The sequence of ossification of the distal tarsal bones is not clear, however.

Contrary to what seen in extant sauropsids, where the calcaneum is the first tarsal element that ossifies (Fröbisch, 2008), the fibulare (the calcaneum homologous) ossifies much later in mesosaurs and aquatic fossil diapsids; in *Hovasaurus boulei* it is suggested that it appears after the c4 does (after Caldwell, 1994). Thus, it may be possible that it is already present in the tarsus of the non-hatched mesosaurid (Fig. 9), but if so, it should have been very small. Considering the presence of only two bones in juvenile individuals, identified as the astragalus and the calcaneum (Figs. 1-3), it is possible that the intermedium and the tibiale (which possibly is a composite bone if it already fused to c4) fuse early in ontogeny, as some previous workers have suggested (e.g. Gegenbaur & Williston, in Schaeffer, 1941). Indeed, the tibiale fuses to c4 in *Proterogyrinus*, suggesting that these bones also ossify early, and this event was proposed as the first step towards the formation of the amniotic astragalus, as both these bones also fuse to the intermedium later (Holmes, 1984).

This pattern of ossification is mostly in agreement with recent discoveries in those fields of paleontology and developmental genetics looking for patterns and processes of vertebrate limb evolution (Caldwell, 2002 and references therein). Moreover, it highlights, at least in basal tetrapods, the potential conservatism of the underlying genetic controls of limb development patterns, exceptions are related to different ecological and functional adaptations (see below).

The astragalus during ontogeny

The astragalus is the largest bone in the mesosaurid tarsus, featuring an L-shaped outline in dorsal view in mature specimens (see Figs. 4, 7).

The shape of the astragalus changes dramatically during ontogeny; mature individuals show a stout, roughly squared bone with broad articulating facets for the crus (Fig. 8 A and C). This bone also possesses a wide, shelf-like latero-distal facet for receiving the centrale or 'navicular' (Figs. 6-7), which can be totally separated from the astragalus, or partially fused so that the free, unfused part of the bone can only be seen on the ventral surface (Fig. 8).

However, the astragalus of immature mesosaurids is a delicate, roughly rounded or maybe subquadrangular bone bearing an evident thick dorso-medial border which developed into very well defined articulating areas during growth, producing a slightly excavated central area in the dorsal margin for the fibula and a broad, medially placed almost sub-triangular surface for the tibia. These thickened margins can be seen even in very small newborn individuals (see Fig. 1-3 C-G).

In his 1993 study, Rieppel stated that the mesosaurid astragalus does not show any evidence of being a fusion of the plesiomorphically separated tarsal elements; to him all the suture-like structures (e.g. delicate grooves or thickenings) seen on the ventral surface correspond to attachments of muscles and tendons, and the medial groove delimitates the passage of the perforating artery. Even though the mesosaur astragalus of post-hatching stages does not show the tripartite structure described in *Captorhinus* (Peabody, 1951; Fox & Bowman, 1966; Kissel, Dilkes & Reisz, 2002 and references therein), it seems to have been derived from the junction of at least three bones, as we can deduce from the tarsus of the non-hatched mesosaurid (Fig. 9) where we interpret although with doubts, that the incipient astragalus is the only bone in

the tarsus, showing suturing for the intermedium, the tibiale and maybe both the proximal centralia (c4+c3). Actually, some of the original joints remained in some specimens, but they show a slightly different pattern from that described by Peabody (1951) because the mediodistal Y-shaped suture for intermedium, c4 and c3 is not as evident in the studied specimens (see figures 3, 6, 8).

The mesosaur 'navicular'

The 'navicular' is a bone present in both synapsid and sauropsid amniotes. In the latter, it is observed at least in their basalmost representatives: a 'navicular' is found in captorhinids, basal diapsids, some Parareptilia and Mesosauridae and in all pelycosaurs (Figs. 8, and 10). Later, it becomes a bone that is only characteristic of derived synapsids and living mammals and it is lost in crown diapsids. In mesosaurs it ossifies at a late stage (at the same time that the foramen for the perforating artery forms) and is separated from the astragalus in most individuals or abuts against the distal margin of this bone, even fusing partially with it in mature individuals (Figs. 6 and 8). That means that the presence of the 'navicular' in mesosaurs is indicative of maturity.

The presence of the 'navicular' in *Mesosaurus* is a novel characteristic, as all but one (Modesto, 1996a, b; 1999) of the previous workers did not mention its presence in descriptions of the mesosaurid tarsus. Indeed, Modesto (1996a,b) described the presence of a lateral centrale only in *Stereosternum* and stated that this bone is never present in *Mesosaurus*. We have enough evidence to confirm that a transversely elongated bone is invariably present distal to the astragalus in all the analysed mature specimens—most frequently representing two sutured

454 bones— identified as the centralia c1 and c2 present in "pelycosaurs" and other basal amniotes.
455 As these bones suture to the astragalus in very mature individuals, as also seems to occur in
456 *Captorhinus aguti* (Peabody, 1951), it becomes difficult to identify its presence in the tarsus, as
457 probably occurred with the specimens studied by Modesto (1996,1999) assigned to *Mesosaurus*
458 *tenuidens*. We first become aware of the presence of a 'navicular' in *Mesosaurus* from an
459 isolated, relatively large astragalus where the fusion of c1 and c2 has not yet been completed (see
460 Fig. 8 for more detail of this condition). It firstly appears as two sutured (but not fused) bones
461 (Figs. 4 and 6 H-I), and there seems to be a reduction in the size of c1, which becomes a pointed
462 medial tip that is not preserved in most individuals because of the fragility of its suture to c2 (see
463 Figs. 3G; 8B-C). As a result, in *Mesosaurus,* the 'navicular' strongly abuts the platform-like
464 facet on the distal margin of the astragalus (Figs. 6P, 8).

465 This variable condition concerning the fusion of centralia 1 and 2 recalls that observed in
466 'pelycosaurs', in which some species show the centralia 1 and 2 as separate bones (e.g.
467 *Ophiacodon*), while others show them fused (e.g. *Haptodus*) (Romer & Price, 1940) (Fig. 10). It
468 is likely that this is an ontogenetic, perhaps heterochronic condition in mesosaurs (L. Gaetano
469 and D. Marjanović, personal communication), but this needs to be tested by analysis of more
470 than one individual of the same species at different stages of development. For instance, the
471 morphology of the c1 in mesosaurids is very similar to that of the putative medial centrale of
472 *Sphenacodon ferox* (according to Henrici et al., 2005), and if it is repositioned medially to the
473 lateral central we can obtain a navicular-like bone in *Sphenacodon*. Thus, the small size of the
474 tarsal bones of early amniotes and the possibility that they can be displaced from their original
475 positions, plus to the fact that the recognition of homologous bones seems to be a difficult
476 endeavor, make it likely that the real nature of the tarsus structure in several taxa could remain

477 obscure. Mesosaurs may provide a good opportunity to revisit and gain a better understanding of
478 the processes that are involved in the origin and early evolution of the amniotic tarsus.
479
480 **Morphological changes supporting an evolutionary transition in the origin of the amniote**
481 **tarsus**
482
483 Although most previous workers (e.g. Carroll, 1964; Berman & Henrici, 2003; O'Keefe
484 et al., 2006; Meyer & Anderson, 2013, and references therein) accepted the composite origin of
485 the astragalus following the contribution of Peabody (1951), the reappraisal of that condition and
486 its significance performed by Rieppel (1993) introduced controversy. This last author rejected
487 the multipartite origin of the astragalus, arguing that there was a lack of unequivocal ontogenetic
488 evidence that would show that the bones which would form the composite astragalus are present
489 in at least some stage of development. He rejected the proposed composite origin of the
490 astragalus by Peabody (1951) mainly based on the fact that this bone derives from a single
491 ossification center in extant reptiles and that, according to Sewertzoff (1908), lizards have just a
492 single block of cartilage close to the distal end of the fibula and tibia where the calcaneum and
493 the astragalus later ossifies. In *Sphenodon punctatus*, the astragalus originates by the
494 condensation of more than one chondrogenic element, but they fuse during the embryonic stage
495 (Rieppel, 1993), and interestingly, there are also two chondrogenic condensations distal to the
496 fibula in pleurodiran turtles (Fabrezi et al. 2009). In *Podocnemis* species for instance, one is the
497 intermedium and the other is an elongated element, postaxially placed, which is interpreted to be
498 the tibiale+c4 (Fabrezi et al. 2009). There is also a connective connection between c4 and the
499 intermedium in *Phrynops hylarii*, showing a tarsal pattern that seems to be consistent with the

basic early amniote tarsal construction as suggested by mesosaurs and other basal, non-amniote taxa.

In lizards, the tarsal formation is not as clear as in turtles. Rieppel (1992a), considered that the proximal cartilage anterior to the fibulare is the astragalus, however, there are not conclusive embryological studies that show the homology of the anterior tarsal cartilages in lizards (Fabrezi, Abdala & Martínez Oliver, 2007). The morphogenetic approach of Shubin and Alberch (1986) seems to be useful to reconstruct the skeletal morphology in lizard limbs, and then, to identify the developmental constrains that can produce deviations in some groups from the otherwise apparently conservative pattern (see Fabrezi, Abdala & Martínez Oliver, 2007).

On the other hand, the presence of more than one cartilage condensation, apparently homologous with the ancestral tetrapod tarsals, has been recently described to be present during early embryonic stages in the development of six different orders of modern birds (Ossa Fuentes, Mpodozis & Vargas, 2015) and also in chameleons (Diaz & Trainor, 2015). However, their homology to the earliest amniote condition is difficult to establish,when the pattern is observed in such very specialized groups. Indeed, in the above mentioned papers, (Ossa-Fuentes, Mpodozis & Vargas, 2015; Diaz & Trainor, 2015) it is suggested that the intermedium and the tibiale (although the latter is not pretty much apparent from the figures provided by Diaz & Trainor, 2015) appear as independent ossifications at very early stages of the development. On the other hand, Ossa-Fuentes, Mpodozis & Vargas (2015) observed that in the six groups that they studied, in contrast to the most common condition in birds (i+fe), the intermedium forms a separate ossification center that later fuses to the ‘astragalus’ (sic) forming the ascending process characteristic of dinosauromorphs. Thus, the ‘astragalus’ should be the tibiale? Moreover, the pattern of ossification that Ossa-Fuentes, Mpodozis & Vargas (2015) suggest, where the fibulare

523 is the first to ossify, followed by the putative intermedium and later by the tibiale, is very
524 different to that currently accepted to occur in basal amniotes.

525 The centralia, which are considered basic components of the astragalus structure, are
526 recognized in stem-lepidosaurs. However, these bones are not detected in dinosauromorphs and
527 in many extant diapsids (e.g., chameleons and birds). Therefore, they must have fused to a
528 different bone than the astragalus or disappeared during the evolution of modern sauropsids as
529 they are not recognizable during the ontogeny of the most advanced taxa".

530 Selective pressures to reduce the number of tarsal bones in the sense that they are an
531 extension of the epipodials, favour stability by strengthening the feet to drive the body forward.
532 Thus, the acquisition of unitary, stout structures instead of several separate, delicate bones was
533 an improvement for sustained locomotion capabilities. Therefore, we have to be cautious
534 regarding these findings, considering the high variability shown by the chameleons' tarsal
535 structure, and the lack of embryological evidence in the fossil taxa for use in comparison.
536 Therefore, as we previously mentioned, the possibility that neomorphic elements are present in
537 such derived groups cannot be ruled out with the available data.

538 Indeed, there are several known examples of tetrapods, possibly stem amniotes, that
539 allow us to deduce the steps of fusion of the tarsal bones leading to the attainment of the amniote
540 condition. Thus, as the embryology of extant lizards suggests, the fusion of these elements in the
541 development of the amniote ankle is produced in the embryonic stage (Fabrezi, Abdala &
542 Martínez Oliver, 2007) and so, it is not possible to address their original ossification centers any
543 more (Gauthier et al., 1988). Rieppel (1993) observed that associations of tarsal bones are
544 common in amphibians and that, while centralia 1 and 2 can be fused or separated, c3 and c4
545 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 may not represent a condition of phylogenetic relevance. However, we can see a real transition from closely related, supposedly non amniote taxa (e.g. *Gephyrostegus*, *Westlothiana*, *Tuditanus*, *Pantylus* (see Ruta, Coates & Quicke, 2003 and Marjanović & Laurin (2015), for the phylogenetic position of these taxa), to the acquisition of the primitive amniotic tarsal configuration (see figure 10). Thus, if we consider the association of the tibiale and c4 observed in some *Proterogyrinus* specimens (Holmes, 1984) and possibly present in the tarsus of the non-hatched mesosaurid (see Fig. 9) as the first step towards the development of the amniotic tarsus (Holmes, 1984), we can reconstruct the succession including *Gephyrostegus* (see Carroll, 1970 as a reference of the tarsal structure in this last taxon) where the tibiale+c4 (and c3?, see O'Keefe et al., 2006) complex is associated with the intermedium to form the composite amniotic astragalus, a configuration that is also present in some microsaurs (e.g. *Tuditanus punctulatus*, Carroll & Baird, 1972; Carroll & Gaskill, 1978 and *Pantylus cordatus,* Carroll, 1968) and possibly in *Westlothiana* (Smithson, 1989 but see Smithson et al. 1994). Within that transformation, the fibulare becomes the calcaneum and c1 and c2 remain as the only centralia present, either as separated bones or fused to form a single element, the 'navicular'.

Phylogenetic context supporting the evolutionary transition

On a phylogenetic point of view, even considering that there is not complete consensus about the relationships of the taxa involved in the transition, their relationships seem to be supported by the most recent cladistics analyses of basal tetrapods: Ruta, Coates & Quicke, 2003; Vallin & Laurin, 2004; Klembara, 2005; Ruta & Coates, 2007; Marjanović & Laurin,

2009, 2015 (see Fig. 11). These phylogenies show *Proterogyrinus* as an embolomere anthracosaur, although the relationships of this taxon are contentious and were not completely resolved (see Ruta, Coates & Quicke, 2003). G*ephyrostegus* is very close to Seymouriamorpha and to microsaurs, a hypothesis supported by the Laurin & Reisz (1997) tree, which also argues that lepospondyls are a monophyletic group closely related to amniotes (see also Marjanović & Laurin, 2015). Otherwise, if microsaurs are paraphyletic to other lepospondyls and to the amniote stem, as other workers suggest (Olori, 2015), they could have been the last phylogenetic intermediaries in our evolutionary transformation series.

It is noteworthy that some taxa which are not classified as amniotes have an amniote-like tarsus or at least developed the large proximal tarsal bones that characterize the amniotic tarsus, the astragalus and the calcaneum (Fig. 10). Notable examples of this feature are the diadectids earlier analysed (Romer & Byrne, 1931; Romer, 1944), although adults show the autapomorphic condition of a fusion between both the proximal bones to produce an astragalocalcaneum bone. Within lepospondyls, the microsaurs *Pantylus* (Carroll, 1968) and *Tuditanus punctulatus* have intriguingly, an amniote-like tarsus (Carroll & Baird, 1972). Moreover, the proterogyrinid *Proterogyrinus scheelei*, *Gephyrostegus bohemicus* and probably *Westlothiana lizziae* also have an amniote-like tarsus (see Holmes, 1984; Smithson, 1989). Because mesosaurids are very basal amniotes (Laurin & Reisz, 1995; Piñeiro et al., 2012b) or basal parareptiles (Modesto, 1996 a-b; Modesto, 1999; Piñeiro, 2004) we explored these taxa in order to find homologies between putative plesiomorphic, non-amniotic tarsi and their corresponding structure in mesosaurids according to the different ontogenetic stages described for the group.

The status of Westlothiana and microsaurs and its role in the transition

592

593 Regarding the condition in *Westlothiana*, Smithson (1989), reconstructed the tarsus as
594 very amniote-like, including within it nine bones (see Smithson, 1989, figure 2d). There were
595 certainly nine bones in the preserved material although they were not preserved in their original
596 anatomical position. But, later, (Smithson et al., 1994) pointed out that the tarsus of *Westlothiana*
597 is indeed very plesiomorphic (or amphibian-like) because it included ten, rather than nine bones
598 (see figure 20A in Smithson et al., 1994). We do not find enough evidence to refute the former
599 reconstruction or for validate the latter, thus, a proposal about the tarsus structure in
600 *Westlothiana* would be very speculative at this stage. Moreover, the renaming of the two large,
601 proximally placed bones originally described as the astragalus and the calcaneum as an
602 intermedium and a fibulare, is also speculative because this last bone is difficult to identify from
603 the preserved specimen, where the foot bones are mostly disarticulated and obscured by the
604 caudal vertebrae (Smithson et al., 1994). Besides, according to these authors, the putative
605 intermedium is L-shaped, a characteristic very frequently found in the astragalus of early
606 amniotes. Despite *Westlothiana* possessing other advanced conditions that may suggest its
607 relation to the amniote clade, it also retains some plesiomorphic features in the skeleton such as a
608 prefrontal-postfrontal contact, excluding the frontal from the orbital margin (Smithson, 1989).
609 Thus, the reconstruction of the real structure of the tarsus in *Westlothiana* may be crucial to an
610 understanding of the evolutionary transition to the origin of the amniotic astragalus as we have
611 figured it out in this contribution. We hope that our paper will encourage new studies on this
612 taxon.

613 Concerning microsaurs, these ecologically diverse, small-bodied tetrapods are credible
614 candidates for being part of the stem leading to the emergence of the earliest amniotes. They

615 develop a tarsus with a very amniote-like morphology, and as was recently demonstrated they
616 even show a similar ossification pattern, with the intermedium (?astragalus) and the fibulare
617 (?calcaneum) being the first tarsal bones to ossify (see Olori, 2015). They are also the only
618 proximal elements in the tarsus as in all amniotes, and naming them as intermedium and fibulare
619 is just arbitrary at this stage, if we have no embryological information to prove their identity. We
620 have to take into account that in mesosaurids the astragalus and the calcaneum are the only
621 proximal tarsal bones in born individuals, despite the former deriving from the fusion of three or
622 four bones.
623
624 *Diadectids*
625
626 Diadectids were recently considered to be amniotes (Berman, 2000), and as such, they
627 would have had an amniote tarsus. Recent discoveries of possible juvenile diadectid tarsi
628 including a putative composite astragalus formed by the intermedium, the tibiale and the
629 proximal centrale (c4, as it was identified) introduced interesting new data to the origin of the
630 amniotic astragalus (Berman & Henrici, 2003). Later, this material was assigned to the species
631 *Orobates pabsti*, a diadectid (Berman et al., 2004). Nevertheless, the holotype specimen of
632 *Orobates* described by Berman & Henrici (2003) and Berman et al. (2004: 29) as having a
633 tripartite astragalus (MNG 10181) was recently subjected to an in-deep study using micro-focus
634 computed tomography scans (Nyakatura et al., 2015), which allowed for a thoughtful anatomical
635 understanding of the specimen. The scanned image and digital reconstruction show that there are
636 seven separated bones in the tarsus of *Orobates*, whose morphology suggests that could be
637 homologized with immature astragalus and calcaneum plus two centralia (c1+c2) and three small

distal tarsals. Indeed, despite the very good preservation of the individual, it was apparently subjected to severe diagenetic distortion; 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, led to the interpretation of Berman & Henrici (2003) about the presence of a composite astragalus in *Orobates*.

Berman & Henrici (2003) also described two associated (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 us of the astragalus and calcaneum of a very young individual, taking into account the ontogenetic stages described here for the very basal amniote *Mesosaurus tenuidens*.

This new configuration matches the pattern of the tarsus already known for diadectids: distinct astragalus and calcaneum in young individuals, which fuse later to produce an astragalocalcaneum in very mature adults. Thus, diadectids have a very amniote-like tarsus. The non-diadectid diadectomorphs (*Tseajaia campi*) do not have a well-defined tarsus, but this can be masked by the not sufficiently good preservation of the specimen feet. Even though, in *Tseajaia campi*, three distinct bones seem to form the proximal line (Moss, 1972), some fusions tending to achieve the amniote-like pattern can be hypothesized to be present: the tibiale fuses to c4 as the evolutionary transition reviewed above suggests, and the intermedium, shown by Moss (1972) as fusing to c4, indeed fuses to c3 (see Figs. 10, 12), supporting the putative incorporation of both centralia into the amniotic astragalus as O'Keefe et al. 2006 have suggested and as it is shown by the tarsus in the non-hatched mesosaurid (Fig.9).

660 Some groups like diadectids and seymouriamorphs for instance, show a high plasticity in
661 producing different patterns often correlated to a different expression of otherwise highly
662 conserved regulatory genes (Shubin, 2002). Therefore, the expression of these genes and the
663 consecutive structure of the tarsus may be regulated by the different ecological pressures to what
664 some have to adapt along the different stages of their development. Juvenile or young adult
665 *Diadectes* show a conservative tarsus, and distinct astragalus and calcaneum were described as
666 being present (Romer & Byrne, 1931; Romer, 1944; Berman & Henrici, 2003). However,
667 astragalocalcaneum fusion is shown to occur in very large and mature individuals, where it
668 would seem that the movement between these bones becomes very limited or null (Romer,
669 1944).
670
671 *Hylonomus lyelli*
672
673 Revising the evidence from other basal amniotes such as *Hylonomus lyelli* (Carroll, 1964;
674 Meyer & Anderson, 2013) we found some inconsistencies related to the identification of the
675 bones figured, perhaps as an attempt to follow the Peabody's (1951) suggestion of a tripartite
676 origin of the astragalus. Thus, Meyer & Anderson (2013), following Carroll (1964, fig. 1),
677 identified the astragalus and calcaneum from a partially disarticulated specimen where the feet
678 are completely disassociated and considered the calcaneum of *Hylonomus* as two times larger
679 than the astragalus. According to the information found in Carroll (1964, p. 72, fig. 8) and based
680 on the ontogenetic succession that we described here for mesosaurs, the calcaneum can
681 sometimes be equal in size to the astragalus or even a little larger, but never that much larger.
682 Thus, we could deduce both that it is an incomplete astragalus missing the intermedium, as

683 Meyer & Anderson proposed in the text and in figure 3 (but this would suggest that the type
684 specimen of *Hylonomus lyelli* belonged to a very young individual and it does not appear to be
685 the case, see figure 1 of Carroll, 1964), or that the bone identified as the calcaneum is the
686 astragalus or that the bone is neither the astragalus nor the calcaneum. We are inclined to accept
687 the last hypothesis because the overall small size of the individual suggests that these bones are
688 much too large to be tarsal bones; they seem to be elements of the pelvic girdle, possibly the
689 pubis (see figure 1 of Carroll, 1964). The well identified astragalus of *Hylonomus lyelli* (see
690 figure 8 of Carroll, 1964) does not show any trace of sutures.
691
692 *Captorhinids*
693
694 Taking into account the previous evolutionary transition in favor of a composite origin of
695 the amniotic astragalus, which of course may also include other taxa, the interpretation of
696 Peabody (1951) and later workers of the presence of more than one ossification center in the
697 astragalus of *Captorhinus* and other basal amniotes seems sensible. However, other extensive
698 descriptions of *Captorhinus* (e.g. Fox & Bowman, 1966) do not provide more conclusive
699 evidence about the structure of the tarsus and, as Rieppel (1993) claimed, it is necessary to have
700 ontogenetic evidence (e.g. articulated skeletons of very young individuals of *Captorhinus* and/or
701 of related taxa) to demonstrate the homology of the bones composing the tripartite astragalus and
702 their presence in the earliest stages of development. Isolated astragali from the Lower Permian of
703 Oklahoma were described by Kissel, Dilkes & Reisz (2002) as belonging to *Captorhinus*
704 *magnus*, showing the putative tripartite structure visible only from the dorsal surface of the
705 bones. However, this feature was discussed by Rieppel (1993) who argued that the putative

706 unclosed sutures should be interpreted as areas of muscular attachment, or grooves for blood
707 vessel irrigation, or fractures.

708 Concerning *Captorhinus*, most of the isolated astragali figured by Peabody (1951) clearly
709 belong to mature animals, according to their size and structure (see Fox and Bowman, 1966, for
710 comparison); the smallest one already shows the same morphology seen in the larger ones. If the
711 astragali shown by Peabody (1951) partially represent an ontogenetic transformation series, they
712 cannot confidently demonstrate that the apparent tripartite structure is derived from the fusion of
713 three or four of the plesiomorphic tarsal bones. A feature that could not support the hypothesis of
714 the tripartite structure is that the sutural lines and groove patterns present in *Captorhinus* as
715 described by Peabody (1951) are only visible on the ventral surface of the bone; alternatively, it
716 suggests that the fusion started on the dorsal surface and was not completed in adult individuals.
717 The same condition can be observed in the large captorhinid *Captorhinus magnus* (Kissel, Dilkes
718 & Reisz, 2002).

719 Fragmentary pedes referred to juvenile and adult individuals of the giant, largest known
720 captorhinid *Moradisaurus grandis* from the Upper Permian of Niger, were figured and described
721 by O'Keefe et al. (2005, 2006). Even though the bones were found in association and it was
722 possible to recognize the identity of some of them, they represent isolated and disarticulated
723 pedes whose referral to *Moradisaurus* can be possible but not accurate, at least no more, than to
724 any other basal tetrapod of the same size. Nevertheless, based on the pes assigned to a juvenile
725 captorhinid, O'Keefe et al. (2006) suggested that the c3 is also a component of the multipartite
726 amniote astragalus, occupying its latero-distal corner. However, the individualization of the
727 constituent bones of the juvenile tarsus and all the possible arrangements show that there is a
728 bone, dorsal to the intermedium that does not belong to the tarsus, unless it is part of the

intermedium yet not totally ossified because the juvenile condition of the specimen. But, that bone is the only that is totally isolated from the rest of the tarsus, which excepting the four distal tarsals, appears as a co-ossified structure.

Even though our reconstruction of the non-hatched *Mesosaurus* tarsus is consistent with the O'Keefe et al. (2006) reconstruction of the *Moradisaurus* tarsus in the fact that the c3 may be part of the astragalus, the arrangement of the bones seems to have been very different in both taxa. Moreover, the putative calcaneum has a very developed notch for the perforating artery, which does not match with the condition in the astragalus, including the evident individualization of the constituent bones. It is also difficult to include the O'Keefe et al. (2006) specimen because their reconstruction does not show an evident fusion between the tibiale and the c4, and because it is a unique, isolated, putatively juvenile pes of *Moradisaurus*, where the identity of the bones is highly subjective. The other fragmentary pes, interpreted to pertain to an adult specimen displays the typical amniotic tarsal structure and the astragalus shows no sign of the composite origin.

The presumable "implicit" relationship between mesosaurids and basal synapsids regarding the structure of their skull and tarsus

Friedrich von Huene (1941) proposed for the first time a phylogenetic relationship between *Mesosaurus* and some basal 'pelycosaurs'. That suggestion was not generally acknowledged by later authors who developed the currently accepted hypothesis that mesosaurids are the basalmost sauropsids (Laurin & Reisz, 1995) or the basalmost parareptiles (Modesto, 1999). More recently, Piñeiro (2004) found some evidence that she understood gave

support to von Huene's hypothesis (1941) but acknowledged that it should be tested in a phylogenetic context. Moreover, the nature of the mesosaurid skull, discussed during more than a hundred years, has been recently reassessed to show the presence of a synapsid-like lower temporal fenestra in *Mesosaurus tenuidens* (Piñeiro et al., 2012c). This contribution gave credit to the observations made by von Huene (1941) about the morphology of the mesosaur skull. Similarly, the tarsus of mesosaurs has been studied by several authors, and here we have demonstrated that its structure is almost identical to that described for basal synapsids, but also it is equivalent to that of basal sauropsids, including one of the basalmost diapsid *Petrolacosaurus kansensis* (Reisz, 1981).

Basal synapsids show a greater development of the calcaneum (Romer & Price, 1940), which in some taxa roughly acquires the size of the astragalus. In contrast to this, the calcaneum of *Mesosaurus* is smaller than the astragalus (although the size differences are less significant in adult individuals), and develops a lateral expansion in the area of the heel, possibly for insertion of extensor tendons including the Achilles tendon (Fig. 7).

Indeed, the tarsus in early amniotes is both structural and morphologically equivalent in the two groups, except that in 'pelycosaurs' there is no evidence for the multipartite formation of the astragalus, thus generating doubts about the homology of these bones in synapsid and sauropsid amniotes (Rieppel, 1993). However, the multipartite original structure can be seen just in very young mesosaurs and it disappears before the achievement of the adult stage; but it seems to be evident in captorhinids, being possibly an heterochronic pattern.

Evolutionary paths for the development of amniote tarsus: the mesosaur contribution

775 The morphological ontogenetic transformation presented here for *Mesosaurus tenuidens*
776 is the most complete known for a basal amniote (cf. Laurin & Reisz, 1995) and as such, it
777 constitutes a relevant database for studies of a different nature. The information provided for this
778 data base on the origin of the amniotic tarsus suggests that, as Peabody (1951) and previous
779 authors (e.g. Holmgren, 1933) have stated, the earliest astragalus originated from at least four
780 ossification centers (taking into account that the tibiale and c4 fuse together early in the
781 ontogeny), near the tibial and fibular distal margins.

782 According to our observations of the non-hatched *Mesosaurus tenuidens* which possesses
783 an astragalus formed by at least four bones, we can say that the mesosaurid astragalus is not a
784 neomorphic as Rieppel (1993) has suggested, unless we consider that once united in the earliest
785 stages of the development, these bones form a new element. Even the evidence taken from taxa
786 such as the embolomere *Proterogyrinus scheelei* Romer, 1970 can provide support for the
787 multipartite hypothesis and the identification of the bones provided in the present work (Holmes,
788 1984).

789 We made several interesting observations that support the already established homologies
790 and possible evolutionary paths towards the origin of the primitive amniotic astragalus.
791 Particularly in *Proterogyrinus* the intermedium has a very similar structure to that of the
792 astragalus of young mature mesosaurs, and the tibiale is clearly sutured against the medial corner
793 formed by c4 and the intermedium. The fibulare is also very similar to the calcaneum of the same
794 stage (see Figs. 1-6), so it is logical to presume that these bones are homologous, as already
795 stated. The main question is what happens to the remaining bones to obtain the mesosaurid (=
796 basal amniote) tarsus consisting of two large proximal elements plus one or two centralia and
797 five distal tarsals. We find evidence for the presence of c3 early in the ontogeny (Fig. 9); it is

possible that it fuses to c4 in the described mesosaur ontogenetic transformation after the c4 fuses to the tibiale. Indeed, based on the structure shown by *Proterogyrinus* (Holmes, 1984), where apparently the tibiale fuses to c4, and taking into account that shown by the tarsus in the captorhinomorph *Labidosaurus* (Williston, 1917) where the intermedium and the tibiale also fuse to c4, we hypothesized three possibilities or combinations: A, the astragalus is just formed by the intermedium+tibiale only, and c4 and c3 undergo a reduction in size until they finally disappear (not plausible, given the probable presence of c4 and c3 in the tarsus of the non-hatched mesosaurid); B, the astragalus is formed by intermedium+tibiale+c4, and c3 is reduced to be lost (not probable given its putative presence in the tarsus of the non-hatched mesosaurid and taking into account the proposal by O'Keefe et al., 2006); C, the astragalus results from the fusion of all bones, i+te+c4+c3 (Figs. 9 and 12A). The last possibility (C) seems to be supported by the materials that we described here, and is consistent with that suggested by O'Keefe et al. (2006), who provided evidence for the inclusion of c3). It does not imply the loss of bones but a re-patterning to produce the amniotic tarsus. Moreover, there are also two possibilities for the formation of the 'navicular': 1, it results from fusion of c1 and c2; 2, it is formed by the c2 after the reduction and loss of c1 (see Fig. 12B). We found probable evidence of some of these fusions (the tibiale+ ?c4+ intermedium, c1+c2) in early stages of *Mesosaurus tenuidens's* development, but not in all individuals.

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, but the evidence from mesosaurs might support just C-1.

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 for a better recognition of intraspecific (ontogenetic) from interspecific variation in mesosaurs and provides more informative characters that can be used in comparative studies of amniote relationships.

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 subadult and adult mesosaurs, 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 non-hatched mesosaurid in an advanced stage of development. These bones seem to be the intermedium and the tibiale, and the later fused to c4; and the c3. Thus, our study rejects the hypothesis that the amniotic astragalus is neomorphic.

Regarding the analyzed ontogenetic series, we could determine that the 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. Moreover, the morphological changes observed in the mesosaur ontogeny could have strong implications in the recognition of until now undocumented, ancestral developmental features of early amniotes.

Current morphological and comparative studies on the mesosaurid skeleton suggest other interesting similarities between mesosaurids and basal synapsids that will be properly described in a forthcoming paper. However, these features are also shared with other basal sauropsids and

taxa that are not even amniotes. For instance, mesosaurs share characters with taxa previously known to be closer to Amniota (Panchen & Smithson, 1988; but see also Smithson et al., 1994) but these hypotheses were not phylogenetically evaluated. These taxa are now considered as stem or crown-tetrapods (Olori, 2015; Marjanović & Laurin, 2015) or their affinities are not yet well defined (e.g. *Westlothiana*), but they still remain close to the earliest amniotes. This commonly shared morphology among apparently unrelated but very basal taxa reflects the primitive nature of mesosaurids, as already noted by Huene (1941) and other paleontologists. The example of the similar tarsal structure observed in mesosaurids, some 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 when the first recognized amniote appeared and walked on the planet.

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Figure captions

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1118 astragalus and calcaneum can be observed close to the crus. See the interpretive drawing
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1129 See text for further descriptions and interpretive drawing in Fig. 2 E-G.

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1138 hatched mesosaurid (see Fig. 9 and text for further descriptions of each the included
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1153 Figure 5. *Mesosaurus tenuidens*, ontogenetic transformation in the tarsus formation. Interpretive
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1158 adult individuals. The formation of the 'navicular' by the fusion of c1 and c2 is shown
1159 through the series, as well as the formation and development of the foramen for the
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1169 young individual, in dorsal, ventral and medial views respectively. The bone shows the
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1172 morphology with respect to the dorsal one. Note that there are no traces of the 'navicular'
1173 preserved along the distal surface of the astragalus, which bears a concave margin. B,
1174 GP-2E 5203, photograph and interpretive drawing of astragalus, calcaneum and incipient
1175 'navicular' of a young individual in dorsal view. Recall on that the 'navicular' is already
1176 united to the astragalus by c2, being formed by c1 and c2 and the suture between them is
1177 still well visible. C, FC-DPV 1479, photographs and interpretive drawings of an isolated
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1182 features different from the dorsal one. The wide and triangular facet for articulation with
1183 the tibia can be seen from the medial view. Anatomical abbreviations: a, astragalus; ca,
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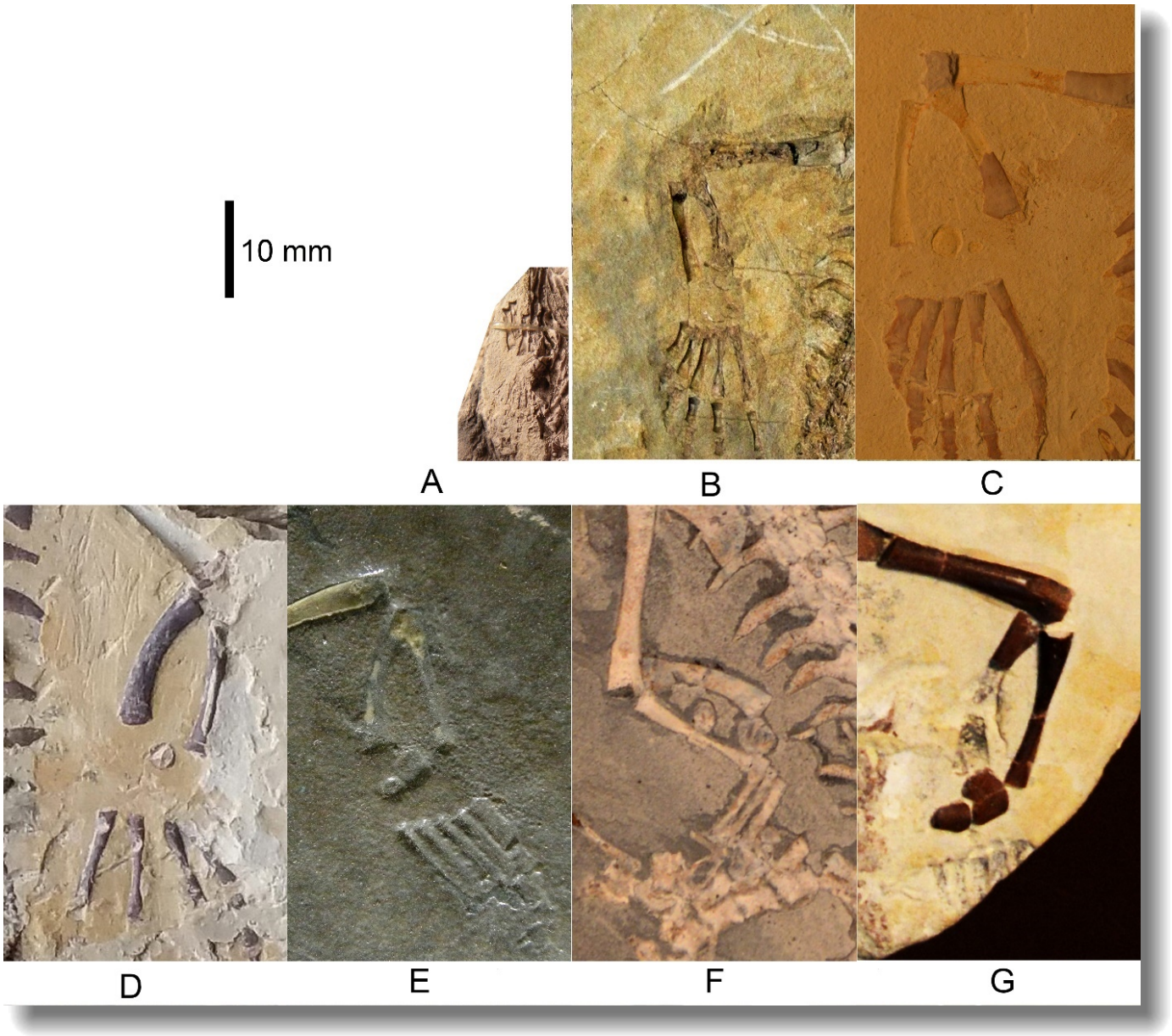

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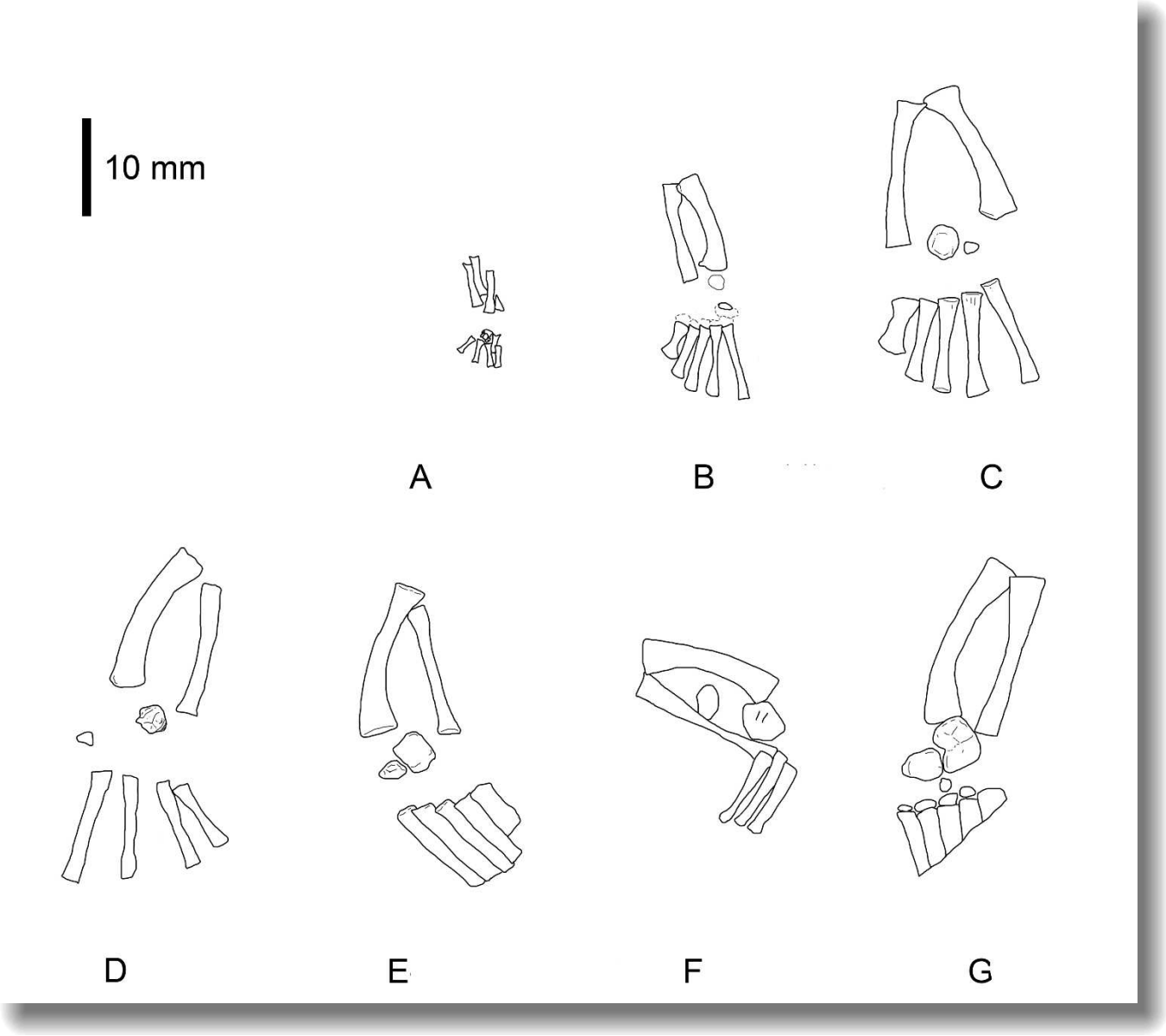

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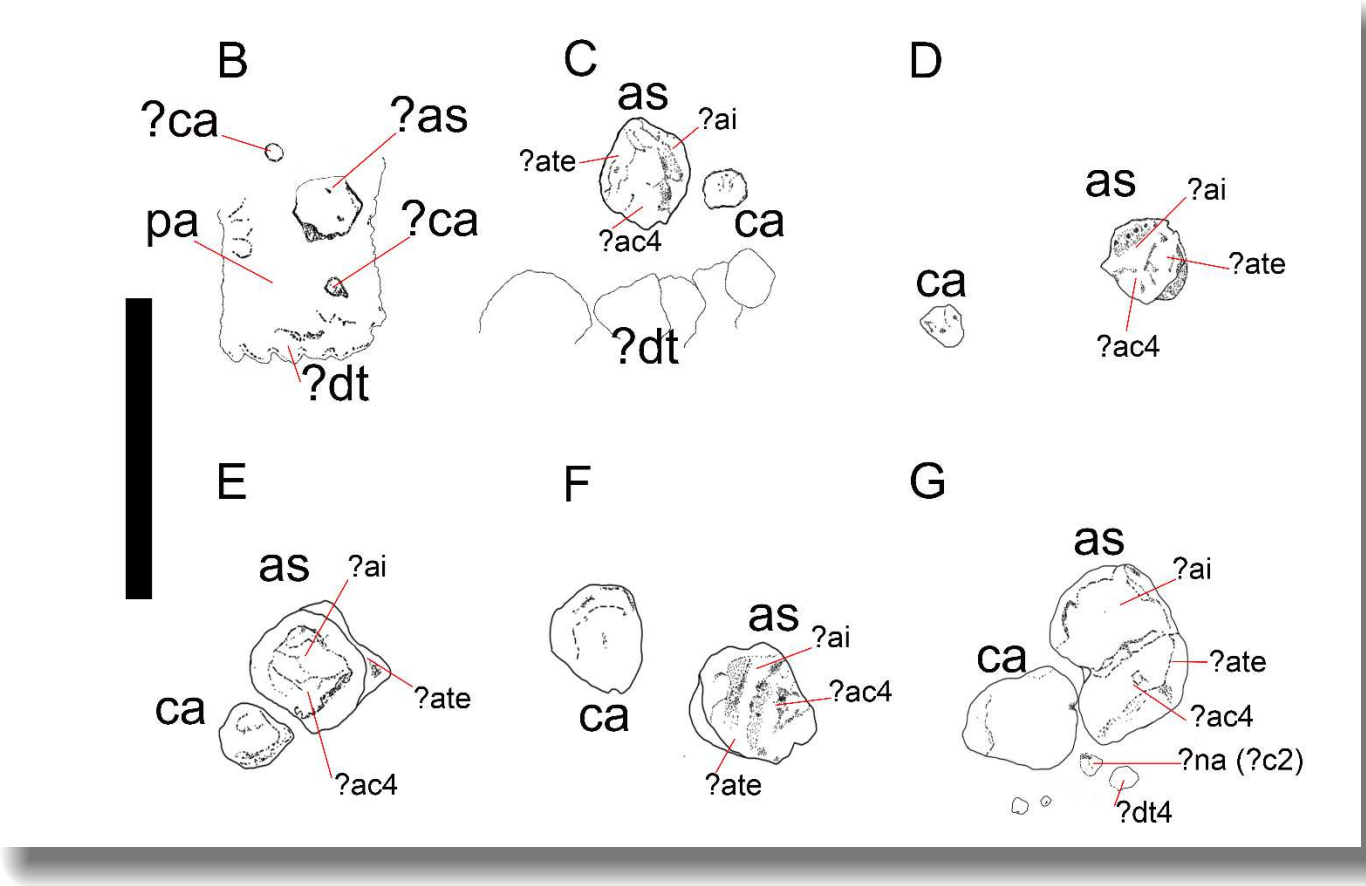

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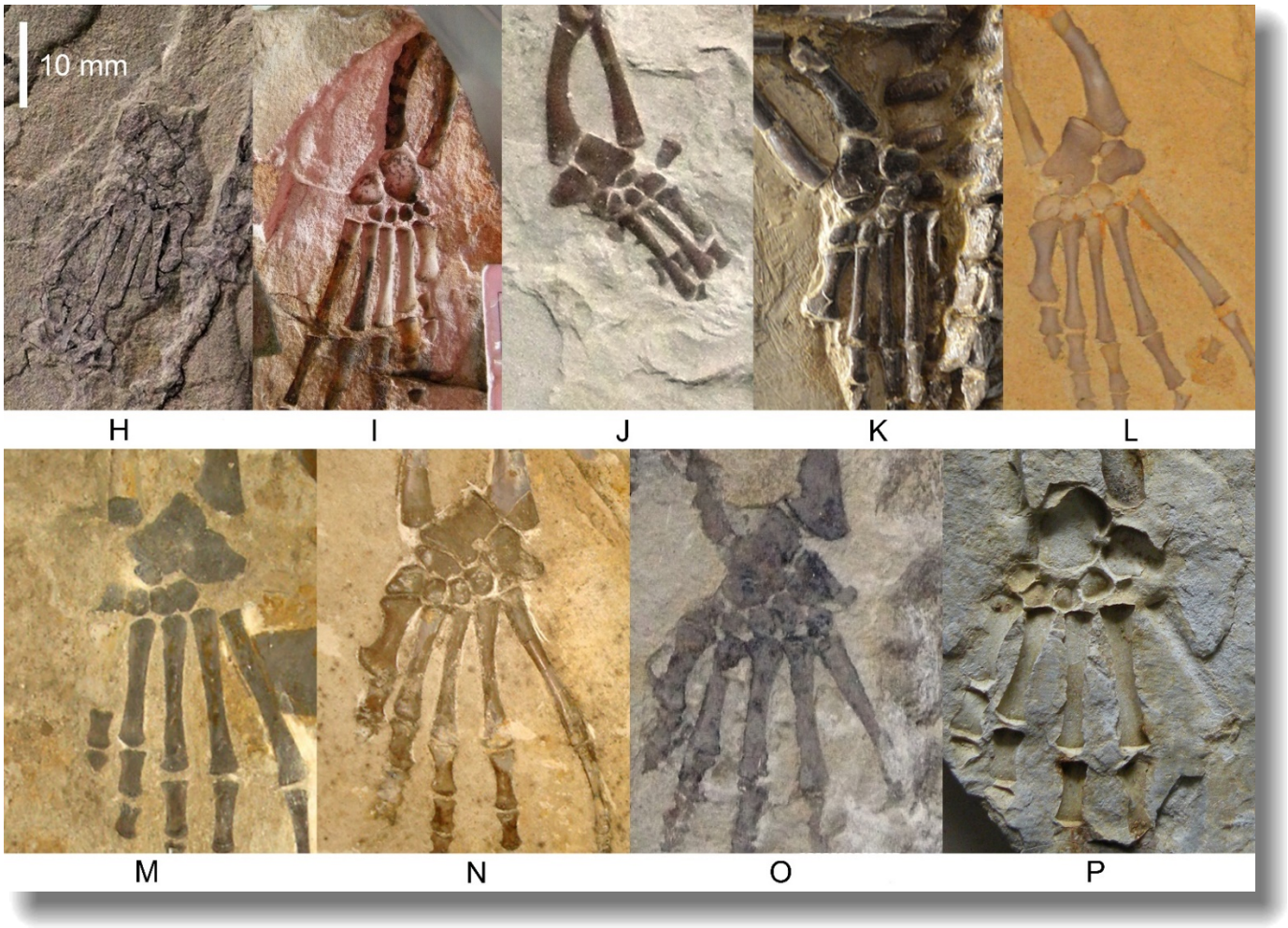

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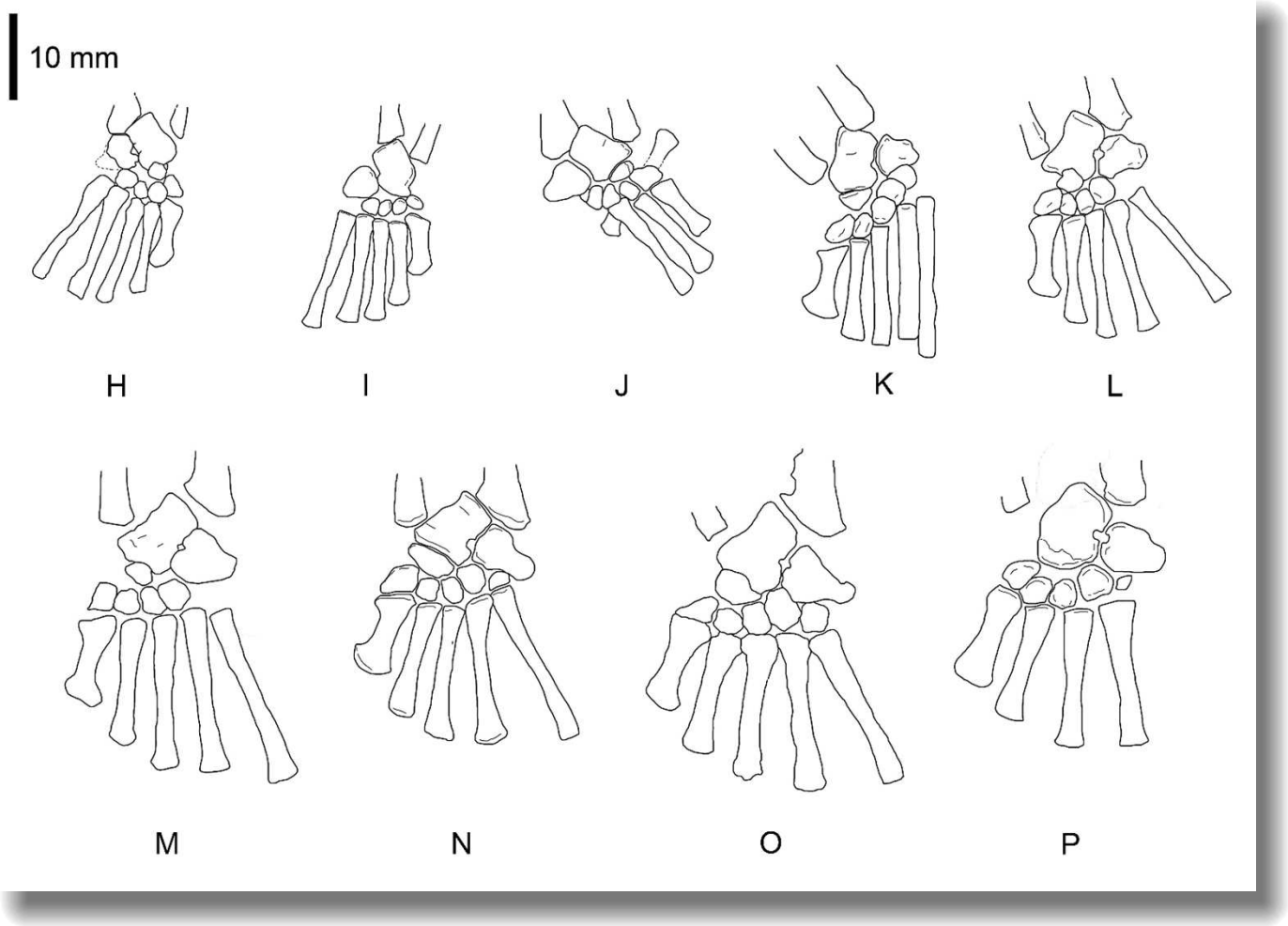

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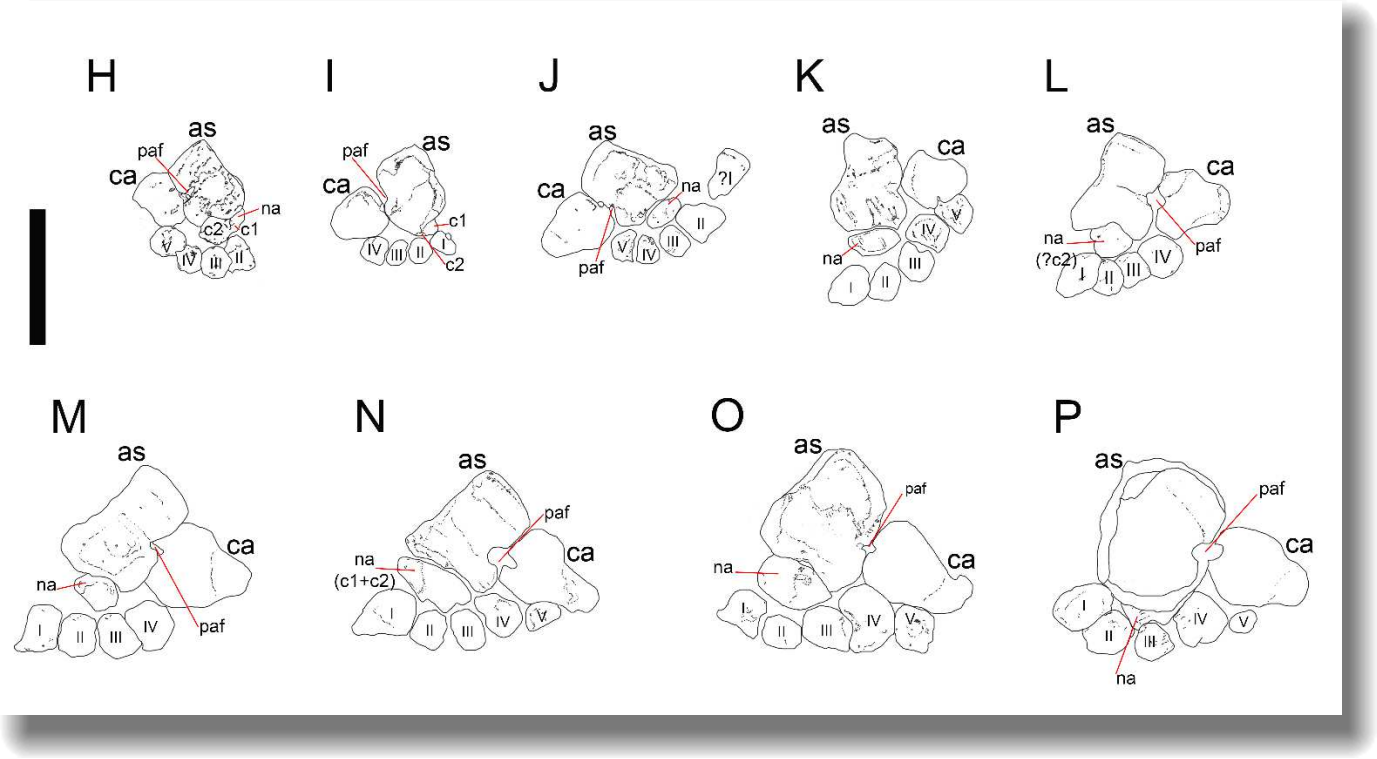

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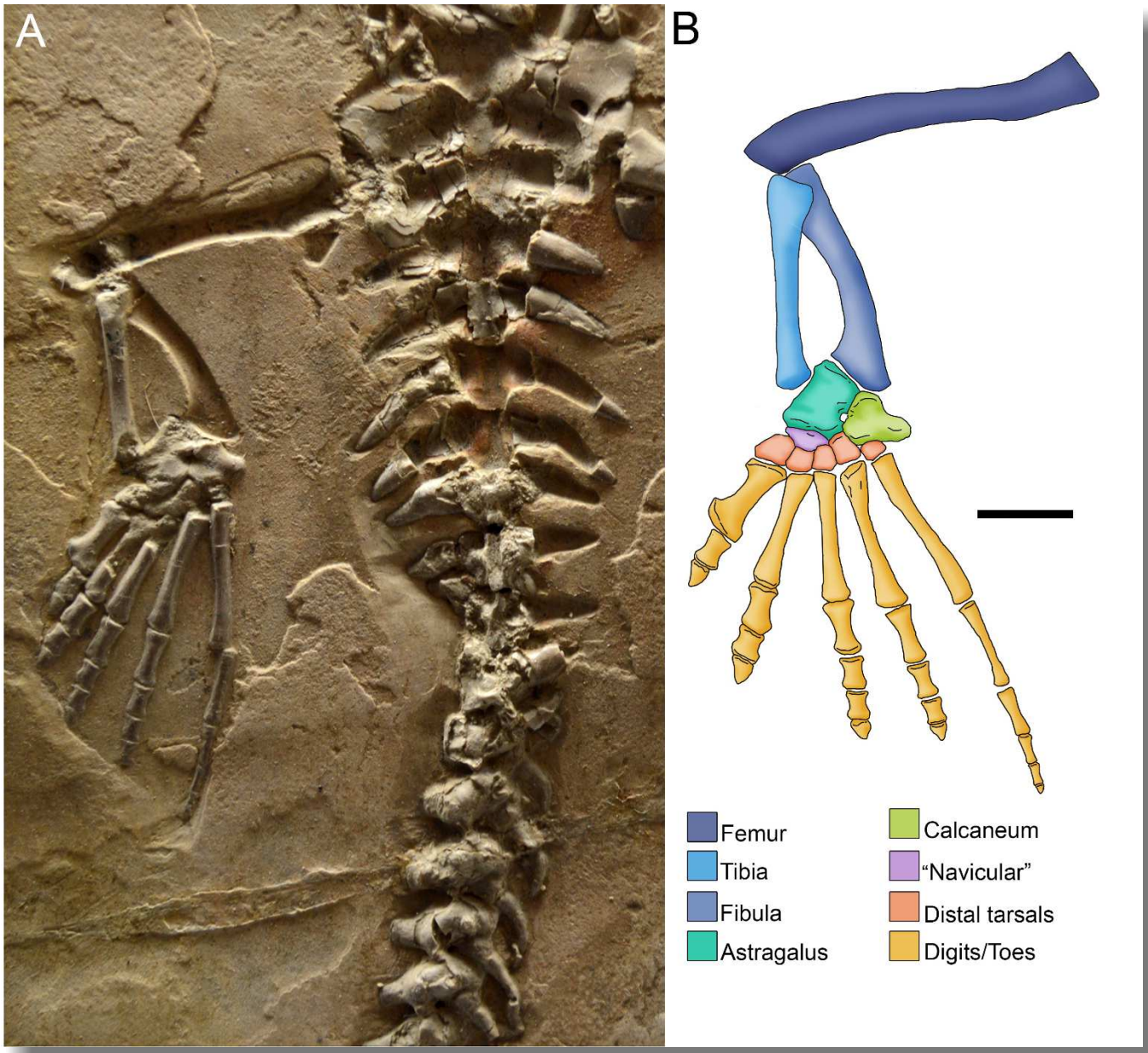

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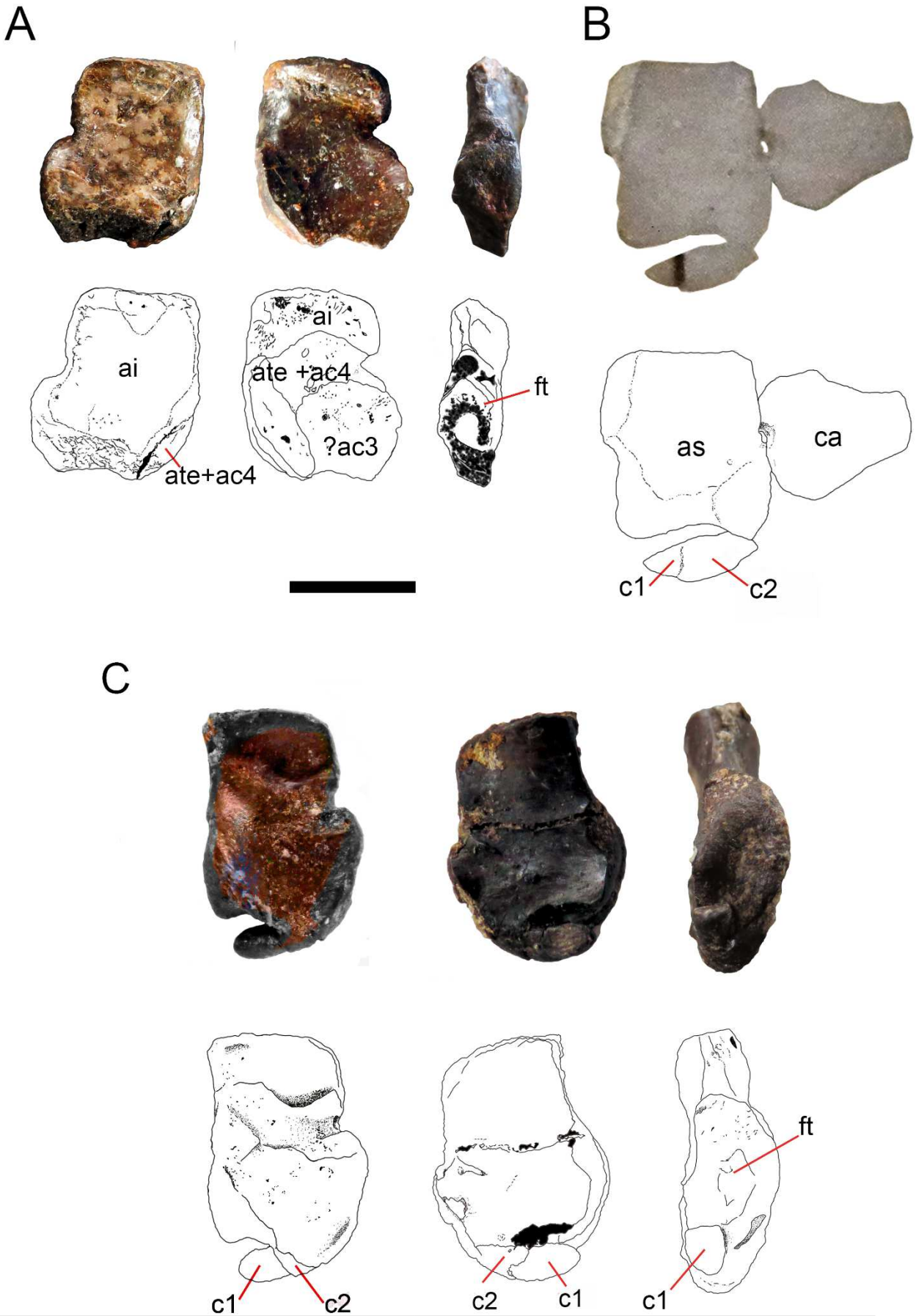

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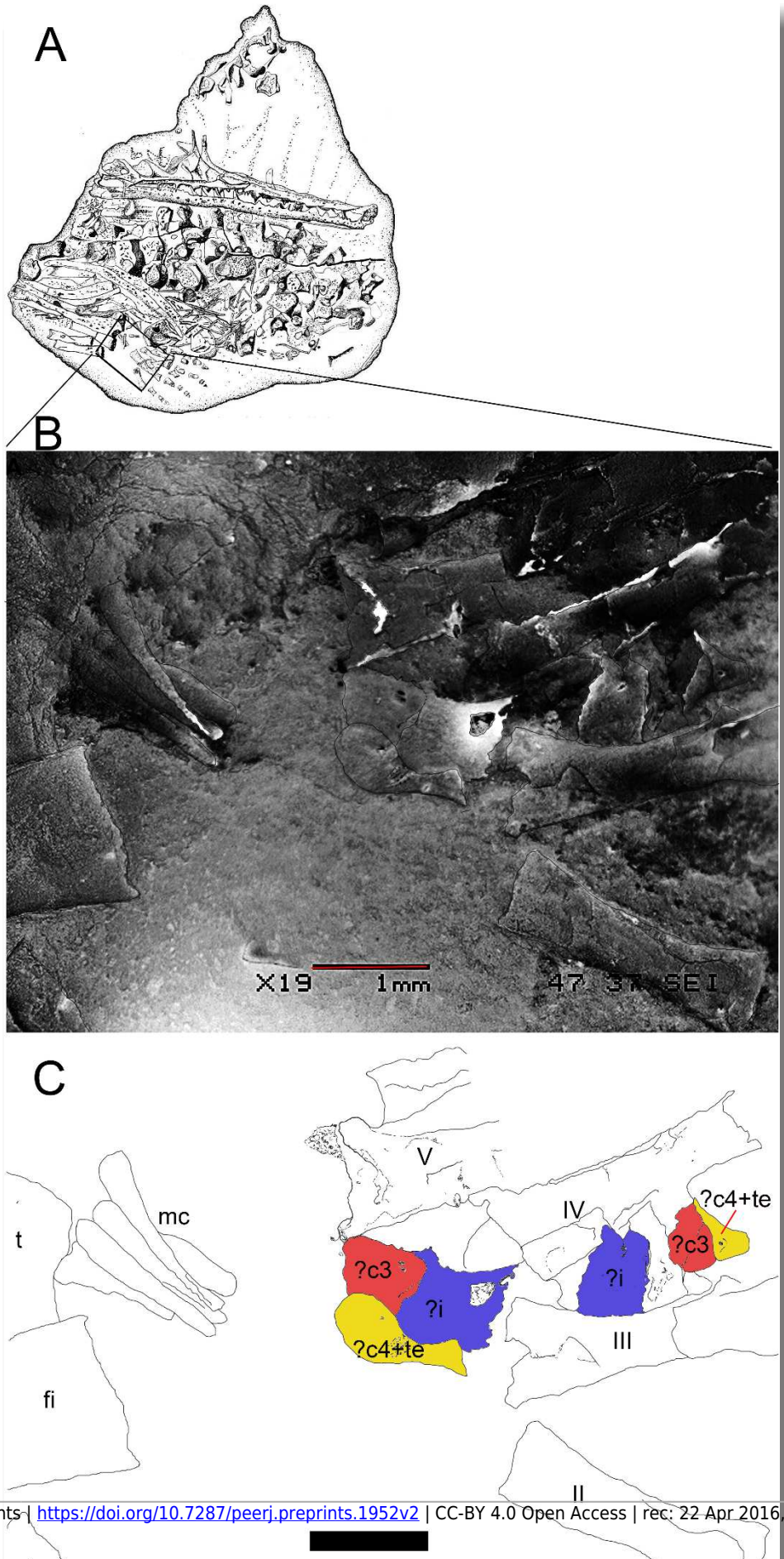

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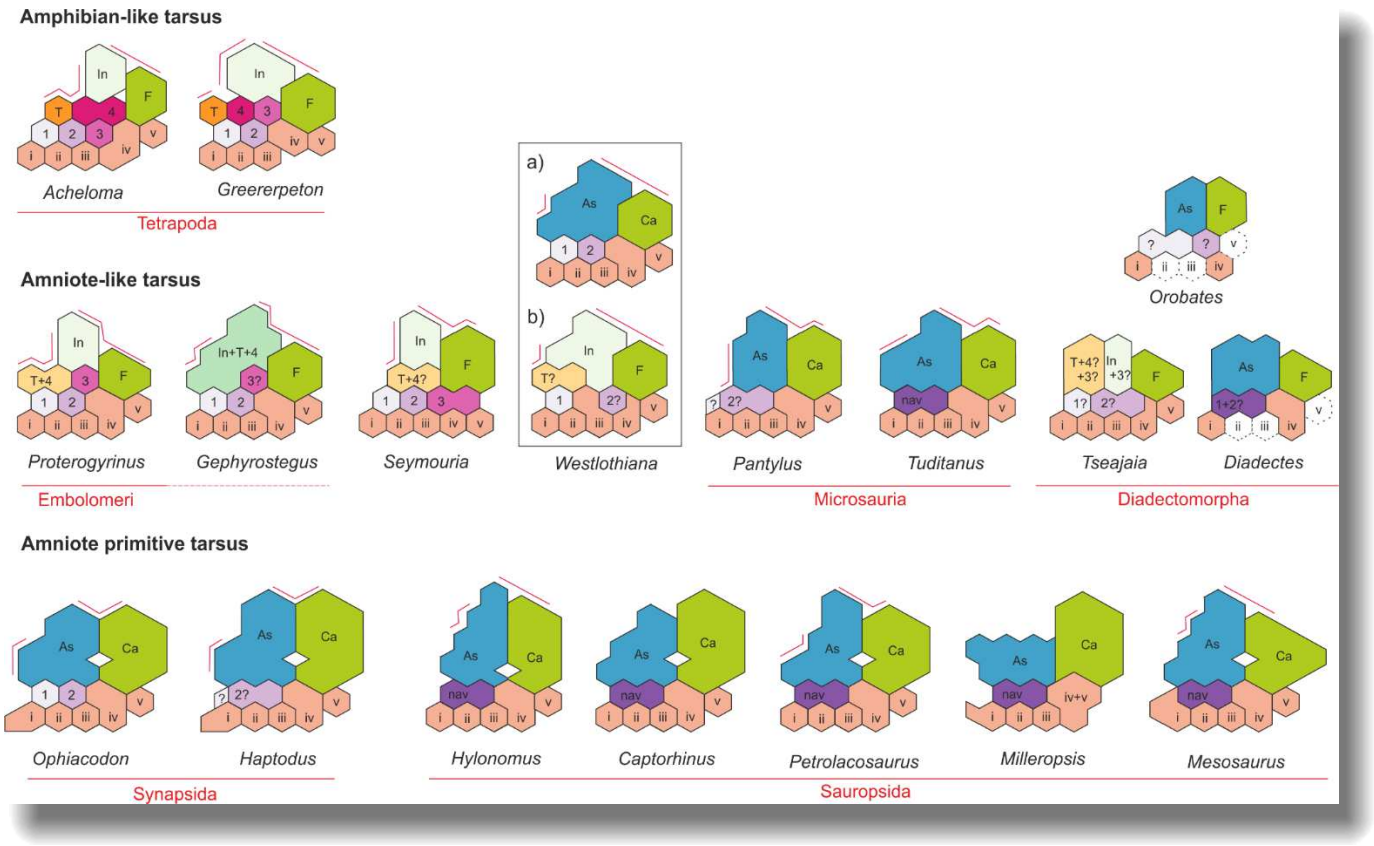

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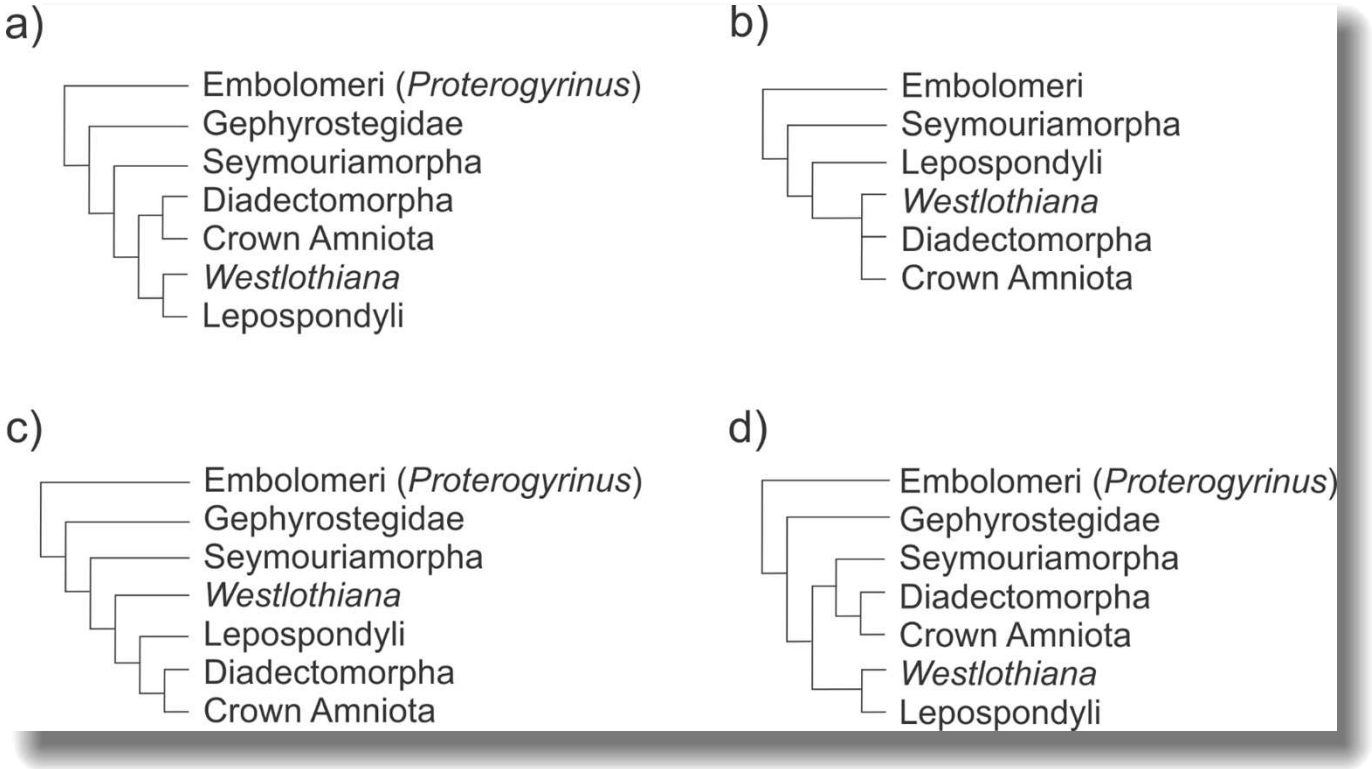

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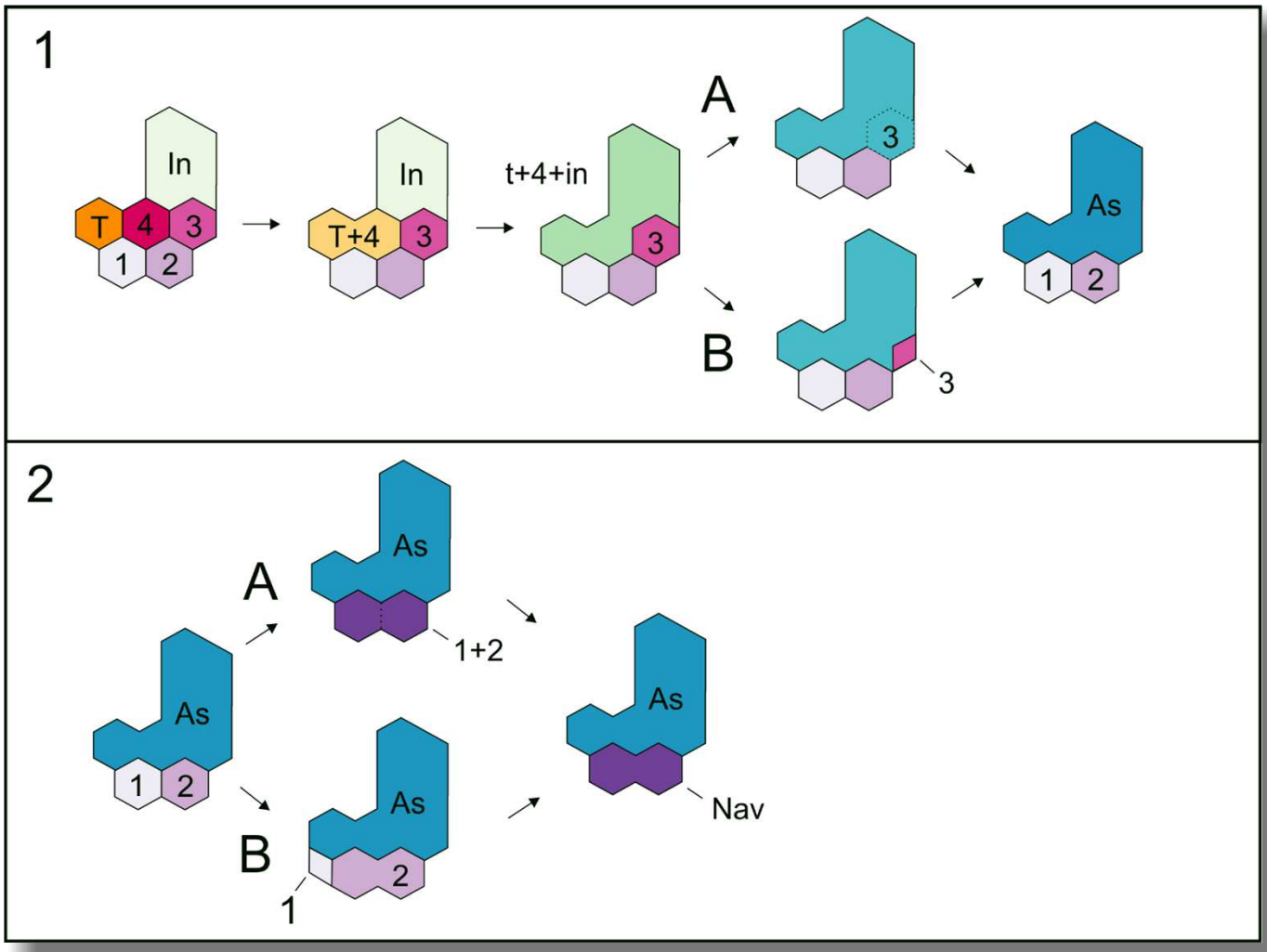

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