



An unusual pattern of limb morphology in the Tithonian marine turtle *Neusticemys neuquina* from the Vaca Muerta Formation, Neuquén Basin, Argentina

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Here, we report an unusual pattern in the manus and pes morphology of the Tithonian marine turtle *Neusticemys neuquina*. We analyse the forelimbs of two previously known specimens and describe the hind limbs of two previously undescribed specimens. *Neusticemys neuquina* is characterized by a relative elongation of both the forelimb and hind limb, compared with stem Chelonioidea, as well as an elongation of the pedal digit V, achieved through the elongation of the bones, as well as a moderate hyperphalangy. The elongation of pedal digit V is the most striking feature of *N. neuquina*, a feature unknown in other turtles. □ *Limb morphology*, *Neusticemys neuquina*, *Tithonian turtle*.

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One of the most frequent skeletal transformations among secondarily aquatic reptiles is the elongation of the limbs. In the particular case of turtles (extant Chelonioidea), which are fully adapted to marine environments, the elongation occurs mainly in the hands, as they are transformed into flippers through an increase in the length of the bones, which are much longer than the hind limbs (Zangerl 1953; Joyce & Gauthier 2004).

Traditionally, it has been postulated that turtles invaded the oceans from continental (terrestrial and/or fresh water) environments. According to Nicholls (1997), the fossil record documents three separate radiations of marine turtles: The earliest, during the Jurassic, was comprised of stem cryptodiran plesiochelyids, which probably inhabited shallow coastal waters but lacked the skeletal modifications of Chelonioidea. The second marine radiation, which occurred during the Late Cretaceous and Tertiary, is seen in pleurodiran Pelomedusoids, which occupied shallow inland seas and coastal areas. Finally, the most successful radiation is seen in Chelonioidea, beginning in the Early Cretaceous. The oldest record of this radiation is well documented by *Santanachelys*, a primitive chelonoid from the Late Aptian or Early Albian of Brazil (Hirayama 1998) (for a differing hypothesis regarding the phylogenetic position of this taxon, see also Joyce 2007). Billon-Bruyat *et al.* (2005) analysed the oxygen isotope signatures on plesiochelyid turtles

from Solnhofen (Tithonian, Germany) and, on the basis of the marine isotopic signature and the cranial anatomy, propose that these turtles were inhabitants of coastal marine environments and depended on the sea for feeding during their entire, or at least the most important, part of their life cycle. In this sense, plesiochelyids represent the oldest marine turtle radiation.

The adaptation of Testudinata to marine life has been interpreted mainly based on the transformations of the limbs, documented within the crown Chelonioidea clade, and stem taxa (see Kear & Lee 2006). Chelonoids are characterized by an elongated fore-flippers without hyperphalangy. Moreover, the phalangeal count for manual digit V in chelonoids (2 phalanges) is lower than in some other chelonians, namely Trionychidae, whose forelimbs are not true paddles, and exhibit the highest phalangeal count among turtles: 2–3–3–5–4 (Walker 1973; Shaffer *et al.* 1997; Richardson & Chipman 2003).

Tithonian fossil records in Patagonia document a different and peculiar episode in the evolution of turtle limbs that occurred much before the oldest known chelonoid, *Santanachelys gaffneyi*. *Neusticemys neuquina*, recovered from Tithonian (Late Jurassic) marine beds of the Vaca Muerta Formation (Neuquén Basin, Argentina) is known from eight specimens which include the carapace and plastron, forelimbs and a mandible (Fernández & de la Fuente 1988, 1993; Gasparini *et al.* 1997; de la Fuente 2007). Two

undescribed specimens provide significant information regarding the hind limb morphology of this taxon. *Neusticemys neuquina* is characterized by a relative elongation of both the fore and hind limbs, which contrasts with stem Chelonoidea and eurysternids (Joyce 2000), as well as an elongation of digit V of the hind limb. This last feature was achieved through the elongation of the bones and the addition of one or two phalanges relative to the condition seen in Chelonoidea and eurysternids. In this contribution we describe the hind limb of these previously undescribed materials of *N. neuquina* and analyse the transformations that comprise fore and hind limbs. The elongations of digit V of the hind limb has never been documented in other known turtles.

Material and methods

MLP 92-IV-10-1. Slab and counterslab with left and right forelimbs, manus and tibia (Fig. 1, 2). The fossil beds of the Vaca Muerta Formation at Los Catutos quarry (38°49' S and 70°12' W) (Neuquén Province, Argentina) are located in the *Windhausenicerias internispinosum* biozone (upper Middle Tithonian, Leanza 1993).

MOZ 0868. Carapace remains and left hind limb and pes (Fig. 3).

MPCA 6769. A peripheral bone and a left? hind limb and pes (Fig. 4). The MPCA 6769 can be confidently assigned to *N. neuquina* based on its anatomy and stratigraphic origin. The corresponding elements in 6769 and MOZ 0868 are thus almost identical in shape and relative size (Table 1).

MOZ 6094. Carapace, lower jaw, right humerus and femur (Gasparini *et al.* 1997; figs 5, 6, E–D). The beds of the Vaca Muerta Formation that produced this marine turtle are exposed on the northern margin of Trincajuera Creek (38°06'45" S and 70°29'45" W), some 12 km east of Loncopué village (Neuquén Province, Argentina). The ammonite *Substeuerocheras koeneni* associated with the turtle suggests an uppermost Tithonian age (Leanza *in* Gasparini *et al.* 1997).

Institutional abbreviations. BSP: Bayerische Staatssammlung für Palaeontologie und Historische Geologie, Munich, Germany; MPCA: Museo Provincial Carlos Ameghino, Cipolletti, Provincia de Río Negro, Argentina; MLP: Museo de La Plata, La Plata, Argentina; MOZ: Museo Profesor Olsacher de Zapala, Provincia de Neuquén, Argentina; QM: Queensland Museum, Brisbane, Australia.

Anatomical abbreviations. ac, astragalocalcaneum; C, carapax; ce, centrale; dc, distal carpal; dt, distal tarsal; F, femur; Fi, fibula; H, humerus; in, intermedium; mc, metacarpal; mt, metatarsal; pi, pisiform; R, radius; ra?, radiale; Ti, tibia; U, ulna; ul, ulnare; I–V, digits.

Description and results

Forelimb

The forelimb of *N. neuquina* is known from the almost complete left and right limbs of the MLP 92-IV-10-1 (Fig. 1) collected as a split slab and counterslab, and the right humerus of MOZ 6094 (Gasparini *et al.* 1997; figs 5, 6, D). In the MOZ 6094, the right humerus is completely preserved, exposed in

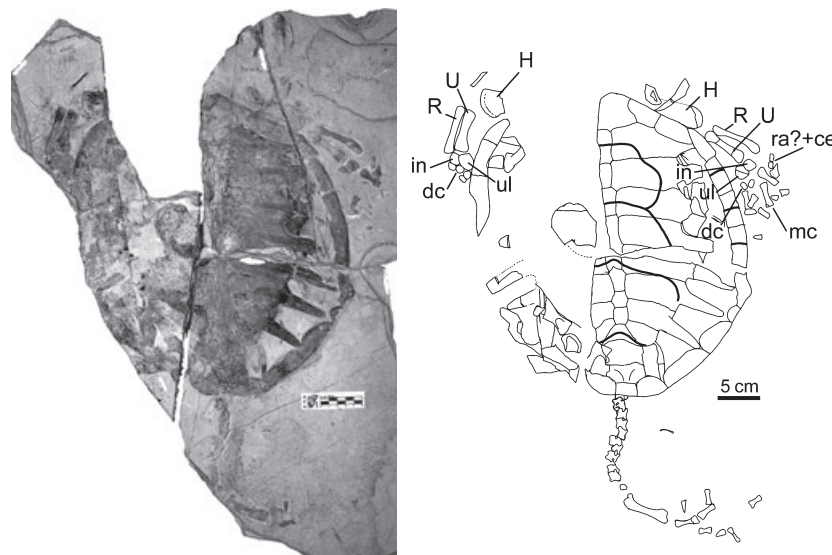


Fig. 1. *Neusticemys neuquina* (Fernández & de la Fuente 1988), slab of the specimen MLP 92-IV-10-1.

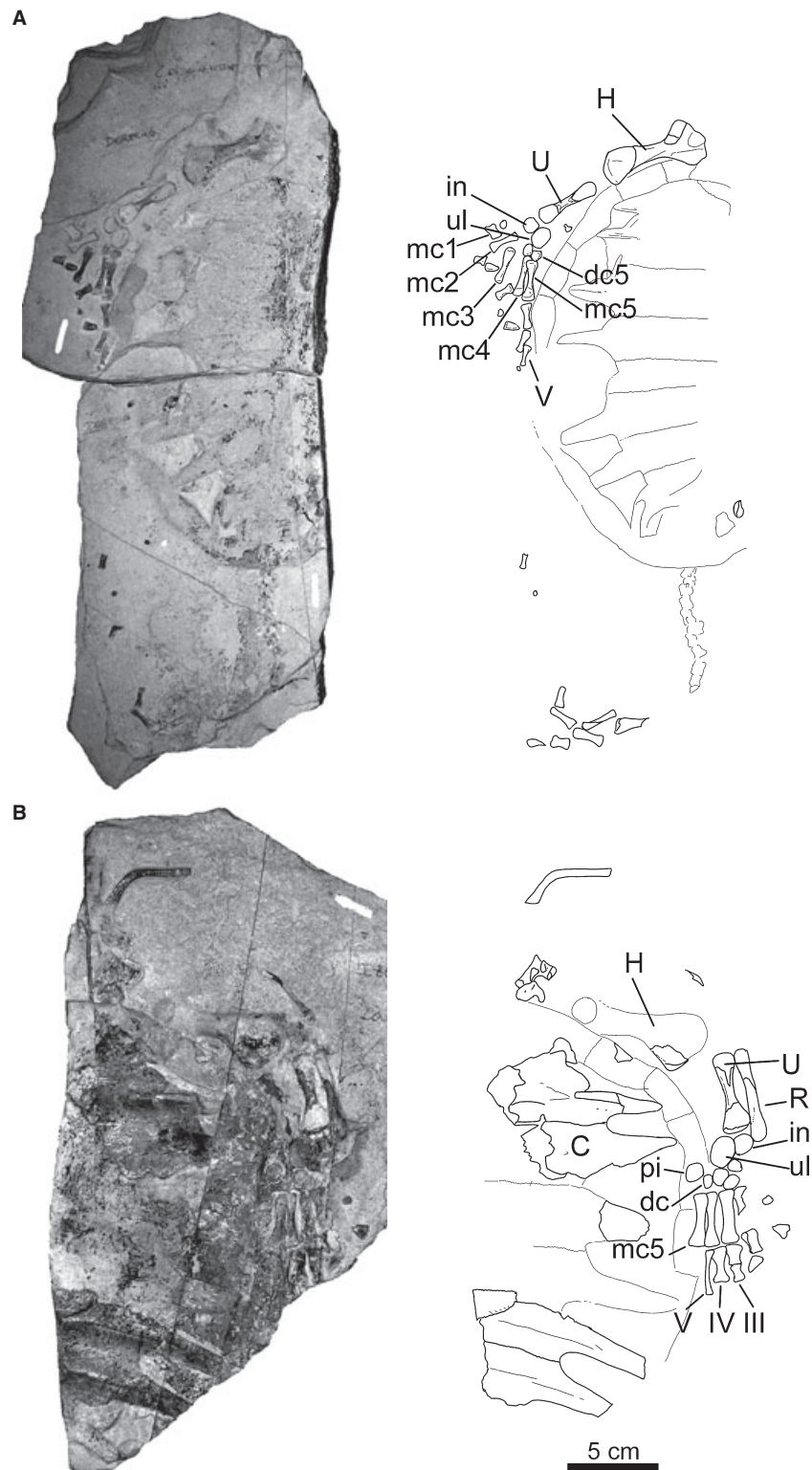


Fig. 2. *Neusticemys neuquina* (Fernández & de la Fuente 1988), counterslab of the specimen MLP 92-IV-10-1.

dorsal view and has no signs of diagenetic compression. This humerus is shorter than the femur (see Table 1) as in freshwater turtles and stem Chelonioidea (e.g. *Toxochelys latiremis*; Zangerl 1953). In MLP

92-IV-10-1, the right humerus was heavily flattened during diagenesis. Nevertheless, the general outline and major features of the bone can be observed, and its morphology is consistent with the morphology of

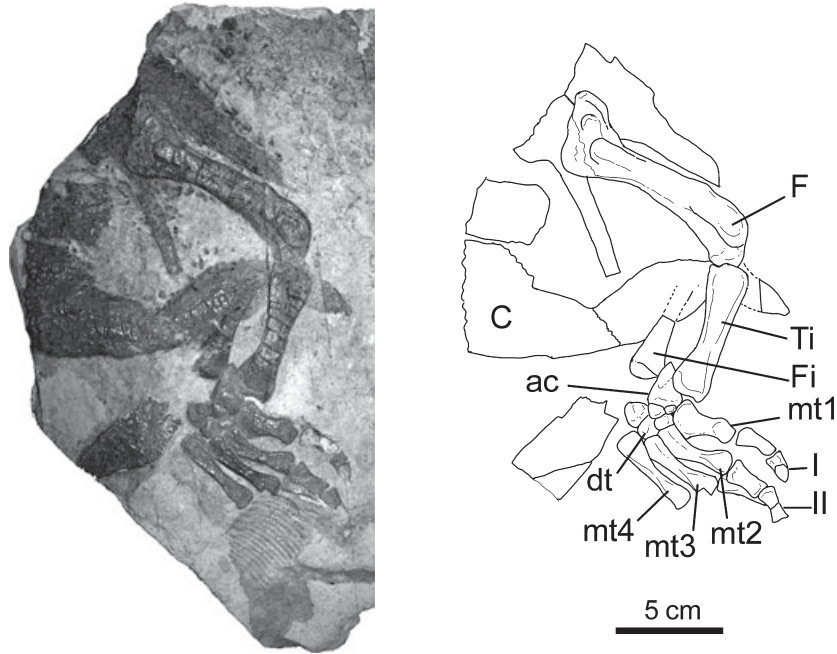


Fig. 3. *Neusticemys neuquina* (Fernández & de la Fuente 1988), slab of the specimen MOZ 0868.

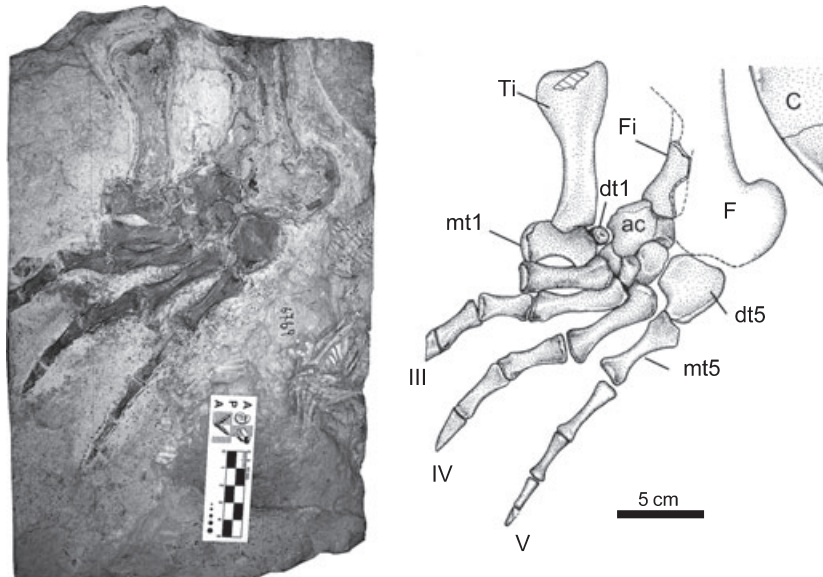


Fig. 4. *Neusticemys neuquina* (Fernández & de la Fuente 1988), slab of the specimen MPCA 6769.

MOZ 6094 humerus. In the MLP 92-IV-10-1, the humerus is 1.60 times longer than the ulna. This ratio falls within the range of observed values in turtles with the exception of *Dermochelys coriacea* (see Joyce & Gauthier 2004). In MOZ 6094, the shaft of the humerus is sigmoidally curved. Both proximal and distal ends of the bone are fairly expanded. The radial process is located at the same level as the caput humeri. In MOZ 6094 the angular relationship between the *caput humeri* and the shaft axis is 120° . The humerus is less expanded distally than

proximally, ending in a flat, rounded articular surface. A marked ectepicondylar furrow can be seen along the posterior third of the humerus mid-dorsal surface of MOZ 6094.

In MLP 92-IV-10-1, the right radius, ulna and carpus are preserved in articulation, while those of the left limb are slightly displaced. The radius is a slender element, longer than the ulna (Table 1). The relatively length between the two zeugopodial elements (1.10) is close to the values of the modern cheloniids, *Eretmochelys imbricata* and *Chelonia mydas*, and

Table 1. Length of bones of the forelimbs and hind limbs of *Neusticemys neuquina*, specimens MOZ 6094, MLP 92-IV-10-1, MPCA 6768 and MOZ 0868.

	MOZ 6094	MLP 92-IV-10-1	MPCA 6768	MOZ 0868
Humerus	125	88		
Radius		61		
Ulna		55		
mc I		16		
mc II		29		
mc III		33		
mc IV		35		
mc V		34		
Femur	134			100
Tibia		56	89.3	63.5
Fibula				54
mt I			39.4	29
mt II			46.7	34
mt III			50.6	39
mt IV			55	42
mt V			46	

significantly different from the values of freshwater turtles published by Zangerl (1953). Both elements lie in the plane of the manus, enclosing a large spatium interosseum. The ulna is a flat element, slightly compressed at the middle of the shaft. Its proximal and distal ends are moderately expanded. The distal end of the radius articulates with the intermedium, and the distal end of the ulna articulates with the intermedium and an enlarged ulnare.

Manus. – The carpals are flattened and have restricted articular surfaces, particularly the distal carpals and the pisiform. On the slab and counterslab of MLP 92-IV-10-1, the carpals elements of the left limb are preserved in articulation. The ulnare, intermedium and pisiform are enlarged and the ulnare is the largest element of the carpus (proximo-distal length of the ulnare 20 mm). The articular facets of the ulnare for the ulna, intermedium, centrale and distal carpal 4 are relatively well defined, while most of the distal edge is gently curved giving the whole element a roughly oval outline. Anterior to the ulnare, and distal to the radius and the ulna, there is an intermedium. The articular facets for the radius, ulna and ulnare are well defined, while the distal edge contacting the distal carpals is gently curved. Antero-distal to the intermedium, there are two bones that are articulated. The most anterior element is roughly rectangular with its main axis directed antero-posteriorly. The length and width of the other element is subequal, and it bears on its proximal edge two well-defined facets for the intermedium and ulnare. These two tightly articulated elements have also been preserved on the right forelimb, although they are displaced from their original location (Fig. 1). The homology of two centrale distal to the intermedium in the autopodium of extant

Testudinata is unclear. Sheil (2003), in his study of skeletogenesis in trionychnid *Apalone spinifera*, identified them as radiale and centrale 3. Sánchez-Villagra *et al.* (2007a), in their analysis of the autopodial development in the chelonioids *C. mydas* and *Caretta caretta*, hypothesized that the radiale is missing in sea turtles, and probably in turtles in general, and described them as centrale. The ossification pattern in these two species differs and the centrale distal to the radius remains cartilaginous in the adults of *C. caretta*, but it is ossified in *C. mydas*. In *N. neuquina* both elements are well ossified.

On the left limb, slightly displaced from the proximal carpus, distal carpals 3–5 are articulated, and form a row with a rounded and enlarged pisiform. The pisiform is placed distal to the ulnare and distal carpal 5, and it is approximately equal in size to the intermedium. The rounded outline of the pisiform contrasts with the angular pisiform of some extant Testudinata, including the cheloniid *C. caretta* and the trionychnid *A. spinifera* (Walker 1973; Sheil 2003). The present specimen resembles the rounded pisiform of the protostegid *S. gaffneyi* (Hirayama 1998). The distal carpals are nearly identical in shape: convex anteriorly and distally. The proximo-distal length of distal carpal 4 is approximately half of the ulnare's length. Distally, the carpals 3–5 articulate with metacarpals 3–5. On the right limb, the carpals have been preserved mainly as imprints in the matrix, except for distal carpals 4–5, which remain articulated with metacarpals 4 and 5 respectively.

All metacarpals from the right limb are completely preserved on the slab and counterslab except for metacarpal 1. Metacarpal 1 is almost complete but its distal portion is broken. Nevertheless, its outline has been preserved imprinted in the sediment. Metacarpals 3–5 are also preserved on the counterslab containing the left limb. Metacarpal 1 is hourglass shaped with concave anterior and posterior margins, and its length and width are subequal. Its length is approximately half of the length of the largest metacarpal (Table 1). Metacarpals 2–5 are slender elements. Excluding metacarpal 1, metacarpal 2 is the shortest and the widest. Metacarpals 3–5 are constrained in the middle of their shaft and their proximal and distal ends are fairly expanded. The expansion is greater on the distal end of metacarpal 5. The most conspicuous feature of the metacarpals is their relative length, in particular the elongation of metacarpal 5. Metacarpals 3–5 are long and subequal in length (Table 1). This is an unusual condition. Even in forms with elongated metacarpals 3–5, such as *Eurysternum wagleri*, *Achelonia formosa*, *Paleomedusa testa* and *Solnhofia parsonsi*, metacarpal 4 is the longest (Joyce 2000), while in *N. neuquina* metacarpal 5 is as long as metacarpal 4.

The first phalanges of digit IV and V of the left limb have been preserved and the outline of the first phalanx of digit III is preserved as a subtle imprint on the matrix. The first phalanx of right digit V is preserved on the counterslab. The distal end of the first phalanx of digit IV is poorly preserved; so, its length cannot be measured. The first phalanx of digit V of the right limb is well preserved and its length (22 mm) is approximately 65% of the length of metacarpal V. On the right counter slab, the second phalanx of digit V is articulated with the distal end of the first phalanx. Striking features of this phalanx are that it is as long as the first phalanx, slender and has a flattened distal end. These characteristics strongly suggest that it is not the last (ungual) phalanx of the digit, and so the phalangeal count for digit V must have been at least three. In Chelonioids, elongation of the forelimb is the result of the increased length of the bones, without hyperphalangy. The phalangeal formula is 2:3:3:3:2 in extant sea turtles as *C. mydas* and *C. caretta*. A phalangeal count for digit V of three is known in Chelydridae, Batagurinae, most of the pleurodires and the Trionychoids (Walker 1973; Richardson & Chipman 2003; Sheil 2003; Sánchez-Villagra *et al.* 2007b), as well as in *S. parsonsi* and *Odontochelys semitestacea* (Joyce 2000; Li *et al.* 2008).

Hind limb

The femora have been preserved in MPCA 6769, MOZ 0868 and MOZ 6094. In the MOZ 0868, the femur was crushed during diagenesis and it is preserved in ventrolateral view, below the carapace, only slightly displaced from its original location, and articulated with the zeugopodial elements. In MPCA 6769, only the proximal shaft is preserved and it was rotated post-mortem by 180° in such way that the *caput femoris* is pointing backwards and the femoral axis lies parallel to the posterior margin of the fibula. Contrary to crown Chelonioids and *Carettochelys insculpta*, the axes of the femur in the MOZ 0868 and the MOZ 6094 are slightly arched. Although an open intertrochanteric fossa is recognized in MOZ 0868, the relative size between both trochanters (major and minor) cannot be determined in the three specimens where the femur is preserved, due to the poor preservation of these structures. Distally, the femur in MOZ 0868 expands and forms a large tibial condyle. Unfortunately, this condyle has been crushed lateral-medially in this specimen.

The complete tibia has been preserved in MOZ 6769, MOZ 0868 and MLP 92-4-10-1, while the distal end of the fibula is partially preserved in only two specimens (MOZ 6769 and MOZ 0868). As is typical in testudinates, the tibia is the stouter of the two bones

and has an expanded head with a broad articular surface, which articulates with the tibial condyle of the femur as is seen in the left tibia of specimen MOZ 0868. The distal end of the fibula, almost as wide as the distal end of the tibia in specimen MLP 92-4-10-1, and narrower (as a result of crushing) in MOZ 0868, articulates with the astragalocalcaneum in the former specimen.

Pes. – The right pes of MPCA 6769 is well preserved and articulated, only the phalanges of digits 1 and 2, and the distal part of digit 3 are missing. The *pes* of MOZ 0868 is also well preserved, although more incomplete. In MPCA 6769, distal to the preserved distal fragment of the fibula, there is a large astragalocalcaneum. A subtle suture separates the more anterior and larger part from a roughly quadrangular smaller distal element. In extant sea turtles (*C. caretta* and *C. mydas*), two elements distal to the fibula fuse early in chondrogenesis, becoming the astragalocalcaneum in adults. These elements have been identified as the fibulare and intermedium (Sánchez-Villagra *et al.* 2007a).

In MPCA 6769 the most anterior part of the astragalocalcaneum, probably corresponding to the intermedium, distally contacts with distal tarsals 1–4. The distal and most posterior border of the astragalocalcaneum is strongly concave and contacts distal tarsal 4. In MOZ 0868, there is a large element composed by two tightly sutured bones. This is tentatively identified as the astragalocalcaneum. The most anterior part is badly preserved and the tibia is overlies it.

The homology of the distal tarsal elements of Testudinata has been revised recently. Traditionally, the hooked element present in the Testudinata tarsus has been identified as a modified fifth metatarsal. Fabrezi *et al.* (2009), in their comprehensive paper describing limb development in pleurodiran turtles, identified the hooked element present in the chelonian tarsus as the fifth distal tarsal. Assuming this hypothesis of homology, the preserved distal tarsal elements in MPCA 6769 are identified as distal tarsal 1–5, and in MOZ 0868, as distal tarsal 1–4 (Fig. 3). Distal tarsal 1 is considerably smaller than the other distal tarsals; its outline is rounded and articulates proximally with the astragalocalcaneum, distally with metatarsal 1 and posteriorly with distal tarsal 2. Distal tarsals 2 and 3 are nearly equal in size, each articulating distally with MT II and III, respectively, and with the astragalocalcaneum proximally. Distal tarsal 2 is roughly rectangular. Distal tarsal 4 is larger than distal tarsals 1–3. In MPCA 6769 it is in its natural position, articulating proximally through a strongly convex border with the astragalocalcaneum, anteriorly with distal tarsal 3, distally with metatarsal 4 and posteriorly with

distal tarsal 5. In MOZ 0868, it is slightly displaced losing most of its natural contact with metatarsal 4. Distal tarsal 5 is only preserved in MPCA 6769, and it is the largest tarsal element. Its maximum proximo-distal length is subequal to its maximum antero-posterior length. Its anterior edge is straight forming a well-defined facet for the articulation with distal tarsal 4. Its antero-distal edge is concave and the proximal end of metatarsal 4 is close to it, although not in contact. Its most postero-distal edge contacts briefly with metatarsal 5. Its posterior edge is gently curved.

The complete metatarsal row is preserved in MPCA 6769. MOZ 0868 preserves only metatarsals 1–4. Nevertheless, a subtle depression on the matrix marks the location of metatarsal 5. The relative lengths of the metatarsals are: IV > III > II = V > I. Metatarsal 1 is more robust than the other metatarsals.

The most striking features of the hind limb of *N. neuquina* are the relative length of the digits and the pedal phalangeal formula. In MPCA 6769, digits 4 and 5 are completely preserved, and digit 5 is long, being subequal in length to digit 4. This condition is unusual for tetrapods in general, and for Testudinata in particular. In all extant Testudinata, digit 5 is reduced in length and ends blind within the skin (Joyce 2007). In most tetrapods, the length of digit 5 is reduced in comparison with digit 4, even highly modified in secondarily aquatic forms such as mosasaurs. One of the few exceptions is *Mesosaurus* spp. (Romer 1956; fig. 193), in which digit 5 is even longer than digit 4. In all known Testudinata, digit 5 is much shorter than digit 4, but, unfortunately, digit 5 is unknown in *O. semitestacea* (Li *et al.* 2008).

In MOZ 0868, there are two phalanges in the first digit. The first phalanx is preserved as an extremely thin sheet of bone, and as imprint on the sediment. The last phalanx is short and robust and, although only the proximal half is preserved, the complete outline of this element is demarcated on the sediment as an imprint. Its pointed distal tip allows its identification as the unguis phalanx. The first phalanx of digit 2 is completely preserved, and it is as long as the first phalanx of digit 1. Only the proximal half of the second phalanx is preserved, but its length can be estimated based on the imprint on the sediment. The second phalanx of digit 2 is slightly shorter than the first phalanx of the same digit, with a moderate constriction in the middle of the shaft. Its distal end is flat, indicating that this phalanx is not the unguis. There is a displaced slender phalanx distal to metatarsal 3 and partially overlapped by the first phalanx of digit 2 that cannot be unequivocally associated with any metatarsal. In MPCA 6769, no phalanges of digits 1 and 2 are preserved. The preaxial phalanges are relatively more robust than the post-axial phalanges. There are three

phalanges in digit 3. Distal to the second phalanx and articulated with it, there is a small fragment of the proximal part of phalanx 3. The first and second phalanges are complete and subequal in length. Digit 4 has three phalanges; the first phalanx is more robust than the rest, and slightly longer than the second. The unguis phalanx is relatively long, its length being about twice the width of the proximal edge. Digit 5 has four slender phalanges; the first phalanx is the longest. Both first and second phalanges have a moderate constriction in the middle of the shaft. The distal phalanx is slender and short, and its length approximately half of the length of the unguis phalanx of digit 4. Its morphology contrasts with the stout, curved and pointing end of the unguis phalanx of the other digits, and suggests that it was not clawed, ending blind within a skin pocket. Based on the available information, *N. neuquina* had at least three phalanges in digits 2 and 3, and its pedal phalangeal formula would be: 2–3?–3?–3–4.

Discussion

Phylogenetic position of Neusticemys neuquina

Neusticemys neuquina was named by Fernández & de la Fuente (1988). It was first tentatively assigned to the genus *Eurysternum* from the European Tithonian (von Meyer 1839) on the basis of three specimens from the lower beds of the Vaca Muerta Formation (Tithonian), outcropping at Cerro Lotena. Fernández & de la Fuente (1993) studied two other specimens from the middle beds of the Vaca Muerta Formation. When comparing the new material with the figure of the lost holotype of *E. wagleri*, type species of the genus *Eurysternum*, Fernández & de la Fuente (1993) proposed the relocation of the Tithonian species within a new genus, *Neusticemys*.

The phylogenetic position of *N. neuquina* is still uncertain. It shares with plesiochelyids and eurysternids (de Broin 1994; Joyce 2000; de Lapparent de Broin 2001) the width of the carapace, the shortening of the nuchal and the first costal bones. Nevertheless, these are primitive characters and therefore not useful to determine phylogenetic relationships. *Neusticemys neuquina* shares derived characters of the shell with the undescribed specimens BSP-AS-1921 and BSP-1952-1-113 from Upper Jurassic of Germany and protostegid from the Lower Cretaceous of Australia as *Notochelone costata* (QM F 33511) (Gaffney 1981). Among these characters are the antero-posterior elongation of the carapace, the presence of broad pleuro-peripheral fenestrae, the loss of the firm sutural contact between the carapace and plastron along the

bridge and the development of broad lateral fenestrae and a large central fenestra on the plastron. Although these character do not suggest a relationship because they characterize shells of marine turtle with pelagic habits (Zangerl 1980), other traits, like the plastral index, are in close similarity to the condition recognized in Dermochelyidae (Hirayama 1998). de Laparent de Broin (2001) tentatively assigned *N. neuquina* and the undescribed Bayern form to Family Protostegidae or 'proto-Protostegidae' based on the general carapace and plastral morphology. Although this statement could be right, it never has been tested in a cladistic framework. One of the main problems to resolve the phylogenetic position of *N. neuquina* is the lack of a skull, a skeletal element that is so important in establishing turtle relationships. In this context we assign *N. neuquina* to Eucryptodira *incertae sedis*.

Neusticemys neuquina as a marine turtle

All *N. neuquina* specimens have been found in marine Tithonian beds of the Vaca Muerta Formation in the Neuquén Basin (Northwestern Patagonia, Argentina). The Vaca Muerta Formation was deposited during a rapid transgression that is biostratigraphically well constrained and which covered the majority of the basin (Legarreta *et al.* 1993; Gasparini *et al.* 1999). Spalletti *et al.* (2000) modelled the Neuquén Basin as a palaeogulf separated from the Pacific by an island arc with frequent gaps. *Neusticemys neuquina* has been found in the southern sector of the basin, in Cerro Lotena, Los Catutos quarry and Trincajuera Creek. None of the eight specimens found in any of the three localities including the four described in this paper are disarticulated, indicating the absence of long post-mortem transport. This taphonomic evidence suggests that *N. neuquina* inhabited this protected marine environment. *Neusticemys neuquina* remains have been found along with other marine reptiles such as the turtle *Notoemys laticentralis*, metriorhynchid crocodiliforms, ophthalmosaurid ichthyosaurs and pliosaurs (Gasparini & Fernández 2005), and no terrestrial faunal elements have been recovered from the Vaca Muerta Formation.

Neusticemys neuquina forelimbs as flippers

Although the Tithonian marine reptile assemblage from the Neuquén Basin is composed mainly of pelagic marine reptiles, the limb morphology of *N. neuquina* does not suggest pelagic habits, or at least, there is no compelling evidence to support this hypothesis. Forelimb proportions of extant turtles closely reflect the habitat to which they are adapted (Joyce & Gauthier 2004). Joyce (2007), in his analysis

of phylogenetic relationships of Mesozoic turtles, recognized two different types of *manus* transformations from more terrestrial basal type: flippers and paddles. In Trionychia the hand is developed into a flipper, has five fingers and all of them are completely encased in the skin. The entire flipper is highly mobile, with well-developed articular surfaces on the metacarpals and phalanges, and can be moderately long, as in trionyichids, or very long, as in carettochelyids. According to Joyce (2007), these flippers resemble the forelimbs of pinnipeds such as otarids. In paddles, the fingers that form the paddles are fully encased by the skin as well, but they are tightly bound to one another with connective tissue, resulting in a stiffened paddle. The distribution of this character (*manus* transformed into paddles) suggests that the presence of short paddles is an autapomorphy of *S. gaffneyi* and a synapomorphy of Panchelonioidea. Within this latter clade, elongate paddles occurs as a synapomorphy of Chelonioidea. Richardson & Chipman (2003), when analysing the variations in phalanx number in amniotes, also distinguished two different types of derived turtle hands: the trionyichids *manus* and the chelonoids flipper. The trionyichid *manus* is characterized by moderate hyperphalangy, but it is not developed into a true 'flippers'. By contrast, in the forelimb of chelonoids, the *manus* is elongated and the entire forelimb transformed into an elongated flipper. Both contributions identified paddles (Joyce 2007) or elongated flippers (Richardson & Chipman 2003) as a characteristic of marine turtles. Broadly applied, as is common in the literature, the term *marine turtles* is used as a synonym for Chelonioidea or Panchelonioidea, which evolutionary history probably originated in the Aptian–Albian, thereby dismissing Jurassic marine turtles such as *N. neuquina* and plesiochelyid turtles from lithographic limestones of western Europe (Billon-Bruyat *et al.* 2005).

Neusticemys neuquina forelimbs could be considered flippers (*sensu* Joyce 2007) as they are elongated as seen in trionyichids. The *manus* is also relatively long (i.e. the *manus* is longer than the humerus), but it is also strongly curved, and the heavy pointed unguis phalanges indicate that at least some digits were not completely included within the skin. The *manus* depicts an unusual pattern, shared with *S. parsonsi*, of a post-axial elongation represented by metacarpal 5, which is as long as metacarpal 4. Articular surfaces of the carpus and the enlarged rounded pisiform indicate restricted movement in this zone of the limb, and that the entire limb was not as highly mobile as a trionyichid flipper. Nonetheless, articular surfaces on the phalanges indicate a certain mobility of the digits and that the whole limb did not act as a stiffened paddle during locomotion. As defined by Joyce

(2007), *N. neuquina* forelimbs, although elongated, do not fit into either the flipper or paddle category but nevertheless differs from aquatic freshwater turtles.

Unusual pes morphology and phalanx formula of Neusticemys

The most striking feature of the limb morphology is the elongation of digit 5 in the hind limbs. Another very peculiar condition of *N. neuquina* is the presence of four phalanges of digit 5. Whether the number of phalanges represents a moderate hyperphalangy is still unclear. In extant sea turtles, the elongation of limbs and transformation into flippers has been achieved through the increase in length of bones without hyperphalangy, and the highest phalanx counts found among Testudinata are in the Trionychidae, whose forelimbs are not true flippers (Richardson & Chipman 2003). Hyperphalangy is defined as a digit morphology wherein the number of phalanges arranged linearly within an individual digit increases above the plesiomorphic condition (Fedak & Hall 2004). If we use this definition when analysing the patterns of changes in the phalangeal numbers within Testudinata evolution, the problem arises that the plesiomorphic condition of the pedal phalangeal formula is still uncertain. Within crown-clade turtles, Sánchez-Villagra *et al.* (2007b) hypothesized that 2–3–3–3–3 is the plesiomorphic phalangeal formula, and that the phalangeal formula 2–2–2–2–2 of *Proganochelys quenstedti* (see Gaffney 1990) and *Palaeochersis talampayensis* (Rougier *et al.* 1995) is a secondary adaptation to terrestrial habits for these two extinct basal taxa. The discovery of *O. semitestacea* provides significant new information. The phalangeal formula of the most basal turtle is higher than the plesiomorphic condition previously proposed for turtles. The phalangeal formula of *O. semitestacea* is 2–3–4–4–3 in the manus and 2–3–4–4–? in the pes. As digit 5 of the pes is not preserved, we cannot be sure that the four phalanges in digit 5 in *N. neuquina* pes represent the additions of only a single phalanx beyond the *O. semitestacea* condition. Recently, Bona & Alcalde (2009) studied the skeletal development of the extant pleurodiran turtle, *Phrynops* spp., and re-interpret the phalangeal pedal formula of this taxon as 2–3–3–3–4 (2–3–3–3–5 in Bona & Alcalde 2009, as they considered the hooked element to be metatarsal 5). Thus, *Phrynops* spp. has the highest phalangeal number on record for digit 5 in turtles.

Regardless of whether the phalangeal formula of *N. neuquina* represents a moderate hyperphalangy or not, the pattern of its limbs is unusual in that it has an elongated posterior border of the manus and pes

(including elongation of digit 5). As Fedak & Hall (2004) pointed out, the distinction between digit length and phalangeal number is important as digit length does not always correlate with an increase in the number of phalanges. In *N. neuquina*, the elongation of the posterior border of the manus is achieved by the increase in length of metacarpal 5 and the first two phalanges of digit 5. Based on evidence of absolute length values of preserved elements of digit 5 and metacarpal 5 (74 mm), conservative estimates of the posterior border of the manus suggest that it was longer than the zeugopodium and slightly shorter than the epipodium. In the pes, regardless of the phalangeal formula, digit 5 is as long as digit 4. This condition is presently unknown in other turtles. Even *Phrynops* spp., with the highest phalangeal number in the post-axial digit, has a shorter digit 5 than digit 4, and metatarsal 5 (identified as phalanx 1 in Bona & Alcalde 2009) is approximately half the length of metatarsal 4. This striking feature is also uncommon among tetrapods, even in forms with elongated limbs that are transformed into paddles, such as in mosasaurs.

No imprints of soft tissues have been preserved on the matrix surrounding the autopodium; so, it is unclear whether the manus and/or foot were/was extensively webbed as in other aquatic turtles that have elongated manus and pes, such as Trionychia.

Palaeoecology

Limb morphology, as an indicator of habitat preference, has been used in the evaluation of competing hypotheses of early turtle evolution, i.e. terrestrial (Lee 1996) versus aquatic origins (Rieppel & Reisz 1999). Joyce & Gauthier (2004) conclude that the turtle stem lineage (e.g. Norian *P. quenstedti*, *P. talampayensis*) lived on land, and that aquatic habits are derived. The recent discovery of *O. semitestacea* from the Carnian (Upper Triassic) of China has changed ideas about turtle origins and the evolution of their body plan (Reisz & Head 2008). A significant topic of discussion is the environment in which basal forms lived. The deposits from which *O. semitestacea* was recovered indicate that the most basal turtle inhabited marginal areas of the sea or river deltas (Li *et al.* 2008). This finding changes our ideas about marine radiation of turtles. *Neusticemys neuquina*, as well as plesiochelyids, can no longer be considered representative of the first radiation of turtles into marine environments (Nicholls 1997). This topic is intimately related to the primitive ecology of turtles. It remains to be resolved whether turtles originated in a terrestrial or an aquatic setting. If turtles evolved in terrestrial environments and are related to pareisaurid and/or procolophonids

(Lee 1993, 1995, 1996) and if *O. semitestacea* was indeed marine *O. semitestacea* must be considered the first attempt of marine colonization, and Late Jurassic plesiochelyids, and *N. neuquina*, may represent a second radiation. It bears mentioning that this 'radiation' also included basal pleurodiran turtles including the Oxfordian *Caribemys oxfordiensis* (Cuba), the Tithonian *N. laticentralis* (Argentina) and the Valanginian *N. zapatensis* Cadena Rueda & Gaffney 2005 (Colombia) (de la Fuente & Iturralde-Vinent 2001; Cadena Rueda & Gaffney 2005). Alternatively, it has been proposed that turtles are primarily aquatic and that they originated in aquatic settings (Rieppel & Reisz 1999; Li *et al.* 2008). More specifically, sedimentological evidence suggests that the most basal turtle inhabits near-shore marine environments. In this evolutionary scenario, *N. neuquina* and plesiochelyids could represent not a radiation to a marine environment, but a lineage that evolved within a coastal marine environment. From these environments, two major ecological shifts probably occurred during evolutionary history of turtles: an extreme transition from marine and/or brackish water to land, and also one to fresh water.

The limb morphology of *N. neuquina* does not differ significantly from that of other aquatic turtles with elongated manus arbitrarily defined here as a manus longer than the humerus, and the number of phalanges above the inferred 'basal condition' (i.e. 2–3–3–3–3), as in *O. semitestacea* and extant Trionychia, but it is considerably different from the pattern of the other more terrestrial, basal Testudinata such as *P. quenstedti* and *P. talampayensis*, characterized by stout and short autopodium (Gaffney 1990; Sterli *et al.* 2007).

Traditionally, limb transformation in turtles has been interpreted somehow straight in the context of the secondary adaptation of one lineage, Pancheloniodea, from some aquatic coastal environment into marine ones (Zangerl 1953; Hirayama 1998; Kear & Lee 2006). The extreme of this adaptation is represented by extant chelonoids, which have elongated limbs transformed into flippers, enlarged lacrimal glands for salt excretion and spend little time out of the sea except at egg-laying time. The oldest record of this is represented by Early Cretaceous (Late Aptian or Early Albian) *S. gaffneyi* (Hirayama 1998). In this scenario, the transformation sequence of limb morphology for a fully pelagic marine adaptation comprised fresh water-transitional marine forms such as toxochelyids and truly marine turtles (i.e. Chelonioidea).

However, recent discoveries permit discussion of an alternative evolutionary scenario, where turtles radiated from shallow marine and/or brackish environments (such as the ones where *O. semitestacea* was

found) into continental environments (i.e. exclusively freshwater environments) as well as terrestrial habits. The Jurassic period is recognized as a key time for turtle evolution (e.g. Danilov & Parham 2008; Anquetin *et al.* 2008; Sterli 2008). The fossil record of Early and Middle Jurassic turtles is still poor and, although recent Jurassic discoveries such as *Condorchelys antiqua*, *Heckerochelys romani* and *Eileanchelys waldmani* bring new insights into the evolution of stem turtles, their limbs are unknown, and so their ecological preference remain unclear. *Eileanchelys waldmani* has been interpreted as an aquatic turtle, the earliest known such species until the discovery of *O. semitestacea*, inhabiting lagoons and lakes of variable salinity (Anquetin *et al.* 2008). Billon-Bruyat *et al.* (2005) confirmed that plesiochelyid turtles are coastal marine inhabitants based on the oxygen isotope compositions of their bones. In broad terms, a putative scenario could be that turtles radiated from nearshore and shallow marine habits into continental environments (either fresh water or terrestrial) and a pelagic marine lifestyle.

Conclusions

Neusticemys neuquina was a Jurassic marine (although not pelagic) turtle, inhabiting protected areas such as the Neuquén Basin. Its forelimbs are relatively long (approximately 67% of the carapace length). As an autapomorphic condition, the pes of *N. neuquina* is characterized by four ossified phalanges of digit 5. Another striking feature of *N. neuquina* pes is the elongation of the digit 5, which is as long as digit 4. Such an elongation of the post-axial digit has never been described for any other known turtle, and it is also uncommon for tetrapods. One of the few tetrapods with this condition is *Mesosaurus* spp., in which digit 5 is even longer than digit 4. There are still large gaps in the evolutionary history of turtles. Recent discoveries of Triassic and Jurassic turtles in marine environments, and the diversity of their limb morphology, as depicted by the unusual manus and pes of *N. neuquina*, indicate that morphological diversity of Jurassic turtles was even greater than previously thought.

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