

Tachypleus syriacus (Woodward)—a sexually dimorphic Cretaceous crown limulid reveals underestimated horseshoe crab divergence times

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Abstract The fossil record represents an important test to molecular divergence estimates, with known occurrences representing minimum divergence times for sister taxa. As such, accurately placing fossils in phylogenies is integral to understanding the patterns and processes that shape the tree of life. The chelicerate order Xiphosura comprises classic archetypes of morphological stasis, with the earliest known Ordovician representatives exhibiting all key morphological characteristics of the group. Molecular studies on the four extant species consistently retrieve a basal split between Limulinae and Tachypleinae, but conflict regarding the relationships of the three Asian species. Molecular divergence estimates using either no or a single fossil calibration point infer a Cretaceous or Palaeogene origin for Limulidae and a Palaeogene or Neogene origin for Tachypleinae and *Tachypleus*. Here, we present male and female specimens of *Tachypleus syriacus* (=‘*Mesolimulus*’ *syriacus*) from the Cretaceous of Lebanon, revealing an anterior scalloped carapace margin in males—a derived condition of sexual dimorphism shared with *Tachypleus tridentatus*. Morphological phylogenetic analysis of total group Limulidae retrieves a monophyletic *Tachypleus* with a minimum divergence time during the Cretaceous, while

crown-group Tachypleinae and Limulidae are both present during the Triassic, showing that molecular clock analyses have significantly underestimated the divergence times for these taxa.

Keywords Calibration · Cretaceous · Limulidae · Molecular clock · *Tachypleus* · Xiphosura

Introduction

Limulidae is a clade of Triassic to Recent horseshoe crabs exhibiting no vestiges of segmentation dorsally in the thoracetron (Riek and Gill 1971) and include all four modern species. Xiphosura (comprising Xiphosurida and its stem lineage sensu Lamsdell (2013)) in general, and limulids in particular, are considered to have a relatively poor fossil record, largely due to their unmineralized exoskeleton and predilection for marginal environments that are not well represented in the stratigraphic record (Babcock et al. 2000). A number of fossil taxa have, however, been assigned to modern genera, *Limulus* in particular (Zinken 1862; Watson 1909; Reeside and Harris 1952); of the thirteen currently valid fossil limulids, five are allied with *Limulus* or *Tachypleus*, although the majority of these allocations rely only on gross similarity. Largely as a consequence of the apparently poor fossil record, there are no published phylogenetic analyses of fossil representatives of Limulidae, with the exception of Fisher’s (1984) tree, which was presented without any accompanying dataset, and so none of these designations have been rigorously tested. Thus, we have no concept of which, if any, of the fossil limulids resolve within the crown group. The uncertainty surrounding the relationships of fossil taxa has had major implications for molecular divergence time estimates of the modern species, which have had difficulties in assigning appropriate fossil

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calibration points, resulting in fossil calibrations either being excluded completely (Avisé et al. 1994) or reduced to a single calibration for the origination of Limulidae in the form of the late Jurassic *Mesolimulus walchi* (Obst et al. 2012). While potentially resolving as part of the sister group to the remaining limulids (Størmer 1952), *M. walchi* is not the oldest known species of *Mesolimulus*, which has a fossil record extending into the Triassic (Vía 1987). The use of multiple fossil calibrations has been shown to drastically improve the accuracy of divergence estimates as they provide hard minimum divergence times for groups (Donoghue and Benton 2007); however, for these to be utilized accurately the affinities of the fossil taxa must be hypothesized with some confidence (Benton et al. 2009). Proving the monophyly of higher taxa is of prime importance here, as is accurate stratigraphic dating. Morphological phylogenetic analyses are the best tests of relationship for fossil taxa, and provide a solid groundwork for future total evidence studies. Core to these morphological analyses are accurate homology statements and recognition of shared morphology through comparative work incorporating both modern and fossil taxa.

Material

'*Mesolimulus*' *syriacus* was described by Woodward (1879) as a species of *Limulus* based on a single specimen from the Upper Cretaceous (Cenomanian; 100 Ma) Hâqel *Konservat-Lagerstätte* of Lebanon and was transferred to the genus *Mesolimulus* by Størmer (1952). Subsequent intense commercial collecting has yielded a wealth of specimens from the three contemporaneous Lebanese *Konservat-Lagerstätten* of Hâqel, Hjoûla, and en Nammoûra (Dalla Vecchia et al. 2002), including abundant fish and crustaceans, aquatic reptiles (such as turtles, dolichosaurs, and varanoids), and pterosaurs (Dalla Vecchia et al. 2001). Among these new discoveries are a number of new specimens of '*Mesolimulus*' *syriacus* from Hâqel

and Hjoûla that provide a more complete picture of the species' morphology and indicate an assignment to the modern genus *Tachypleus* (see Figs. 1, 2 and 3). While the details of the prosomal appendages and opisthosomal opercula are unknown, a number of dorsal features signal clear affinity to the extant *Tachypleus* species, including the manner in which the ophthalmic ridge angles inwards anterior to the lateral eyes, while the presence of ancillary posterior spines located dorsolaterally to the telson insertion is a trait shared with *Tachypleus tridentatus*. Therefore, '*Mesolimulus*' *syriacus* should be transferred to the genus *Tachypleus* as *Tachypleus syriacus* comb. nov. All material is accessioned at the Natural History Museum, London (BMNH NHM) or the Museo Civico di Storia Naturale di Milano (MSNM).

Systematic palaeontology

Xiphosura Latreille 1802 (= Merostomata Dana 1852)

Xiphosurida Latreille 1802

Limulina Richter and Richter 1929

Limuloidea Zittel 1885

Limulidae Zittel 1885

Tachypleinae Pocock 1902

Tachypleus Leach 1819

Type species. *Limulus gigas* Müller 1785.

Other species. *Tachypleus decheni* (Zinken 1862), *T. gadeai* (Vía Boada and De Villalta 1966), *T. syriacus* (Woodward 1879), *T. tridentatus* Leach 1819.

Emended diagnosis. Moveable lateral spines of opisthosoma different in sexes, long and equal in male and young female, posterior 3 short and apically acute in adult female. Emended from Størmer (1955).

T. syriacus (Woodward 1879) (Figs. 1, 2, 4a, 5 and 6)

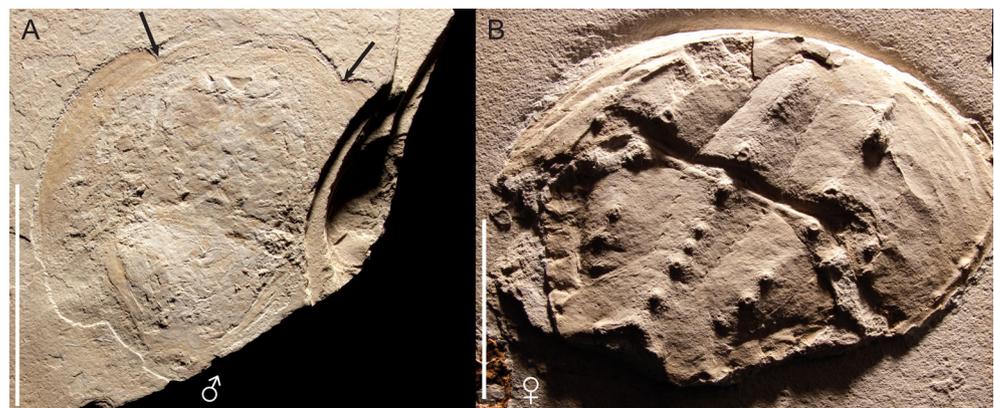
1879 *Limulus syriacus* n. sp.; Woodward, p. 554, pl. 26 fig. 6.

1952 *Mesolimulus* (?) *syriacus* (Woodward); Størmer, p. 637, fig. 1 m

1955 *M. syriacus* (Woodward); Størmer, p. 22, fig. 16.5

1987 *M. syriacus* (Woodward); Vía, p. 285

Fig. 1 *T. syriacus* (Woodward). **a** BMNH NHM IA 187, male. Cretaceous, Lebanon. **b** BMNH NHM IA 188, female. Cretaceous, Lebanon. Arrows indicate the lateral extent of the anterior scalloped margin in the male. Scale bars represent 50 mm



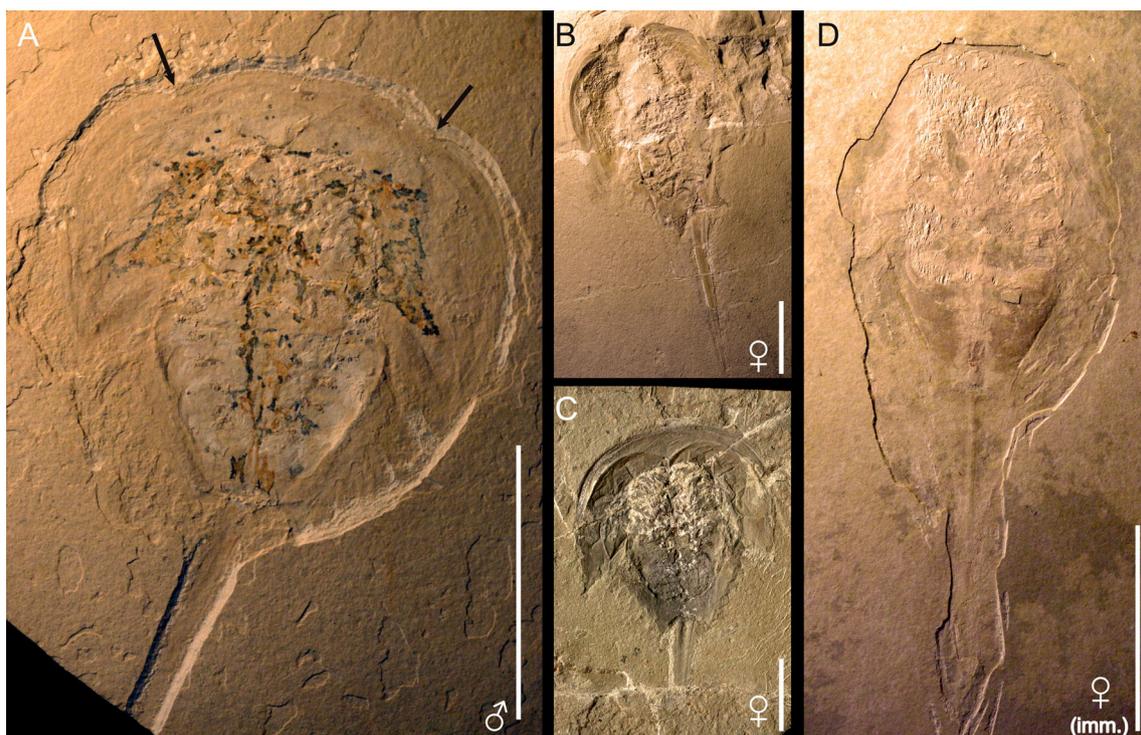


Fig. 2 *T. syriacus* (Woodward). **a** MSNM i9352, male. Cretaceous, Lebanon. **b** MSNM i25083, female. Cretaceous, Lebanon. **c** MSNM i27468, female. Cretaceous, Lebanon. **d** MSNM i9351, immature

female. Cretaceous, Lebanon. *Arrows* indicate the lateral extent of the anterior scalloped margin in the male. *Scale bars* represent 50 mm

2011 *M. syriacus* (Woodward); Feldmann et al., p. 1340
Holotype. BMNH NHM 59783 (immature female) (Fig. 4a).
Referred material. BMNH NHM IA 187 (adult male) (Figs. 1a, 5 and 6), BMNH NHM IA 188 (adult female) (Fig. 1b), MSNM i9352 (adult male) (Fig. 2a), MSNM i2083 (adult female) (Fig. 2b), MSNM i27468 (adult female) (Fig. 2c), MSNM i9351 (immature female) (Fig. 2d).
Locality, horizon and age. Hâqel and Hjoûla *Konservat-Lagerstätten*, Lebanon, late Cretaceous (Cenomanian).
Diagnosis. *Tachypleus* with a series of axial and pleural nodes on dorsal surface of thoracetrone; large axial spine on anterior thoracetrone margin; pleura of free lobe extending beyond thoracetrone margin; ancillary posterior spines located dorsolaterally to the telson insertion on the thoracetrone; males with raised anterior prosomal arch resulting in scalloped morphology of carapace anterior margin.
Description. BMNH NHM 59783. Complete specimen preserving carapace, thoracetrone and telson as dorsal compression, dorsal surface of thoracetrone and carapace exfoliated to reveal internal structures (Fig. 4a). Anterior-most portion of carapace and left portion of carapace and thoracetrone missing where limestone slab has broken away. Maximum preserved length of specimen 197 mm, maximum preserved width 82 mm. Carapace preserved length 43 mm, preserved width 82 mm (estimated total width 128 mm). Raised crescentic structure, 11 mm long by 4 mm wide, may represent lateral eye located 34 mm from carapace lateral margin and 28 mm

from carapace posterior. Genal spine preserved on right, 22 mm wide at base, extending for 23 mm beyond carapace posterior. Thoracetrone 52 mm long, preserved width 60 mm (estimated total width 70 mm). Opisthosomal doublure 11 mm wide at posterior, anterior width unclear. An ovate gut trace, 7 mm wide and 17 mm long, is preserved centrally in the anterior half of the thoracetrone. Bands of striated muscle tissue are preserved to the right, and occur along the length of the thoracetrone. Right margin of thoracetrone preserves at least five fixed epimera (the anterior-most are at least partially obscured by the carapace posterior) and six moveable spines, the anterior two of which are only faintly preserved; the third and fourth

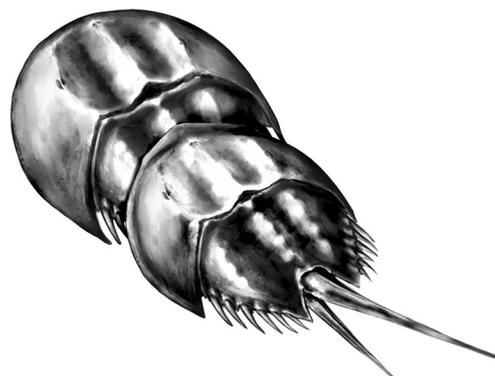


Fig. 3 Idealized reconstruction of male and female *T. syriacus* in amplexus. Drawing by Antony Lamsdell

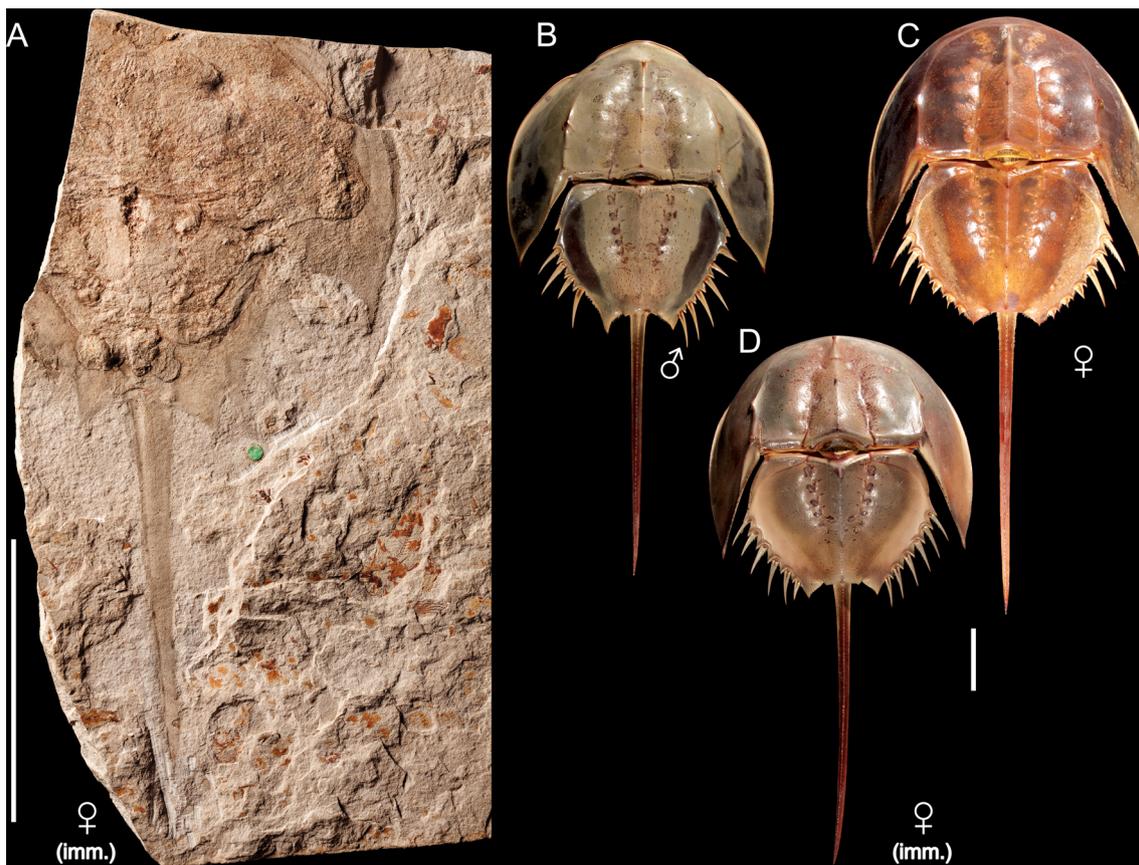


Fig. 4 *T. syriacus* (Woodward) and *T. tridentatus* Leach. **a** *T. syriacus* – BMNH NHM 59783, immature female (holotype). Cretaceous, Lebanon. **b** *T. tridentatus*—YPM IZ 055581, adult

male. Recent, Japan. **c** *T. tridentatus*—YPM IZ 002430 CR, immature female. Recent, Japan. **d** *T. tridentatus*—YPM IZ 055576, adult female. Recent, Japan. Scale bars represent 50 mm

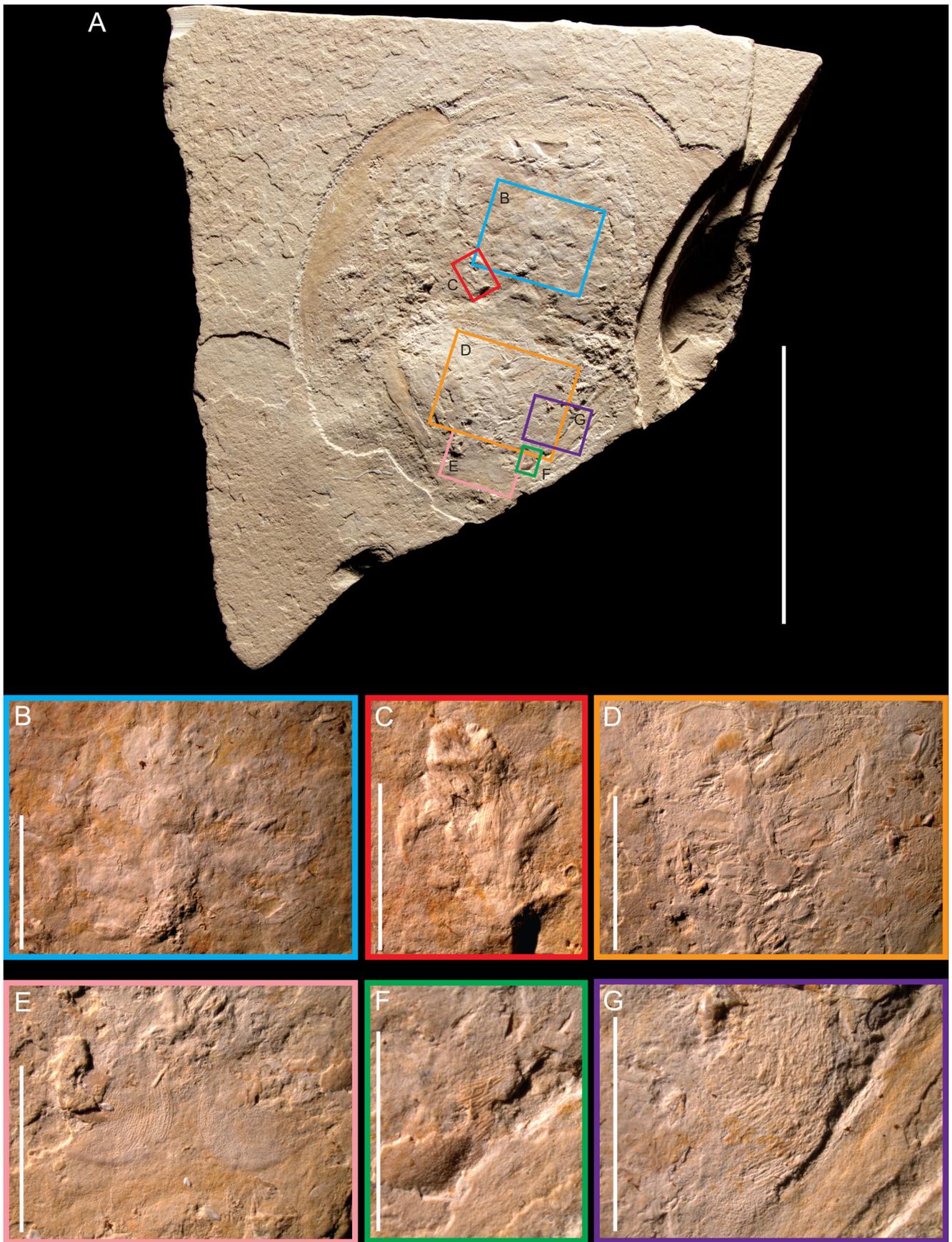
moveable spines are 11 mm long, while the fifth is 12 mm and the sixth is 14 mm. Telson insertion 15 mm wide, terminal pleura each 9 mm long, 12 mm wide at base. Telson 10 mm wide at base, 102 mm long. Evidence of central keel on telson.

BMNH NHM IA 187. Carapace and thoracetron seen in dorsal view, dorsal surface of thoracetron and central region of carapace exfoliated to reveal ventral structures (Figs. 1a, 5, and 6). Specimen located on edge of limestone slab, missing telson and rightmost regions of carapace and thoracetron margin. Maximum preserved length of specimen 94 mm, maximum preserved width 87 mm. Carapace 52 mm long, preserved width 75 mm (estimated total width 94 mm). Anterior carapace margin scalloped, arch 50 mm wide, extending 10 mm anteriorly from base of notches. Marginal rim 2 mm wide. Ventral prosomal structures faintly preserved as chalky infill, including limb insertions, mouth, and anterior triangular extension of doublure. Genal spine preserved on left, 18 mm wide at base, extending posteriorly 21 mm from carapace posterior. Thoracetron 39 mm long, 52 mm wide at prosomal joint. Telson insertion 10 mm wide, terminal pleura each 4 mm long, 7 mm wide at base. Opisthosomal doublure 7 mm wide anteriorly, narrowing to 5 mm wide at posterior. Lateral margins of thoracetron poorly preserved, fixed and

moveable spines obscured. Ventral opisthosomal structures preserved in the same manner as ventral prosomal structures. Six pairs of opercula insertion points located medially on thoracetron, surrounded by strands of biological material that may be muscle fibers. Posterior-most pair of book gills preserved as small, foliated structures abutting the opisthosomal doublure.

BMNH NHM IA 188. Carapace and thoracetron preserved in three-dimensional relief in oblique dorsal view (Fig. 1b). Telson is missing from specimen and lateral regions of free lobes have broken off, presumably retained on counterpart. Maximum preserved length of specimen 109 mm, maximum preserved width 132 mm. Carapace 49 mm long, width 132 mm, although the oblique angle and slight flattening has

Fig. 5 *T. syriacus* (Woodward)—BMNH NHM IA 187, details of soft tissue preservation. **a** Whole specimen. Colored boxes indicate the boundaries of panels **b–g** which are marked with corresponding colored borders. **b** Mouth with associated soft-tissue preservation around the prosomal appendage insertions. **c** Muscle tissue. **d** Central region of the opisthosoma showing muscle fibers and heavily sclerotized ventral surface of the thoracetron. **e** Posterior-most pair of book gills. **f** Foliated fragments of book gills. **g** Fibrous tissue most likely representing opercula muscles. Scale bars **a** 50 mm; **b, d, e, g** 10 mm; **c, f** 5 mm



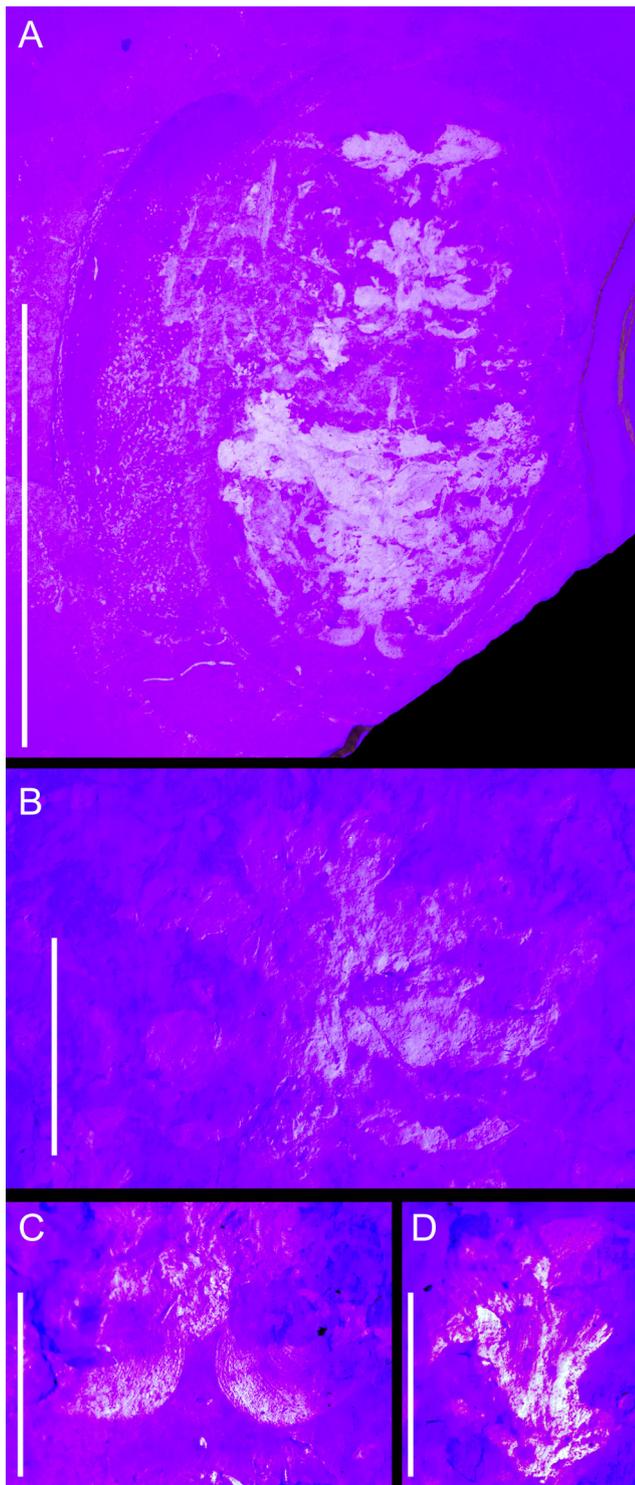


Fig. 6 *T. syriacus* (Woodward)—BMNH NHM IA 187, soft tissue fluorescence under UV light. **a** Whole specimen. **b** Mouth with associated soft-tissue preservation around the prosomal appendage insertions shown in Fig. 5b. **c** Posterior-most pair of book gills shown in Fig. 5d. **d** Muscle tissue shown in Fig. 5c. Scale bars: **a** 50 mm; **b**, **c** 10 mm; **d** 5 mm

resulted in some warping of the specimen. Anterior margin of carapace rounded. Marginal rim 3 mm wide. Cardiac lobe

40 mm long, 26 mm wide at posterior, narrowing to 15 mm at anterior. Dorsal keel present on cardiac lobe. Lateral eye preserved on right side, 8 mm long by 5 mm wide. Eye located 29 mm from carapace posterior, 26 mm from carapace center. Ophthalmic ridge associated with lateral eye, effaced anterior to cardiac lobe. Series of nodes or short spines present on carapace, distal portions of spines consistently broken off, present axially at posterior of cardiac lobe, on apex of ophthalmic ridge associated with lateral eye, and at posterior limits of ophthalmic ridges. Genal spines preserved, uncompressed example on right, 39 mm wide at base, extending posteriorly 36 mm from carapace posterior. Thoracetrion 60 mm long, 67 mm wide at prosomal joint, greatest width of 86 mm located just prior to genal spine termination. Telson insertion 18 mm wide, terminal pleura each with a preserved length of 9 mm, distal portions missing, both 19 mm wide at base. Free lobes at thoracetrion anterior missing lateral portions but appear to extend across thoracetrion width as in modern species, missing portion due to upward inflection of lobe. Axial region of thoracetrion with evidence of keel, axial spines or nodes also present, first (and largest) located at thoracetrion anterior, four smaller axial spines located further along thoracetrion. Series of spines in line with ophthalmic ridge also present on thoracetrion, most anterior spine located on free lobe, three further spines present further back. Six pairs of apodemal pits present flanking the axis. Ancillary posterior spines located dorsolaterally to the telson insertion. Lateral margins of thoracetrion poorly preserved but show some evidence of fixed pleural spines on right side. Sockets for at least four pairs of moveable spines are also evident.

MSNM i9352. Almost complete specimen in ventral view, missing distal portion of telson (Fig. 2a). Maximum preserved length of specimen 126 mm, maximum preserved width 83 mm. Carapace 46 mm long, 126 mm wide. Anterior carapace margin scalloped, arch 49 mm wide, extending 8 mm from base of notches. Genal spines 20 mm wide at base, extending posteriorly 17 mm from carapace posterior. Thoracetrion 35 mm long, 42 mm in width at prosomal joint, maximum width 52 mm located just prior to genal spine termination. Opisthosomal doublure 6 mm wide anteriorly, narrowing to 4 mm wide at posterior. Six pairs of moveable opisthosomal spines preserved, each 7 mm long by 2 mm wide. Telson with a preserved length of 46 mm, 7 mm wide at base.

MSNM i2083. Complete specimen preserved in three-dimensional relief in dorsal view (Fig. 2b). Dorsal surface is badly preserved and affords no detail of prosomal or opisthosomal structures. Specimen total length 266 mm, maximum width 113 mm. Carapace 81 mm long, 113 mm wide. Anterior margin of carapace rounded. Genal spines 32 mm wide at base, extending posteriorly 27 mm from carapace posterior. Thoracetrion 63 mm long, maximum width 72 mm. Lateral thoracetrion margin not preserving moveable spines. Telson 122 mm long, 14 mm wide at base.

MSNM i27468. Almost complete specimen in ventral view preserved in three-dimensional relief, missing posterior half of telson (Fig. 2c). Specimen total preserved length 198 mm, maximum width 140 mm. Carapace 90 mm long, 140 mm wide. Anterior margin of carapace rounded. Prosomal doublure with anterior triangular extension. Genal spines 32 mm wide at base, extending posteriorly 27 mm from carapace posterior. Thoracetron 63 mm long, 81 mm wide at prosomal joint, maximum width 99 mm located just prior to genal spine termination. Opisthosomal doublure 23 mm wide anteriorly, narrowing to 18 mm wide at posterior. Insertion points for six pairs of moveable spines present at thoracetron margin but spines themselves not preserved. Six sets of opercula present within thoracetron cavity. Telson insertion 23 mm wide, terminal pleura each with a length of 18, 27 mm wide at base. Telson with preserved length of 45 mm, 14 mm wide at base.

MSNM i9351. Poorly preserved specimen in ventral view with evidence of prosomal appendages (Fig. 2d). Specimen total length 158 mm, maximum width 62 mm. Carapace 54 mm long, 62 mm wide. Anterior carapace margin rounded. Prosomal doublure with anterior triangular extension. Outlines of five pairs of prosomal appendages visible, individual podomeres indistinct and appendage terminations not preserved but otherwise appear as in other xiphosurids. Thoracetron 32 mm long, maximum width 38 mm. Telson 72 mm long, 3 mm wide at base.

Remarks. The holotype (BMNH NHM 59783) exhibits no reduction in the three posterior pairs of moveable opisthosomal spines (of which it has six pairs, rather than five as originally reported (Woodward 1879)), and would at first appear to be a male. This assumption is potentially supported by the size of the specimen, which is comparable to (although slightly larger than) the male specimens (BMNH NHM IA 187, MSNM i9352). However, the holotype was shown to have a rounded anterior carapace margin in its initial description (Woodward 1879), suggesting that the specimen must represent a female. Corroborating this observation is now unfortunately impossible, as when initially described the holotype was missing part of the left side of the carapace and thoracetron and in the time since its initial description the specimen appears to have been further damaged, now also missing the carapace anterior. Although we are unable to verify the lack of scalloping, the original description is otherwise accurate; there are no discrepancies among the given measurements, and the available morphology of the carapace, thoracetron and telson is as reported. Therefore, we tentatively accept the original interpretation of the holotype carapace having a rounded anterior margin. Further evidence for the holotype representing a female stems from the proportions of the thoracetron, which is broader and of a greater size compared to the carapace than in males. It seems likely that the holotype specimen does in fact represent a female, but a juvenile individual rather than a full adult. This conclusion is supported by

the occurrence of a second immature specimen (MSNM i27468) of similar size which clearly preserves a rounded anterior carapace margin. Juvenile female *T. tridentatus* retain undifferentiated moveable opisthosomal spines to molt stages which are equal in size to adult males (Fig. 4b–d); this explains the female morphology of the carapace and thoracetron as well as the lack of opisthosomal spine differentiation given the size of the specimen.

A number of specimens, including the holotype and the male described herein, exhibit preservation of internal soft-tissue structures such as muscle and book gill lamellae (Fig. 4). While book gill lamellae are shed along with the external cuticle during molting, the presence of muscle tissue indicates that these specimens represent mortalities. The preservation, including muscular tissue, is similar to that reported in *Mesolimulus walchi* from the Upper Jurassic (150 Ma) Solnhofen limestone (Briggs and Wilby 1996; Wilby and Briggs 1997) and Nusplingen Plattenkalk (Briggs et al. 2005) of southern Germany; while the fidelity of soft-tissue preservation in these specimens is not to the same extent as the Nusplingen material, it is well worth noting the potential for soft-tissue preservation in arthropods at Hâqel and Hjoûla. This adds to the range of taxa with exceptional preservation known from these *Konservat-Lagerstätten*, soft tissue previously being reported in fish (Capasso et al. 2009) and cephalopods (Jattiot et al. 2015).

Sexual dimorphism

The Lebanese specimens exhibit clear signs of sexual dimorphism, a condition readily recognized among modern taxa but notoriously hard to convincingly diagnose in fossil arthropods (Hughes and Fortey 1995). All four species of modern horseshoe crab exhibit size dimorphism, with males consistently having smaller prosomal dimensions than females (Loveland and Botton 1992) (Fig. 7) and similar size distributions have been shown in '*Limulus*' *decheni*, from the Palaeogene (Eocene; 56 Ma) of Germany (Hauschke and Wilde 2004). Males also have a curved anterior prosomal arch that allows easier mounting of the female during mating; in *T. tridentatus* this structure is extremely pronounced and visible dorsally in the form of an anterior scalloping of the prosomal margin (Botton et al. 1996) (Fig. 7a). Female *Tachypleus* show a dimorphic reduction of the three posterior pairs of moveable opisthosomal spines (Fig. 7a and b), again apparently increasing the ease of mounting for males. Two clear size classes are evident among the *T. syriacus* specimens, with the smaller individuals interpreted as representing males and immature specimens, while the larger members of the population comprise mature females. Corroboration for this comes from a number of smaller specimens that show clear scalloping of the anterior prosomal margin (Figs. 1a and 2a) identical to that of *T. tridentatus* males (Fig. 7a); this scalloping is absent in all

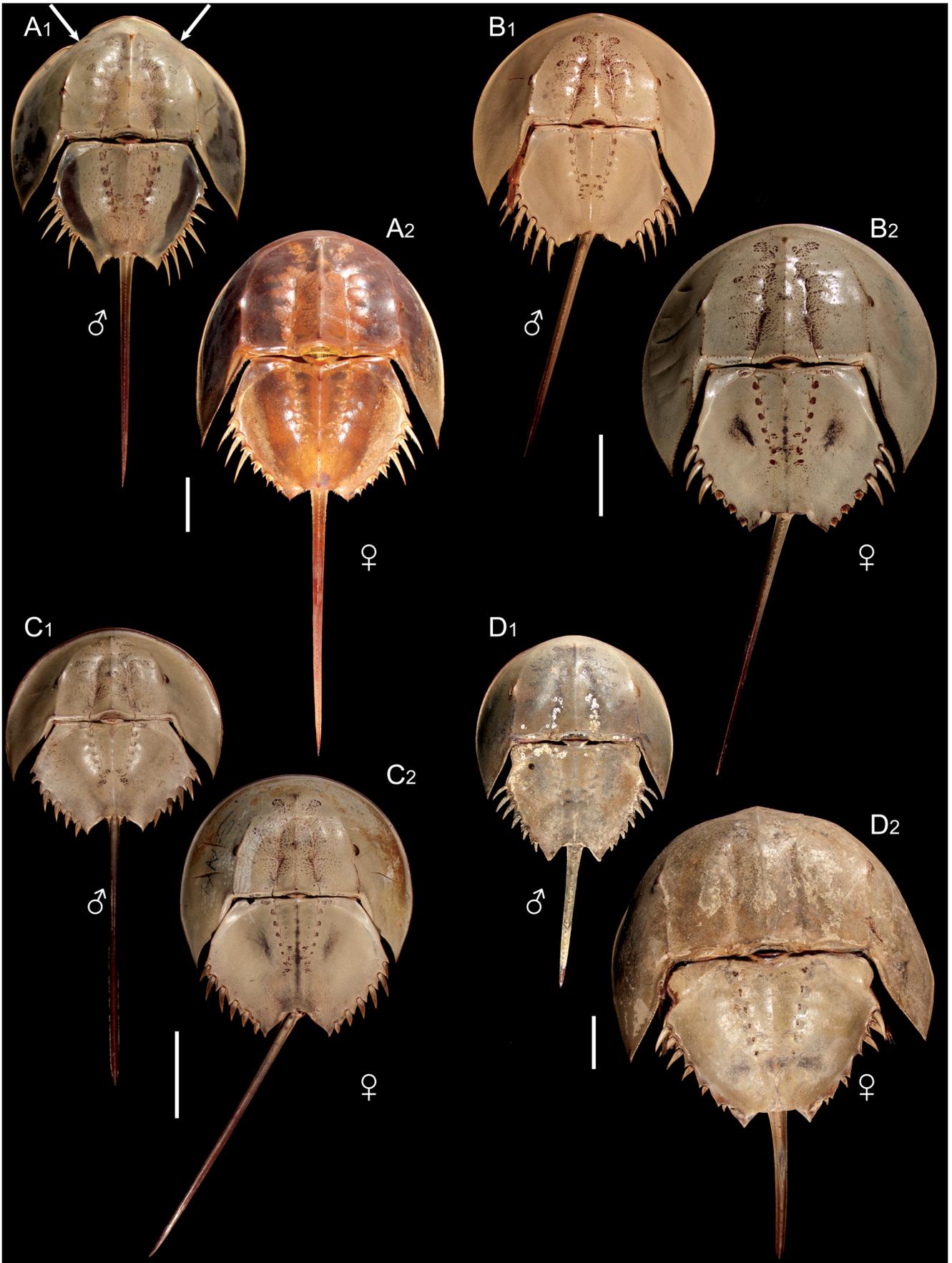


Fig. 7 Extant Limulidae. **a** *Tachypleus tridentatus*—YPM IZ 055581, male (*a*₁), and YPM IZ 055576, female (*a*₂). Recent, Japan. The male exhibits a pathology concerning the moveable spines on the thoracetron; the foremost spine on the *right* is partially fused to the fixed pleural spine, while the two posterior moveable spines on the *left* have not been expressed at all. **b** *Tachypleus gigas*—YPM IZ 055578, male (*b*₁), and YPM IZ 055570, female (*b*₂). Recent, Singapore. **c** *Carcinoscorpius rotundicauda*—YPM IZ 055595, male (*c*₁), and YPM IZ 055574, female (*c*₂). Recent, Singapore. **d** *Limulus polyphemus*—YPM IZ 055605, male (*d*₁), and YPM IZ 070174, female (*d*₂). Recent, United States. The female has sustained damage to the telson, which has been broken off some way before its natural termination. Arrows indicate the lateral extent of the anterior scalloped margin in the male *Tachypleus tridentatus*. Scale bars represent 50 mm

larger specimens (Figs. 1b and 2b–c), including the holotype (Fig. 4a). This scalloping is clearly a derived condition, as it is absent from all other extant species (Fig. 7b–d) and the only other extinct species where both sexes are clearly known (Hauschke and Wilde 2004), and suggests that *T. syriacus* and *T. tridentatus* are sister taxa. One potential conflict to this assignment, however, is the presence of elongate posterior opisthosomal moveable spines on the holotype of *T. syriacus*, which is marginally larger than the male specimens, was described as lacking a scalloped margin (Woodward 1879), and has general body proportions indicating that it is a female. The lack of reduction of the three posterior pairs of moveable spines would appear to preclude the species from *Tachypleus*, however immature females of *T. tridentatus* retain full-length opisthosomal spines at body sizes in excess of those attained by mature males (Fig. 4b–d). The *T. syriacus* holotype is markedly smaller than the largest females, and so it appears that the type specimen is in fact an immature female, accounting for the undifferentiated opisthosomal spines. This in some way explains the species' previous assignment to *Mesolimulus*, as immature individuals of the modern genera exhibit transitory plesiomorphic morphological conditions that resemble those expressed in the extinct genus. Failure to recognize juvenile or immature individuals as such has been shown to negatively impact the chances of accurately pinpointing their phylogenetic position (Lamsdell and Selden 2013; Campione et al. 2013), and frequently results in stemward slippage of the taxon in question. This can also translate in purely taxonomic schemes as assignments to more basal genera, as appears to have been the case here. Post-embryonic ontogeny has been shown to have a marked impact on the morphology of Palaeozoic xiphosurans (Haug et al. 2012), and the case of the *T. syriacus* holotype shows that these issues extend to fossil limulids also.

Phylogenetic analysis

Incorporating *T. syriacus* into a morphological phylogenetic analysis of fossil and recent limulids confirms its position as the sister taxon to *T. tridentatus* (Fig. 8), united by the

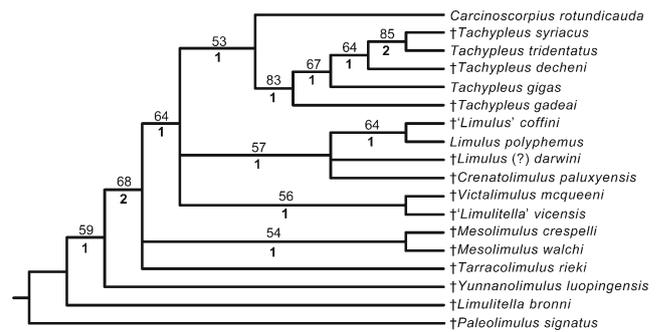


Fig. 8 Single most parsimonious tree (Tree length 22, Consistency Index 0.909, Retention Index 0.958, Rescaled Consistency Index 0.871) from the dataset in Table S1, also available in the public database Morphobank (Project 1228), analyzed through implicit enumeration with all characters unordered and of equal weight in TNT (Goloboff et al. 2008). *Paleolimulus signatus* was utilized as the outgroup. Branch support is shown for each node; regular numbers above the node are jackknife values retrieved from 1000 replicates with 33 % deletion, numbers in bold beneath the node are Bremer support values

dimorphic scalloping of the male prosoma and the presence of ancillary posterior spines located dorsolaterally to the telson insertion. *Tachypleus decheni* also resolves within *Tachypleus*, sharing with *T. syriacus* and *T. tridentatus* the ancillary spines but lacking the dimorphic carapace margin (Hauschke and Wilde 2004). *Tachypleus gigas* and *T. tridentatus* are shown to be congeneric, contra some molecular studies (Shishikura et al. 1982; Xia 2000; Kamaruzzaman et al. 2011) but in agreement with the recent analysis of Obst et al. (2012), with *Carcinoscorpius* resolving as the sister taxon to a monophyletic *Tachypleus*. This has major ramifications for the divergence estimates of the extant xiphosurids; *T. tridentatus* and *Tachypleus gigas* are estimated to have diverged either during the Neogene (Avisé et al. 1994) or during the late Palaeogene/early Neogene (Obst et al. 2012), however the position of *T. syriacus* shows that this split occurred by at least the late Cretaceous (Fig. 9). This also falsifies the proposed Palaeogene (Obst et al. 2012) or Palaeogene/Neogene (Avisé et al. 1994) age for the *Tachypleus/Carcinoscorpius* split, however, the placement of the Triassic aged *T. gadeai* (Via Boada and De Villalta 1966) at the base of the *Tachypleus* clade pushes back the minimum divergence time even further. By extension, the divergence of Tachypleinae and Limulinae must also have occurred by the Triassic, rather than during the Cretaceous (Obst et al. 2012) or Palaeogene (Avisé et al. 1994) as previously estimated.

Crown-group Xiphosurida is revealed to include a large number of Mesozoic and Cenozoic limulids, with the Cretaceous *Crenatolimulus* (Feldmann et al. 2011) resolving within Limulinae alongside *Limulus*, which itself has a fossil record potentially extending to the late Jurassic (Kin and Błazejowski 2014). These, combined with the numerous extinct species now assigned to *Tachypleus*, expand the total number of confirmed crown-group species from four to ten. It is currently unclear whether the clade comprising *Victalimulus mcqueeni*

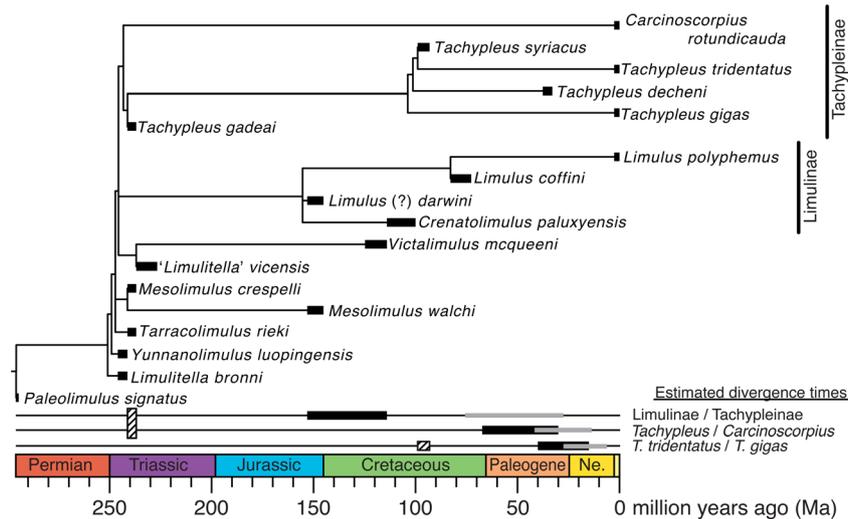


Fig. 9 Chronologically correlated phylogeny of fossil and extant Limulidae. Species ranges are plotted against the geological timescale, with time shown in millions of years (Ma). Estimated divergence times for Limulinae/Tachypleinae, *Tachypleus/Carcinoscorpius* and *Tachypleus tridentatus/Tachypleus gigas* are shown immediately above the geological timescale, with divergence estimates from Avise et al.

(1994) shown in gray and Obst et al. (2012) in black. The hatched boxes show the minimum possible divergence times based off fossil data, with the occurrence of *T. syriacus* inferring a Cretaceous divergence for *Tachypleus tridentatus/Tachypleus gigas* while *T. gadeai* suggests a Triassic divergence for both *Tachypleus/Carcinoscorpius* and Limulinae/Tachypleinae

Riek and Gill 1971 and '*Limulitella*' *vicensis* (Bleicher 1897) also forms part of the crown group; at present, it resolves in a polytomy with Tachypleinae and Limulinae, and so it is equivocal whether its affinities lie closer to either subfamily or if it represents the sister clade to the crown group. Irrespective of this, the results presented here show that even the closest estimates have drastically underestimated modern limulid divergence times, with the divergence of *T. tridentatus* and *Tachypleus gigas* offset by some 50 million years, that of Tachypleinae and Limulinae by 175 million years, and pushes back the origin of crown Limulidae by 90 million years (Obst et al. 2012) (Fig. 9).

Discussion

Modern xiphosurans have been intensively studied for around 150 years, spurred on by their archaic appearance (Renwick 1968) and importance as both a biomedical and economic resource (Botton and Ropes 1987). While early questions regarding their position within the arthropod tree (Lankester 1881) and the number of extant species (Waterman 1958) are long since resolved, the application of molecular phylogenetic methods to the group has raised new uncertainty about the interrelationships of the Asian species (Shishikura et al. 1982; Avise et al. 1994; Xia 2000; Kamaruzzaman et al. 2011; Obst et al. 2012). The most recent molecular analysis, comprising a broad study of multiple loci across multiple populations, retrieved a topology congruent with the traditional taxonomy of a monophyletic *Tachypleus* with *Carcinoscorpius* resolving as its sister group (Obst et al. 2012). This is

corroborated by the morphological data as exemplified by the analysis presented here, and demonstrates the importance of using multiple loci in molecular analyses; molecular analyses of felids utilizing ancient DNA have also been shown to converge with morphologically derived topologies as multiple loci are incorporated into the studies (Giribet et al. 2001; Barnett et al. 2005).

The placing of fossil taxa on the limulid tree provides an explanation for the conflict among molecular studies, with long branch lengths inferred for each of the modern species based on the revised fossil-based divergence time estimates. Scenarios such as this are known to be susceptible to long-branch attraction (Bergsten 2005), with the phenomenon documented in a number of real datasets (Anderson and Swofford 2004). While most long-branch attraction is considered to be due to the occurrence of fast-evolving species this is clearly not the case among limulids, which already exhibited relatively slow rates of molecular change based on the underestimated divergence times (Obst et al. 2012). It is possible that the long-branch issues are caused due to the deep divergence times and subsequent loss of the majority of species within the genera: this results in long branches through poor taxon sampling. Another possibility is that the different xiphosurid lineages exhibit varying evolutionary rates, resulting in long-branch attraction through heterotachy (Philippe et al. 2005). Evidence for this comes from the major overlap of molecular divergence time estimates for *T. tridentatus/T. gigas* and *Tachypleus/Carcinoscorpius*, events which the fossil record shows were separated by at least 145 million years, suggesting some degree of rate asymmetry occurs within the Tachypleinae. These issues can be somewhat mitigated

through sampling multiple loci (Yang and Yoder 2003), thereby sampling a number of different molecular rates, and including fossil taxa in order to split up the long branches in question (Wiens 2005), and may explain why our morphological analysis and the multi-loci analysis of Obst et al. (2012) are in agreement in regard to tree topology.

The combination of fossil and molecular data has the potential to form a powerful toolkit with which to tackle longstanding macroevolutionary issues (Strotz and Allen 2013; Garwood et al. 2014; Longrich et al. 2015). Fossils represent a unique information resource that can be invaluable for informing on patterns of biogeography, recognizing patterns of character acquisition, and inferring the timing of divergences. Ascertaining the phylogenetic placement of fossils is an integral step in any of these processes and requires detailed comparative studies of morphology. Such studies also constitute the first step towards broader, total evidence analyses. The recognition of a derived condition of sexual dimorphism in *T. syriacus* demonstrates that the two modern *Tachypleus* species must have diverged prior to the late Cretaceous, while the first comprehensive phylogenetic analysis of living and fossil Limulidae shows that crown-group xiphosurids originate early in the Mesozoic, some 90 million years prior to molecular clock estimates. The new analysis also supports previous claims of morphological and ecological stasis within Limulidae, revealing a widespread distribution for both Limulinae and Tachypleinae throughout the Mesozoic with an apparent high degree of niche conservatism, as the earliest crown-group limulids occur in environments identical to those of their modern counterparts (Diedrich 2011). How these Mesozoic and Cenozoic species have responded to changing climate, as well as the patterns of survival and extinction exhibited by stem-group limuloid clades, are valuable sources of information for modern conservation efforts, especially given current fears over declining horseshoe crab populations in both Asia (Shin et al. 2009; Mishra 2009) and America (Shuster and Botton 1985; Faurby et al. 2010) and the subsequent impact on migrating shorebirds (Baker et al. 2004; Karpanty et al. 2006; Niles et al. 2009) and loggerhead turtles (Seney and Musick 2007; Witherington et al. 2009) that rely on the American populations as a source of food. The fossil record shows how these creatures have responded to habitat loss in the past and can be used to predict how modern species will respond to current and future environmental changes and guide conservation efforts.

Data accessibility

Detailed descriptions of specimens along with the phylogenetic data matrix and character list can be found in the electronic supplementary material. The phylogenetic dataset is also available from the public database Morphobank (Project 1228): <http://morphobank.org/permalink/?P1228>.

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Author contributions J.C.L. conducted the phylogenetic analysis and photographed and described the specimens. S.C.M. provided the new specimens and locality information. Both authors contributed intellectually to the study and to the completion of the manuscript.

Conflict of interest The authors declare that they have no conflict of interest.

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