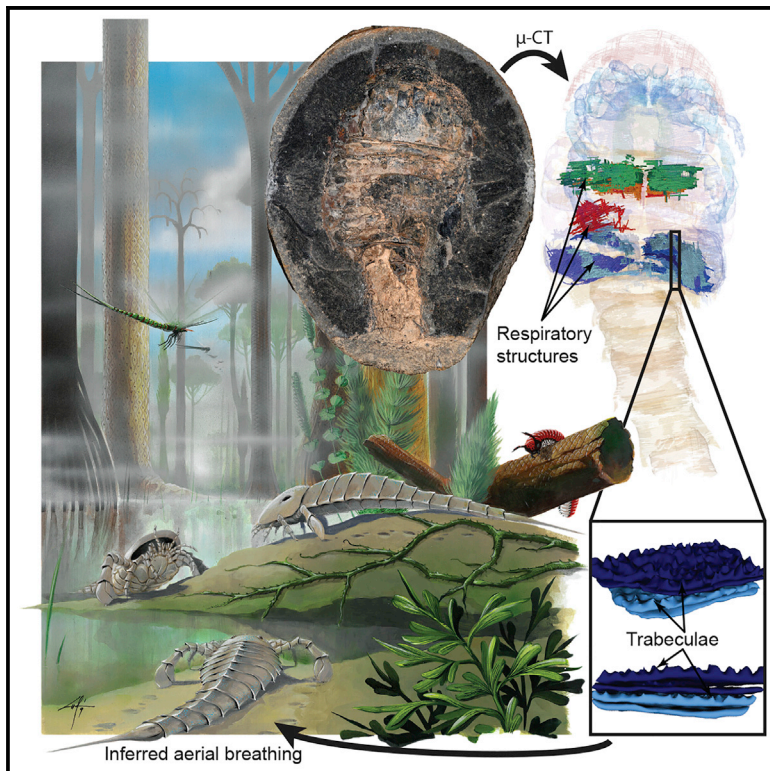


Current Biology

Air Breathing in an Exceptionally Preserved 340-Million-Year-Old Sea Scorpion

Graphical Abstract



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In Brief

Lamsdell et al. use computed tomography to study the respiratory organs of a three-dimensional Carboniferous eurypterid. Trabeculae on the respiratory lamellae indicate that the animal was capable of breathing air. Evidence from spermatophores in Silurian species and amphibious trackways demonstrates air breathing was common to all eurypterids.

Highlights

- A three-dimensionally preserved eurypterid, *Adelophthalmus pyrrhae*, is described
- Computed tomography reveals the presence of trabeculae on the respiratory lamellae
- Occurrence of trabeculae indicates eurypterids were capable of breathing air
- Data from eurypterids suggest horseshoe crabs were not secondarily aquatic



Report

Air Breathing in an Exceptionally Preserved 340-Million-Year-Old Sea Scorpion

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SUMMARY

Arachnids are the second most successful terrestrial animal group after insects [1] and were one of the first arthropod clades to successfully invade land [2]. Fossil evidence for this transition is limited, with the majority of arachnid clades first appearing in the terrestrial fossil record. Furthermore, molecular clock dating has suggested a Cambrian-Ordovician terrestrialization event for arachnids [3], some 60 Ma before their first fossils in the Silurian, although these estimates assume that arachnids evolved from a fully aquatic ancestor. Eurypterids, the sister clade to terrestrial arachnids [4–6], are known to have undergone major macroecological shifts in transitioning from marine to freshwater environments during the Devonian [7, 8]. Discoveries of apparently subaerial eurypterid trackways [9, 10] have led to the suggestion that eurypterids were even able to venture on land and possibly breathe air [11]. However, modern horseshoe crabs undertake amphibious excursions onto land to reproduce [12], rendering trace fossil evidence alone inconclusive. Here, we present details of the respiratory organs of *Adelophthalmus pyrrhae* sp. nov. from the Carboniferous of Montagne Noire, France [13], revealed through micro computed tomography (μ -CT) imaging. Pillar-like trabeculae on the dorsal surface of each gill lamella indicate eurypterids were capable of subaerial breathing, suggesting that book gills are the direct precursors to book lungs while vascular ancillary respiratory structures known as *Kiemenplatten* represent novel air-breathing structures. The discovery of air-breathing structures in eurypterids indicates that characters permitting terrestrialization accrued in the arachnid stem lineage and suggests the Cambrian-Ordovician ancestor of arachnids would also have been semi-terrestrial.

RESULTS

Systematic Paleontology

- Chelicerata Heymons, 1901 [14]
- Eurypterida Burmeister, 1843 [15]
- Eurypterina Burmeister, 1843 [15]
- Adelophthalmoidea Tollerton, 1989 [16]
- Adelophthalmidae Tollerton, 1989 [16]
- Adelophthalmus* Jordan in Jordan and von Meyer, 1854 [17]
- Adelophthalmus pyrrhae* sp. nov.

Etymology

Adelophthalmus pyrrhae is named after Pyrrha of Thessaly, daughter of Epimetheus and Pandora in Greek mythology, who along with her husband Deucalion cast stones that turned into babies to repopulate the earth after a great flood, which is a reference to the nodular mode of preservation of the holotype.

Holotype

Hunterian Museum, University of Glasgow, (GLAHM) A23113, almost complete specimen lacking telson (Figure 1).

Location and Age

Lower Carboniferous (Middle to Late Tournaisian) Lydiennes Formation(?), St. Nazaire Group, Montagne Noire region, France. Specific details about the source locality for the specimen are lacking; however, the Lydiennes Formation is the only geological unit in the region to produce fossil-bearing phosphatic nodules. The Lydiennes Formation is made up of black siliceous rocks, primarily described as radiolarian cherts, as well as black shales, and is characterized by abundant phosphate nodules, typically ~5 to 6 cm long, bearing exceptionally preserved, permineralized fossils [18, 19]. The localities of the Lydiennes Formation range from classic localities, interpreted as an offshore basin with well-formed nodules, to more recently described nearshore localities typically with poorly formed nodules [20, 21]. The fossils in the Lydiennes phosphatic nodules include cephalopods, arthropods, and abundant plants, which are *in situ* in the lower Lydiennes Formation [18–24].

Diagnosis

Adelophthalmus with appendages II–V bearing a pair of spines venterodistally on each podomere; lacking any lateral reduction



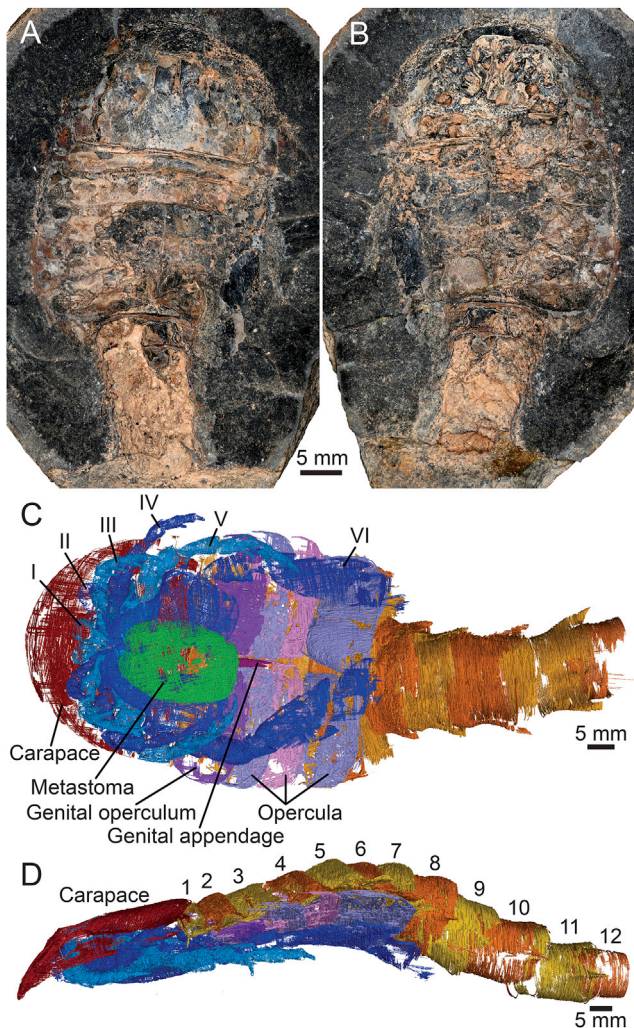


Figure 1. Photographs and Digital Segmentation of *Adelophthalmus pyrrhae*

(A and B) Photograph of the part (A) and counterpart (B) of the phosphatic nodule containing *Adelophthalmus pyrrhae*.

(C and D) Digital segmentation of *Adelophthalmus pyrrhae* specimen in ventral (C) and left lateral (D) view. Labels show major aspects of the morphology, with Roman numerals indicating the prosomal appendage pair and Arabic numerals the body segment.

See also [Figures S1](#) and [S2](#) and [Tables S1](#) and [S2](#).

of the anterior opisthosomal segment; epimera lacking on preabdominal segments, present on postabdominal segments.

Description

The fossil is preserved within a phosphate nodule approximately 71 mm long and 55 mm wide and is visible where the nodule has been split medially, exposing the eurypterid along its dorsal plane ([Figures 1A](#) and [1B](#)). Almost the entire eurypterid is preserved, with the exception of the telson. Externally, a portion of the book gills is visible on the sixth body segment, where the split has crossed through the operculum into the branchial chamber, revealing six overlapping lamellae that appear semicircular in shape attaching obliquely to the midline of the body. A more

complete view of the eurypterid's morphology is afforded through micro computed tomography (micro-CT) scanning, permitting digital reconstruction of the entire specimen preserved within the nodule ([Figures 1C](#), [1D](#), [S1](#), and [S2](#)). This reveals details of the external ventral morphology, including prosomal appendages, the metastoma, genital appendage, and opercula, as well as the structure of the respiratory organs and gut. The specimen is interpreted as a carcass based on the retracted position of the prosomal appendages and the lack of opisthosomal curvature or telescoping [[25](#)]. A full description of the specimen can be found in the [Supplemental Information](#).

Remarks

All six pairs of prosomal appendages are preserved, including the chelicera, which are short and robust, and the distally expanded paddle of appendage VI. Appendages II–V all bear a pair of spines ventrodistally on each podomere; this, combined with the presence of postabdominal epimera and the lack of any lateral reduction in the anterior opisthosomal segment, indicates that *Adelophthalmus pyrrhae* has a close affinity to the American species *Adelophthalmus mazonensis* and *Adelophthalmus mansfieldi* [[8](#)]. As in other eurypterids, the mesosomal opisthosomal appendages of somites VIII–XIII are highly modified into broad, medially fused opercular plates that cover the entirety of the ventral sternites. The first two opercula are further fused into a single functional unit called the genital operculum, which bears the genital appendage. Posterior to the genital operculum are four more opercula, although the penultimate operculum—corresponding to the fifth dorsal tergite—is absent from the specimen and appears to have been lost due to taphonomic processes, potentially having broken off separately when the nodule was opened and subsequently been lost.

The opercula enclose the book gills within a branchial chamber that is defined dorsally by the abdominal sternites ([Figures 2A–2E](#)). Four pairs of book gills are preserved, corresponding to each of the preserved opercula ([Figures 2A](#) and [2B](#)), indicating a total of five pairs of book gills in life, as in *Xiphosura* [[26](#)]. The genital operculum bears only a single pair of book gills, located on the posteriormost of the two fused opercula, representing the appendages of somite IX. The book gills are horizontally oriented ([Figures 2C](#) and [2E](#)) and fragmentary, with only the book gills of the sixth operculum preserved in their entirety ([Figure 2F](#)); these are oval, attach close to the midline of the body, and consist of six lamellae. The number of lamellae in the anterior gills is unclear; however, the amount of fragmentary material within the branchial chamber indicates a higher lamella count and that these lamellae also bore trabeculae. Further evidence that the anterior book gills had more lamellae comes from a specimen of the Ordovician eurypterid *Onychopterella augusti* that exhibits four sets of book gills (interpreted here as belonging to segments 2–5), each with 45 lamellae [[27](#)]. The gills in *Onychopterella* were interpreted as being vertically stacked rather than horizontally oriented, as indicated by *Adelophthalmus pyrrhae*. This apparent difference, however, is taphonomic; the lamellae of *Adelophthalmus pyrrhae* are deflected into a more vertical orientation laterally by the curvature of the opercula ([Figure 2B](#)), and specimens of the Cretaceous xiphosurid *Tachypleus syriacus* show that the lateral margins of the book gills can be preserved so as to appear vertically stacked [[28](#)]. Although the gill macrostructure

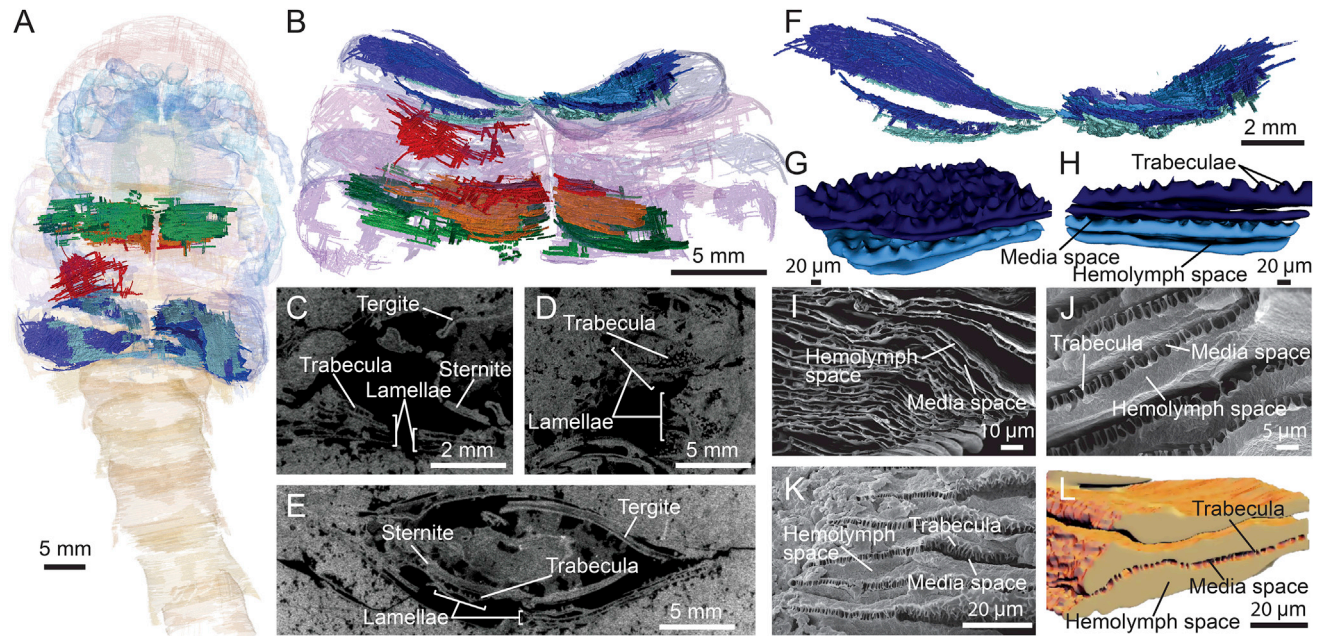


Figure 2. Respiratory Organs in *Adelophthalmus pyrrhæ* and Other Chelicerates

(A) Digital segmentation showing the location of the preserved gill lamellae.
 (B) Anterodorsal view of gill lamellae above the opercula.
 (C) Scan image of *Adelophthalmus pyrrhæ* showing lamellae with trabeculae of the sixth body segment in lateral view.
 (D) Scan image of *Adelophthalmus pyrrhæ* showing trabeculae of lamellae located on the fourth and sixth body segments in dorsal view.
 (E) Scan image of transverse cross section of *Adelophthalmus pyrrhæ* showing fragments of the lamellae of the third body segment displaying trabeculae.
 (F) Digital segmentation of the lamellae of book gills of *Adelophthalmus pyrrhæ* located on the sixth body segment.
 (G and H) Digital segmentation showing the trabeculae on two lamellae of *Adelophthalmus pyrrhæ* book gills located on the sixth body segment.
 (I) SEM image showing the book gills of the extant xiphosurid *Limulus polyphemus*. The trabeculae-like structures visible inside the hemolymph space are pillar cells, which are also found in arachnid book lungs [29, 36].
 (J) SEM image showing the book lungs of the extant spider *Aculepeira ceropegia*.
 (K) SEM image showing book lungs of the extant scorpion *Euscorpium carpathicus*.
 (L) Digital segmentation of the book lungs of the Devonian trigonotarbid arachnid *Palaeocharinus* sp. Image in (I) courtesy of Y. Suzuki, (K) courtesy of L. Prendini, and (J) and (L) courtesy of G. Scholtz.

is xiphosuran in appearance, the microstructure is markedly arachnid in nature. The dorsal surface of each lamella is covered with regularly spaced 0.15-mm-tall, 0.05-mm-wide pillar-like trabeculae (Figures 2C–E) projecting up into the media space between lamellae, with a clear hemolymph space within each lamella (Figures 2G and 2H). Trabeculae are commonly found in pulmonate arachnids and are a terrestrial adaptation for air breathing, serving to keep the lung lamellae from collapsing together and eliminating the media space, which would suffocate the organism [29, 30, 31]. Trabeculae are absent in horseshoe crabs (Figure 2I), the gills of which collapse out of water and are not efficient at air oxygen transfer, rendering them incapable of subaerial breathing [32], nor have trabeculae been described in any of the fossil xiphosurans [28, 33] or stem eucheelicerates [6, 34, 35] preserving gills. The presence of trabeculae in *Adelophthalmus pyrrhæ* is therefore direct evidence that eurypterids were able to breathe in subaerial environments through their main respiratory organs. The trabeculae exhibit regular spacing and possess a morphology comparable to the trabeculae found in arachnids, which comprise a conical base extending into a narrow pillar (Figures 2J and 2K). The majority of the trabeculae within the specimen are represented by the broad

conical base, with very few retaining the pillar structure (Figures 2G and 2H); however, this preservation is identical to that of the trabeculae in an exceptionally preserved Devonian trigonotarbid, which also preferentially preserves the base of the trabeculae but preserves the pillars in irregular shapes and widths (Figure 2L). When sufficiently preserved, the trabeculae of *Adelophthalmus pyrrhæ* exhibit a differentiation into anterior proximal trabeculae attached to both lamellar surfaces and posterior distal trabeculae attached only to the dorsal surface of the ventral lamella (Figure 2C), a distribution also observed in modern arachnids [29, 31].

DISCUSSION

Eurypterids are known to utilize a dual respiratory system (Figure 3A), with gills on the opercula supplemented by vascular structures located on the ventral surface of the body wall termed *Kiemenplatten* [11, 13]. These *Kiemenplatten* have been tentatively suggested to act as ancillary respiratory structures for putative amphibious excursions [11]. The occurrence of trabeculae on the book gills indicates that these too were active respiratory organs in air and confirms that eurypterids were fully capable of

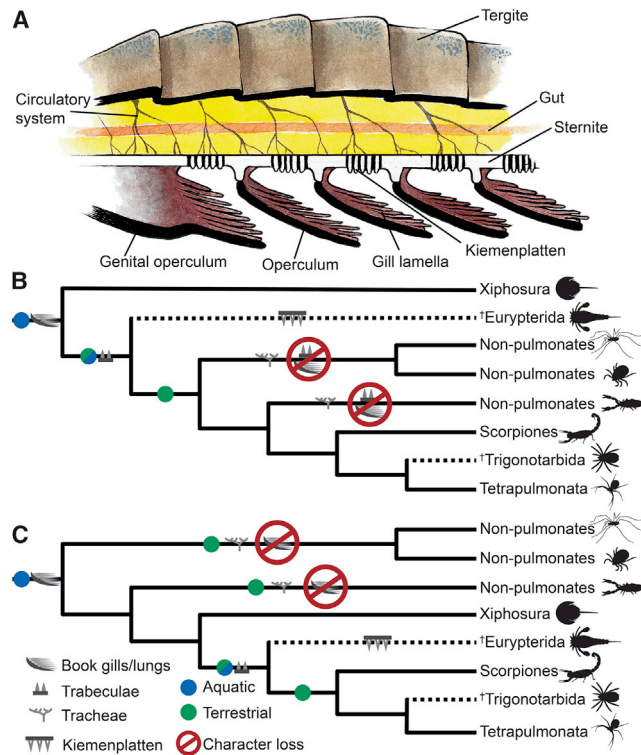


Figure 3. Respiratory Organ Structures in Eurypterids and Other Chelicerates

(A) Reconstruction in lateral cross section of the respiratory system of eurypterids as exemplified by *Adelophthalmus pyrrhae*.

(B) Inferred evolution of terrestrialization and respiratory structures in euchelicerates, with simplified phylogeny for a monophyletic Arachnida derived from Lozano-Fernandez et al. [3].

(C) Alternative hypothesis for the evolution of terrestrialization and respiratory structures in euchelicerates with a polyphyletic Arachnida following the tree topology suggested by Noah et al. [42]. Note that, in this scenario, spermatophores would have to be secondarily lost in Xiphosura. See also Figure S3.

persisting in terrestrial environments for extended periods (Figure S3). The low number of respiratory lamellae in the posterior book gills of *Adelophthalmus pyrrhae* is puzzling but may be a desiccation resistance strategy to reduce overall surface area while promoting subaerial gas exchange as seen in amphibious and terrestrial crustaceans [37]. Interestingly, amphibious crustaceans also maintain some gills with a higher surface area for aquatic respiration [37]. Despite these adaptations for subaerial breathing, eurypterids had a predominantly aquatic life habit, as indicated by the diversity of species (including *Adelophthalmus pyrrhae*) with their posterior pair of prosomal appendages modified into a broad swimming paddle and their abundant aquatic fossil record. It has also been suggested that the eurypterid's method of masticating food via appendicular gnathobases would have been unable to function on land [38], thereby limiting the amount of time that eurypterids could have spent out of their usual aquatic environment. Instead, the semi-terrestriality may have allowed eurypterids to move between ephemeral pools to reproduce in sheltered creche environments, as indicated by the spatial segregation between adults and juveniles observed

in the fossil record [39]. Further support for this interpretation comes from the discovery that eurypterids, like arachnids, possessed spermatophores [40]. Spermatophore-mediated reproduction may have permitted female eurypterids to store sperm for up to several months as in modern arachnids [41], permitting time for migration to creche environments to reproduce after mating. The presence of spermatophores also opens up the possibility that eurypterids were capable of transferring sperm in terrestrial environments.

Molecular divergence estimates indicate that arachnids occupied terrestrial environments during the early Ordovician or late Cambrian [3]. Until now, such a time frame for terrestrialization would have required an almost saltationist transition from an aquatic to terrestrial life habit. Some Silurian scorpions have been proposed to be aquatic and exhibit a stepwise acquisition of terrestrial characteristics [43, 44]; however, this would necessitate two terrestrialization events within Arachnida [45], and the aquatic nature of these scorpions has been debated [2, 46]. Similar disconnects between molecular estimates and the fossil record of myriapods were recently resolved by the recognition that the aquatic Cambrian-Triassic euthycarcinoids are stem myriapods [47]. Similarly, the solution to the discrepancies in the projected and observed timing of arachnid terrestrialization may lie within their stem lineage. The discovery of air-breathing adaptations in the eurypterids, the arachnid sister group, indicates that terrestrial adaptations accrued in a stepwise pattern along the arachnid stem lineage (Figure 3B), culminating in the modifications for preoral digestion, including a preoral cavity formed from the basal articles of the pedipalp and anteroventrally directed mouth that characterize arachnids [38] (which *Adelophthalmus pyrrhae* lacks; Figure S2). Critically, the occurrence of subaerial breathing can be inferred across all eurypterids based on trackway evidence of terrestrial excursions in *Stylonurina* [9, 10]; the occurrence of *Kiemenplatten* across Eurypterida, including records from the early Silurian [48]; and the morphological evidence from *Adelophthalmus pyrrhae*, indicating that terrestrial adaptations were likely inherited from the common ancestor of eurypterids and arachnids. The Cambrian-early Ordovician ancestor of arachnids and eurypterids would therefore have been semi-terrestrial, corresponding to the molecular clock estimates for terrestrialization within the group, with the radiation and diversification of arachnids occurring fully within a terrestrial setting.

Recent molecular phylogenetic work has suggested that xiphosurans are in-group arachnids and are secondarily aquatic [42, 49] but has been rebutted based on further molecular analyses [50] and assessment of whole-genome duplication events [51]. Eurypterids have not been considered in these studies and were assumed to have a life habit similar to horseshoe crabs. As such, our revised understanding of eurypterid respiration has important ramifications for the suggestion that horseshoe crabs (and eurypterids) are secondarily aquatic. The semi-aquatic nature of eurypterids could be indicative of a lineage in the process of either leaving or returning to the water; however, other aspects of their morphology, including the lack of terrestrial feeding capabilities and the absence of paired apoteles [38], are more indicative of an organism with greater affinity to the aquatic rather than terrestrial realm. Crucially, the ancillary respiratory *Kiemenplatten*, which would not have functioned in subaqueous

media [11], are distinct to any respiratory structure in Arachnida. This strongly indicates that eurypterids were experimenting with modes of terrestrial respiration and were in the process of terrestrializing rather than returning to aquatic environments. This in turn suggests that horseshoe crabs evolved from fully aquatic ancestors. Assuming a single terrestrialization event for Arachnida therefore necessitates that non-pulmonate arachnids lost their book lungs (Figure 3B), with tracheae evolving multiple times among non-pulmonates, as indicated by their occurrence on different body segments in different groups [26]. Alternatively, arachnids may have invaded land multiple times [42]; however, this scenario still necessitates that non-pulmonates lost their respiratory lamellae and independently developed tracheae (Figure 3C). Nevertheless, the discovery of trabeculae in the book gills of eurypterids demonstrates that terrestrialization in at least pulmonate arachnids occurred as the final step of a protracted series of character acquisitions within the arachnid stem lineage and that eurypterids represent a truly unique example of semi-terrestriality as part of a broader evolutionary trajectory toward the invasion of land.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at <https://doi.org/10.1016/j.cub.2020.08.034>.

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AUTHOR CONTRIBUTIONS

J.C.L. and V.E.M. designed the project; J.C.L., V.E.M., O.A.P.-F., and M.J.H. performed the research; V.E.M., O.A.P.-F., and M.J.H. processed the data; J.C.L. interpreted the data and wrote the manuscript; and V.E.M. and M.J.H. contributed to the writing of the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological Samples		
<i>Adelophthalmus pyrrhae</i>	Hunterian Museum, University of Glasgow	GLAHM:A23113
Deposited Data		
DICOM image stacks	This paper	Morphosource, project ID P977
Software and Algorithms		
ImageJ	[52]	https://imagej.nih.gov/ij/
VGStudio MAX v. 3.2	Volume Graphics, Heidelberg, Germany	https://www.volumegraphics.com/en/products/vgstudio-max.html
Zerene Stacker	Zerene Systems, Richland, WA, USA	https://www.zereneystems.com/cms/stacker
Phoenix DATOS x 2	GE Sensing & Inspection Technologies, Hürth, Germany	https://www.bakerhughesds.com/inspection-technologies/radiography-ct/x-ray-computed-tomography/phoenix-datosx

RESOURCE AVAILABILITY

Lead Contact

Further information and requests for resources should be directed to and will be fulfilled by the Lead Contact, James Lamsdell (james.lamsdell@mail.wvu.edu).

Materials Availability

The holotype and only known specimen of *Adelophthalmus pyrrhae* is accessioned in the Hunterian Museum of the University of Glasgow under the accession number GLAHM:A23113.

Data and Code Availability

DICOM image stacks of the scan data are available through MorphoSource, project ID P977.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Specimen Repositories

The experimental subject is a fossilized specimen of the eurypterid *Adelophthalmus pyrrhae* (GLAHM:A23113) preserved within a phosphate nodule from the Lower Carboniferous (Middle to Late Tournaisian) Lydiennes Formation(?), St. Nazaire Group, Montagne Noire region, France. The specimen is housed in the Hunterian Museum of the University of Glasgow, Glasgow, United Kingdom

METHOD DETAILS

Description of GLAHM A23113

Body length of holotype and only known specimen > 71.4 mm. Carapace trapezoid, 17.6 mm long, 29.9 mm wide. Lateral eyes not clearly preserved but may be positioned centrimedially. Ocelli not preserved but inflated ocellar area may be located centrally on carapace. Prosomal appendages preserved in their entirety, with measurements shown in [Table S1](#). Chelicera composed of three podomeres: a non-spiniferous peduncle, a fixed finger, and a free finger. Appendages II-V are *Hughmilleria* type, bearing conical spines from the third to penultimate podomeres. The number of podomeres in each appendage increases posteriorly, with appendage II having seven podomeres, appendage III eight, appendage IV eight, and appendage V nine. Appendage VI is expanded into a swimming paddle, *Adelophthalmus* type, with nine podomeres. The first podomere of each post-cheliceral appendages is the coxa, which bear spiniferous gnathobases proximally. The coxa of appendage VI is greatly expanded and bears an anterior 'ear', which is subquadrate in shape. The metastoma is positioned centrally between the coxa of appendage VI and overlaps them significantly, creating a rudimentary oral chamber. The metastoma is 14.5 mm long by 10.2 mm wide, oval in shape, with an angular anterior notch.

The opisthosoma is composed dorsally of twelve tergites, the measurements of which are shown in [Table S2](#). The first tergite is not truncated laterally as in some *Adelophthalmus* species. Tergites 1-6 with rounded lateral margins. Tergites 7-12 fused with sternites

to form ankylosed segments, bearing angular lateral epimera. Ventral opisthosomal structures well preserved. Genital operculum, comprising the fused opercula of the first and second opisthosomal segments, 8.7 mm long and 31.4 mm wide, with an anterior median inflection. Opercula of third, fourth, and sixth opisthosomal segments are preserved, with the operculum of the fifth segment being absent. Each operculum is 8 mm long by 30 mm wide and is overlapped by the preceding operculum for about half its length. Type A genital appendage present on genital operculum, 7.3 mm long by 1.3 mm wide, extends to posterior of subsequent operculum. Book gill lamellae preserved on the posterior of the two fused genital opercula and each preserved postgenital operculum. Anterior book gills are fragmentary, with the total number of lamellae unclear but with a total number > 15. The posteriormost pair of book gills are well-preserved and comprise only six sets of lamellae. Lamellae of all book gills bear pillar-like trabeculae on their dorsal surface. Internal fore- and hindgut preserved. Foregut extends anterior from mouth to midway along appendage VI, curving ventrally and opening to oral region at the anterior margin of the metastoma. Midgut appears to expand but is poorly preserved. Hindgut narrow, extends posteriorly from the seventh opisthosomal segment. The telson is not preserved, nor is any indication of cuticular ornament.

Specimen Imaging and Computed Tomography

GLAHM:A23113 was photographed with a Canon EOS 60D DSLR camera with Canon EF 100mm f/2.8 Macro IS USM lens on a StackShot macro rail system (Cognisys, Traverse City, MI, USA); composite images were produced using Zerene Stacker (Zerene Systems, Richland, WA, USA). Micro-CT was performed with a Phoenix | tome | x μ CT scanner (General Electric Company, Fairfield, CT, USA) at the Microscopy and Imaging Facility at the American Museum of Natural History. Reconstruction of the CT images was done using Phoenix DATOS | x 2 (GE Sensing & Inspection Technologies, Hürth, Germany) and segmentation was performed in VGStudio MAX v. 3.2 (Volume Graphics, Heidelberg, Germany).

QUANTIFICATION AND STATISTICAL ANALYSIS

Specimen measurements were taken directly from the specimen and Micro-CT images using the software ImageJ and the linear measurement tool. No statistical analyses were undertaken in this study.