Microanatomy of Early Devonian book lungs

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1. INTRODUCTION

The book lungs of an exceptionally preserved fossil arachnid (Trigonotarbida) from the Early Devonian (approx. 410 Myr ago) Rhynie cherts of Scotland were studied using a non-destructive imaging technique. Our three-dimensional modelling of fine structures, based on assembling successive images made at different focal planes through the translucent chert matrix, revealed for the first time fossil trabeculae: tiny cuticular pillars separating adjacent lung lamellae and creating a permanent air space. Trabeculae thus show unequivocally that trigonotarbid fossils were fully terrestrial and that the microanatomy of the earliest known lungs is indistinguishable from that in modern Arachnida. A recurrent controversy in arachnid evolution is whether the similarity between the book lungs of Pantetrapulmonata (i.e. spiders, trigonotarbid, etc.) and those of scorpions is a result of convergence. Drawing on comparative studies of extant taxa, we have identified explicit characters (trabeculae, spines on the lamellar edge) shared by living and fossil arachnid respiratory organs, which support the hypothesis that book lungs were derived from a single, common, presumably terrestrial, ancestor.

Keywords: Trigonotarbida; respiration; Tetrapulmonata; Scorpiones; terrestrialization

2. MATERIAL AND METHODS

Fossil book-lung morphology was resolved in a remarkable new trigonotarbid specimen (figure 1a) preserved in a polished thin section of Rhynie chert held at the Palaeobotany Section of the University of Münster, Germany (PBM 3530). This fossil can be assigned to Palaeochcarinus based on comparisons with previous trigonotarbid descriptions (e.g. Fayers et al. 2005). The translucent chert matrix allows the analysis of cuticular surfaces and under optimal conditions this even includes internal, cuticle-lined structures within the arthropods (Dunlop et al. 2003), as well as fine (cellular) details of plants and their spores (Wellman et al. 2006).

To study the book lungs, we developed a new, non-destructive approach to imaging morphological details in Rhynie material; a methodology which we hope can be more widely applied to other fossils from this locality in future. For a similar, but destructive, approach to imaging morphological details in Rhynie material; a methodology which we hope can be more widely applied to other fossils from this locality in future. For a similar, but destructive, method involving grinding through nodules to reveal their contents at successive planes and then reconstructing the enclosed animals three-dimensionally, see Sutton et al. (2005). Three-dimensional reconstructions of the lungs were generated using the software package BrainVue. A series of 600 micrographs of the lung (figure 1c) was taken through a Zeiss Axioplan 2 microscope with a 100× (oil immersion) lens; focusing as far as possible through the specimen in its translucent chert matrix with a step width of 0.1 μm. This yielded a stack depth of 60 μm (electronic supplementary material 1). Volume-rendering (figure 1f) created a three-dimensional image of this stack of virtual sections, including all voxels with a higher impermeability to light than an (adjustable) threshold. A more detailed, three-dimensional reconstruction of three lung lamellae, plus the atrial wall, was obtained by specifically tracing the outlines of these structures throughout the stack using the Isar2 Measurement Pro tool. Again, these stacked images were combined and subjected to contour surface rendering to yield a single model of a region of the fossil lung, which we include here as a three-dimensional, virtual animation (figure 1f; electronic supplementary material 2). The terminology for lung structures and lung orientation, with respect to the proximal region (i.e. where haemolymph would have entered) and the distal lamella edge (at the atrium where the air enters), follows Scholtz & Kamen (2006) and Kamenz & Prendini (in press).

3. RESULTS

In gross morphology, the lungs of this approximately 5 mm long Palaeochcarinus sp. specimen (figure 1a) are very similar to those of Recent Tetrapulmonata
In the preserved plane of section, probably towards the midline of the body, the fossilized book lungs (figure 1b) identified here have a length of approximately 300 µm and a height of approximately 250 µm. Up to 34 individual lamellae can be resolved, of which 25 reveal the distal lamellar edge, i.e. the edge projecting into the atrial chamber where the air enters the lung lamellae. Individual lamellae are orientated more or less horizontally within the body and expand from approximately 2 µm in the proximal region up to approximately 6 µm at the distal lamellar edge, i.e. where they join the atrium.

For the first time, we have been able to identify crucial details within these fossilized respiratory organs, namely trabeculae (figure 1c)—narrow pillars of cuticle which traverse the air space between adjacent lamellar walls—and cuticular spines projecting into the atrium from the lamellar edge of the lung (figure 1c). These trabeculae are rod-like, regularly spaced and of a consistent height (approx. 2 µm). Trabeculae in the proximal region of the lung are fused to two adjacent lamellae. Thus, they traverse the entire air space and represent the so-called ‘bridging trabeculae’ (cf. Kamenz & Prendini in press). These bridging trabeculae can also occur up to the distal lamellar edge, but here only in the median region of the lung. A few trabeculae near the air-filled atrium remain free at the opposite end and thus represent ‘papillate trabeculae’ (sensu Kamenz & Prendini in press). Spines on the lamellar edges occur frequently and project in several directions from the lamella itself. Overall, these fossil characters match in great detail the corresponding structures in both modern scorpions and tetrapulmonates (figure 1d,e). This three-dimensional reconstruction (using Bitplane Imaris) of the fossil lung in PBM 5350 showing a distal lamellar region. Images generated from a digitally combined stack of photographs taken at successive planes of focus: reconstruction of the surface of the lamellae and their trabeculae; trabeculae are shown here only between two lamellae on the polished plane of section (see also electronic supplementary material 1 and 2). Lamellae are fused towards the midline of the body. Note that these spines resolved on the lamellar edges would have been more pointed in life, but the reconstruction programme tends to blur them artificially. A, air sac; At, atrium; Bl, book lung; Bt, bridging trabeculae; Cc, chelicera; E, epidermis cell; F, region of lamellar fusion; H, haemolymph cell; L, lamella (haemolymph space); Lc, leg coxae; N, nucleus; O, opisthosoma; P, prosoma; Pc, pillar cells; Pp, pedipalp; S, spine; Sp, spiracle; St, sternite; T, trabecula; ant, anterior; post, posterior.

4. DISCUSSION
The significance of our results is twofold. First, trabeculae and lamellar spines provide conclusive...
evidence that these were the lungs of fully terrestrial, air-breathing animals. Neither structure is seen in the book gills of horseshoe crabs (Scholtz & Kamenz 2006), and thus the lamellae in the Rhynie fossils are undoubtedly those of a functional lung and not, for example, a gill within a brachial chamber. Trabeculae maintain an air space within the lungs and have never been recorded from (aquatic) arachnid outgroups with lamellate respiratory organs, i.e. Xiphosura (Recent) or Eurypterida (extinct). Lamellar spines in book lungs may function to prevent dust or other particles from clogging up the entrance of individual lamellae. Thus, we conclude that the book lung had already achieved its modern fine morphology at an early stage of life on land back in the Early Devonian. In many arachnids, it has remained essentially unchanged ever since. Physiologically, however, the book lung is not as efficient (at least for small animals) as a branching tracheal system (Anderson & Prestwich 1985). Thus, lungs appear, in certain arachnids, to have evolved into tracheal systems—for instance, within spiders on multiple occasions (Bromhall 1987; Hilken 1998). Such tracheae may well represent adaptations to more active modes of life. Hypotheses of respiratory organ evolution in spiders, including ways in which lung lamellae might have evolved into tracheal tubes, are summarized by Levi (1967).

Second, a major controversy in arachnid evolution is whether their common ancestor was aquatic (Stormer 1976; Selden & Jeram 1989) or terrestrial (Scholtz & Kamenz 2006). The ‘aquatic’ hypothesis is based on the (disputed) notion that early scorpions were marine. This hypothesis was elaborated in the greatest detail by Kjellesvig-Waering (1986), although a restudy of his key specimens does not always support his interpretations and conclusions (cf. Dunlop et al. in press). If correct, marine scorpions would imply that book lungs must have evolved at least twice, i.e. in scorpions and, convergently, in the stem lineage of pantetrapulmonates. Book lungs, in, for example, spiders, do occur in topologically different segments compared with those in scorpions. Despite this positional difference, detailed comparison of lung morphology in modern representatives of both lung-bearing arachnid groups revealed at least three (apomorphic) features in common. That these structures are indeed likely to be homologous—and not just independent adaptations to a terrestrial lifestyle—is indicated by their specific characteristics (shape, position, arrangement, etc.), which differ from unequivocally convergent cases of terrestrialization in other arthropods such as crustaceans (see, for discussion, Scholtz & Kamenz 2006). Chief among these characters are the presence of bridging trabeculae in a specific arrangement within the air space and of marginal spines (Scholtz & Kamenz 2006). These data support a single origin for the lungs and implicitly a terrestrial common ancestor of the Arachnida. The book-lung microanatomy in Palaeocharinus revealed here makes a crucial contribution to this debate. Trigonotarbid resolve convincingly as the sister group of Tetrapulmonata (Selden et al. 1991; Shultz 2007). Were trigonotarbids to lack trabeculae and spines, this would be a powerful argument for convergence; namely that fully modern book lungs appeared within the pantetrapulmonate line, independent of those of scorpions. By contrast, the presence of these detailed book-lung structures in a more than 400 Myr-old fossil supports the hypothesis that lungs in arachnids evolved only once, as proposed by Scholtz & Kamenz (2006), and that common apomorphies (bridging trabeculae, spines) can be found in living and, based on our results, fossil lung-bearing arachnid forms.

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Selden, P. A., Shear, W. A. & Bonamo, P. M. 1991 A spider and other arachnids from the Devonian of New York,


