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Microanatomy of Early Devonian book lungs

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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 trigonotarbids 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, trigonotarbids, 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

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1. INTRODUCTION

The world-famous Rhynie and Windyfield cherts of Aberdeenshire, Scotland, UK, currently dated as Early Devonian (Pragian, *ca* 410 Myr ago), represent the most completely known Palaeozoic terrestrial/freshwater ecosystem (Rice *et al.* 2002). The original community is thought to have lived in and around a series of pools in a putative hot springs environment. Fossils were preserved through being inundated by hot, silica-rich water trapping plants and animals *in situ* within sinters which precipitated out of the water and eventually solidified into cherts (Fayers & Trewin 2004). Exceptionally preserved arthropod fossils from these cherts have made a vital contribution to our understanding of early life on land. This is largely thanks to their three-dimensional preservation of external and even internal morphological details. Terrestrialization is widely seen as one of the key events in Earth history and this fundamental ecological shift from water to air undoubtedly made significant new demands on the physiology of early land animals, particularly their respiration. In respect of this, we reinvestigated one of the most important discoveries at Rhynie, the fossilized lungs (Claridge & Lyon 1961) found in the extinct (Silurian–Permian) arachnid order Trigonotarbida. Closely related to spiders, trigonotarbids resolve at the base of the Pantetrapulmonata (Shultz 2007), which, as its name implies, is a clade of arachnids defined by the presence of two pairs of book lungs; one pair of which was subsequently modified into tracheae in more derived spiders (Levi 1967; Bromhall 1987). These Rhynie respiratory organs are still the oldest putative record of a lung in any animal group. However, previous accounts (Claridge & Lyon 1961; Størmer 1976) figured only their gross morphology, with stacks of thin, sheet-like lamellae which give the book lung its name. Having established a suite of phylogenetically informative characters in the lung microanatomy of Recent arachnids (Scholtz & Kamenz 2006; Kamenz & Prendini in press), our principal aim here was to use newly available specimens prepared from slices of Rhynie chert to test whether these apomorphies could be resolved, three-dimensionally, in exceptionally preserved Devonian arachnids too; fossils crucially dating from the early phases of arthropod terrestrialization.

2. MATERIAL AND METHODS

Fossil book-lung morphology was resolved in a remarkable new trigonotarbid specimen (figure 1*a*) 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 *Palaeocharinus* sp. 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) or fungi (Taylor *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, 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 Bitplane Imaris. A series of 600 microphotographs of the lung (figure 1*c*) 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 1*f*) 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 Imaris 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 1*f*; 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 & Kamenz (2006) and Kamenz & Prendini (in press). In addition, we sectioned the book lungs of Recent species of Amblypygi and Scorpiones (figure 1*d*,*e*; for details of the method, see Scholtz & Kamenz 2006).

3. RESULTS

In gross morphology, the lungs of this approximately 5 mm long *Palaeocharinus* sp. specimen (figure 1*a*) are very similar to those of Recent Tetrapulmonata

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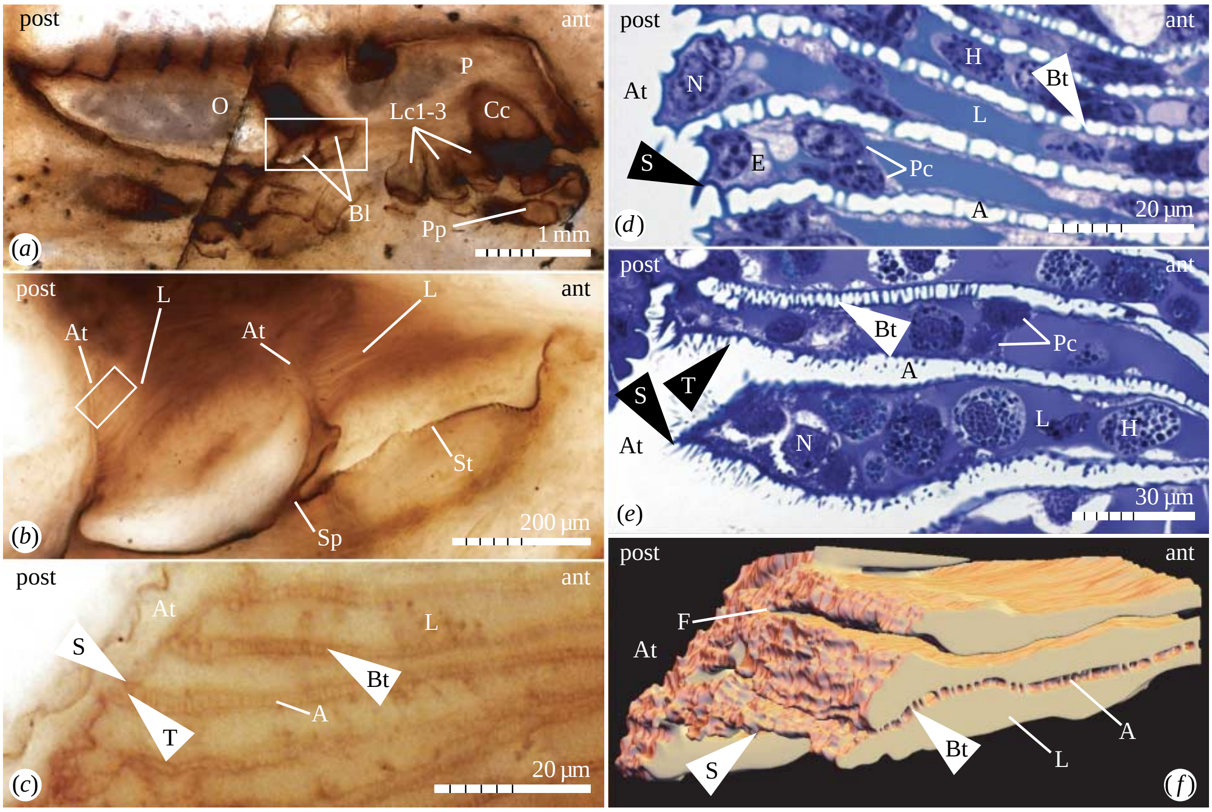

Figure 1. Fine structure of the book lungs in Devonian and Recent arachnids. (*a*) Longitudinal section through an almost complete *Palaeocharinus* sp. (Arachnida: Trigonotarbida), PBM 3530, from the Early Devonian Rhynie cherts of Scotland. (*b*) Same specimen, close-up of the book lungs—see box in (*a*). (*c*) Detail of the distal parts of medial lamellae revealing bridging trabeculae traversing the air space and spines from the lamellar edge—see box in (*b*). (*d*) Comparative transverse histological section through the book-lung lamellae of a Recent tetrapulmonate arachnid, *Damon* sp. (Amblypygi; 1.5 µm) stained with methylene blue–azure II revealing the same pattern of trabeculae and spines. (*e*) Comparative longitudinal histological section through the book-lung lamellae of a Recent scorpion, *Heterometrus longimanus* (1.5 µm) stained with methylene blue–azure II revealing the same pattern of trabeculae and spines. (*f*) Three-dimensional reconstruction (using BITPLANE IMARIS) of the fossil lung in PBM 3530 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.

(i.e. Araneae, Amblypygi and Uropygi; cf. Scholtz & Kamenz 2006). In the preserved plane of section, probably towards the midline of the body, the fossilized book lungs (figure 1*b*) 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 1*c*)—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 1*c*). 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 1*d*,*e*; e.g. Scholtz & Kamenz 2006; Kamenz & Prendini in press).

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 (Størmer 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. Trigonotarbids 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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