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Morphology and evolution of spider book lungs (Araneae)

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Abstract. Book lungs are an iconic character for arachnids, yet previous accounts of their morphology in spiders (Arachnida: Araneae) are based on sporadic reports from a few species using a diverse suite of terminologies. Here, we focus on the fine structure of spider book lungs using standardized terminology and a wider taxon sampling of seven species studied as histological sections and forty species studied with scanning electron microscopy. All spider lungs share a similar basic morphology, which also matches the ground pattern for arachnids in general. This includes a spiracle opening into an atrium with a folded wall from which stacked lamellae containing pillar cells project into a haemolymph sinus. The air spaces are separated by proximal trabeculae, which span the space completely, and distal trabeculae which originate from the lamellar dorsal surface. Within this framework, several differences amongst spider lungs could be identified. Distal trabeculae can be pilate or reticulate. Lamellar margins pointing into the atrium can be echinate, arbuscular-reticulate or arbuscular-reticulate-echinate. The atrium wall can be psilate, verrucate or arbuscular-reticulate. The character states identified here offer new perspectives for apomorphies of major spider clades. Reticulate distal trabeculae are only seen in the Mygalomorphae investigated here. The arbuscular-reticulate condition on the lamellar margin is only seen in representatives of Opisthothelae; with a further modification to arbuscular-reticulate-echinate in the Ctenidae. With one notable exception, an arbuscular-reticulate atrium wall is seen in the Araneomorphae sampled. These data are further compared to other pulmonate arachnids. Book lung fine structure in the earliest branching spider clade – the Mesothelae – matches the condition observed for the closely related Amblypygi (whip spiders).

Key words. Arachnida, Araneae, book lung, morphology, Scorpiones, Amblypygi, Uropygi.

1. Introduction

With more than 48,000 species (World Spider Catalog 2018), spiders (Arachnida: Araneae) are a megadiverse arachnid group. Spiders belong to a larger clade which also includes whip spiders (Amblypygi), whip scorpions (Thelyphonida) and schizomids (Schizomida). All of these arachnids have a ground pattern of one pair of book lungs each on the second and third opisthosomal segments. This entire four-lunged arachnid clade has been referred to in the literature as Megoperculata (Weygoldt & Paulus 1979) or Tetrapulmonata (Shultz 1990, 2007). Relationships within tetrapulmonates are still under investigation. The Labellata hypothesis (Weygoldt & Paulus 1979) recognizes Araneae + Amblypygi, while the Pedipalpi hypothesis (Shultz 1990, 2007) recognizes Amblypygi + (Thelyphonida + Schizomida); see these authors for details. Scorpions (Scorpiones) also have book lungs, in this case one pair each on opisthosomal segments four to seven. Book lungs are thus widely recognized (e.g. Westheide & Rieger 2007) as a typical textbook character for arachnids. Note that the lungs on the third segment are reduced in many spiders (Schmitz 2016; see below) and also in schizomids. Furthermore, most of the remaining arachnid groups respire via tracheal systems. These tracheae open on different segments in different groups, which implies a homoplastic character within arachnids. In palpigrades (Palpigradi) and certain mites (e.g. Acari: Astigmata) the respiratory organs have been lost completely.

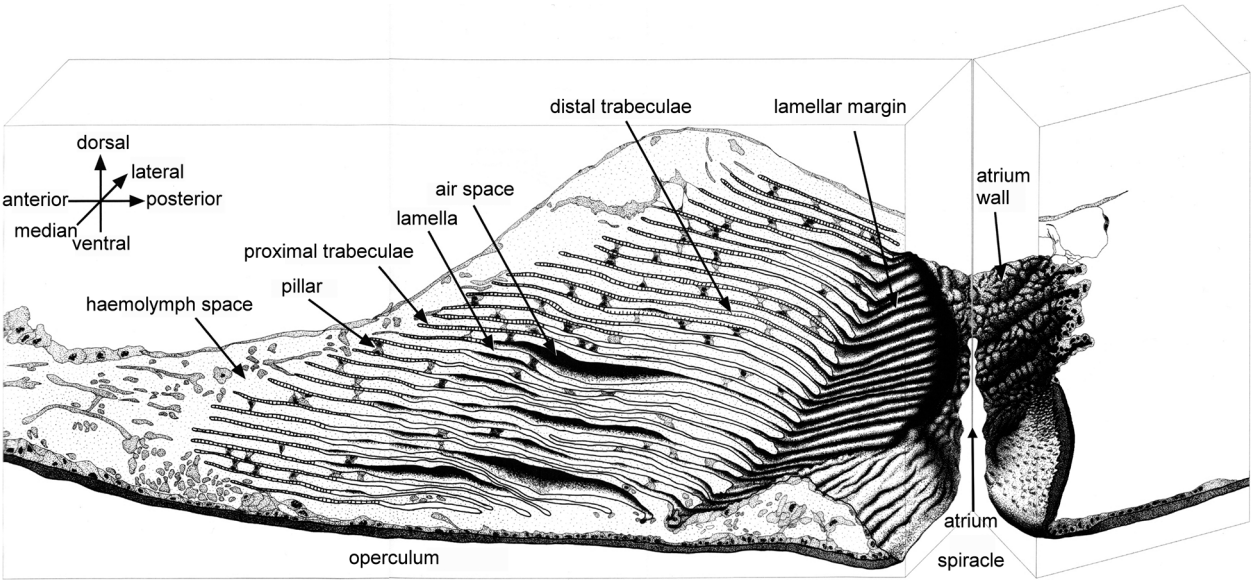

Fig. 1. Semi-schematic overview (sagittal view) of the elements of an arachnid book lung (modified after KAMENZ & PRENDINI 2008).

Book lungs are derived from the ectoderm and have a lining of cuticle shed during the moulting process (WEHNER & GEHRING 2007). Each lung is composed of multiple stacked leaf-like lamellae through which the haemolymph flows (Fig. 1). A very thin (ca. 0.03 µm: REISINGER et al. 1991) cuticle separates the lamellae from the adjacent air spaces and gas exchange takes place across this cuticle (e.g. PAUL & FINCKE 1989). The haemolymph spaces contain epithelial pillars which counteract the pressure in the fluid, while the air spaces are prevented from collapsing under haemolymph pressure by a series of cuticular projections usually termed trabeculae (e.g. REISINGER et al. 1991). The air spaces open posteriorly into an atrium, which in turn opens to the environment through a slit-like spiracle. This basic morphology is quite conservative across all pulmonate arachnids. Nevertheless, there is debate in the literature about whether different lineages of arachnids moved from water onto land independent of one another, and thus whether the book lungs of scorpions and tetrapulmonates are convergent. SCHOLTZ & KAMENZ (2006) argued that the book lungs of all pulmonate arachnids share several unique features, namely (a) fused proximal cuticular trabeculae within the air space, (b) spines on the lamella margins and (c) the shape of the pillars in the haemolymph space. This implies that the book lung evolved only once in a, presumably terrestrial, arachnid common ancestor; an interpretation consistent with the Arachnopulmonata hypothesis (SHARMA et al. 2014; GIRIBET 2018; HOWARD et al. 2019) which groups scorpions in a clade with the tetrapulmonate arachnids. Yet, KAMENZ (2009) – and especially KAMENZ et al. (2005) and KAMENZ & PRENDINI (2008) for scorpions – did recognise a degree of diversity in the fine structure of the lungs. Specifically, they used scanning electron micrographs (SEM) to recognize taxon-specific differences in (a) the surface of the lamellae, (b) the lamellar margins, and (c) the atrium wall. All of these characters may be phylogenetically informative.

That a great diversity of lung structures is found within the ca. 2000 species of scorpions begs the question whether a similar degree of diversity exists within the spiders, which have more than twenty times as many species. Araneae can be broadly divided into the Mesothelae, which retain opisthosomal segmentation, and Opisthothelae in which this segmentation has been reduced. Opisthothelae are further divided into Mygalomorphae (e.g. tarantulas, trap-door spiders) and the Araneomorphae which encompasses most of the spider species. Two pairs of book lungs are retained in mesotheles and mygalomorphs, and among the araneomorphs in hypochilid and gradungulid spiders. In most araneomorphs, however, the second pair of lungs have been replaced by tracheae. Furthermore, in a handful of araneomorphs such as the small-sized Caponiidae und Symphytognathidae, the first pair of lungs have also been reduced and replaced by tracheae. Thus spiders are the only arachnid taxon in which a transition from lungs to tracheae can be documented based on phylogenetic inference (LEVI 1967; WESTHEIDE & RIEGER 2007; SCHMITZ 2016) and structural and ontogenetic data (PURCELL 1909; RAMÍREZ 2000, 2014).

The fine structure of the book lungs has been comprehensively documented for all major scorpion groups (KAMENZ & PRENDINI 2008), and also studied in whip spiders and whip scorpions (SCHOLTZ & KAMENZ 2006). The latter paper only included representatives of Liphistiidae and Aranaeidae, and in general there are only sporadic SEM studies of spider lung morphology. Beginning with the earliest branching clades, there is published data for the mesothele Liphistiidae (HAUPT 2003) and the mygalomorph Theraphosidae (REISINGER et al. 1990, 1991). Araneomorph spiders studied include Araneidae, Cybaeidae and Agelenidae (MOORE 1976), Agelenidae again (HEXTER

Table 1. Summary of the species studied, methods used and the character states in the fine structure of the book lungs observed. See Supplementary Figs. S1 – S46 for images. Methods: Scanning electron microscopy (SEM), histological sections (H), examination of exuviae (E) and computed micro tomography (μCT). Note that histology alone did not provide details of the lung fine structure. Distal trabeculae (TR) can be pilate (1) or reticulate (2). Lamellar margins (MA) can be echinate (1), arbuscular-reticulate (2) or arbuscular-reticulate-echinate (3). The atrium wall (AT) can be psilate (1), verrucate (2) or arbuscular-reticulate (3).

TAXON	METHOD(S)	TR	MA	AT
Mesothelae				
Liphistius sp. (Liphistiidae)	SEM	1	1	2
Opisthothelae				
Mygalomorphae				
Atypus piceus (Sulzer, 1776) (Atypidae)	SEM, H	2	2	2
Linothele megatheloides Paz & Raven, 1990 (Dipluridae)	SEM, H	2	2	2
Fufius sp. (Cyrtaucheniidae)	SEM	2	2	2
Stasimopus sp. (Ctenizidae)	SEM	2	2	1
Gorgyrella sp. (Idiopidae)	SEM	2	2	1
Acanthogonatus francki Karsch, 1880 (Nemisiidae)	SEM	2	2	2
Brachypelma albopilosum Valerio, 1980 (Theraphosidae)	SEM, E	2	2	2
Grammostola rosea (Walckenaer, 1837) (Theraphosidae)	SEM, H	2	2	2
Theraphosa blondi (Latreille, 1804) (Theraphosidae)	SEM, E	2	2	2
Araneomorphae				
Hypochilus thorelli Marx, 1888 (Hypochilidae)	SEM	1	2	1
Progradungula otwayensis Milledge, 1997 (Gradungulidae)	SEM	1	2	3
Kukulkania hibernalis (Hentz, 1842) (Filistatidae)	SEM	1	2	3
Pholcus phalangioides (Fuesslin, 1775) (Pholcidae)	SEM, H	1	2	3
Loxosceles laeta (Nicolet, 1849) (Sicariidae)	SEM	1	2	3
Entelegynae				
Gandanameno sp. (Eresidae)	SEM	1	2	3
Eriauchenius workmani O.P.-Cambridge, 1881 (Archaeidae)	SEM	1	2	3
Araneus diadematus Clerck, 1757 (Araneidae)	SEM	1	2	3
Tetragnatha extensa (Linnaeus, 1758) (Tetragnathidae)	SEM	1	2	3
Nephila sp. (Nephilidae)	SEM	1	2	3
Neriene radiata (Walckenaer, 1841) (Linyphiidae)	SEM	1	2	3
Parasteatoda tepidariorum (C.L. Koch, 1841) (Theridiidae)	SEM, μCT	1	2	3
RTA-clade				
Anyphaena accentuata (Walckenaer, 1802) (Anyphaenidae)	SEM	1	2	3
Heteropoda maxima Jäger, 2001 (Sparassidae)	SEM, E	1	2	3
Heteropoda venatoria (Linnaeus, 1767) (Sparassidae)	SEM	1	2	3
Micrommata virescens (Clerck, 1757)	SEM	1	2	3
Thanatus coloradensis Keyserling, 1880 (Philodromidae)	SEM	1	2	3
Xysticus sp. (Thomisidae)	SEM	1	2	3
Selenops radiatus Latreille, 1819 (Selenopidae)	SEM	1	2	3
Marpissa radiata (Grube, 1859) (Salticidae)	SEM	1	2	3
Haplodrassus sp. (Gnaphosidae)	SEM	1	2	3
Nomisia sp. (Gnaphosidae)	SEM	1	2	3
Eratigena atrica (C.L. Koch, 1843) (Agelenidae)	SEM	1	2	3
Cheiracanthium punctorium (Villers, 1789) (Eutichuridae)	SEM	1	2	3
Cupiennius salei (Keyserling, 1877) (Ctenidae)	SEM	1	3	3
Oxyopes lineatus Latreille, 1806 (Oxyopidae)	SEM	1	2	3
Pisaura mirabilis (Clerck, 1757) (Pisauridae)	SEM	1	2	3
Dolomedes okefinokensis Bishop, 1924 (Pisauridae)	SEM, E	1	2	3
Hogna inominata (Simon, 1886) (Lycosidae)	SEM	1	2	3
Trochosa terricola Thorell, 1856 (Lycosidae)	SEM	1	2	3

1982), Lycosidae (Schmitz & Perry 2000) and Salticidae (Hill 1977). However, these earlier studies often only focused on the trabeculae on the lamellar surface and did not document the margins of the lamellae or the fine structure of the atrium wall. In a wider sense, book lung fine structure has never been used as a phylogenetic character

for resolving spider relationships. At best, even comprehensive cladistic analyses using morphology, such as CODDINGTON & LEVI (1991), GOLOBOFF (1993), GRISWOLD et al. (1999) and RAMÍREZ (2000), scored lungs only as a simple presence/absence character.

Here, we attempt a comprehensive comparative study of spider book lung morphology using broad taxon sampling (Table 1) and investigation of the three lung characters – trabeculae, lamellae margins and atrium – previously shown in scorpions to be a valuable source of variation. Our primary goals were to reconstruct the ground pattern of the spider book lung and to investigate whether particular character complexes may be informative for the delimitation and/or the phylogeny of the major spider groups.

2. Material and methods

Material. A principal aim of the present study was to significantly increase the taxon sampling for spider book lung morphology through studying a range of species from groups with many plesiomorphic characters (i.e. mesotheles and mygalomorphs) and those showing a number of derived characters (araneomorph lineages). The histological data assembled here is based on semi-thin sections derived from seven species from six of the traditional families. For scanning electron microscopy (SEM) of the lungs' fine structure, data was gathered from 40 species (Table 1). The *in situ* orientation of the lung lamellae was also investigated in one species using micro-computed tomography (μCT). Several spiders were collected locally by the first author, with identifications following BELLMANN (2006) or NENTWIG et al. (2018). Non-European species were obtained from academic colleagues (see Acknowledgments), or commercial biological suppliers (Terraristika Hamm, Matthias Köhler, Stefan Kurtsiefer). The correct nomenclature and familial position of each species was checked against the WORLD SPIDER CATALOGUE (2018). Sampling covering all major spider subgroups was impractical within the confines of this project, but to achieve wide coverage we adopted the 'exemplar approach' (e.g. YEATES 1995; PRENDINI 2001) in which studies of given species allow us to make inferences about the general condition in higher taxa. Species were thus chosen (Table 1) to reflect most of the major groups (e.g. after CODDINGTON 2005) and for each species 1–3 individuals were usually studied. Terminology for book lung structures largely follows SCHOLTZ & KAMENZ (2006), but some additional (novel) terms proved necessary as outlined in the Results.

Fixation. Tissue samples were fixed with glutaraldehyde, osmium tetroxide and Sörensen's buffer following the protocols of KARNOVSKY (1965). In brief, saccharose was added to the Sörensen's buffer to create a washing buffer solution for tissues of terrestrial arthropods. Saccharose renders the osmotic concentration of the solution more like that of the target tissue, reducing deformation at the cellular level. The washing buffer solution and glutaraldehyde solution were combined to form the initial fixative. Spiders were anesthetized with carbon dioxide, decapitated with a razor blade and placed in the glutaraldehyde solution. The chitinous arthropod exoskeleton is not very permeable, thus the spiders' opisthosoma was first perforated with a needle to allow the fixative to permeate better into the body. All body parts were fixed in case they were necessary for subsequent study or confirmation of the species identification. Samples were stored in a refrigerator at 4°C to slow down the decay process and give the fixative time to work. After 24–48 hours the body was cut with a razor blade in the pedicel region (i.e. between the prosoma and opisthosoma) and in transverse section across the posterior end of the opisthosoma. This also facilitated the fixative entering the body. An additional sagittal cut was also made along the long axis of the opisthosoma. After three days, fixation was complete and the original solution was replaced by 70% ethanol. Specimens destined for histological study underwent further fixation (see below), while for SEM work the fixation process was here complete. All specimens, and parts thereof, were stored in the refrigerator.

Semi-thin sections. For histology excess tissue was removed from the lung region, since smaller samples are better suited for subsequent fixation and embedding. Specimens were washed three times in washing buffer solution and then incubated in screw top-specimen jars in a fume cupboard for 2–3 hours using 2% osmium tetroxide (OsO_4) as a fixative. Afterwards, samples were washed twice for ca. 10 minutes in washing buffer solution to remove any remaining OsO_4. Specimens were subsequently embedded in the epoxy resin araldite to give them the necessary stability for sectioning. First, any water remaining in the samples was removed by dehydration in increasing concentrations of alcohol which was finally replaced with propylene oxide (Epoxypropan). Final embedding in araldite using standard techniques took place a day later. In brief, warm (45°C) araldite was placed in a desiccator for ca 20 minutes to extract any air bubbles. Samples were placed in moulds, covered in the prepared araldite and warmed to 60°C. After ca. 30 minutes the position of the samples in the moulds was checked and after 3–4 hours the araldite was viscous enough that the samples maintained their position. The temperature was raised to 70°C, the araldite hardened completely in ca. 12–16 hours, and the embedded samples could then be removed from their moulds.

Semi-thin sections were made using standard histological techniques on a Leica Ultracut UCT ultramicrotome, with glass knives derived from a Leica EM KMR 2 device. The araldite block containing the sample was trimmed using a file and razor blade to remove excess plastic before being mounted on the microtome. The glass knife was angled at 6° to the object and a series of 1.5 μm thin sections was created automatically by the microtome and transported directly into an adjacent water bath. The

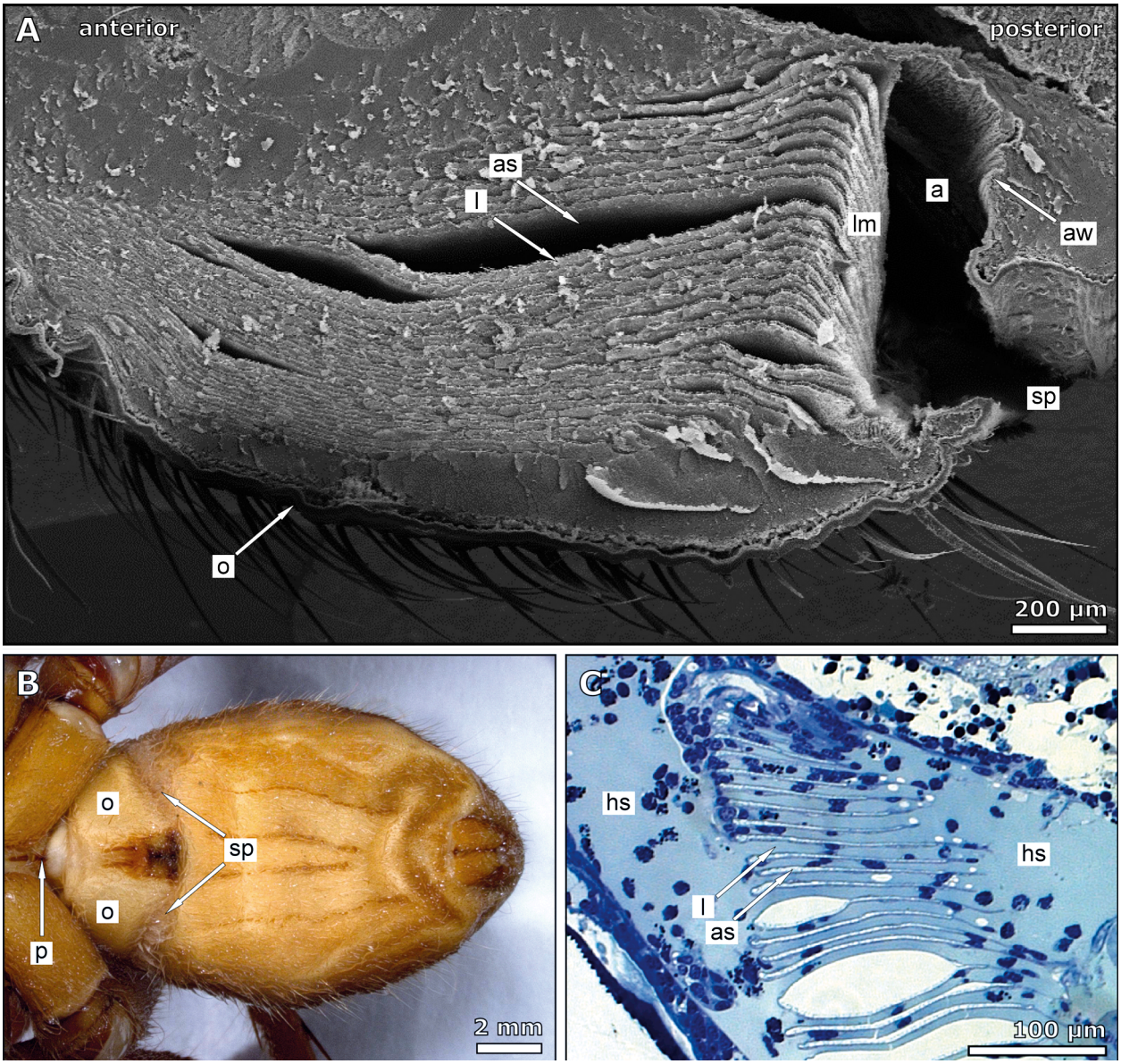

Fig. 2. Aspects of book lungs. **A**: SEM (sagittal fracture) of a book lung of *Gorgyrella* sp. (compare with Fig. 1). **B**: Position of book lungs in the opisthosoma of *Heteropoda venatoria*. **C**: Histological transverse section through a book lung of *Grammostola rosea* with partly inflated distal air spaces (as). — ***Abbreviations***: a: atrium, aw: atrium wall, hs: haemolymph sinus, l: lamella, lm: lamellar margin, o: operculum, p: petiolus, sp: spiracle.

resulting sections were transferred onto a slide with a fine paintbrush. Once 20 sections were in position, the slide was placed on a Leica HI 1220 hotplate at 70°C where the evaporation of the water flattened any raised parts of the sections and attached them firmly to the slide. Specimens were stained using methylene-blue-azure II which was generously pipetted onto the slide before it was again placed on the hotplate at 70°C for 2 minutes. Excess stain was washed off with distilled water, the slides were dried on the hotplate, and a coverslip was attached using Histokit. The histological preparations were studied using an Axioskop 2 plus light microscope and digital photographs were taken with an AxioCam HRC device.

Scanning Electron Microscopy (SEM). Specimens for SEM were trimmed under a stereomicroscope to remove unnecessary tissue around the book lung. The lung was then cut twice in a sagittal (i.e. anterior–posterior) plane near the median and lateral regions of the spiracle opening. In other words, the lines of section were perpendicular to the long axis of the slit-like spiracle. It was found to be better to section the lungs when still wet from storage in alcohol as they become fragile and tend to crumble once they have dried, which makes a controlled cut difficult. Water was removed prior to critical point drying by placing the specimens (twice for 20 minutes) in successively more concentrated solutions of ethanol. Critical point drying was carried out with a BAL-TEC 030 device using CO_2 to extract any remaining fluid, a method which prevents the tissue from shriveling. Lung fragments were attached in the desired orientation to SEM stubs and then sputter-coated with gold – including rotation of the sam-

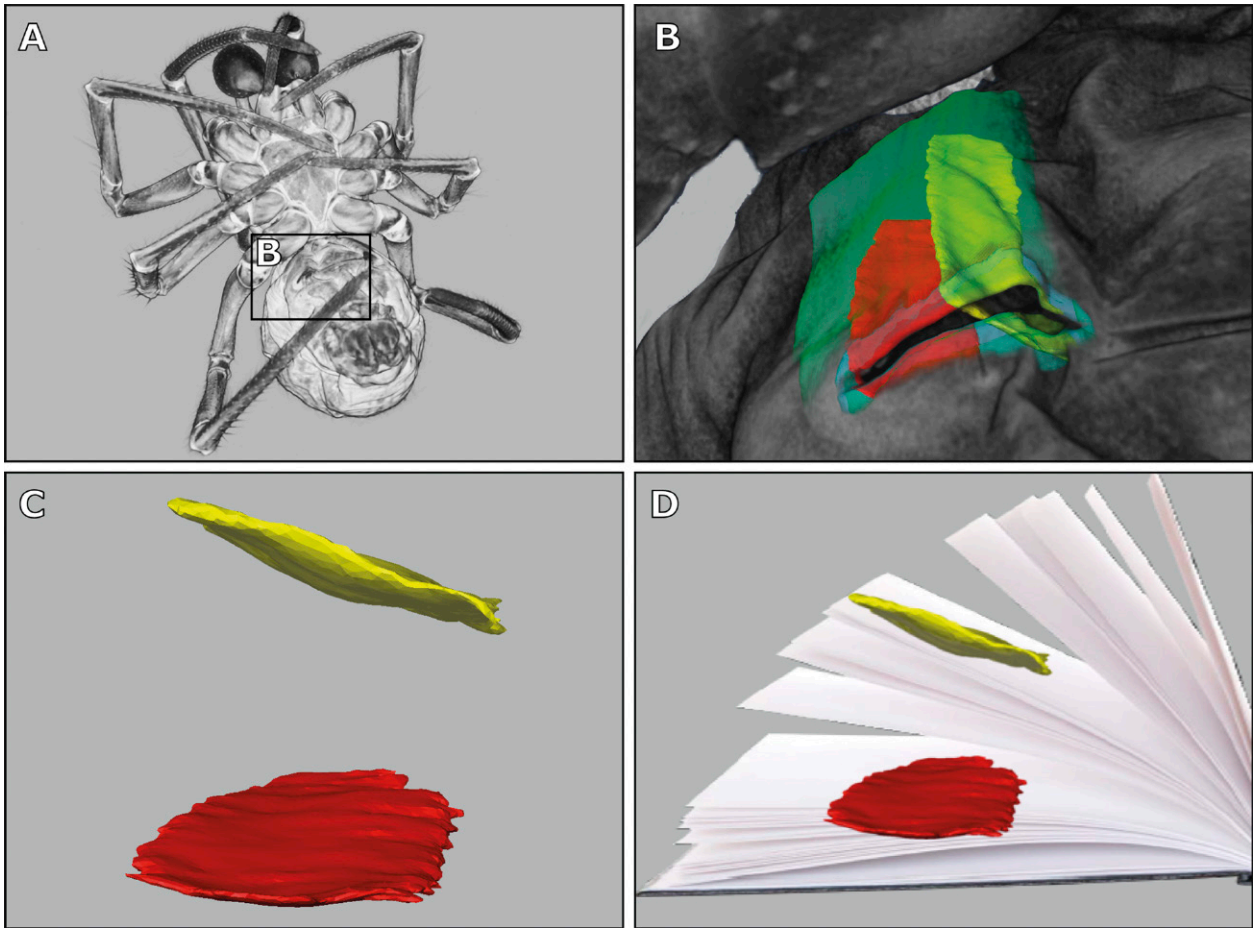

Fig. 3. Spatial relationships of book lungs and the lamellae based on a µCT-scan of *Parasteatoda tepidariorum*. **A**: Overview, the frame marks the position of the right book lung seen in B. **B**: The outline (green), the spiracle (blue), and a median (yellow) and a lateral (red) lamella of the right book lung. **C**: The two lamellae of B isolated. **D**: The lamellae mounted onto the pages of an open book.

ple to achieve a uniform coating – for three minutes using a BAL-TEC SCD 005 device. The specimens were then examined and digitally photographed using a LEO 1450 VP (Zeiss) scanning electron microscope. In some cases it was also possible to use the moulted skin (exuvia) of a spider for SEM, as these cuticle-lined organs are also shed during moulting. These are marked with an 'E' in Table 1. Exuviae for study were dried for three days in a desiccator with silica gel to remove any remaining water and then dissected, mounted and sputter-coated as above. Unless stated otherwise, adult animals were used for SEM.

Micro-Computed Tomography (µCT). In order to obtain information about the *in situ* position of the book lungs, and the orientation of the lamellae in life, a µCT scan of *Parasteatoda tepidariorum* (Theridiidae) (see HOFFMANN 2014) was used. For technical details see SCHOLTZ & BRENNEIS (2016). Applying the 3D reconstruction software Amira, the visible margins of the lungs and their lamellae could be delimited and marked in the individual images and then transformed into a three-dimensional model.

Deposit of material. All material of this investigation including histological sections, SEM-preparations, and preserved animals will be stored in the collection Arachnida and Myriapoda of the Museum für Naturkunde, Leibniz Institute for Evolution and Biodiversity Science, Berlin.

3. Results

Gross morphology. The book lungs of all spider species studied here are similar in basic construction. Spiders thus express a gross morphology consistent with the ground pattern given in the Introduction (Fig. 1). Despite their greater diversity in terms of species number, it is fair to say that spider lungs are more homogeneous than the lungs of scorpions (cf. KAMENZ & PRENDINI 2008). However, some differences in the cuticular fine structures (e.g. distal trabeculae, lamellar margins and the atrium wall) were observed among the material available and these are elaborated below. In overview, spider book lungs are situated in the anterior ventral region of the opisthosoma (Fig. 2). Each lung is covered by a more or less elliptical, plate-like operculum and opens through a narrow spiracle along the posterior margin of this plate. The spiracles are largely perpendicular to the

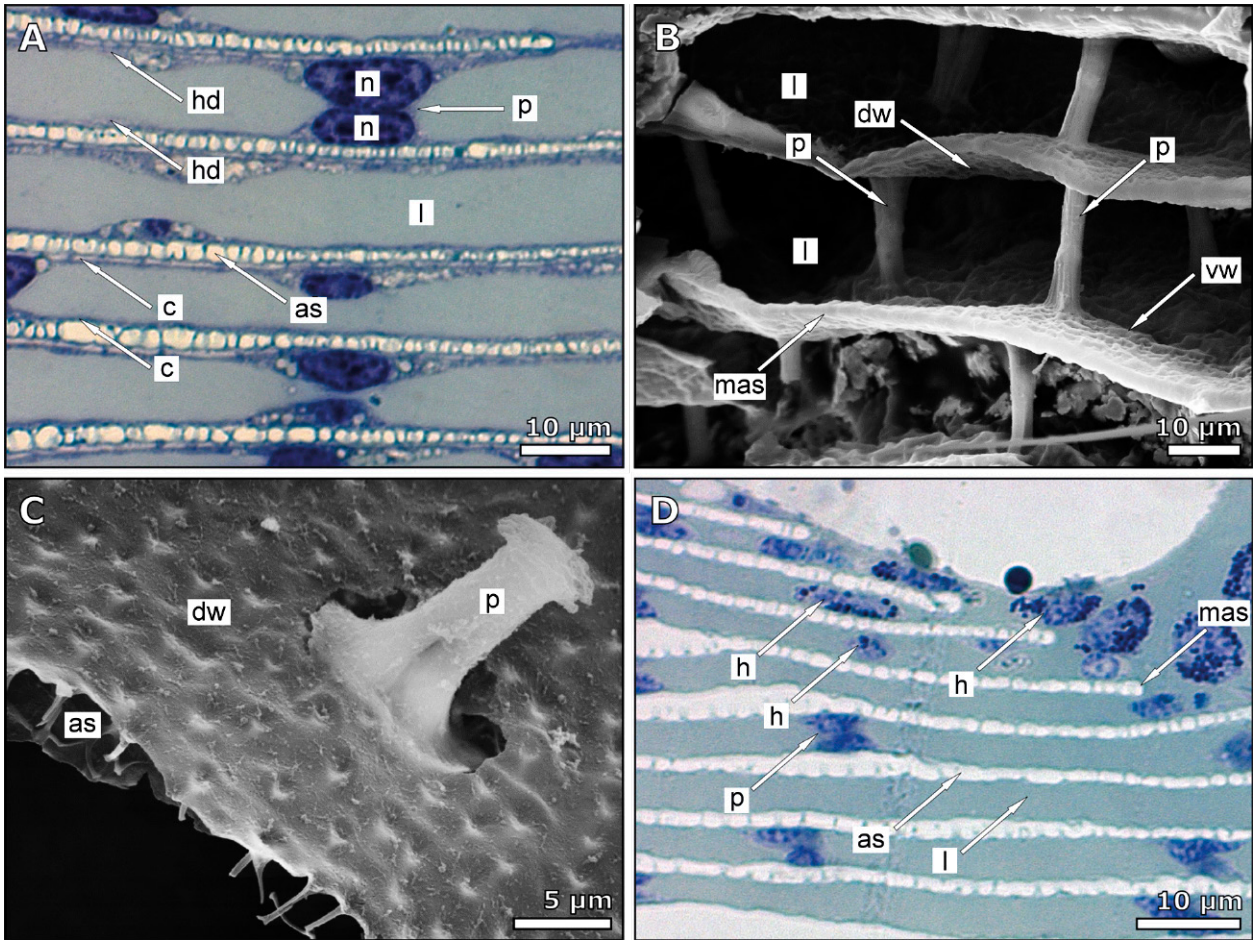

Fig. 4. The morphology of the lamellae. **A**: Histological transverse section of several lamellae (l) of the proximal region of a book lung of *Grammostola rosea*. **B**: SEM micrograph (fracture) of lamellae (l) of *Stasimopus* sp. **C**: SEM (fracture) of one lamella (ventral view with the ventral wall removed) of *Stasimopus* sp. **D**: Histological transverse section of several lamellae (l) in the distal region of a book lung of *Pholcus phalangioides*. — ***Abbreviations***: as: air space, c: cuticle, dw: dorsal wall of lamella, h: granular haemolymph cell, hd: hypodermis, mas: margin of air space, n: nucleus of pillar cell, p: pillar, vw: ventral wall of lamella.

long axis of the body and open into an atrium. A large number of blind-ending sacs project anteriorly from this atrium into a large haemolymph sinus, forming a series of haemolymph-filled lamellae separated by the air sacs. The posterior margins of the lamellae, i.e. those facing into the atrium, form the lamellar margins and the atrium itself is delimited by the atrium wall.

Orientation of the lamellae. The *in situ* position of the lamellae was studied in *Parasteatoda tepidariorum* (Theridiidae) using µCT (Fig. 3). The orientation of the individual lamellae is largely dependent on their position within the lung, which can be visualized as an 'open book' with its pages all at slightly different angles (Fig. 3). In detail, the lamellae in the middle of the lung are orientated at an angle of about 45° relative to the spiracle, while the angle towards the lateral margins of the lungs is successively reduced down to about 20°. This change is due to the height of the air spaces increasing from a medial to a lateral position. All these orientations are relative to the slit-like spiracle. Since the spider's opisthosoma is rounded and tapers anteriorly towards the pedicel, the spiracles of at least the anterior book lung pair may be drawn somewhat up the sides of the opisthosoma, with the consequence that the lamellae in transverse section may express a largely horizontal orientation. The stacked lamellae maintain a constant distance from the rounded body wall. For this reason each lamella is slightly offset compared to the next one. The height of the lamellae remains constant (Fig. 3).

Pillar cells. Dorsally and ventrally each lamella has a thin layer of epithelial cells, the hypodermis (Foelix 2011) (Fig. 4), which is covered by a thin cuticle which separates the haemolymph space from the air space (Fig. 4). All spiders studied here have epithelial pillar cells (*sensu* Reisinger et al. 1991) within the lamellae. These are composed of at least two cells projecting from the hypodermis on each side of the lamella which meet in the middle and form a solid unit which presumably holds the lamellar walls in a certain distance (Fig. 4). The cells are narrowest at their contact point in the middle and in the histological preparations each cell reveals pale cytoplasm and a more darkly stained nucleus (Fig. 4A,D). No significant differences in the morphology of the pillar cells was observed among the species studied which

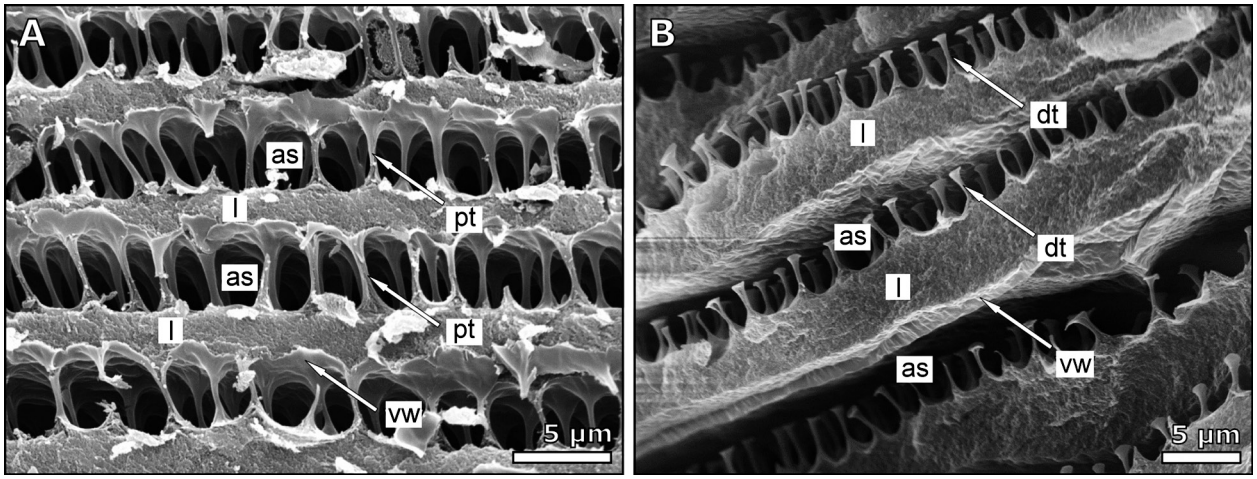

Fig. 5. SEM micrographs of sagittal fractures of the book lung of trabeculae of *Aculepeira ceropegia*. **A**: Anterior region with proximal trabeculae (pt). **B**: Posterior region with distal trabeculae (dt). — ***Abbreviations***: as: air space, l: lamella, vw: ventral wall of lamella.

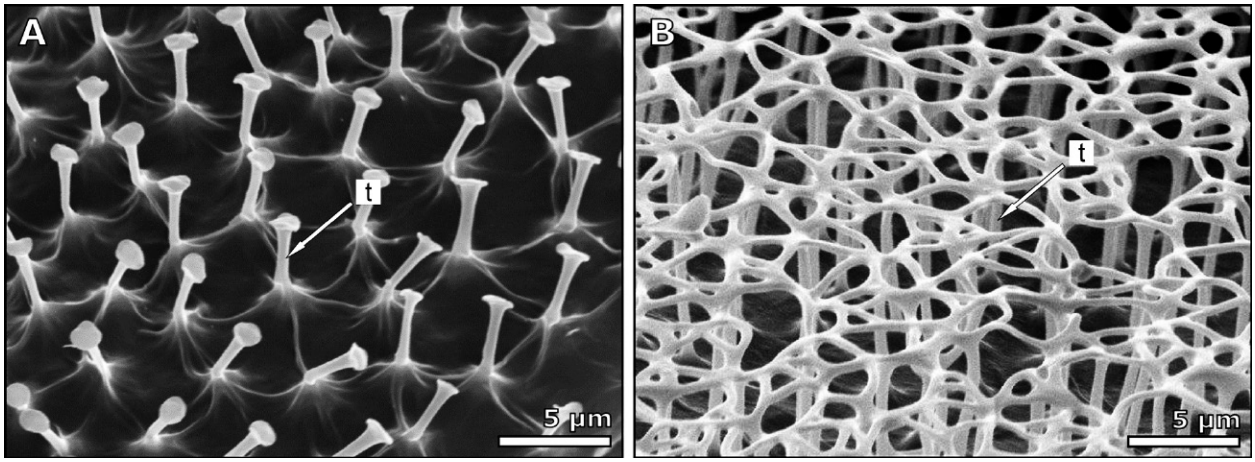

Fig. 6. SEM micrographs of the character states of the trabeculae. **A**: Pilate trabeculae (t) of *Nephila* sp. **B**: Reticulate trabeculae (t) of *Grammostola rosea*.

suggests that they are uniform across Araneae. The histological preparations also revealed granular haemocytes (FOELIX 2011) as irregular haemolymph cells containing darkly stained granular areas (Fig. 4D).

Trabeculae. In all spiders studied here, the dorsal side of each lamella bears cuticular trabeculae which project up into the air space (Figs. 1, 5). As the pillar cells (see above) stabilize the haemolymph space, so trabeculae maintain a gap in the air space and prevent adjacent lamellae from collapsing against one another. The ventral surface of the lamellae in all the spiders studied here is smooth and lacks any fine structure. As noted above, the height of the air spaces increases from medial to lateral parts of the lungs. The height of the air spaces does not increase from the anterior part of the lung moving towards the (posterior) atrium, however there is a doubling of the height of the lamella. For example in *Aculepeira cerupegia* (Araneidae), the air spaces are consistently ca. 5 µm high, but the lamellae – containing the haemolymph – double in height from 2.5 µm at the anterior end to 5 µm at the posterior end.

We can also recognise a proximal lamellar area as the anterior and median part of an individual book lung lamella, and a distal lamellar area as its posterior and lateral parts. In this scheme we can also refer to **proximal** and **distal trabeculae** respectively (Figs. 1, 5). The proximal trabeculae – also referred to as bridging trabeculae (SCHOLTZ & KAMENZ 2006) – span the air spaces more or less perpendicular to the lamellar surface and are firmly attached both dorsally and ventrally to the cuticle sheets of the lamellae. These column-like trabeculae are thinnest in the middle, but widen noticeably as they approach the lamellar surfaces (Fig. 5A). These proximal trabeculae were similar in all the material studied and thus, like the pillar cells, are probably uniform across Araneae.

Distal trabeculae emerge from the dorsal surface of the lamellae and project into the air space (Fig. 5B). They become thinner from the base, but unlike the proximal trabeculae they end free in the lumen of the air space and do not attach to the opposite wall. The air space does, however, become thinner in an anterior direction up to the point that the distal trabeculae are replaced by proximal (bridging) trabeculae. The transition from proximal

to distal trabeculae can be observed in histological preparations; especially those in which insufficient fixation has inflated the air spaces (Fig. 2). This artefact is only visible in the distal area where the trabeculae are free at the tip, and not in the proximal area where the trabeculae are connected to both sheets of cuticle. Furthermore, in SEM preparations the distal areas of the air spaces can be finely teased apart with a needle, but attempting this in the proximal area damages the connected sheets of cuticle.

Significantly, the distal trabeculae of spiders occur in two quite distinct morphologies. For these we propose two alternative character states. (1) **Pilate trabeculae** (Fig. 6A) are free-standing structures with a star-shaped base which project upwards as narrow and slightly tapering columns, ending in a variable (but often flattened and disc-like) apical terminus. By contrast, (2) **reticulate trabeculae** (Fig. 6B) are similar in having a star-shaped base again projecting up into a column, but here the apical tips branch out and connect to branches from adjacent trabeculae to form an irregular network sheet within the air space. Among the taxa sampled (Table 1), reticulate trabeculae were only found in mygalomorph spiders.

Lamellar margins. The lamellar margins form the posterior border of the individual lamellae going into the atrium and connect the dorsal and ventral lamellar walls (Figs. 1, 2). In transverse section a thickening of the haemolymph space towards the lamellar margins is visible and cellular structures such as granular haemocytes are found more often. The surface of the lamellar margins is invariably ornamented with cuticular projections which can vary in their structure between different taxa (see below). These marginal structures extend as a band – whose width varies between species – onto the dorsal surface of the lamellae and the transition into the typical dorsal trabeculae here can be either smooth or sudden. In most cases the marginal structures also extend briefly onto the (otherwise smooth) ventral surface of the lamellae. In a few cases the marginal structures gradually disappear ventrally or as in *Gorgyrella* sp. (Idiopidae) extend for some distance across the ventral lamellar surface.

We recognise three character states for the lamellar margins. (1) **Echinate** margins express a series of spiny projections; all of about the same length, but noticeably longer than wide (Fig. 7A). (2) **Arbuscular-reticulate** margins are connected, trabeculae-like structures in which column-shaped projections narrow towards their tips, where they then branch out to either form arc-shaped networks or end freely. The lamellar margins are covered dorsally and ventrally, but may be free in places (Fig. 7B). (3) **Arbuscular-reticulate-echinate** consist of a smaller number of robust thorns pointing into the atrium with fine, arc-shaped branches creating a network between adjacent thorns (Fig. 7C). *Liphistius* sp. (Mesothelae: Liphistiidae) expresses echinate margins, but most of the spiders studied (Table 1) have arbuscular-reticulate margins. Uniquely among the species available for SEM study, *Cupiennius salei* (Ctenidae) has arbuscular-reticulate-echinate lamellar margins. The same morphology was also detected in the closely related species *Cupiennius getazi* (Küntzel 2014).

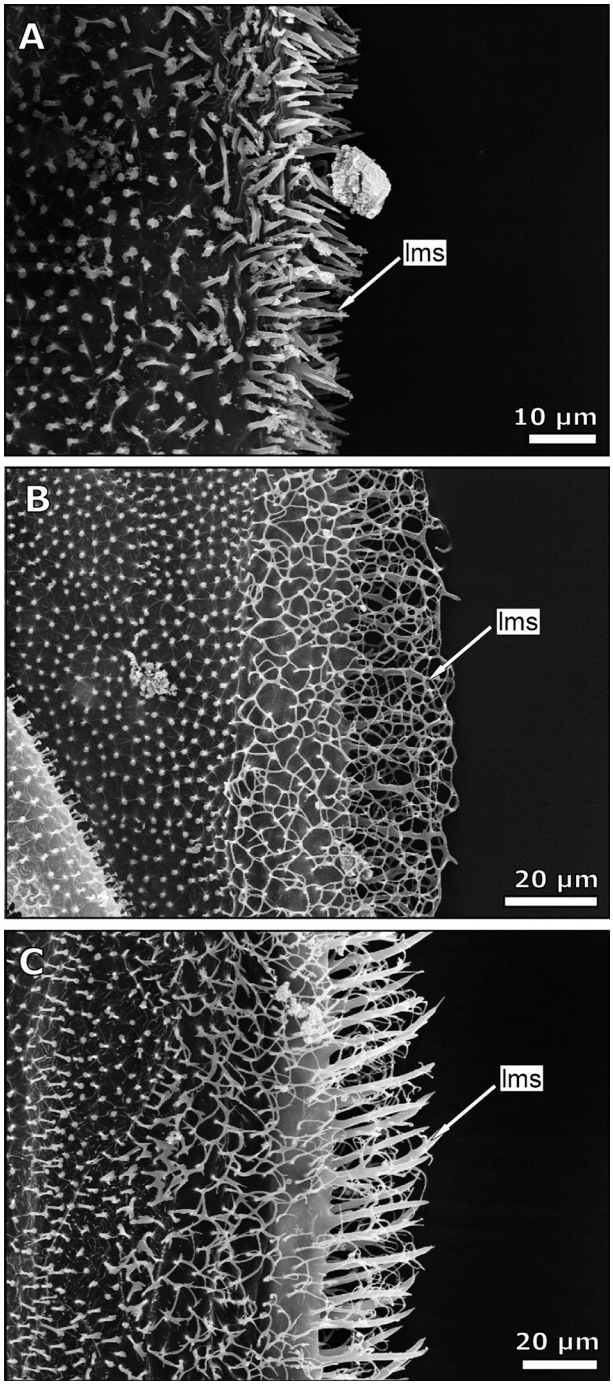

Fig. 7. SEM micrographs of the character states of the lamellar margin, dorsal views. **A**: Echinate (*Liphistius* sp.). **B**: Arbuscular-reticulate (*Hogna inominata*). **C**: Arbuscular-reticulate-echinate (*Cupiennius salei*). — ***Abbreviation***: lms: structures of the lamellar margin.

Atrium wall. Both the histological sections and the SEM results reveal the atrium wall as a thin undulating or folded cuticular structure (Figs. 1, 2). The surface of the atrium facing into the air space can show a variety

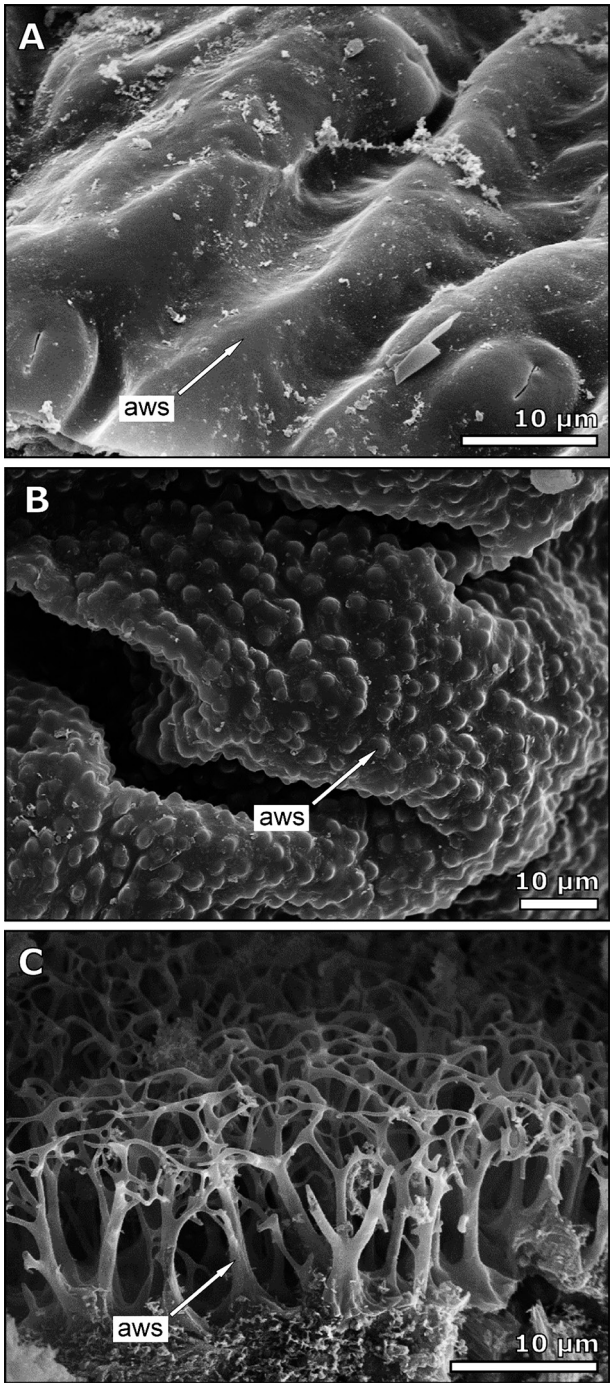

Fig. 8. SEM micrographs of the character states of the atrium wall. **A**: Psilate (*Gorgyrella* sp.). **B**: Verrucate (*Liphistius* sp.). **C**: Arbuscular-reticulate (*Xysticus*). — ***Abbreviation***: aws: structures of the atrium wall.

of morphologies. We recognize three character states. (1) A **psilate** atrium wall is smooth without any visible fine structure (Fig. 8A). (2) A **verrucate** atrium wall is densely ornamented with small, wart-like projections, widest at their bases (Fig. 8B). (3) An **arbuscular-reticulate** atrium wall bears connected networks of trabeculae-like structures, the column-shaped projections branching out apically and almost invariably connected to adjacent branches (Fig. 8C). Most of the mesothele and mygalomorph spiders express either psilate or verrucate atrium walls (Table 1), and the atrium of *Hypochilus thorelli* (Hypochilidae) is also psilate. By contrast all of the remaining spiders studied have an **arbuscular-reticulate** atrium wall.

Fine structures related to growth stadia. To test whether the lung character states identified above are stable across juvenile and adult spiders, a number of species were chosen for which multiple postembryonic instars were available. Body length (BL) was measured from the front of the prosoma to the end of the opisthosoma, excluding the spinnerets. We examined five instars of the theraphosids *Brachypelma albopilosum* (BL 13, 24, 33, 46 and 62 mm (adult)) and two of *Theraphosa blondi* (BL 21 and 82 mm (adult)), as well as two instars of the sparassid *Heteropoda maxima* (BL 8 and 25 mm). In *B. albopilosum* and *T. blondi* the older (i.e. larger) animals had a higher number of apically networked reticulate trabeculae. However, in none of the three species studied were there any significant differences in the prominence of the trabeculae or the morphology of the atrium wall.

The lamellar margins of both instars of *H. maxima* are identical, but in the two tarantulas some differences between older and younger animals could be observed. In particular, we observed differences in the branching structures from the lamellar margins. In some areas these form fully developed networks, in others they only partly branch, and in some cases there are areas with only very short, pointed projections. Different animals within an ontogenetic sequence may show differences in the extent to which the margins of the lamellae express areas with these three patterns of marginal ornamentation; although it should be added that these areas merge smoothly into one another.

Fine structures related to body size. Finally, to test whether body size differences between adults of closely-related species influence the character states we selected three groups for which small and large species were available. From Sparassidae we used *Micrommata virescens* (BL 12 mm), *Heteropoda venatoria* (BL 17 – 34 mm) and *H. maxima* (BL ca. 46 mm; see also JÄGER 2001). From Pisauridae we used *Pisaura mirabilis* (BL 13 mm) and *Dolomedes okefinokensis* (BL 32 mm). From Lycosidae we used *Trochosa terricola* (BL ca. 10 mm) and *Hogna inominata* (BL ca. 25 mm).

Unsurprisingly, larger spiders have larger book lungs both in terms of the length and width of the lamellae, and in the total number of lamellae. Among other species examined, the jumping spider *Marpissa radiata* (Salticidae) with a body length of 7 mm has about 30 lamellae in each lung. By contrast, the giant tarantula *Theraphosa blondi* with a body length of 82 mm has more than 200 lamellae in each lung (NK, pers. obs.). The size and prominence of the lung's fine structures does appear to be influenced by body size. For example, the spines on the lamellar margins in the pisaurid *Dolomedes okefinokensis* are about twice as large, and noticeably more robust at the base, compared to those in its smaller rela-

tive *Pisaura mirabilis*. A similar pattern is observed for the structures on the atrium wall. In general, trabeculae of larger spiders tend to be thinner and more elongate; for example comparing the sparassids *Micrommata virescens* and the larger *Heteropoda venatoria*. In species with pilate trabeculae the knob at the tip of each projection tends to be more prominent, enlarged and disc-like. However, there is no difference in the basic structures of the lungs between closely related large and small species. In other words, the character states identified in Table 1 for the trabeculae, marginal spines and atrium wall are not size-dependent, neither within nor between species.

4. Discussion

General morphology. The present study largely confirms previous observations about the basic structure of the spider book lung (e.g. Kaestner 1929). All have ventro-lateral spiracles opening into an atrium with folded walls from which air spaces project, separated by stacked lamellae within a haemolymph sinus. The exact number and shape of the lamellae is influenced by the size of the animal and the shape of its opisthosoma respectively. All spiders have an anterior and medial lamellar area bearing proximal trabeculae. These proximal trabeculae attach to both sides of the adjacent lamellae and span the air space. They were previously reported from *Eurypelma californicum* by Reisinger et al. (1991) – now probably *Aphonopelma hentzi* (see Nentwig 2012) – as well as *Liphistius trang* und *Araneus diadematus* by Scholtz & Kamenz (2006), and were also figured by Felgenhauer (1999). Furthermore, all of the species studied here as histological preparations revealed pillar cells within the lamellae; again previously figured by Reisinger et al. (1991) and Scholtz & Kamenz (2006) in the species noted above, as well as in *Tegeneria* sp. by Foelix (2011). Trabeculae and pillar cells maintain a constant distance within the air and haemolymph spaces respectively. In *Atypus piceus* and *Cupiennius getazi* differences in the height of the lamellae and the air spaces were observed. However, these are probably artefacts of fixation and/or preparation.

As well as the features noted above which are common to all the studied spiders, we recognise several character states (Table 1, Fig. 9) relating to the fine structure of the lungs. Distal trabeculae can be pilate (1) or reticulate (2). Lamellar margins can be echinate (1), arbuscular-reticulate (2) or arbuscular-reticulate-echinate (3). The atrium wall can be psilate (1), verrucate (2) or abuscular-reticulate (3). Several of these character states have been previously mentioned in the literature, and to facilitate comparative studies we summarize alternative names used by earlier authors in Table 2. We should note that some of these terms, such as interlamellar hairs, seem less appropriate as the structures in question are neither hairs nor setae. Some confusion with regards to translation of German terms is also possible, thus we suggest adopting the categories listed above which derive directly from the observed morphology.

The relative size of these fine structures is to some extent dependent on the size of the animal. Lager spiders not only have larger lungs with more lamellae, the fine structures can be more than twice the size of the corresponding structures in smaller species. However, it is important to stress that larger species do not differ qualitatively from smaller ones and the categories listed in Table 1 were recognisable in all the spiders studied, and appear to be generally size-independent. That said, for those taxa where ontogenetic series were available some differences in the fine structure of the lamellar margins were observed between different instars. This should be borne in mind in future studies where it may be helpful to record whether data was derived from juvenile and/or adult specimens.

Trabeculae. Pilate trabeculae were observed here in the mesothele spider *Liphistius* sp. (Table 1), and were also recorded in the literature by Haupt (2003) in *Liphistius malayanus* und *Liphistius trang*. Pilate trabeculae were also observed in all araneomorph spiders studied here (Table 1). This distribution is further confirmed by diverse literature records for other araneomorphs (e.g. Kästner 1929; Peters 1929; Moore 1976; Hexter 1982; Hill 1977; Schmitz & Perry 2000); all of whom figured pilate trabeculae, albeit under a variety of different names (Table 2). By contrast, reticulate trabeculae were only observed in the mygalomorph spiders studied here (Table 1). Reticulate trabeculae in mygalomorphs were also reported in the literature, namely in the atypid *Atypus piceus* in Kästner (1929) and the theraphosids *Grammostola* sp. in Peters (1969) and *Eurypelma californicum* in Reisinger et al. (1990). The monophyly of the Mygalomorphae has been convincingly demonstrated in the literature based on morphological features such as loss of the anterior median spinnerets and reduction of the anterior lateral spinnerets; see e.g. Raven (1985) for these and other diagnostic characters. It was also recovered as monophyletic by Wheeler et al. (2017) and Fernández et al. (2018) in their comprehensive molecular spider phylogenies. Mygalomorphs thus represent one of the stable groups within spiders which Agnarsson et al. (2013) referred to as 'benchmark clades'. We propose that reticulate trabeculae could be an additional apomorphy for Mygalomorphae (Fig. 9A).

Lamellar margins. Echinate lamellar margins were observed here only in the mesothele spider *Liphistius* sp. (Table 1), and were also recognised by Haupt (2003: "spines") in *Liphistius malayanus* und *Liphistius trang*. By contrast, almost all of the opisthothele spiders examined here revealed arbuscular-reticulate margins. This is also supported by several records in the literature, including *Atypus piceus* and *Araneus diadematus* in Kästner (1929), *Grammastola* sp. in Peters (1969), *Argyroneta aquatica* in Moore (1976) and *Eurypelma californicum* in Reisinger et al. (1991). See again Table 2 for the di-

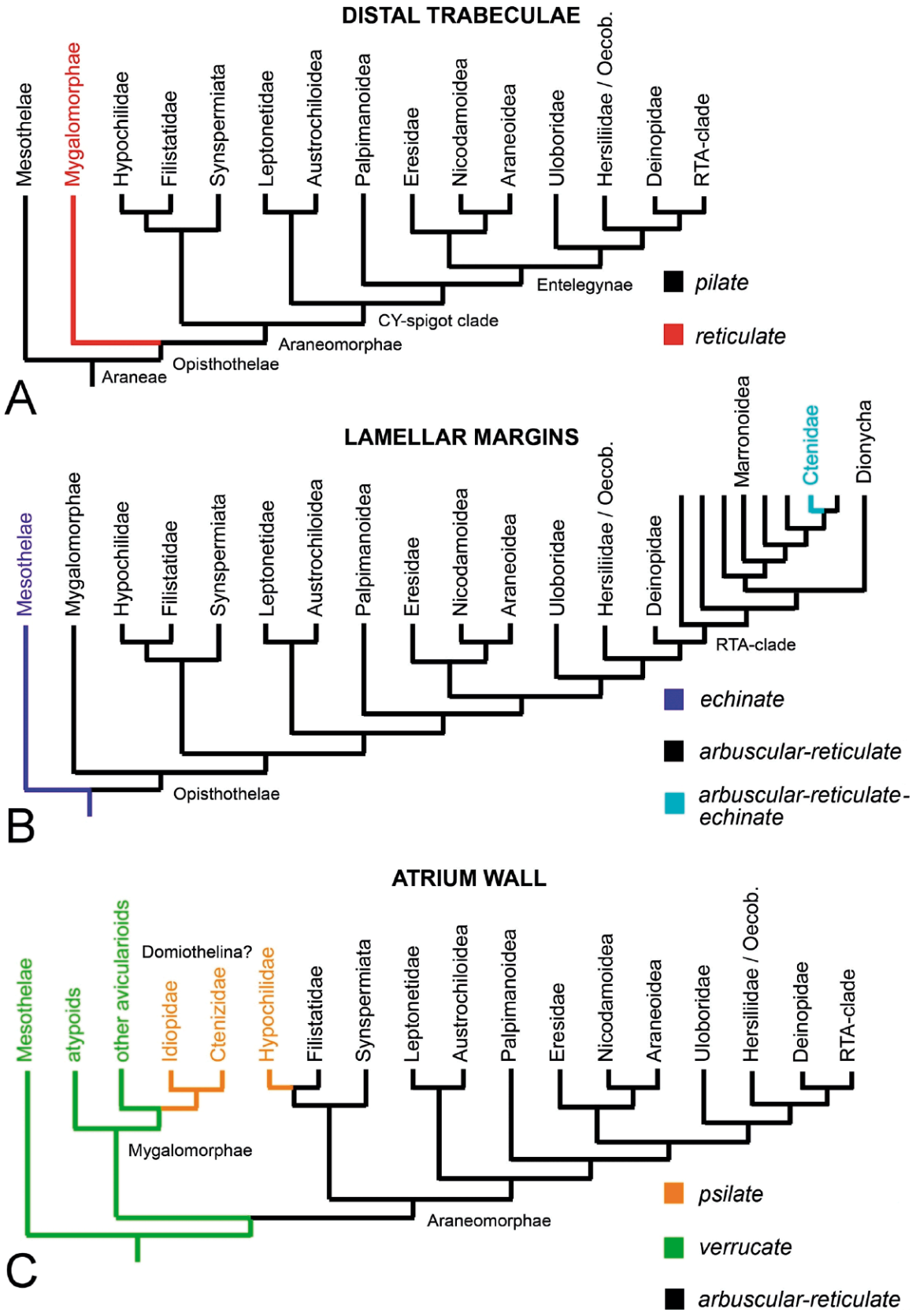

Fig. 9. Book-lung character-states mapped on consensus cladograms of Araneae, primarily after WHEELER et al. (2017) and FERNÁNDEZ et al. (2018). Individual groups mostly collapsed into their larger clades. **A**: Distal trabeculae: the reticulate condition is potentially apomorphic for mygalomorphs, all other spiders being pilate, which is also the ancestral condition for Araneae. **B**: Lamellar margins: the echinate condition is only seen in mesotheles. Based on out-group comparison this seems the ancestral state for Araneae. The arbuscular-reticulate condition is likely to be apomorphic for opisthotheles, with an additional arbuscular-reticulate-echinate condition in ctenids. **C**: Atrium walls: verrucate walls are seen in mesotheles and most mygalomorphs and this can be assumed to be the ancestral aranean state, psilate walls occur convergently in at least two mygalomorph groups (perhaps corroborating Domiothelina) and in hypochilids, and arbuscular-reticulate walls in all remaining spiders being an apomorphy of Araneomorphae.

Table 2. Alternative terminologies in the literature for the character states identified here for fine structures within the book lungs.

FINE STRUCTURE	ALTERNATVE NAME	SOURCE
distal trabeculae		
pilate trabeculae	"Stiftchen"	Kästner (1929); Peters (1969)
	nail-headed spigots	Moore (1976)
	buttressed studs	Hill (1977)
	interlamellar hairs	Hexter (1982)
	spike-like struts	Schmitz & Perry (2000)
	pedestals	Felgenhauer (1999)
	simple trabeculae	Kamenz (2009)
	internal spacers	Ramírez (2014)
reticulate trabeculae	"Stiftchen mit verbundenen Seitenzweigen"	Kästner (1929)
	networked trabeculae	Kamenz (2009)
lamellar margins		
echinate	spines, thorns	Kamenz (2009)
arbuscular-reticulate	"netzartig verflochtene Säulchen"	Kästner (1929)
	"Reuse"	Peters (1969)
	mesh surface filter	Moore (1976)
	network of irregular struts	Hill (1977)
	looping networks	Hexter (1982)
	branching and anastomosing arcuate bows	Reisinger et al. (1990); Kamenz (2009)
	mesh of cuticular extensions	Ramírez (2014)
arbuscular-reticulate-echinate	—	
atrium wall		
psilate	—	
verrucate	slightly grained warts	Reisinger et al. (1990)
arbuscular-reticulate	air filter	Moore (1976)
	network of irregular cuticular struts	Hill (1977)
	larger complex hairs	Hexter (1982)
	arcuate bows	Kamenz (2009)
	mesh of cuticular extensions	Ramírez (2014)

vergent terminologies used in these studies. SEM images of *Otagoa nova* (Desidae; images by Charles Griswold) and *Uliodon* cf. *frenatus* (Zoropsidae; images by Martín Ramírez) in the 'Morphobank' database also reveal this character state. Opisthothelae is another well-supported benchmark clade (see also Wheeler et al. 2017), with clearly defined morphological apomorphies such as loss of opisthosomal segmentation and posteriorly positioned spinnerets; see especially Platnick & Gertsch (1976). We suggest that arbuscular-reticulate lamellar margins are apomorphic for Opisthothelae and differentiate most living spiders from the mesotheles (Fig. 9B).

There are no obvious differences in the lamellar margin between the major clades of opisthothele spiders *sensu* Coddington (2005), Wheeler et al. (2017) or Fernández et al. (2018). Mygalomorphs and araneomorphs – and their subgroups – are essentially identical for this character. We should note that our definition of arbuscular-reticulate margins was formulated in such a ways as to encompass a range of morphologies from tree-like structures, fully branching at their apex, through to irregular, pointed projections. As noted in the Results, a degree of variability was observed in this character state which may be partly related to ontogeny (see also above). At the same time we do not consider this variation sufficient to warrant subdivision into further character states as these encompass a morphological gradient which could not be unequivocally defined and delimited into meaningful categories.

An intriguing exception is the Ctenidae, or wandering spiders, for which a third character state (arbuscular-reticulate echinate) could be recognised and explicitly defined in the two species studied. Whether this is functionally significant is unclear. Ctenids are fairly large spiders and active hunters which no longer spin webs for prey capture. An arbuscular-reticulate echinate lamellar margin might be an apomorphy of Ctenidae (Fig. 9B). Nevertheless, it would be interesting to test this character in closely related taxa from within the broader RTA-clade of spiders. In the Wheeler et al. (2017) tree, 'core Ctenidae' is the sister-group to a clade including Oxyopidae, Pisuaridae, Trechalidae and Lycosidae, while Fernández et al (2018) recovered two separate lineages of ctentids nested among pisaurids and lycosids. Three of these putative outgroups were examined in the present study (oxyopids, pisaurids and lycosids: Table 1) and all show the arbuscular-reticulate lamellar margins as the other opisthothele spiders.

Atrium wall. A psilate (i.e. smooth) atrium wall was not previously recognised in the literature. It was observed here (Table 1) in two mygalomorphs, *Gorgyrella* sp. (Idiopidae) and *Stasimopus* sp. (Ctenizidae) as well as in *Hypochilus thorelli* (Hypochilidae) (Fig. 9C). A verrucate atrial wall was seen in the mesothele *Liphistius* sp. and SCHOLTZ & KAMENZ (2006: p. 11) described the atrium wall in *Liphistius trang* as being "without any cuticular structure", albeit based only on histological data. These wart-like structures have a diameter of ca. 0.5 – 1.5 µm and may thus be difficult to resolve in a histological section which is only 3 µm thick. A verrucate atrial wall was also observed in representatives of the remaining mygalomorph spiders (Table 1) – i.e. taxa belonging to the Atypidae (Atypoidea) and Dipluridae, Cyrtaucheniidae, Nemesiidae and Theraphosidae (all Avicularioidea) – and can also be seen in REISINGER et al.'s (1990) SEM images of the theraphosid *Eurypelma californicum*. The verrucate wall thus occurs widely among mygalomorphs, but does not appear to support any of the currently recognised clades here. Interestingly, the corresponding psilate condition is restricted to representatives of Idiopidae and Ctenizidae and could help corroborate the putative mygalomorph clade Domiothelina (cf. GOLOBOFF 2003; CODDINGTON 2005). However, this character state would in this case be convergent with the condition in mesotheles (Fig. 9C) and we should caution that other authors have questioned the monophyly of Domiothelina (e.g. HEDIN & BOND 2006; WHEELER et al. 2017).

Within araneomorphs, arbuscular-reticulate structures on the atrium wall were found in all non-hypochilid spiders studied. Non-hypochilids were traditionally referred to as Neocribellatae (e.g. CODDINGTON 2005), although subsequent molecular phylogenies (see below) have not recovered this clade and usually recognise instead (Hypochilidae + Filistatidae), itself sister-group to a wider clade named Synspermiata, which encompasses many of the groups previously referred to as haplogynes (WHEELER et al. 2017; FERNÁNDEZ et al. 2018). The arbuscular-reticulate atrial structures have also been figured in the literature, for example from *Araneus diadematus* and *Segestria senoculata* in KÄSTNER (1929), *Argyroneta aquatica* in MOORE (1976), *Sicarius* sp. in RAMÍREZ (2014), and *Tegenaria* sp. in HEXTER (1982). Again, different authors used different names for these structures (Table 2). The presence of an arbuscular-reticulate atrium wall as an putative morphological apomorphy for Neocribellatae is significant given that several recent molecular analyses (e.g. AYOUB et al. 2007; AGNARSSON et al. 2013; BOND et al. 2014; GARRISON et al. 2016; WHEELER et al. 2017; FERNÁNDEZ et al. 2018) did not recover the traditional palaeocribellate/neocribellate split at the base of the araneomorph spiders; see AGNARSSON et al. (2013) for a discussion of what factors may underlie this result. Yet, based on the recent molecular analyses, the arbuscular-reticulate atrium appears as an apomorphy for Araneomorphae (Fig. 9C).

Comparing spiders with other arachnids. SCHOLTZ & KAMENZ (2006) inferred the homology of the book lungs across all pulmonate arachnids based on the shared presence of a spiracle leading into an atrium with a folded atrial wall and stacked lamellae associated with the vascular system. Further specific structures seen in the lungs of all arachnids are the proximal trabeculae, pillar cells within the lamellae and spines on the lamellar margins. These marginal spines were identified here in *Liphistius*, as the echinate character state (Table 1). Their presence supports the general hypothesis that lungs have a single origin (see also Arachnopulmonata *sensu* SHARMA et al. 2014), although the present work differs from SCHOLTZ & KAMENZ (2006) – who sampled only two spider species – in recognising further characters in the fine structure of the lamellar spines within Araneae as elaborated above.

To reconstruct the ground pattern of the spider lung, we can draw on whip spiders (Amblypygi) as an outgroup. These animals were interpreted by some authors (e.g. WEYGOLDT & PAULUS 1997) as the sister-group of Araneae, but see e.g. SHULTZ (1990, 2007) for an alternative view. The lungs of 16 species of whip spider were studied in the thesis of KAMENZ (2009), who described pilate trabeculae, echinate lamellar margins and a verrucate atrium wall. In other words, like the mesothele spider *Liphistius* (Table 1), they would score 1 1 2 for their book lung fine structures. We might also note that extinct arachnid order Trigonotarbida forms the sister-group of the Tetrapulmonata as the clade Pantetrapulmonata *sensu* SHULTZ (2007). Exceptionally preserved fossils of trigonotarbids from the Early Devonian (ca. 410 Ma) Rhynie chert include the oldest known book lungs which also clearly show pilate trabeculae and probably echinate lamellar margins (KAMENZ et al. 2008). This character distribution supports the hypothesis that the book lung of *Liphistius* reflects the ground pattern for Araneae in general (see Fig. 9). It would thus seem reasonable to score structures like reticulate trabeculae in mygalomorphs, arbuscular-reticulate lamellar margins in opisthotheles, and arbuscular-reticulate atrium walls in araneomorphs as apomorphic, and potentially diagnostic, character states (Fig. 9). According to this view, psilate atrium walls evolved convergently in hypochilids and in the lineage leading to ctenizids and idiopids (Fig. 9).

Scorpions can also express both pilate trabeculae and echinate lamellar margins (SCHOLTZ & KAMENZ 2006) as well as a verrucate atrium wall (KAMENZ 2009). This further suggests that the 1 1 2 (i.e. pilate trabeculae / echinate margins / verrucate atrium) character combination reflects the ground pattern both of (Pan)tetrapulmonata and perhaps of Arachnopulmonata in general. However, as noted above scorpions express a diversity of lung fine structures; even more so than that identified here for spiders. Relationships among the scorpions remain a source of controversy, but most phylogenies agree that the Buthidae is a basal lineage (e.g. STOCKWELL 1989; SOLEGLAD & FET 2003; KAMENZ et al. 2005; PRENDINI & WHEELER 2005). All buthids express proximal trabeculae similar to those of spiders and whip spiders and a verrucate atrium wall. However, instead of having distal trabeculae there

is a character of raised, anastomosing cuticular lines which Kamenz & Prendini (2008) termed "venation". If we accept trabeculae as part of the arachnid ground pattern then the replacement of distal trabeculae with venation in buthid scorpions would have to be interpreted as a derived character state for this group. See Kamenz & Prendini (2008) for further discussions, and further examples of morphological diversity among scorpions.

Finally, whip scorpions (Thelyphonida) present another complex situation. They have reticulate trabeculae (our state 2) on the lamellar surfaces, the lamellar margin structures can be echinate or arbuscular-reticulate (our states 1 and 2) or alternatively they have so-called 'spiny rhombuses', and a variety of conditions for the atrium wall are observed; including states termed arbuscular-reticulate, 'bristles', 'mushrooms' and 'networked mushrooms' (Kamenz 2009). It may be useful to compare this data with the phylogeny of whip scorpions and schizomids recently published by Clouse et al. (2017), but this is beyond the scope of the present study. Our working hypothesis is that the reticulate trabeculae of whip scorpions are convergent with those of mygalomorph spiders. Similarly, we presume that the arbuscular-reticulate atrium wall of whip scorpions is convergent with that of most araneomorph spiders.

The book lung paradox. The comparative studies on book lung fine structures in scorpions and spiders revealed substantial differences concerning the degrees of diversity between these two arachnid groups. Scorpiones comprise about 2,000 species whereas the number of Araneae is, with more than 48,000 species, nearly 24 times larger. Dunlop et al. (2008) counted the number of known arachnid fossil species from the Paleozoic to the Cenozoic. According to these authors, there are 111 fossil scorpion species and 979 fossil spiders. Thus, the species number of spiders is again much higher than that of scorpions. The fact that the ratio between spiders and scorpions measures only half of the Recent species diversity between the two groups might be due to the incompleteness of the fossil record. In stark contrast to this, the morphology of spider book lungs is rather homogeneous compared to that of scorpions. This is true for the shapes of the trabeculae, the structures at the distal lamella edges, and those of the atrium wall. Already the number of character states reflects this diversity (or disparity as some authors prefer).

Kamenz et al. (2006) and Kamenz & Prendini (2008) described four character states for trabeculae or lamellar surface (simple trabeculae, branched trabeculae, slender venation, ribbed venation), seven character states for the lamellar edge (bristles, spines, thorns, smooth/wrinkled, meandering, arcuate bows, padded), and twelve for the inner margin of the spiracle (hillocks, subconical, hairlike, flattened, scaly, chisel-like, hexagonal tiles, treelike, subtree-like, polygonal columns, clublike, spiked macelike). In contrast to this, for spiders we discriminate between two character states for trabeculae, three for the lamellar edge, and three for the atrial wall. Thus, the ratio between morphological diversity and species number differs greatly between the two groups. In fact the real figure might be even greater since it has to be stated that in a way character states simplify the matter. They have to be necessarily typological and do not reflect the 'real' diversity. As a character state structures are grouped together that have some characteristic features in common and minor differences have to be neglected. This is shown in the figures of Kamenz et al. (2006) and Kamenz & Prendini (2008) and also in those of this publication. Nevertheless, the number of character states is a useful proxy for the estimation of morphological differences in book lung morphology.

The estimated age of crown-group Araneae and Scorpiones shows a corresponding range (Wolfe et al. 2016) – anatomically modern groups of both orders appear in the late Carboniferous – and one can assume the extinction rate of scorpion species is similar to that of spider species. This means that spiders underwent a much higher rate of speciation than scorpions. At the same time, the radiation of spiders did not involve morphological diversification at a comparable pace. The resulting pattern are largely conserved book lung fine structures. Consequently, speciation in scorpions implies a more fundamental and rapid change of book lung morphology.

The reasons can be twofold. One can be sought in external factors leading to different adaptations; the other explanation could be based on internal structural constraints that led to less diversification in spiders. Both external and internal causes are difficult to evaluate. The diversity of lifestyles is difficult to compare. At first sight however, scorpions do not occupy a greater variety of habitats than spiders. Both groups are found in arid desert areas as well as humid rain forests. On the contrary, the range of scorpions is more restricted since spiders occupy moderate and cold climate regions in which scorpions do not exist. Hence, one would expect just the opposite pattern with a greater diversity of spider book lung structures. Yet, the adaptive value of the various character states is elusive. Likewise, internal factors leading to a conserved pattern in spiders and a variable in scorpions are problematic to consider. The gross morphology of the book lungs is very much alike between the two groups. Hence, the key to understanding morphological diversity must be looked for at a different place. We might also note that most spiders developed tracheae as an additional adaptation for terrestrial respiration (reviewed by Schmitz 2016), replacing in many species the second pair of book lungs. If spiders came to rely on their trachea as the primary means of gas exchange, there may have been less evolutionary pressure to modify the older – and now perhaps somewhat redundant – lung system.

Future perspectives. The present study was only able to sample part of the living spiders' diversity. Given that the lungs of all spiders studied so far have a similar gross morphology, histological methods are probably less useful in future. They are time-consuming and offer less information about the spatial orientation of structures in

three-dimensions. Scanning electron microscopy proved the more effective method to look for informative fine structures within the lungs, and could be applied fairly easily to other taxa. This would allow us to test the hypotheses proposed here relating to character states which appear to be apomorphies for major clades. For example, a study of the remaining mygalomorphs would allow us to check whether all of them have reticulate trabeculae. Examination of further putatively basal araneomorphs, especially Austrochilidae and Gradungulidae, could test the hypothesis that a psilate or arbuscular-reticulate atrium wall correlates to the traditional concepts of Paleocribellatae and Neocribellatae respectively, and thus to what extent lung morphology fits the latest molecular trees (e.g. WHEELER et al. 2017; FERNÁNDEZ et al 2018). The unusual lamellar margins found in wandering spiders (Ctenidae) show that unique morphologies can occur in smaller subgroups and wider taxon sampling may reveal additional specific morphological character states. All of the characters identified here (Table 1, Fig. 9) should be scored into future phylogenetic matrices.

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File 1: küntzel&al-spiderbooklungs-asp2019-electronicsupplement-1.pdf — **Fig. S1.** SEM; ***Liphistius* sp.** Schiödte, 1849 – Liphistiidae. **A** Trabeculae on lamella surface, dorsal view. **B** Atrium wall, anterior view. **C** Lamellar margin structures, posterior view. **D** Lamellar margin structures, dorsal view. — **Fig. S2.** SEM; ***Linothele megatheloides*** Paz & Raven, 1990 – Dipluridae. **A** Trabeculae on lamella surface, dorsal view. **B** Atrium wall, anterior view. **C** Lamellar margin structures, posterior view. **D** Lamellar margin structures, dorsal view. — **Fig. S3.** SEM; ***Acanthogonatus francki*** Karsch, 1880 – Nemesiidae. **A** Trabeculae on lamella surface, dorsal view. **B** Atrium wall, anterior view. **C** Lamellar margin structures, posterior view. **D** Lamellar margin structures, dorsal view. — **Fig. S4.** SEM; ***Brachypelma albopilosum*** Valerio, 1980 – Theraphosidae; body length 1.3 cm. **A** Trabeculae on lamella surface, dorsal view. **B** Atrium wall, anterior view. **C** Lamellar margin structures, posterior view. **D** Lamellar margin structures, ventral view. — **Fig. S5.** SEM; ***Brachypelma albopilosum*** Valerio, 1980 – Theraphosidae; body length 2.4 cm. **A** Trabeculae on lamella surface, dorsal view. **B** Atrium wall, anterior view. **C** Lamellar margin structures, posterior view. **D** Lamellar margin structures, dorsal

view. — **Fig. S6.** SEM; ***Brachypelma albopilosum*** Valerio, 1980 – Theraphosidae; body length 3.3 cm. **A** Trabeculae on lamella surface, dorsal view. **B** Atrium wall, anterior view. **C** Lamellar margin structures, posterior view. **D** Lamellar margin structures, dorsal view. — **Fig. S7.** SEM; ***Brachypelma albopilosum*** Valerio, 1980 – Theraphosidae; body length 4.6 cm. **A** Trabeculae on lamella surface, dorsal view. **B** Atrium wall, anterior view. **C** Lamellar margin structures, posterior view. **D** Lamellar margin structures, dorsal view. — **Fig. S8.** SEM; ***Brachypelma albopilosum*** Valerio, 1980 – Theraphosidae; body length 6.2 cm. **A** Trabeculae on lamella surface, dorsal view. **B** Atrium wall, anterior view. **C** Lamellar margin structures, posterior view. **D** Lamellar margin structures, dorsal view. — **Fig. S9.** SEM; ***Grammostola rosea*** (Walckenaer, 1837) – Theraphosidae. **A** Trabeculae on lamella surface, dorsal view. **B** Atrium wall, anterior view. **C** Lamellar margin structures, posterior view. **D** Lamellar margin structures, dorsal view. — **Fig. S10.** SEM; ***Theraphosa blondi*** (Latreille, 1804) – Theraphosidae; body length 2.1 cm. **A** Trabeculae on lamella surface, dorsal view. **B** Atrium wall, anterior view. **C** Lamellar margin structures, posterior view. **D** Lamellar margin structures, dorsal view. — **Fig. S11.** SEM; ***Theraphosa blondi*** (Latreille, 1804) – Theraphosidae; body length 8.2 cm. **A** Trabeculae on lamella surface, dorsal view. **B** Atrium wall, anterior view. **C** Lamellar margin structures, posterior view. **D** Lamellar margin structures, dorsal view. — **Fig. S12.** SEM; ***Atypus piceus*** (Sulzer, 1776) – Atypidae. **A** Trabeculae on lamella surface, dorsal view. **B** Atrium wall, anterior view. **C** Lamellar margin structures, posterior view. **D** Lamellar margin structures, dorsal view. — **Fig. S13.** SEM; ***Fufius* sp.** Simon, 1888 – Cyrtaucheniidae. **A** Trabeculae on lamella surface, dorsal view. **B** Atrium wall, anterior view. **C** Lamellar margin structures, posterior view. **D** Lamellar margin structures, dorsal view. — **Fig. S14.** SEM; ***Gorgyrella* sp.** Purcell, 1902 – Idiopidae. **A** Trabeculae on lamella surface, dorsal view. **B** Atrium wall, anterior view. **C** Lamellar margin structures, posterior view. **D** Lamellar margin structures, dorsal view. — **Fig. S15.** SEM; ***Stasimopus* sp.** Simon, 1892 – Ctenizidae. **A** Trabeculae on lamella surface, dorsal view. **B** Atrium wall, anterior view. **C** Lamellar margin structures, posterior view. **D** Lamellar margin structures, dorsal view. — **Fig. S16.** SEM; ***Hypochilus thorelli*** Marx, 1888 – Hypochilidae. **A** Trabeculae on lamella surface, dorsal view. **B** Atrium wall, anterior view. **C** Lamellar margin structures, posterior view. **D** Lamellar margin structures, dorsal view. — **Fig. S17.** SEM; ***Progradungula otwayensis*** Milledge, 1997 – Gradungulidae. **A** Trabeculae on lamella surface, dorsal view. **B** Atrium wall, anterior view. **C** Lamellar margin structures, posterior view. **D** Lamellar margin structures, dorsal view. — **Fig. S18.** SEM; ***Kukulcania hibernalis*** (Hentz, 1842) – Filistatidae. **A** Trabeculae on lamella surface, dorsal view. **B** Atrium wall, anterior view. **C** Lamellar margin structures, posterior view. **D** Lamellar margin structures, dorsal view. — **Fig. S19.** SEM; ***Pholcus phalangioides*** (Fuesslin, 1775) – Pholcidae. **A** Trabeculae on lamella surface, dorsal view. **B** Atrium wall, anterior view. **C** Lamellar margin structures, posterior view. **D** Lamellar margin structures, dorsal view. — **Fig. S20.** SEM; ***Loxosceles laeta*** (Nicolet, 1849) – Sicariidae. **A** Trabeculae on lamella surface, dorsal view. **B** Atrium wall, anterior view. **C** Lamellar margin structures, posterior view. **D** Lamellar margin structures, dorsal view. — **Fig. S21.** SEM; ***Gandanameno* sp.** Lehtinen, 1967 – Eresidae. **A** Trabeculae on lamella surface, dorsal view. **B** Atrium wall, anterior view. **C** Lamellar margin structures, posterior view. **D** Lamellar margin structures, dorsal view. — **Fig. S22.** SEM; ***Eriauchenius workmani*** O. P.-Cambridge, 1881 – Archaeidae. **A** Trabeculae on lamella surface, dorsal view. **B** Atrium wall, anterior view. **C** Lamellar margin structures, posterior view. **D** Lamellar margin structures, dorsal view. — **Fig. S23.** SEM; ***Haplodrassus* sp.** Chamberlin, 1922 – Gnaphosidae. **A** Trabeculae on lamella surface, dorsal view. **B** Atrium wall, anterior view. **C** Lamellar margin structures, posterior view. **D** Lamellar margin structures, dorsal view. — **Fig. S24.** SEM; ***Nomisia* sp.** Dalmas, 1921 – Gnaphosidae. **A** Trabeculae on lamella surface, dorsal view. **B** Atrium wall, anterior view. **C** Lamellar margin structures, posterior view. **D** Lamellar margin structures, detail. — **Fig. S25.** SEM; ***Cheiracanthium punctorium*** (Villers, 1789) – Miturgidae. **A** Trabeculae on lamella surface, dorsal view. **B** Atrium wall, anterior view. **C** Lamellar margin structures, posterior view. **D** Lamellar margin structures, dorsal view. — **Fig. S26.** SEM; ***Anyphaena accentuata*** (Walckenaer, 1802) – Anyphaenidae. **A** Trabeculae on lamella surface, dorsal view. **B** Atrium wall, anterior view. **C** Lamellar margin structures, posterior view. **D** Lamellar margin structures, transverse section, detail. — **Fig. S27.** SEM; ***Marpissa radiata*** (Grube, 1859) – Salticidae. **A** Trabeculae on lamella surface, dorsal view. **B** Atrium wall, anterior view. **C** Lamellar margin structures, posterior view. **D** Lamellar margin structures, dorsal view. — **Fig. S28.** SEM; ***Xysticus* sp.** C. L. Koch, 1835 – Thomisidae. **A** Trabeculae on lamella surface, dorsal view. **B** Atrium wall, anterior view. **C** Lamellar margin structures, posterior view. **D** Lamellar margin structures, ventral view. — **Fig. S29.** SEM; ***Thanatus coloradensis*** Keyserling, 1880 – Philodromidae. **A** Trabeculae on lamella surface, dorsal view. **B** Atrium wall, anterior view. **C** Lamellar margin structures, posterior view. **D** Lamellar margin structures, dorsal view. — **Fig. S30.** SEM; ***Selenops radiatus*** Latreille, 1819 – Selenopidae. **A** Trabeculae on lamella surface, dorsal view. **B** Atrium wall, anterior view. **C** Lamellar margin structures, posterior view. **D** Lamellar margin structures, dorsal view. — **Fig. S31.** SEM; ***Heteropoda maxima*** Jäger, 2001 – Sparassidae; body length 0.8 cm. **A** Trabeculae on lamella surface, dorsal view. **B** Atrium wall, anterior view. **C** Lamellar margin structures, posterior view. **D** Lamellar margin structures, dorsal view. — **Fig. S32.** SEM; ***Heteropoda maxima*** Jäger, 2001 – Sparassidae; body length 2.5 cm. **A** Trabeculae on lamella surface, dorsal view. **B** Atrium wall, anterior view. **C** Lamellar margin structures, posterior view. **D** Lamellar margin structures, dorsal view. — **Fig. S33.** SEM; ***Heteropoda venatoria*** (Linnaeus, 1767) – Sparassidae. **A** Trabeculae on lamella surface, dorsal view. **B** Atrium wall, anterior view. **C** Lamellar margin structures, posterior view. **D** Lamellar margin structures, dorsal view. — **Fig. S34.** SEM; ***Micrommata virescens*** (Clerck, 1757) – Sparassidae. **A** Trabeculae on lamella surface, dorsal view. **B** Atrium wall, anterior view. **C** Lamellar margin structures, posterior view. **D** Lamellar margin structures, dorsal view. — **Fig. S35.** SEM; ***Tegenaria atrica*** C. L. Koch, 1843 – Agelenidae. **A** Trabeculae on lamella surface, dorsal view. **B** Atrium wall, anterior view. **C** Lamellar margin structures, posterior view. **D** Lamellar margin structures, dorsal view. — **Fig. S36.** SEM; ***Cupiennius salei*** (Keyserling, 1877) – Ctenidae. **A** Trabeculae on lamella surface, dorsal view. **B** Atrium wall, anterior view. **C** Lamellar margin structures, posterior view. **D** Lamellar margin structures, dorsal view. — **Fig. S37.** SEM; ***Pisaura mirabilis*** (Clerck, 1757) – Pisauridae. **A** Trabeculae on lamella surface, dorsal view. **B** Atrium wall, anterior view. **C** Lamellar margin structures, posterior view. **D** Lamellar margin structures, dorsal view. — **Fig. S38.** SEM; ***Dolomedes okefinokensis*** Bishop, 1924 – Pisauridae. **A** Trabeculae on lamella surface, dorsal view. **B** Atrium wall, anterior view. **C** Lamellar margin structures, posterior view. **D** Lamellar margin structures, dorsal view. — **Fig. S39.** SEM; ***Trochosa terricola*** Thorell, 1856 – Lycosidae. **A** Trabeculae on lamella surface, dorsal view. **B** Atrium wall, anterior view. **C** Lamellar margin structures, posterior view. **D** Lamellar margin structures, dorsal view. — **Fig. S40.** SEM; ***Hogna inominata*** (Simon, 1886) – Lycosidae. **A** Trabeculae on lamella surface, dorsal view. **B** Atrium wall, anterior view. **C** Lamellar margin structures, posterior view. **D** Lamellar margin structures, dorsal view. — **Fig. S41.** SEM; ***Oxyopes lineatus*** Latreille, 1806 – Oxyopidae. **A** Trabeculae on lamella surface, dorsal view. **B** Atrium wall, anterior view. **C** Lamellar margin structures, posterior view. **D** Lamellar margin structures, dorsal view. — **Fig. S42.** SEM; ***Araneus diadematus*** Clerck, 1757 – Araneidae. **A** Trabeculae on lamella surface, dorsal view. **B** Atrium wall, anterior view. **C** Lamellar margin structures, posterior view. **D** Lamellar margin structures, dorsal view. — **Fig. S43.** SEM; ***Neriene radiata*** (Walckenaer, 1841) – Linyphiidae. **A** Trabeculae on lamella surface, dorsal view. **B** Atrium wall, anterior view. **C** Lamellar margin structures, posterior view. **D** Lamellar margin structures, dorsal view. — **Fig. S44.** SEM; ***Parasteatoda tepidariorum*** (C. L. Koch, 1841) – Theridiidae. **A** Trabeculae on lamella surface, dorsal view. **B** Atrium wall, anterior view. **C** Lamellar margin structures, transverse section. **D** Lamellar margin structures, dorsal view. — **Fig. S45.** SEM; ***Tetragnatha extensa*** (Linnaeus, 1758) – Tetragnathidae. **A** Trabeculae on lamella surface, dorsal view. **B** Atrium wall, anterior view. **C** Lamellar margin structures, posterior view. **D** Lamellar margin structures, dorsal view. — **Fig. S46.** SEM; ***Nephila* sp.** Leach, 1815 – Nephilidae. **A** Trabeculae on lamella surface, dorsal view. **B** Atrium wall, anterior view. **C** Lamellar margin structures, posterior view. **D** Lamellar margin structures, dorsal view. — DOI: 10.26049/ASP77-2-2019-05/1