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Morphology of the tracheal system of camel spiders (Chelicerata: Solifugae) based on micro-CT and 3D-reconstruction in exemplar species from three families

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A R T I C L E I N F O

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ABSTRACT

We studied the tracheal system of exemplar species representing three families of Solifugae Sundevall, 1833, i.e., *Galeodes granti* Pocock, 1903, *Ammotrechula wasbaueri* Muma, 1962 and *Eremobates* sp., using μ CT-imaging and 3D-reconstruction. This is the first comparative study of the tracheal system of Solifugae in 85 years and the first using high-resolution nondestructive methods. The tracheal system was found to be structurally similar in all three species, with broad major tracheae predominantly in the prosoma as well as anastomoses (i.e., connections between tracheal branches from different stigmata) in the prosoma and opisthosoma. Differences among the three species were observed in the presence or absence of cheliceral air sacs, the number of tracheae supplying the heart, and the ramification of major tracheae in the opisthosoma. The structure of the tracheal system with its extensive branches and some anastomoses is assumed to aid rapid and efficient gas exchange in the respiratory tissues of these active predators. The large diameter of cheliceral tracheae (air sacs) of taxa with disproportionally heavier chelicerae suggests a role in weight reduction, enabling solifuges to reach greater speeds during predation. The air sacs may also permit more rapid and efficient gaseous exchange, necessary to operate the musculature of these structures, thereby improving their use for predation in an environment where prey is scarce.

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1. Introduction

Terrestrial chelicerates (Arachnida) respire using book lungs, tracheae, or a combination thereof. Many authors regard book lungs as the plesiomorphic condition (Dunlop, 1997; Scholtz and Kamenz, 2006; Kamenz et al., 2008), whereas a tracheal respiratory system is considered derived. Tracheae evolved at least twice independently within Arachnida, regardless of which hypothesis for arachnid phylogeny is adopted (e.g., Weygoldt and Paulus, 1979; Wheeler and Hayashi, 1998; Giribet et al., 2002; Shultz, 1990, 2007; Pepato et al., 2010; Regier et al., 2010; Sharma et al., 2014), and

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probably more than once within acariform mites (Alberti and Coons, 1999). The independent evolutionary origins of tracheae in spiders and other tracheate arachnids (*sensu* Weygoldt and Paulus, 1979) is supported not only by their relative phylogenetic positions, but also by the morphological differences among their respective tracheal respiratory systems.

The tracheae of spiders open through a single stigma situated medially on the ventral surface of the opisthosoma, or through stigmata emerging from the atrium of the former book lungs (Schmitz, 2013, 2016). The tracheae may be simple or branched, with a brush-like appearance, but do not anastomose (Bromhall, 1987). Many spider taxa possess both tracheae and book lungs but, in some derived taxa, book lungs have been completely replaced by tracheae (e.g., Opell, 1998). The tracheae of most spiders float freely in the hemolymph and do not reach the tissues (Foelix, 2010; Strazny and Perry, 1987; Schmitz and Perry, 2000;







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Schmitz, 2016). Hemocyanins function as oxygen-carrying proteins that provide the convective transport of respiratory gases from the tracheal endings to the tissues, and *vice versa*.

Unlike spiders, tracheate arachnids (i.e., "Acari", Ricinulei, Solifugae, Pseudoscorpiones, and Opiliones) possess paired stigmata on the prosoma, the opisthosoma, or both (Beier, 1931; Kästner, 1931a; Alberti and Coons, 1999; Coons and Alberti, 1999; Höfer et al., 2000; Klußmann-Fricke and Wirkner, in press). These taxa rely exclusively on tracheae for respiration. Their complex tracheal system comprises major tracheal branches (sometimes anastomosing), from which minor branches emerge and ramify through the body to the oxygen-consuming tissues. Tracheate arachnids do not possess hemocyanins (Markl, 1986; Rehm et al., 2012) that would function as oxygen-binding carrier proteins and thus, in the absence of the hemolymph providing a convective system, the tracheae transport the respiratory gases directly to and from the tissues.

Among tracheate arachnids, the camel spiders or Solifugae Sundevall, 1833 (ca. 1000 species; Levin, 2013) are unusual because of their highly active lifestyle (Roewer, 1934; Punzo, 1993). Their active hunting is associated with comparatively high rates of metabolism and oxygen consumption (Lighton and Fielden, 1996; Lighton et al., 2001). The complex tracheal system of Solifugae presumably evolved to support the high oxygen demand of their tissues. The tracheae of solifuges extend throughout the body, reaching into the peripheral regions. Major branches ramify and anastomose with contralateral tracheae forming an extensively interconnected respiratory network (Kästner, 1931a). The remarkable similarity of the complex tracheal system of solifuges to that of insects has been cited as an example of morphological convergence (Wigglesworth, 1983).

The morphology of the solifuge tracheal system was previously described by Kittary (1848), Bernard (1896), Kästner (1931a) and Roewer (1934). Kästner's (1931a) illustration of the major tracheal branches remains the most detailed and is frequently referred to (Millot and Vachon, 1949; Hennig, 1967; Klann, 2009; Punzo, 2012; Hsia et al., 2013; Westheide and Rieger, 2013). Kästner's (1931a) analysis of the tracheal system was based on dissection and illustration of three exemplar species (*Galeodes araneoides* Pallas, 1772, *Galeodes caspius* Birula, 1890, and a species of *Solpuga* Lichtenstein, 1796).

In the present study, we reinvestigate the tracheal system of three species of Solifugae using modern non-invasive imaging techniques (µCT-imaging and 3D-reconstruction), document the respiratory system in situ, and provide an analysis of the fine branches and ramifications of the tracheal system of Solifugae. We hypothesize that the tracheae function not only as the sites for gaseous exchange but also as a convective system because solifuges lack hemocyanins as oxygen-carrying proteins. We predict that the functional demands on oxygen transport in tracheae and the mechanical principles of flexible tubing systems may have selected for a tracheal system that is in many respects analogous to the tracheal respiratory system of insects. The present study aims to clarify the organization of the major branches of the tracheal system of Solifugae, and detect morphological disparity among three families of this arachnid order. The finer branches of the tracheal system and their connection to various tissues are analyzed in a companion paper in this issue of Arthropod Structure & Development (Franz-Guess and Starck, 2016).

2. Material and methods

2.1. Taxon sample and material examined

We studied exemplar species representing three solifuge families to investigate morphological disparity in the tracheal system across the order: Galeodidae Sundevall, 1833 was represented by two female *Galeodes granti* Pocock, 1903 from Egypt, preserved in ethanol in the collection of the Biocenter at the Ludwig-Maximilians-Universität München (inventory number P-24-GG6 and P-24-GG7). Ammotrechidae Roewer, 1934 was represented by one female *Ammotrechula wasbaueri* Muma, 1962 obtained alive from Arizona, U.S.A., from www.bugsofamerica.com, and now stored at the Zoologische Sammlung Universität Rostock (inventory number ZRSO So 012). Eremobatidae Kraepelin, 1901 was represented by seven adult males of a species of *Eremobates* Banks, 1900 also obtained alive from Arizona, U.S.A., from www.bugsofamerica.com, and now stored at the Zoologische Sammlung Universität Rostock (inventory numbers ZSRO So 010–011 and ZRSO So 013–017).

2.2. Micro-computed tomography (μ CT)

Specimens of *G. granti* were stained using a solution of 100% ethanol containing 1% iodine for μ CT-imaging (Metscher, 2009). Both specimens were retained in the staining solution for 15 days to ensure thorough penetration of cuticle and tissue. Live specimens of *A. wasbaueri* and *Eremobates* sp. were exposed to OsO₄ vapor (EMS-ElectronMicroscopySciences, Hatfield, PA, U.S.A.) for 20 min to thoroughly stain the tracheal walls. OsO₄ stains the waxes in the epicuticle because it has a particularly high affinity for fatty acids. Furthermore, due to the thin walls of the tracheae, at least some of the OsO₄ also penetrates the tracheae and stains the surrounding tissues (i.e. the tracheal epithelium). The stained specimens were frozen at -20 °C, thawed, and the legs removed prior to scanning as described by Iwan et al. (2015).

µCT-imaging was performed with a Phoenix nanotome® (Phoenix|x-ray, GE Sensing & Inspection Technologies, Alzenau, Germany) high-resolution µCT-system in normal resolution mode for G. granti. High-resolution mode was used for A. wasbaueri and Eremobates sp. The program datos x acquisition (target: molybdenum, mode: 0–1; performance: 8–13 W; number of projections: 1080-2400; detector timing: 1000-1500 ms) was used for both resolution modes. Specimens of A. wasbaueri and Eremobates sp. were scanned at the Microscopy and Imaging Facility of the American Museum of Natural History, New York, resulting in a voxel size of 2-10 µm. The software datos|x 2 reconstruction (General Electric Deutschland Holding GmbH, Frankfurt-am-Main, Germany) and VGStudio MAX 2.2.6 (Volume Graphics GmbH, Heidelberg, Germany) were used for image processing. Specimens of G. granti were scanned at the Zoologische Staatssammlung, Munich, using two different settings, resulting in two different resolutions to provide a single complete overview (voxel size $25 \ \mu m$) and two more detailed scans (voxel size 5 μm). The image data obtained for G. granti (voxel size 5.5 µm and 25 µm) was converted to TIF-format using Fiji (Image] 1.49°) for further analysis.

2.3. 3D-reconstruction

The body surface, tracheal system, prosomal ganglion, heart, and intestinal tract of *G. granti* were reconstructed using surface rendering in Amira 6.0.0 (Mercury Computer Systems Inc., Chelmsford, MA, USA). The respective tissues of each μ CT-image were labeled using a graphic pen. Starting at the prosomal stigmata, all connecting tracheal structures were marked in cyan (Fig. 1). The digestive system, excluding the midgut diverticula, was marked in brown, the heart in red, and the prosomal ganglion in yellow. Areas without any visible connection to previously reconstructed organs, due to image and preservation artifacts, were marked in a pale version of the respective color. Selected μ CT-images and respective labeling are provided in Fig. 1 to document how segmented images



Fig. 1. Examples of original μ CT-images documenting the segmentation of tracheae for 3D-reconstruction of *Galeodes granti*. (**A**) Cross-section through the base of the chelicerae, the rostrum and the coxae of the pedipalps (original μ CT-image# 1502). The strong musculature of the chelicerae is evident in cross section, the esophagus is in the middle of the triangular rostrum and, depending on the anatomical orientation, the musculature of the pedipalpal coxae and trochanters are sectioned longitudinally. Cross sections of the distal pedipalpal segments are evident in the periphery. (**B**) Same section as in A but with tracheae labeled cyan. Dark areas around tracheae represent hemolymph-filled spaces (**C**) Cross-section through the third prosomal segment (original μ CT image# 1194). The prosomal stigma is on the dextral side of the image, the H-shaped apodeme of the thrid sternite is evident medially, supporting the anterior part of the midgut with adjoining prosomal midgut diverticula. (**D**) Same section as in C but with tracheae labeled cyan. Dark areas around tracheae represent hemolymph-spaces in this region are smaller than in B. The lateral cheliceral, main posterior prosomal and ventral prosomal tracheae are present in addition to all tracheae of the legs and pedipalps. (**E**) Cross-section through the fourth opisthosomal segment, posterior to the second opisthosomal stigmata (original μ CT image# 347). The opisthosoma is almost entirely filled with midgut diverticula. The dark space dorsal to the midgut diverticula is a preservation artifact. (**F**) Same section as in E but with tracheae labeled cyan. Aside from small hemolymph spaces, only the tracheae of the fourth leg and the main opisthosomal tracheae II are present. Abbreviations: a: atrium; ac: anterior cheliceral trachea; p: entrieve for section as in a but with tracheae of the fourth leg and the main opisthosomal tracheae II are present. Abbreviations: a: atrium; ac: anterior cheliceral trachea; p: edipalp1; pc: posterior cheliceral; mc:

translate into raw data for the 3D-reconstruction. Surfacereconstructions of the tracheal system and body surface of *A. wasbaueri* and *Eremobates* sp. were produced using VGStudio MAX 2.2.6 (Volume Graphics GmbH, Heidelberg, Germany) or Imaris 7.0.0 (Bitplane©, Zurich, Switzerland). The complete sets of μ CTimages are deposited with MorphoBank (O'Leary and Kaufman, 2012) at http://morphobank.org/permalink/?P2422.

2.4. Image processing

Figures were arranged into plates using Adobe Illustrator CS2 (Adobe Systems Incorporated). Bitmap images were embedded into Corel Draw X3 files and digitally edited using Corel Photo Paint X3. The interactive PDF was created using Adobe Acrobat 9 Pro Extended (Adobe Systems Incorporated).

3. Results

The description of the tracheal morphology is based on the reconstruction of *G. granti* with differences observed in the tracheal systems of the other exemplar species noted where relevant.

3.1. Prosoma

3.1.1. Stigmata and major tracheae

The network of major tracheae (>100 μ m in diameter) is most prominent in the prosoma. Fifteen major tracheal trunks can be identified in the prosoma (Figs. 2, 6; Table 1). The prosomal stigmata of the tracheal system open laterally and ventrally between the second and third legs (Figs. 2-4) in the sejugal furrow. Three main tracheal trunks, the main anterior prosomal (map) trachea, the main posterior prosomal (mpp) trachea, and the lateral prosomal trachea (lp), originate from an atrium immediately internal to each stigma. The main anterior prosomal trachea extends to the anterior segments, whereas the lateral prosomal tracheae and the main posterior prosomal trachea extend to the posterior segments. The main posterior prosomal trachea connects to the tracheal system of the opisthosoma. The dextral and sinistral main anterior prosomal tracheae anastomose immediately anterior to the prosomal ganglion (map, mpp; Figs. 2–4, 6). The lateral prosomal tracheae are less developed in A. wasbaueri and *Eremobates* sp.

3.1.2. Cheliceral tracheae

The anterior, posterior and lateral cheliceral tracheae supply the chelicerae (Figs. 3, 6). The anterior and posterior cheliceral tracheae originate from the main anterior prosomal trachea anterior to the prosomal ganglion and dorsal to the anastomosis of the main anterior prosomal trachea: the lateral cheliceral trachea emanates dorsally from the main anterior prosomal trachea, slightly anterior to the origin of the tracheae of the second walking leg. The lateral cheliceral tracheae extend first dorsal and then anterior to the chelicerae. The lateral cheliceral tracheae of A. wasbaueri and Eremobates sp. originate from the main anterior prosomal trachea directly at the stigmata. The lateral cheliceral tracheae of both species are much more pronounced than those of G. granti and, in A. wasbaueri, give rise to a pair of tracheae which extend anteromedially and then curve ventrally along the midline of the body. These tracheae continue in parallel to the anterior anastomosis of the main anterior prosomal tracheae where they anastomose with the latter. The cheliceral tracheae of all three species branch extensively on entering the chelicerae, forming various tracheal tubes that extend into the cheliceral musculature. These cheliceral tracheae do not anastomose. Their main branches display some expansion (i.e., the diameter is wider than at their origin),

appearing inflated compared to other tracheae. These cheliceral air sacs are smaller (Fig. 3B) in *A. wasbaueri*, and only the anterior and posterior cheliceral tracheae are inflated and situated in the anterior part of the chelicerae and both cheliceral fingers. The anterior and posterior cheliceral tracheae are not inflated in *Eremobates* sp. (ac, pc; Fig. 3C).

3.1.3. Ganglion tracheae

The prosomal ganglion trachea (pg; Figs. 4, 6) originates from the main anterior prosomal trachea in the segment which bears the second leg. It first extends medially and then bends ventrally to enter the prosomal ganglion (pg; Figs. 2, 3B, C, 4). The dorsal prosomal trachea, which originates anterior to the prosomal ganglion trachea, extends dorsally, alongside the prosomal ganglion and toward the middle of the prosoma, where it ramifies further (dp, pg; Figs. 2-4, 6) supplying the extrinsic musculature of the chelicerae. The ventral prosomal trachea originates from the lateral prosomal trachea, immediately posterior to the prosomal stigma, and extends ventrally towards the prosomal ganglion (for a clear view of the origin of the ventral prosoma trachea, rotate Fig. 3A ventrally using the interactive 3D-image function). The ventral prosomal tracheae of A. wasbaueri and Eremobates sp. are less pronounced, and do not reach as far anteriorly, as in *G. granti* (vp; Figs. 3, 4, 6). The general pattern of tracheal supply to the prosomal ganglion is similar in all three species.

3.1.4. Lateral and posterior prosomal trachea

The lateral prosomal trachea (lp: Figs. 4, 6) and the main posterior prosomal trachea (mpp. Figs. 2-4, 6) originate at the prosomal stigma and supply the posterior parts of the prosoma. The lateral prosomal trachea divides into anterior and posterior branches. The anterior branch extends ventrally and becomes the ventral prosomal trachea (lp, vp; Figs. 2–4, 6). The posterior branch extends obliquely dorsally and posteriorly where it ramifies further, supplying the area posterior to the meso- and metapeltidium. In both specimens of G. granti studied, the sinistral lateral prosomal trachea was more prominent than the dextral (lp; Fig. 4). The main posterior prosomal trachea extends straight posteriorly, giving rise to the tracheae of the third and fourth legs. The main posterior prosomal trachea continues to the posterior border of the prosoma where it connects to the main lateral opisthosomal trachea (mpp, mlo; Fig. 3). The lateral prosomal tracheae of A. wasbaueri and Eremobates sp. are less pronounced than those of G. granti (lp; Fig. 3).

3.1.5. Pedipalp and leg tracheae

The tracheae supplying the pedipalps, legs I and II originate segmentally from the main anterior prosomal trachea whereas the tracheae of legs II and IV emanate from the main posterior prosomal trachea (map, mpp; Figs. 2–4, 6). Several anterior and a single posterior pedipalpal tracheae originate from the anterior end of the main anterior prosomal trachea. All pedipalpal tracheae extend through the entire length of the pedipalp into the tarsus (ap, pp; Figs. 2, 3). The anterior pedipalpal trachea extends to the tip of the pedipalp without branching, whereas the posterior pedipalpal trachea branches shortly after its origin into two main trunks, merges again in the femur and branches again in the tibia (ap, pp; Figs. 2, 3). The trachea of leg I originates immediately posterior to the posterior pedipalpal trachea from the main anterior prosomal trachea (11, pp, map; Figs. 2-4, 6), and only one trachea extends through all segments of the leg to the tarsus. The trachea of leg II branches from the main anterior prosomal trachea, immediately anterior to the prosomal atrium (12, Figs. 2–4, 6). As with leg I, only one trachea extends to the tarsus: although originating as two, the smaller branch only reaches the trochanter where the two



Fig. 2. Lateral (A), dorsal (B) and ventral (C) views of 3D-reconstruction of major tracheal branches (cyan) and central nervous system (yellow), digestive tract (brown) and heart (red) of *Galeodes granti* based on µCT serial images. (**A**) One pair of stigmata in the prosoma, two pairs of stigmata at the posterior margin of the third and fourth opisthosomal segments, respectively, and a single stigma in the fifth opisthosomal segment indicated by red arrows. Each prosomal stigma opens into one of the main anterior prosomal tracheae, and these two tracheae lead to the other prosomal tracheae. Each of the three opisthosomal stigmata continues into a main opisthosomal trachea and these lead to the other opisthosoma as are the tracheae. Each of the chelicerae, pedipalps and legs. The cheliceral tracheae form air sacs in the anterior half of the chelicerae. The leg and pedipalpal tracheae extend into the tarsi of their respective extremities. (**C**) The bilateral pattern of tracheae in the prosoma and opisthosoma is evident in relation to the fused central ganglia that extend lateral branches to the prosomal and opisthosomal tracheae, is indicated by red arrows. Each opisthosomal tigma opens into one of the main opisthosomal stigmata, each opening into one of the main anterior prosomal tracheae, is indicated by red arrows. Each opisthosomal tigua opens into one of the main opisthosomal tracheae. The tracheae of the prosomal and opisthosomal tigua opens into one of the main opisthosomal stigmata, each opening into one of the main anterior prosomal tracheae, is indicated by red arrows. Each opisthosomal trachea and the and poisthosomal trachea and opisthosomal connect to one another through the main posterior prosomal trachea and the main lateral opisthosomal trachea. The tracheae of the prosoma and opisthosoma connect to one another through the main noterior prosomal trachea; weight in a posterior prosomal tracheae; generation prosomal trachea; generati and teres in prosomal trachea; pei porsomal tracheae; pei opis

branches merge. The tracheae of legs III and IV are extensions of the main posterior prosomal trachea. Two main tracheal trunks enter the legs and merge in the femur (13, 14; Figs. 2, 3). All main tracheae from legs I–IV extend into the tarsus. A similar situation was

observed at the base of the appendages of *A. wasbaueri* and *Eremobates* sp. but the full extent of the tracheae in the legs of these species is unknown because the legs were removed prior to scanning.

Newly proposed terminology for major trachese in prosoma of Solifurate compared with traditional terminology of Kästner (1931a)

New terminology	Abbreviation	Terminology of Kästner (1931a
Main anterior prosomal trachea	map	Truncus principalis
Anterior cheliceral trachea	ac	Ramus cheliceralis
Posterior cheliceral trachea	рс	Ramus cheliceralis accessorius
Lateral cheliceral trachea	lc	Ramus lateralis anterior
Prosomal ganglion trachea	pg	Ramus cerebralis
Dorsal prosomal trachea	dp	Ramus dorsalis
Ventral prosomal trachea	vp	Ramus transversalis
Lateral prosomal trachea	lp	Ramus posterior lateralis
Main posterior prosomal trachea	mpp	Ramus posterior medialis
Anterior pedipalpal trachea	ар	Ramus pedipalpi accessori
Posterior pedipalpal trachea	рр	Ramus pedipalpi
Leg trachea I	11	Ramus pedis I
Leg trachea II	12	Ramus pedis II
Leg trachea III	13	Ramus pedis III
Leg trachea IV	14	Ramus pedis IV

3.2. Opisthosoma

3.2.1. Stigmata and major tracheae

Table 1

The stigmata of the opisthosoma are situated medially at the posterior margin of sternites III–V (red arrows in Figs. 2, 3, 5, 6). The stigmata of opisthosomal segments III and IV are paired and situated in close proximity. The stigma on opisthosomal segment V is unpaired. A main opisthosomal trachea originates from each stigma supplying a distinct region of the opisthosoma; i.e., main opisthosomal trachea I primarily supplies opisthosomal segments I-III as well as the heart, main opisthosomal trachea II primarily supplies opisthosomal segment IV, and main opisthosomal trachea III primarily supplies opisthosomal segment V-VII. In the ammotrechid and eremobatid exemplars, however, the main opisthosomal trachea II supplies the entire opisthosoma posteriorly to the fourth opisthosomal segment. In all species studied, the main opisthosomal tracheae I-III are connected by way of the main lateral opisthosomal trachea which runs bilaterally along the midgut through the entire opisthosoma and forms anastomoses at various points with different branches there of (mlo, mo1-3; Fig. 5; Table 2). Anteriorly, the main lateral opisthosomal tracheae merge into the main posterior prosomal tracheae, thus establishing continuous lateral tracheal trunks that extend from the anterior tracheal anastomosis to the posterior end of the opisthosoma. At the base of the main opisthosomal tracheae as well as along their length, smaller tracheae branch off dorsolaterally along the body wall (Fig. 5), supplying each opisthosomal segment with additional smaller lateral tracheae.

3.2.2. Main opisthosoma trachea I and genital tracheae

Directly posterior to the atrium, the main opisthosomal trachea I divides into multiple anterior and a single posterior branches which extend anteriorly and dorsally. The genital trachea originates from the anterior branch of main opisthosomal trachea I and extends anteroventrally, dividing into two major branches before connecting to the main lateral opisthosomal trachea and entering the prosoma through the diaphragm (g, mlo; Figs. 2, 3, 5). The main lateral opisthosomal trachea of leg IV in the last segment of the prosoma (mlo, g, 14; Figs. 2, 3, 6). The main lateral opisthosomal trachea therefore constitutes the connection between the tracheal networks of the prosoma and opisthosomal.

3.2.3. Heart trachea

The posterior branch of the main opisthosomal trachea I extends dorsally, ramifies before it connects to the main lateral opisthosomal trachea and continues after making the connection (mo1, mlo; Figs. 2, 3, 5, 6). On the sinistral side of the body, the posterior branch ramifies before connecting with the main lateral opisthosomal trachea whereas, on the dextral side, the posterior branch gives rise to the heart trachea, before connecting with the main lateral opisthosomal trachea (mlo, h; Figs. 2, 3, 5, 6). The heart trachea is situated immediately ventral to the heart (h; Fig. 2). As it extends anteriorly, the heart trachea divides once prior to connecting to the main lateral opisthosomal trachea in the first opisthosomal segment (h, g; Figs. 2, 3A, 6). Posteriorly, the heart trachea branches in each opisthosomal segment (h; Fig. 5). The morphology of the heart trachea is distinctly different in *A. wasbaueri* and *Eremobates* sp. (see 3.2.6).

3.2.4. Main opisthosomal trachea II

The main opisthosomal trachea II originates at the stigma of the fourth opisthosomal segment and divides into three major branches, the anterior, posterior and ventral branches (mo2; Figs. 2, 3, 5, 6). The anterior branch extends dorsally and divides once before connecting to the main lateral opisthosomal trachea and continuing dorsally (mo2, mlo; Figs. 2, 3, 5, 6). The posterior branch of the main opisthosomal trachea II in *G. granti* extends posterodorsally and, as with the anterior branch, connects to the main lateral opisthosomal trachea and continues dorsally (mo2, mlo; Figs. 2, 3, 5, 6). The ventral branch of the main opisthosomal trachea II in *G. granti* extends posteriorly, giving rise in each segment to small lateral tracheae which extend along the body wall between adjacent segments (mo2; Fig. 5).

3.2.5. Main opisthosomal trachea III

The main opisthosomal trachea III originates from the unpaired stigma on the fifth opisthosomal segment and extends dorsally, where it bifurcates into two major branches (mo3; Figs. 2, 3, 6) halfway to the connection of the main lateral opisthosomal trachea. Immediately prior to the connection, the two branches give rise to additional side branches before the anterior branch connects to the main lateral opisthosomal trachea slightly posterior to the connection of the main lateral and the main lateral opisthosomal trachea (mo3, mo2, mlo; Figs. 2, 3, 6). The posterior branch also connects to the connection of the anterior branch. The lateral branches of the main opisthosomal trachea III continue dorsally (mlo, mo3; Figs. 2, 3, 6).

3.2.6. Differences among the three species

The connection of the prosomal tracheal network with the opisthosomal tracheal network is similar in *G. granti* and *A. wasbaueri*, but differs in *Eremobates* sp. (Figs. 3, 5), in which the genital trachea is the sole connection between the prosomal and



Fig. 3. Dorsal views of 3D-reconstructions of major tracheal branches of *Galeodes granti* (**A**), *Ammotrechula wasbaueri* (**B**) and *Eremobates* sp. (**C**). One pair of stigmata (red arrows) is present in the prosoma of all three species along with two pairs of stigmata and a single stigma (red arrows) in the opisthosoma. The general, bilateral pattern of tracheae is similar in all three species, but important differences are described in the text. Especially prominent are the air sacs in the anterior, posterior and lateral cheliceral tracheae of *G. granti* and *A. wasbaueri* that are missing in *Eremobates* sp. Whereas the tracheal supply of the heart is provided by only one major heart trachea in *G. granti*, two major tracheae are present in *A. wasbaueri* and *Eremobates* sp. Abbreviations: ac: anterior cheliceral trachea; ap: anterior pedipalpal trachea; as: air sac; dp: dorsal prosomal trachea; g: genital trachea; h: heart trachea; 11–14: leg I–IV tracheae; lc: lateral cheliceral trachea; lp: lateral prosomal trachea; map: main anterior prosomal trachea; mp: main anterior prosomal trachea; pp: posterior pedipalpal trachea; vp: ventral prosomal trachea; Disruptions in course of main lateral opisthosomal trachea; of *G. granti* are image and preservation artifacts.

opisthosomal tracheal networks (g; Fig. 3). The heart is supplied by the posterior branch of the main opisthosomal trachea I in *A. wasbaueri* and *Eremobates* sp. These tracheae are offset, with the branch on the sinistral side of the body extending anteriorly and the branch on the dextral side extending dorsally (h; Fig. 3B, C). The posterior branch of the main opisthosomal trachea II supplies the posterior part of the heart in both species. However, the dextral branch supplies the heart in *Eremobates* sp., whereas the sinistral branch supplies the heart in *A. wasbaueri* (mo2; Fig. 3B, C). The tracheal supply of the heart differs markedly among the three species: the heart is supplied by a single trachea in *G. granti*, whereas it is supplied by multiple tracheae in *A. wasbaueri* and *Eremobates* sp. The anterior branch of main opisthosomal trachea II does not ramify before connecting to the main lateral opisthosomal trachea in *A. wasbaueri* and *Eremobates* sp. (mo2, mlo; Fig. 3). The posterior branch of the main opisthosomal trachea II continues posteriorly to supply the heart in *A. wasbaueri* and *Eremobates* sp., whereas it connects to the main lateral

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Fig. 4. Dorsal view of 3D-reconstruction of major tracheal branches in the prosoma of *Galeodes granti*, between mid-chelicerae and leg III (blue square, inset). Red arrows indicate location of prosomal stigmata. Sinistral and dextral anterior prosomal tracheae anastomose in the pedipalp segment. The anterior, posterior and lateral cheliceral tracheae give rise to several smaller tracheal branches entering the chelicerae. The dextral lateral prosomal trachea is less pronounced than the sinistral lateral prosomal trachea, however. Abbreviations: ac: anterior cheliceral trachea; ap: anterior pedipalpal trachea; dp: dorsal prosomal trachea; 11–14: leg I–IV tracheae; lc: lateral cheliceral trachea; pp: posterior pedipalpal trachea; mp: main noterior prosomal trachea; pp: posterior pedipalpal trachea; pc: posterior cheliceral trachea; pp: posterior pedipalpal trachea; mp: main posterior pedipalpal trachea; not cheliceral trachea; pg: prosomal ganglion trachea; pp: posterior pedipalpal trachea; mp: main posterior pedipalpal trachea; not cheliceral trachea; pg: prosomal ganglion trachea; pp: posterior pedipalpal trachea; mp: main posterior pedipalpal trachea; not cheliceral trachea; mp: most meetion previously reconstructed tracheae in *G. granti*, due to image and preservation artifacts, indicated in pale cyan



Fig. 5. Dorsal view of 3D-reconstruction of major tracheal branches in the opisthosoma of *Galeodes granti*, segments III–V (blue square). The positions of the opisthosomal stigmata are indicated by red arrows. Except for the heart tracheae, all side branches of the main opisthosomal tracheae I–III are connected to the main lateral opisthosomal tracheae. The heart is supplied by one heart trachea. Abbreviations: g: genital trachea; h: heart trachea; I3 and I4: leg III and IV tracheae; mlo: main lateral opisthosomal trachea; mo1–mo3: main opisthosomal trachea I–III.

Table 2

Newly proposed terminology for major tracheae in opisthosoma of Solifugae compared with traditional terminology of Kästner (1931a).

New terminology	Abbreviation	Terminology of Kästner (1931a)
Main lateral opisthosomal trachea	mlo	Truncus longitudinalis
Genital trachea	g	Ramus genitalis
Heart trachea	h	Ramus pericardialis
Main opisthosomal trachea I	mo1	Truncus stigmaticus I
Main opisthosomal trachea II	mo2	Truncus stigmaticus II
Main opisthosomal trachea III	mo3	Truncus stigmaticus III



Fig. 6. Schematic drawings of the tracheal network of Solifugae, in lateral view of the dextral side (A) and in dorsal view (B), based on *Galeodes granti*. (A) Asterisks indicate positions of the prosomal pair of stigmata, the two opisthosomal pairs of stigmata and the single distal opisthosomal stigma. Major tracheal branches supply the rest of the body from there. The distal end of the main posterior prosomal trachea connects with the main lateral opisthosomal tracheae, creating an anastomosis between the prosoma and the opisthosoma. Further anastomoses occur where the sinistral and dextral sides connect at the anterior ends of the main anterior prosomal trachea and the heart trachea, and at the main opisthosomal trachea III. (B) The tracheal network is organized bilaterally with one prosomal and two opisthosomal anastomoses. The tracheal system is also asymmetric because only the side branch of the main dextral opisthosomal trachea I continues into the heart trachea. Air sacs form prominent structures within the chelicerae. The tracheae of the pedipalps and the legs extend all the way into the tarsi. Overall, the complexity of the tracheal network is greater in the prosoma than in the opisthosomal trachea; an anterior prosomal trachea; p: genital trachea; p: lateral prosomal trachea; ic: lateral cheliceral trachea; ip: lateral prosomal trachea; main anterior prosomal trachea; mo: main anterior prosomal trachea; pp: posterior pedipalpal trachea; vp: ventral prosomal trachea III. (B) The trachea interior prosomal trachea; mo: main anterior prosomal and two opisthosomal anastomoses. The tracheal system is also asymmetric because only the side branch of the main dextral opisthosomal trachea is continues into the heart trachea. Air sacs form prominent structures within the chelicerae. The tracheae of the pedipalps and the legs extend all the way into the tarsi. Overall, the complexity of the tracheal network is greater in the prosoma than in the opisthosomal. Coveral, the call prosomal trachea; p: lateral pros

opisthosomal trachea in *G. granti* (mo2; Fig. 3). The ventral branch of main opisthosomal trachea II takes the same course in *G. granti, A. wasbaueri* and *Eremobates* sp. However, an additional branch of the ventral branch of the main opisthosomal trachea II connects to the main lateral opisthosomal trachea, thus further interconnecting the opisthosomal tracheal network, in *A. wasbaueri* (mo2, mlo; Fig. 3).

The ramification of the main opisthosomal trachea III and the connection of the anterior lateral branch to the main lateral opisthosomal trachea in *A. wasbaueri* resemble those of *G. granti*. However, the posterior branch of the main opisthosomal trachea III does not connect to the main lateral opisthosomal trachea, but continues posteroventrally to the midgut, parallel to the ventral branch of the main opisthosomal trachea II in *A. wasbaueri* (mo3, mlo, mo2; Fig. 3). In *Eremobates* sp., the first bifurcation of the main opisthosomal trachea III into two lateral branches occurs soon after the main opisthosomal trachea III originates at the stigma (mo3; Fig. 3). The connections of the anterior and the posterior lateral branches to the main lateral opisthosomal trachea in *Eremobates* sp. are similar to those of *G. granti*, however. Altogether, the configuration of the main opisthosomal trachea III differs markedly among the three species.

The results presented here are summarized in Fig. 6 which presents schematic illustrations of the main tracheal branches of *G*.

granti in dorsal and lateral views. These illustrations are presented to aid understanding of the complex pattern of major tracheae in Solifugae, and serve as a basis for comparison in future studies. *G. granti* was selected as representative in these illustrations because it is the species for which we have the most complete data for the entire tracheal system.

4. Discussion

4.1. Stigmata

The stigmata of Solifugae are situated on the prosoma and opisthosoma. Prosomal stigmata are rare in chelicerates, besides Solifugae occurring only in Ricinulei and some Acari (Kästner, 1931b), but not in other tracheate arachnids (Bromhall, 1987; Höfer et al., 2000; Edwards et al., 2009). The unpaired stigma on the fifth opisthosomal segment is unique to solifuges. Stigmata are paired in all other tracheate arachnids. Because the branching pattern of the trachea originating from the unpaired stigma (mo3) is similar to that of the tracheae originating from the paired stigmata (mo1, mo2) of opisthosomal segments III and IV, and under the assumption of "serial homology" (homomorphy), the single stigma on the fifth opisthosomal segment is probably derived from paired stigmata (Figs. 3, 6). The stigmata on opisthosomal segments III and IV are topographically so close to one another that their atria are separated by only a thin septum (Kästner, 1931a) hence the merger of two stigmata is plausible. A similar mechanistic explanation was also suggested for the occurrence of unpaired stigmata in various spider taxa (Levi, 1967; Bromhall, 1987). It may be further speculated that the merger of paired stigmata and paired tracheae into a single unit might have occurred due to a reduced demand for oxygen in the rear part of the opisthosoma (which contains mostly midgut gland and the fecal sac), or in response to the increased efficiency of gas transport through the extensive tracheal network. A reduction in the number of stigmata reduces water loss, an adaptation to arid conditions (Cloudsley-Thompson, 1970; Edney, 1977; May, 1985).

4.2. Tracheal anastomoses

The course of the tracheae in solifuges leads from the stigmata to all regions of the body (Figs. 2, 3, 6). This is important because solifuges lack hemocyanins as an oxygen-carrying protein, and tracheae are therefore required to transport oxygen directly to the tissues. Anastomoses are present anteriorly in the prosoma, between the prosoma and opisthosoma, anteriorly in the opisthosoma, and in the area of the unpaired stigma, indicating that the tracheae of each tracheal tree are interconnected (Figs. 2, 3, 6). The connection between prosoma and opisthosoma comprises two paired branches in G. granti and Eremobates sp. whereas it comprises only the genital trachea in A. wasbaueri. Despite this apparent difference, the presence of tracheal anastomoses might increase ventilation efficiency because not only are the prosoma and opisthosoma connected but so are the sinistral and dextral halves of the body. Consequently, respiratory gases are distributed evenly throughout. Interconnections between tracheae are known in Solifugae, some Acari (Woodring and Galbraith, 1976), Opiliones (Höfer et al., 2000), and insects (Chapman, 1998). In insects, however, anastomoses are more numerous than in chelicerates because every lateral tracheal tree is connected to the tracheae of adjacent body segments. The tracheae of solifuges are embedded within the musculature and organs and the hemolymph space is reduced to small areas surrounding the tracheae. A flexible structure of tracheal tubes is therefore necessary to avoid constraining the

tracheae and ensure unobstructed oxygen transport to the organs they supply.

4.3. Air sacs

The largest tracheae extend from the prosomal stigmata into the anterior part of the prosoma, where they form air sacs within the chelicerae (Figs. 2, 3, 6). This previously undocumented feature appears to be unique to camel spiders. The tracheae of tracheate arachnids are usually thin tubules, which decrease in diameter along their length and after branching (Beier, 1931; Woodring and Galbraith, 1976; Höfer et al., 2000; Schmitz and Perry, 2000). The largest air sacs were observed in G. granti, the largest of the exemplar species studied. The smaller size of the air sacs in A. wasbaueri and their absence in Eremobates sp. may be related to their smaller body size and the more slender shape of their chelicerae. The absence of air sacs in Eremobates may also be explained by the fact that the specimens examined were adult males, the chelicerae of which are highly modified (as in other members of the family Eremobatidae; Bird et al., 2015). Functional interpretations for the large air sacs of G. granti include improved lever ratios for cheliceral action due to the increased space required to accommodate larger musculature (Van der Meijden et al., 2012) for subduing prey, i.e., other arthropods like scorpions and even small lizards (Banta and Marer, 1972). Air sacs also reduce the weight of large chelicerae, which may explain why they are most pronounced in G. granti (average body length 55 mm), less pronounced in A. wasbaueri (average body length 20 mm) and absent in *Eremobates* sp. (average body length 15 mm). Air sacs might also function for oxygen-storage or as bellows to enhance tracheal ventilation, thus playing a role during gas exchange. Solifuges display discontinuous gas exchange similar to insects (Lighton and Fielden, 1996; Lighton, 1998), where air sacs are known to function like bellows (Chapman, 1998; Resh and Cardé, 2009). In addition, movements that might aid in ventilating air sacs and using them for air storage, were proposed for solifuges (Nogge, 1976). Such movements would represent another potential similarity to insects. However, no further data on ventilation movements in solifuges are currently available.

4.4. Opisthosomal tracheae

Compared to the complex network of tracheae in the prosoma, the opisthosoma, which is almost entirely filled with mid-gut diverticula, possesses a rather simple network of major tracheal branches and no air sacs (Figs. 2–6). The tracheal network of the opisthosoma nevertheless displays a greater degree of ramification than previously described (Bernard, 1896; Kästner, 1931a). The extent of branching as well as the development of the tracheal system within the opisthosoma varies greatly among the Araneae (Bromhall, 1987; Schmitz and Perry, 2000, 2002) from simple tracheal tubes, e.g., in orb-weaving spiders (Bromhall, 1987), to highly complex opisthosomal and prosomal tracheal systems, e.g., in the water spider, Argyroneta aquatica (Crome, 1953). Tracheate arachnids (e.g., harvestmen or mites) exhibit less ramification, however, and the tracheal network in the opisthosoma is less pronounced (Woodring and Galbraith, 1976; Höfer et al., 2000). The unequal tracheal supply of prosoma and opisthosoma might be related to the distribution of energy-expensive tissues, i.e., all locomotor musculature and the nervous system occur in the prosoma (Klußmann-Fricke and Wirkner, in press). The tracheal branches associated with the heart differ between G. granti, in which the heart is exclusively supplied by a single trachea, and A. wasbaueri and *Eremobates* sp., in which it is supplied by multiple tracheae. Another difference among the exemplar species examined is the origin of the heart tracheae from the anterior opisthosomal stigmata. Whereas the branch on the dextral side of the body supplies the heart in *G. granti* and *A. wasbaueri*, the branch on the sinistral side supplies the heart in *Eremobates* sp. Whether this pattern is taxon-specific or represents intraspecific variation like the course of certain arteries in scorpions (Klußmann-Fricke et al., 2012) or decapod crustaceans (Vogt et al., 2009) requires confirmation.

5. Conclusions

Morphological analysis of the tracheal systems of exemplar species representing three families of Solifugae reveals that efficient gas transport is made possible by an extensive network of tracheae reaching throughout the body. The tracheal network may also reduce body weight, especially in the large chelicerae, where air sacs are present, enabling solifuges to reach greater speeds with less energy investment, thereby increasing their chance of catching prey. The large diameter of the tracheae in the chelicerae may also permit rapid and efficient gaseous exchange necessary to operate the musculature of these massive structures, thereby improving their use for predation in an environment where prey is scarce.

Tracheae evolved several times independently within arthropods, at least twice in Hexapoda and Myriapoda (e.g., Dohle, 2001; Richter and Wirkner, 2004). Tracheae are also thought to have evolved at least twice within Arachnida, i.e., in spiders and tracheate arachnids, and probably several times among "Acari" (Alberti and Coons, 1999). The hypothesis that tracheal systems evolved independently in different arachnid taxa seems reasonable considering that tracheae exist in different forms, i.e., tube- or sieve-tracheae, among tracheate arachnids (e.g., Beier, 1931; Kästner, 1931a; Levi, 1967; Bromhall, 1987; Höfer et al., 2000; Talarico et al., 2011), and originate from different positions on different body segments. The intensive branching and formation of anastomoses in solifuges appears to be independently evolved. However, due to the lack of congruence among phylogenetic hypotheses concerning arachnid relationships, it is presently impossible to draw further conclusions concerning the evolution of tracheae in Solifugae.

Author contributions

The study was designed by J. Matthias Starck for *G. granti* and independently by Lorenzo Prendini for *A. wasbaueri* and *Eremobates* sp. The investigation and data analysis was conducted by Sandra Franz-Guess for *G. granti*, and Bastian-Jesper Klußmann-Fricke for *A. wasbaueri* and *Eremobates* sp. The results were analyzed and discussed by all authors. The manuscript was drafted by Sandra Franz-Guess. Critical revisions were performed by J. Matthias Starck. Final revisions were performed by all authors.

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References

- Alberti, G., Coons, B., 1999. Acari: Mites. In: Harrison, F.E., Locke, M. (Eds.), Microscopic Anatomy of Invertebrates, Vol. 8C. Wiley-Liss, New York, pp. 515–1265.
- Banta, B.H., Marer, P.J., 1972. An attack by a solpugid on an iguanid lizard hatchling. Brit. J. Herpetol. 4, 266–267.
- Beier, M., 1931. Ordnung Pseudoscorpionidae (Afterscorpione). Handbook of Zoology Online. De Gruyter, Berlin.
- Bernard, H.M., 1896. IV. The comparative morphology of the Galeodidae. Trans. Linn. Soc. Lond. Ser. 2, Zool. 6, 305–417.
- Bird, T.L., Wharton, R.A., Prendini, L., 2015. Cheliceral morphology in Solifugae (Arachnida): primary homology, terminology, and character survey. Bull. Am. Mus. Nat. Hist. 916, 1–356.
- Bromhall, C., 1987. Spider tracheal systems. Tissue Cell 19, 793–807. http:// dx.doi.org/10.1016/0040-8166(87)90020-6.
- Chapman, R.F., 1998. The Insects: Structure and Function, fourth ed. Cambridge Univ. Press, Cambridge.
- Cloudsley-Thompson, J.L., 1970. Terrestrial invertebrates. Comp. Physiol. Thermoregul. 1, 15–77.
- Coons, B., Alberti, G., 1999. Acari: Ticks. In: Harrison, F.E., Locke, M. (Eds.), Microscopic Anatomy of Invertebrates, vol. 8B. Wiley-Liss, New York, pp. 267–514.
- Crome, W., 1953. Die Respirations-und Circulationsorgane der Argyroneta aquatica Cl. (Araneae), 2. Wiss. Z. Humboldt-Univ., Berlin, pp. 53–83.
- Dohle, W., 2001. Are the insects terrestrial crustaceans? A discussion of some new facts and arguments and the proposal of the proper name "Tetraconata" for the monophyletic unit Crustacea + Hexapoda. In: Ann. Soc. Entomol. France, 37, pp. 85–103.
- Dunlop, J.A., 1997. The origins of tetrapulmonate book lungs and their significance for chelicerate phylogeny. In: Proc. 17th Eur. Colloq. Arachnol., Edinburgh, pp. 9–16.
- Edney, E.B., 1977. Water Balance in Land Arthropods. Springer Verlag, Berlin.
- Edwards, K.T., Goddard, J., Varela-Stokes, A.S., 2009. Examination of the internal morphology of the ixodid tick, *Amblyomma maculatum* Koch (Acari: Ixodidae); a "how-to" pictorial dissection guide. MidSouth Entomol. 2, 28–39.
- Foelix, R., 2010. Biology of Spiders, third ed. Oxford Univ. Press, Oxford.
- Franz-Guess, S., Starck, J.M., 2016. Histological and ultrastructural analysis of the respiratory tracheae of *Galeodes granti* (Chelicerata: Solifugae). Arthropod Struct. Dev. 45 (5), 452–461.
- Giribet, G., Edgecombe, G.D., Wheeler, W.C., Babbitt, C., 2002. Phylogeny and systematic position of Opiliones: a combined analysis of chelicerate relationships using morphological and molecular data. Cladistics 18, 5–70.
- Hennig, W., 1967. Wirbellose II. Gliedertiere. reprinted 1994, first ed. Gustav Fischer Verlag, Jena.
- Höfer, A.M., Perry, S.F., Schmitz, A., 2000. Respiratory system of arachnids II: morphology of the tracheal system of *Leiobunum rotundum* and *Nemastoma lugubre* (Arachnida, Opiliones). Arthropod Struct. Dev. 29, 13–21.
- Hsia, C.C.W., Schmitz, A., Lambertz, M., Perry, S.F., Maina, J.N., 2013. Evolution of air breathing: oxygen homeostasis and the transitions from water to land and sky. Compr. Physiol. http://dx.doi.org/10.1002/cphy.c120003.
- Iwan, D., Kamiński, M.J., Raś, M., 2015. The last breath: a μCT-based method for investigating the tracheal system in Hexapoda. Arthropod Struct. Dev. 44, 218–227.
- Kamenz, C., Dunlop, J.A., Scholtz, G., Kerp, H., Hass, H., 2008. Microanatomy of early devonian book lungs. Biol. Lett. 4, 212–215.
- Kästner, A., 1931a. Ordnung Solifugae (Walzenspinnen). Handbook of Zoology Online. De Gruyter, Berlin.
- Kästner, A., 1931b. Ordnung Ricinulei. Handbook of Zoology Online. De Gruyter, Berlin.
- Kittary, M., 1848. Anatomische Untersuchung der gemeinen (*Galeodes araneoides*) und der furchtlosen (*Galeodes intrepida*) Solpuga. Bull. Naturf. Ges. Moskau Band. 21, 307–371.
- Klann, A.E., 2009. Histology and Ultrastructure of Solifuges. Ernst-Moritz-Arndt-Universität, Greifswald.
- Klußmann-Fricke, B.-J., Wirkner, C.S., 2016. Same but different: analogy between tracheal and vascular supply in the CNS of different arachnids. Org. Div. Evol.
- Klußmann-Fricke, B.-J., Prendini, L., Wirkner, C.S., 2012. Evolutionary morphology of the hemolymph vascular system in scorpions: a character analysis. Arthropod Struct. Dev. 41, 545–560.
- Levi, H.W., 1967. Adaptations of respiratory systems of spiders. Evolution 21, 571-583. http://dx.doi.org/10.2307/2406617.
- Levin, S.A., 2013. Encyclopedia of Biodiversity, second ed. Academic Press, Amsterdam.
- Lighton, J.R.B., 1998. Notes from underground: towards ultimate hypotheses of cyclic, discontinuous gas-exchange in tracheate arthropods. Am. Zool. 38, 483–491. http://dx.doi.org/10.1093/icb/38.3.483.
- Lighton, J.R.B., Fielden, L.J., 1996. Gas exchange in wind spiders (Arachnida, Solphugidae): independent evolution of convergent control strategies in solphugids and insects. J. Insect Physiol. 42, 347–357. http://dx.doi.org/10.1016/ 0022-1910(95)00112-3.
- Lighton, J., Brownell, P., Joos, B., Turner, R., 2001. Low metabolic rate in scorpions: implications for population biomass and cannibalism. J. Exp. Biol. 204, 607–613.
- Markl, J., 1986. Evolution and function of structurally diverse subunits in the respiratory protein hemocyanin from arthropods. Biol. Bull. 171, 90–115.

May, M.L., 1985. Thermoregulation. Compr. Insect Physiol. Biochem. Pharmacol. 4, 507–552.

- Metscher, B.D., 2009. MicroCT for comparative morphology: simple staining methods allow high-contrast 3D imaging of diverse non-mineralized animal tissues. BMC Physiol. 9, 11. http://dx.doi.org/10.1186/1472-6793-9-11.
- Millot, J., Vachon, M., 1949. Ordre des Solifuges. Traite Zool, VI. Masson et Cie, Paris, pp. 482–519.
- Noge, G., 1976. Ventilationsbewegungen bei Solifugen (Arachnida, Solifugae). Zool. Anz. 196, 145–149.
- O'Leary, M.A., Kaufman, S., 2011. MorphoBank: phylophenomics in the "cloud". Cladistics 27 (5), 529-537.
- Opell, B.D., 1998. The respiratory complementarity of spider book lung and tracheal systems. J. Morphol. 236, 57–64.
- Pepato, A.R., da Rocha, C.E., Dunlop, J.A., 2010. Phylogenetic position of the acariform mites: sensitivity to homology assessment under total evidence. BMC Evol. Biol. 10, 235.
- Punzo, F., 1993. Diet and feeding behavior of the solpugid, *Eremobates palpisetulosus* (Solpugida: Eremobatidae). Psyche 100, 151–162. http://dx.doi.org/10.1155/ 1993/13429.
- Punzo, F. 2012. The Biology of Camel-spiders: Arachnida, Solifugae. Kluwer Academic, Boston.
- Regier, J.C., Shultz, J.W., Zwick, A., Hussey, A., Ball, B., Wetzer, R., Martin, J.W., Cunningham, C.W., 2010. Arthropod relationships revealed by phylogenomic analysis of nuclear protein-coding sequences. Nature 463, 1079–1083. http:// dx.doi.org/10.1038/nature08742.
- Rehm, P., Pick, C., Borner, J., Markl, J., Burmester, T., 2012. The diversity and evolution of chelicerate hemocyanins. BMC Evol. Biol. 12, 19. http://dx.doi.org/ 10.1186/1471-2148-12-19.
- Resh, V.H., Cardé, R.T., 2009. Encyclopedia of Insects. Academic Press.
- Richter, S., Wirkner, C.S., 2004. Kontroversen in der phylogenetischen Systematik der Euarthropoda. In: Richter (Ed.), Kontroversen in Der Phylogenetischen Systematik Der Metazoa. Sitzungsberichte Der Gesellschaft Naturforschender Freunde Zu Berlin, 43, pp. 73–102.
- Roewer, C.F., 1934. Solifugae, Palpigradi. In: Roewer, C.F. (Ed.), Dr. H.G. Bronn's Klassen und Ordnungen des Tierreichs, 5. Akademische Verlagsgesellschaft, Leipzig, pp. 1–723.
- Schmitz, A., 2013. Tracheae in spiders: respiratory organs for special functions. In: Nentwig, W. (Ed.), Spider Ecophysiology. Springer Verlag, Berlin, pp. 29–39.
- Schmitz, A., 2016. Respiration in spiders (Araneae). J. Comp. Physiol. B 186, 403-415.

- Schmitz, A., Perry, S.F., 2000. Respiratory system of arachnids I: morphology of the respiratory system of *Salticus scenicus* and *Euophrys lanigera* (Arachnida, Araneae, Salticidae). Arthropod Struct. Dev. 29, 3–12.
- Schmitz, A., Perry, S.F., 2002. Respiratory organs in wolf spiders: morphometric analysis of lungs and tracheae in *Pardosa lugubris* (L.) (Arachnida, Araneae, Lycosidae). Arthropod Struct. Dev. 31, 217–230. http://dx.doi.org/10.1016/ S1467-8039(02)00045-2.
- Scholtz, G., Kamenz, C., 2006. The book lungs of Scorpiones and Tetrapulmonata (Chelicerata, Arachnida): evidence for homology and a single terrestrialisation event of a common arachnid ancestor. Zoology 109, 2–13.
- Sharma, P.P., Kaluziak, S.T., Pérez-Porro, A.R., González, V.L., Hormiga, G., Wheeler, W.C., Giribet, G., 2014. Phylogenomic interrogation of Arachnida reveals systemic conflicts in phylogenetic signal. Mol. Biol. Evol. 31, 2963–2984.
- Shultz, J.W., 2007. A phylogenetic analysis of the arachnid orders based on morphological characters. Zool. J. Linn. Soc. 150, 221–265.
- Shultz, J.W., 1990. Evolutionary morphology and phylogeny of Arachnida. Cladistics 6, 1–38. Strazny, F., Perry, S.F., 1987. Respiratory system: structure and function. In: Nentwig, W. (Ed.), Spider Ecophysiology. Springer Verlag, Berlin, pp. 78–94.
- Talarico, G., Lipke, E., Alberti, G., 2011. Gross morphology, histology, and ultrastructure of the alimentary system of Ricinulei (Arachnida) with emphasis on functional and phylogenetic implications. J. Morphol. 272, 89–117.
- Van der Meijden, A., Langer, F., Boistel, R., Vagovic, P., Heethoff, M., 2012. Functional morphology and bite performance of raptorial chelicerae of camel spiders (). J. Exp. Biol. 215, 3411–3418. http://dx.doi.org/10.1242/jeb.072926.
- Vogt, G., Wirkner, C.S., Richter, S., 2009. Symmetry variation in the heartdescending artery system of the parthenogenetic marbled crayfish. J. Morphol. 270, 221–226.
- Westheide, W., Rieger, G., 2013. Spezielle Zoologie. Teil 1: Einzeller und Wirbellose Tiere, third ed. Springer Verlag, Berlin.
- Weygoldt, P., Paulus, H.F., 1979. Untersuchungen zur Morphologie, Taxonomie und Phylogenie der Chelicerata II. Cladogramme und die Entfaltung der Chelicerata. J. Zool. Syst. Evol. Res. 17, 177–200.
- Wheeler, W.C., Hayashi, C.Y., 1998. The phylogeny of the extant chelicerate orders. Cladistics 14, 173–192.
- Wigglesworth, V.B., 1983. The physiology of insect tracheoles. Adv. Insect Physiol. 17, 85-148.
- Woodring, J.P., Galbraith, C.A., 1976. The anatomy of the adult uropodid *Fuscouropoda agitans* (Arachnida; Acari), with comparative observations on other Acari. J. Morphol. 150, 19–58. http://dx.doi.org/10.1002/jmor.1051500103.