– Take good care of my fungi – Fungus-spore carrying organs in *Tryopodendron* ambrosia beetles

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Abstract: Pilz-züchtende Ambrosia-Käfer (Coleoptera: Scolytinae) sind durch ihre Symbiose mit mutualistischen Pilzen gekennzeichnet, welche ihnen als essentielle Nahrungsquelle dienen. Die Käfer kultivieren aktiv einen oder mehrere ihrer Nahrungspilze (Ambrosia-Pilze) in ihren Tunnelsystemen, die sie im Xylem toter oder stark geschwächter Bäume anlegen. Die Ambrosia-Pilze besiedeln die Brutsysteme und bilden dabei asexuelle Fruchtkörper an den Tunnelwänden, welche die Hauptnahrungsquelle der Käfer und Larven sind. Zur Übertragung der Ambrosia-Pilzsporen aus dem Geburtsnest in ein neu gegründetes Nest besitzen die Weibchen paarweise angeordnete, ektodermale Organe – sogenannte Mycetangien. Da sich die Pilzzucht innerhalb der Scolytinae mehrmals unabhängig entwickelte, unterscheiden sich die Käfer stark hinsichtlich Lage und Struktur der Mycetangien. Für viele heimische Ambrosia-Käfer sind die Morphologie und Lage dieser Strukturen noch nicht untersucht.
In dieser Studie analysierten wir erstmalig die Mycetangien von *Tryopodendron laeve* Eggers, einem relativ seltenen Europäischen Ambrosia-Käfer. Mittels zerstörungsfreier Röntgentomographie (μCT) entdeckten wir ein gepaartes, röhrenförmiges, pleural-prothorakales Mycetangium, welches den bereits bekannten Strukturen anderer *Trypodendron*-Arten ähnelt. Die Morphologie des gefundenen Mycetangiums wurde mit den bereits beschriebenen Organen von *Tryopodendron domesticum*, *T. signatum* und *T. lineatum* verglichen. Des Weiteren untersuchten wir mit Hilfe der Rasterelektronenmikroskopie (REM) vermutliche tracheale Strukturen in der Nähe der Mycetangien. Diese Tracheen, die sowohl bei Weibchen als auch bei den Männchen vorhanden sind, könnten zur Belüftung der Pilzsporenorgane dienen.

Keywords: Scolytidae, *Trypodendron*, mycetangium, mycangium, Ascomycota, *Phialophoropsis*

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Introduction

The bark beetle genus *Trypodendron* (Coleoptera: Curculionidae, Scolytidae) includes 14 species worldwide, from which at least four are common inhabitants of European forests. The European species, *Trypodendron lineatum* (Olivier 1795), *T. domesticum* (Linnaeus 1758), *T. signatum* (Fabricius 1787) and *T. leave* (Eggers 1939) are classified as fungus-farming ambrosia beetles (Francke-Grosmann 1956, Bussler & Schmidt 2008). They bore tunnels in the wood of recently dead or weakened trees and cultivate species-specific fungi of the genus *Phialophoropsis* (Ascomycota: Ceratocystidaceae) within their galleries (Francke-Grosmann 1956, 1966, Mayers & al. 2015, Lehenberger & al. 2019). As the *Trypodendron* species overwinter either within their galleries (*T. domesticum*, Parini & Petercord 2006), the organic layer on the ground (*T. lineatum*, Oranen 2013) or under the bark of their host trees

(*T. signatum* & *T. laeve*, Schwenke 1974, Bussler & Schmidt 2008) they depend on species-specific, pairwise arranged spore-carrying organs to transport and store their fungal symbionts during hibernation and for transmission into a newly excavated breeding gallery in spring. These organs have evolved independently several times within the Scolytidae (Six 2003) and have been termed mycetangia (Sg. mycetangium) (Giese 1967) or mycangia (Sg. mycangium) (Batra 1963). Mycetangia typically are invaginations of the integument, lined with glands or secretory cells (Batra 1963, Levieux & al. 1991).

Although the ecology is well studied for the European *Trypodendron* species (Francke-Grosmann 1956, Nakashima & al. 1992, Mayers & al. 2015, Lehenberger & al. 2019), only schematic figures from the mycetangia were provided up to date (Francke-Grosmann 1956). Mycetangia in *Trypodendron* have been described as paired, tubular, pleural-prothoracic organs (Francke-Grosmann 1956, Batra 1967, Mayers & al. 2015). The structure is similar for at least three of the European species; slight differences were only noticed regarding the size and shape (Francke-Grosmann 1956; Fig. 1 and 2). Only female *Trypodendron* species are known to carry the mycetangia (Francke-Grosmann 1956). No investigation has so far examined the mycetangial structures of *T. laeve*.

Here, we examined the mycetangial structure of an adult female *T. laeve*, using non-destructive X-ray tomography (µCT). Moreover, we investigated putative tracheal structures close to the beetles´ mycetangia in both female and male *T. domesticum, T. signatum* and *T. lineatum* using scanning-electron-microscopy (SEM).

Materials and Methods

Beetle collection: Beetles were collected at the end of March 2017 in the Bavarian Forest National Park (Bavaria, Grafenau). We used pheromone-baited traps ("Lineatin Kombi" by Witasek). Subsequently, caught beetles were individually stored in 1.5 ml Eppendorf Tubes filled with a piece of damp tissue paper. During sampling, beetles were stored at 4 °C. Beetles were finally identified in Freising (TU Munich) under lab-conditions (Grüne 1979, Bussler & Schmidt 2008) and stored alive (3 – 6°C) until usage.

Morphological description: For Scanning-Electron-Microscopy (JEOL/EO, Version 1.000), we first prepared one male and one female of each beetle species (*T. domesticum*, *T. lineatum, T. signatum*). Therefore, we made putative tracheal structures visible by carefully pushing the pronotum away from the mesonotum (similar to Bateman & al. 2016), using minute pins (Bioform). Additionally, we investigated the prothorax of some beetles without removing their heads. In the latter case, we just cut off the mesothorax and the front legs. Subsequently, beetles were air-dried for at least 24 h.

For the micro-Computed Tomography (µCT) we investigated only one female *T. leave* specimen. The beetle was dehydrated by an ethanol (absolut) dehydration series (30 %, 50 %, 70 %, 80 %, 90 %; each step for 20 min) and finally stored within 100 % EtOH, containing 1% iodine (Alba-Tercedor & Hunter 2014). Afterwards, the beetle was air-dried for 24 h within a sterile hood. The measurements were implemented at the chair of process systems engineering (TU Munich, Freising). Here we used the XCT-1600 HR V1.1 computer tomograph (Matri-Technologies, software: MatriX MIPS-NDT) with a range of 1 µm and 2000 projections. The data were analyzed and visualized (images and videos) using the software MAVI V1.4.1.

Results and Discussion

The µCT examination of a female *T. laeve* revealed a paired, tubular, pleural-prothoracic mycetangium (see Fig. 3a – e). Therefore, we can conclude that all four European *Trypodendron* species have mycetangial structures with similar morphologies (see Fig. 1 and 2; Francke-Grosmann 1956, Batra 1967, Mayers & al. 2015). However, the mycetangium of *T. laeve* is more similar to the one from *T. lineatum* than to those of *T. domesticum* or *T. signatum* (Francke-Grosmann 1956). It is likely that we detected a possible opening (exit or entrance) of *T. laeve´*s mycetangium located behind the procoxa. This assumption was made due to a visible bulge in the lower part of the mycetangium, ending near the procoxa (see Fig. 3d). It seems to directly end in the beetle´s inner cuticula. Similar structures have not been described by Franke-Grosmann (1956) for any of the other *Trypodendron* species. However, such variations within a scolytine genus would not be unusual. The presence and type of mycetangia can vary within a genus, though mycetangia types are often genus-specific (Six 2003). It is noteworthy that the µCT-preparation

method could have influenced the beetle´s inner structures, however. Through the storage of the beetles within ethanol and the drying before the measurement, these structures might have been affected. Even though the beetles were slowly dehydrated, a slight change of the inner structures cannot be completely excluded.

The putative tracheal structures were found to be similar for all of the three investigated *Trypodendron* species and were observed in both females and males (see Fig. 3 f & g). Slight differences were only noticed regarding the size, shape and location. They all showed a conspicuous large, sclerotized surface with thin and fine edges, which are covered with fine eyelash-like hairs. Furthermore, all tracheal structures were located within the lower part of the prothorax. These structures may serve the aeration of the beetle´s mycetangia, but this hypothesis needs to be investigated in the future in more detail.

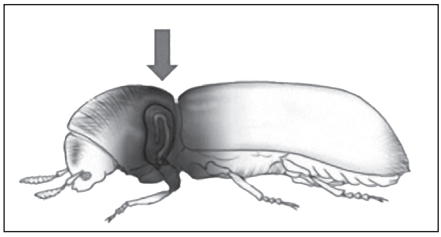

Fig 1: Location of the prothoracid mycetangium within Tryopodendron females.

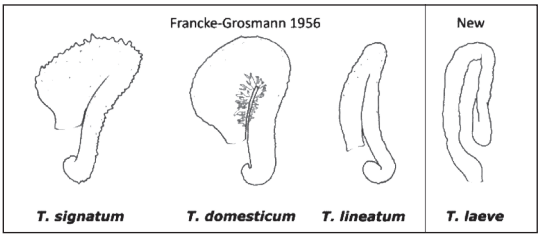

Fig 2: Drawings of mycetangia of Trypodendron spp. (modified from Francke-Grosmann (1956)) and the newly described mycetangium of T. leave.

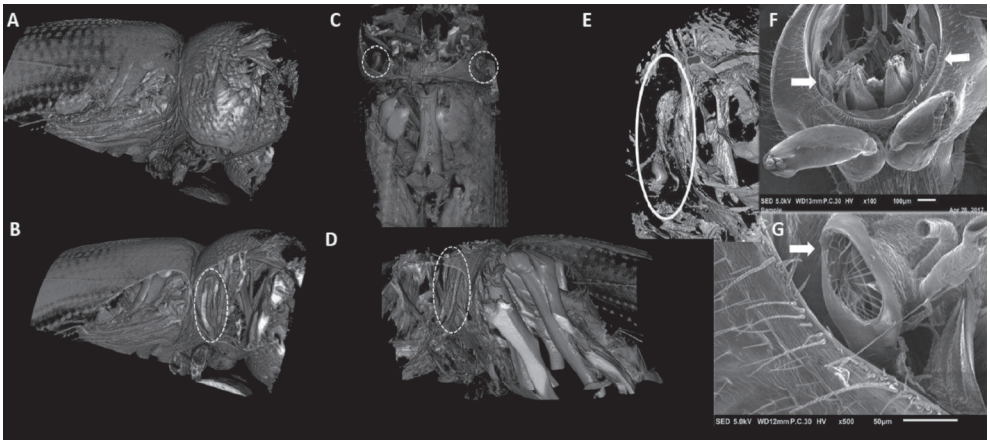

Fig 3: µCT images of *T. laeve*. A: Entire beetle, B: Right side view, C: View from above D: Left side view, notice the bulge at the lower end, E: Complete Mycetangium; Mycetangial structures are marked with a white circle; F & G: Putative tracheal structure of *T. domesticum*. Structures are indicated by arrows.

Conclusions

The µCT-examination of the ambrosia beetle *T. laeve* revealed a paired, pleural-prothoracic mycetangium. This kind of mycetangium is typical for the whole scolytine ambrosia beetle genus *Trypodendron*. Additionally, using scanning-electron-microscopy, we investigated putative tracheal structures close to the beetles´ mycetangia and provide the first SEM images of these structures in T. domesticum, T. signatum and T. lineatum.

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