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Revisiting trends in morphology of antennal sensilla in scarabaeoid beetles

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Abstract

Phytophagous scarab beetles associated with angiosperms have characteristically enlarged lamellate antennae and exhibit a striking morphological variation of sensilla. In this study, we compared the morphology of antennal surface of 62 species Scarabaeoidea using SEM microscopy, particularly also in light of their evolution in association with angiosperms. We investigated the correlation of antennal sensilla morphology, i.e., their structure and distribution, with species diversity and lineage diversification rates. A high diversity of sensilla was observed but also multiple transitional forms, even on the same antennomere. We interpreted this as evidence for a high evolutionary plasticity. We recognized clear patterns of convergence and repeated evolution of certain types of placoid sensilla. One main tendency found in the phytophagous Pleurostict chafers was a shift from sensilla trichodea to placoid-like sensilla, apparently also enhanced by the increase of the lamellate antennal surface, either by size or number of the lamellae. This trend occurred not only in the Pleurosticts, but also in Glaphyridae, a second angiosperm-associated lineage of Scarabaeoidea. However, our results suggest no direct relation between species diversity or the rate of diversification and general sensilla morphology, i.e., the origin of placoid sensilla. This could be explained not only by species-poor lineages also possessing placoid sensilla but also by otherwise successful and species rich groups having sensilla trichodea (e.g., dung beetles). Results further reveal the need to refine current phylogenetic hypotheses by more comprehensive taxon sampling and to expand the molecular characterization of pheromones and odor binding proteins to better understand the role of chemical communication in scarab diversification.

Keywords Coleoptera · Scarabaeidae · Antennal morphology · Sensilla · Evolution

Introduction

Angiosperm plants and their associated herbivores have an important share of terrestrial biodiversity. Several insect groups tracked the rise of the angiosperms, and close coevolutionary interactions with their angiosperm hosts are regarded as a key factor promoting the extraordinary diversity of insects (Ehrlich and Raven 1964). Others have

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diversified in conjunction with the subsequent rise of mammals using their dung (e.g., Ahrens et al. 2014). Key factors promoting global diversification were increased productivity and growth rates of angiosperms due to modification of leaf vein density (de Boer et al. 2012); the evolutionary rise of ectomycorrhiza enhancing chemical weathering of soils (Taylor et al. 2011, 2012); and the promotion of soil nutrient release by angiosperm litter that is easily decomposed (Berendse and Scheffer 2009). This autocatalytic 'litter revolution' resulted not only in a boom of suitable soil habitats, with abundant food resources under various ecological and biogeographical conditions, but also in the development and diversification a complex intestinal endosymbiont microflora (Zhang and Jackson 2008; Andert et al. 2010; Salem and Kaltenpoth 2022). This general environmental change caused by the rise of the angiosperms is likely also to have affected the intra- and interspecific chemical communication, and in consequence all structures involved in it, such as antennal sensilla in arthropods. Food availability in

an increased three-dimensional space provided by the tall angiosperms and deep soil layers, made it more difficult not only to find the right food resources but also mating partners (Leal 1998; Schiestl 2010; Hansson and Stensmyr 2011; Hanks and Millar 2016).

Beetles (Coleoptera) are among the most ecologically diverse insect groups. Phytophagy arose approximately ten times in Coleoptera and may have promoted the largest radiation of beetles, the 'Phytophaga' (Hunt et al. 2007; McKenna et al. 2019). While most of the herbivore species (> 100,000) are adapted to specific host plants (e.g., leaf beetles, longhorns and weevils) (Farrell 1998; Marvaldi et al. 2002), Pleurostict scarabs (Scarabaeidae) (Erichson 1847; Ahrens et al. 2014) are polyphagous; nevertheless, they are one of the most diverse phytophagous beetle groups with about 25,000 species (Scholtz and Grebennikov 2005). They belong to the superfamily Scarabaeoidea, which also include stag beetles, dung beetles, and a number of smaller other groups which are all characterized by a club-like, pectinate antenna (Fig. 1BB), a feature which increases the antennal surface. Some of the plant-feeding scarab Pleurostict species can be crop pests (Jackson and Klein 2006). Despite their unspecific phytophagy and rather similar life style of adults as well as larvae, their morphological evolution over millions of years resulted in divergent body shapes—evidence of past evolutionary pressures in consequence of their soil digging activities and specialized feeding habits (Eberle et al. 2014). To find food and conspecifics for reproduction is vital for their survival and evolutionary success (expressed by the patterns of diversification and morphospace divergence). Therefore, we expect similar trends for the evolution of chemical sense and communication being, among others, expressed in the functionality, density, and morphology of antennal sensilla (Meinecke 1975; Leal 1998). However, detailed comparative studies on antennal morphology in relation to their evolution are lacking, as in most other insects.

Here, we investigate in detail the morphology of antennae and their sensilla in the context of the evolution of Scarabaeoidea. A particular morphological diversity is known in most herbivorous Pleurostict lineages (Meinecke 1975; Bohacz et al. 2020) being expressed by the size and shape of antennal lamella, the type, number, density, and spatial distribution of sensilla. Therefore, we were interested whether the structure of the antennal sensilla is linked to species diversity and speed of diversification of lineages. We hope to obtain deeper insight into the evolution of scarab beetles and the drivers of their diversification.

Chemical communication influences the antennal morphology of insects through selection pressures (Elgar et al. 2018). As in all insects, the antennae are the primary sensory structures in scarabs (Crowson 1981; Chapman 1998). Olfactory sensilla are located on the (unilateral) lamellae, i.e., leaf-like

extensions of the terminal three to seven antennomeres (club) which are spread only by haemolymph pressure (Pass 1980). The number of antennal lamellae may differ between lineages, species, and sex, particularly, in herbivore Pleurostict scarabs (Ahrens and Vogler 2008). Odorant signals can be detected from conspecifics (Nikonov et al. 2002), from microbial processes and from secondary plant metabolisms (de Bruyne and Baker 2008). It is known that several of the used pheromones are produced by intestinal endosymbiont bacteria of the beetles (Hoyt et al. 1971).

If the successful evolution of scarabs in association with angiosperms is mediated by chemical communication and thus by the evolution of advanced antennal sensilla, we would expect particular morphological tendencies in sensilla in association with herbivorous feeding. Furthermore, species diversity and speed of diversification in such lineages with advanced and modified sensilla would be higher compared to that of lineages with unmodified, sensilla trichodea.

Materials and methods

Sampling, preparation, and scanning electron microscopy

We examined 62 species including all major lineages of Scarabaeoidea (Suppl. Table 1). All species examined in this study are deposited in the collections of the Zoological Museum Alexander Koenig Bonn (ZFMK). The antennal lamellae were prepared for SEM as follows: specimens were separately softened in warm water overnight. The following day the antennae were separated from the head with a forceps. For subsequent dehydration, the separated antennal lamellae were treated in an ascending alcohol series (with ethanol solutions of 50%, 70%, 90% and 100%). applying each ethanol solution for five minutes. Some samples were cleaned in an ultrasonic cleaner (Emmi 30; EN IOS 9001:2000). Thereafter each antenna was placed in an acetone filled vial for three minutes, air dried on a microscope slide, and mounted on pin stubs. Finally, they were coated in a Cressington sputter coater (108 auto). SEM photographs of coated specimens were taken with a Zeiss Gemini (SmartSEM, V05.00.05, SEM Type: Supra 55 VP). Some SEMs were obtained with a Zeiss EVO 40 XVP Scanning Electron Microscope at MUSE (Trento, Italy) after gold coating on pieces previously cleaned using a sonicator. Sensilla terminology and classification used here follows Bohacz et al. (2020) and Meinecke (1975).

Patterns of antennal sensilla morphology and its relation to species diversity and diversification rates

The link between plasticity pattern of antennal sensilla morphology (Suppl. Tables 2, 3) and their successful
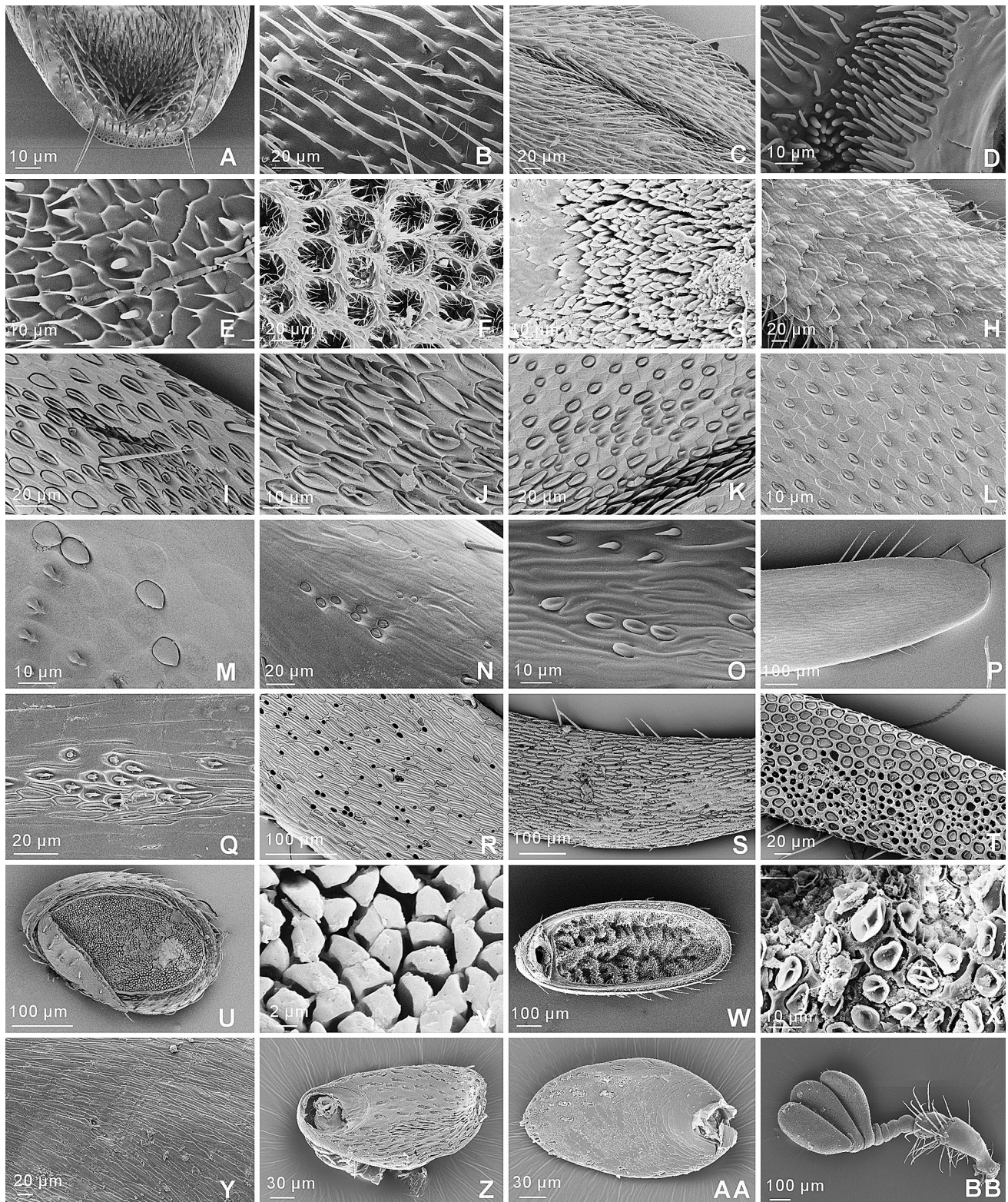
Fig. 1 **A** *Synodendron cylindricum*; **B** *Typhoeus typhoeus*; **C** *Aphodius fossor*; **D** *Onthophagus fracticornis*; **E**, **F** *Odontaeus armiger*; **G** *Codocera ferruginea*; **H** *Xylonychus piliger*; **I** *Automolius angustulus*; **J**, **L** *Liparetrus* sp.; **K** *Liparetrus obscurus*; **M** *Diphucephala* sp.; **N**, **P** *Camenta innocua*; **O** *Triodontella raymondi*; **Q** *Tanyproctus* sp.; **R** *Miotemna* sp.; **S** *Astaena tridentata*; **T** *Raysymmela pallipes*; **U**, **V** *Glaresis handlirschi*; **W**, **X** *Orphnus* sp.; **Y** *Amphicoma bezdekorum*; **Z**, **AA** *Podolasia pilosa*; **BB** *Phaenognatha jenseni*. **A–T**, **V**, **X**, **Y** Antennal lamella surface showing sensilla morphology; **U**, **W**, **Z** second lamella, distal view; **AA** third lamella, basal view; **BB** complete antenna

co-evolution with angiosperms was investigated at two levels. In the absence of species level phylogenies, diversification is often modeled using two different approaches: diversification rates and raw values of species richness (Magallón and Sanderson 2001). We calculated the correlation between the general type of sensilla variation (hair-shaped sensilla vs. placoid sensilla vs. both occurring types mixed; Table 1) with, and a) extant species numbers (of examined lineages) and b) the net diversification rates (Magallón and Sanderson 2001) of selected lineages (of those we were able to examine the sensilla morphology and of which we had molecular data to include the lineages into the phylogeny). In the context of a birth-and-death model of diversification and since extinction is negligible (due to the lack of data), the maximum-likelihood estimate of diversification rate was obtained as $r = \log(n)/t$ for the stem group age (Magallón and Sanderson 2001). We did not consider crown group ages for the calculation of diversification rates, since sampling of molecular phylogenies is yet very incomplete, and current sampling (Ahrens et al. 2014; Gunter et al. 2016; Šípek et al. 2016; Neita Moreno et al. 2019; Eberle et al. 2019) does not reflect complete extant crown groups (but only limited parts of them).

For this purpose, the antennal sensilla morphology was mapped on the recent molecular tree of Neita Moreno et al. (2019) which represents an extended sampling compared to the analysis of Ahrens et al. (2014) which includes four markers widely used in beetle systematics (Hunt et al. 2007; Bocak et al. 2014). We did not consider more recent trees with phylogenomic data (Zhang et al. 2018; McKenna et al. 2019; Cai et al. 2022), due to their more limited taxon sampling. Coding for sensilla and their distribution was very roughly done as follows (Table 1): (1) sensilla trichodea (covering almost 100% of the lamellar surface), (2) placoid sensilla (covering almost 100% of the lamellar surface); (3) both types (covering in relation of ca 50–50% of the lamellar surface); (4) polyedric sensilla; (5) undefined sensilla type (homology uncertain). Sensilla types can be characterized even at finer scales (Altner and Prillinger 1980; Meiniecke 1975; Bohacz et al. 2020; see Suppl. Tables 2, 3), however, many lineages and taxa share multiple minor or transitional types, even within the same species, which make a summarizing analysis in relation to successful evolution very complex. Therefore, we applied for this study a more simplified scheme (see also Scholtz 1990), to reveal prominent and principal patters.

Speciation rates of the clades including stem lineages were calculated based on the time tree (and divergence times) of Ahrens et al. (2014) and number of extant species from Schoolmeesters (2021) and Ahrens (unpublished data). Speciation or diversification rates are an estimate of the rate of change in species numbers over time using clade age and species richness data, typically modeled as some form of birth–death process (Nee et al. 1994; Morlon 2014). While such analyses try to capture speciation and extinction processes more accurately, they can produce misleading results when diversity is not constant or unbounded through evolutionary time (Rabosky 2009; Wiens 2011), particularly when the fossil record is poor (Magallón and Sanderson 2001). This is known to be the case for insect diversification where there have been rapid bursts of speciation through time (Cornwallis et al. 2021).

Results

Our results demonstrate that the majority of early lineages of Scarabaeoidea have trichoid antennal sensilla (sensilla trichodea; Fig. 1A–H; Suppl. Fig. 3). This includes Lucanidae, Geotrupidae, Hybosoridae, Ochodaeidae, Belohinidae, Pleocomidae, and Passalidae (Suppl. Figs. 1A–H, 3A–C, M–R, 5I–N). In most cases, all antennomeres of the antennal club have such sensilla, on both sides. In Bolboceratidae and Glaresidae, we found, in addition to trichoid antennal sensilla, highly modified sensilla, which were in case *Glaresis* polyedric with fine pores (Fig. 1U, V; Suppl. Fig. 3D–I; see also Anton and Beutel 2012) and multi-auricular in Bolboceratidae (Fig. 2F; Suppl. Fig. 5A–H; see also Meinecke 1975). In some genera of Ceratocanthinae (Hybosoridae) the sensilla trichodea occur in different shapes, with more specialized shorter setae often lumped inside alleged "sensory areas" such as the case of the genus *Pterorthochaetes* (Fig. 3S–T). In Belohinidae there is a distinctive U-shaped sensory area composed of setae-like sensilla on the outer side of the last antennomere (Suppl. Fig. 3M–N). The highest variation of sensilla morphology we found in Scarabaeidae, of which, however, the monophyly (Timmermanns et al. 2016; Zhang et al. 2018; Song and Zhang 2018; McKenna et al. 2019; Ayivi et al. 2021) is still subject to some controversy since some molecular phylogenies did not confirm monophyly (Hunt et al. 2007; Bocak et al. 2014; Ahrens et al. 2014; Cai et al. 2022; see Fig. 3 based on Neito Moreno et al. 2019).

Within Scarabaeidae, saprophagous and dung-associated taxa (the clade Scarabaeinae+Aphodiinae) still possess sensilla trichodea (Fig. 3H, G). In Pleurostict lineages including its sister clade Orphninae [+ Allidiostomatinae according to Neito Moreno et al. (2019), supposed sister to Orphninae, but their sensilla could not being yet examined here] we see a clear shift to strong modifications of sensilla morphology from sensilla trichodea to an auriculate and/or placoid style (Fig. 3; Suppl. Fig. 2A–I). This strongly coincides with the transition to herbivorous feeding, i.e., the direct association with angiosperms (Fig. 3, arrow; see also Ahrens et al. 2014). In a few lineages throughout Scarabaeoidea, the sensilla are situated unilaterally on the lamellae (e.g., *Acanthocerodes*; Suppl. Fig. 3Q–R) or in a kind of closable pocket

Table 1 Simplified sensilla type distribution, species number (* based on Ahrens, unpublished data, otherwise Schoolmeesters 2021), age and diversification rate of selected investigated scarab lineages

Clade	Main type sensilla*	Species number	Age-stem lineage[1]	Diversification rate[2]
Ablaberini	**2**	**230***	**99.28**	**0.0238**
Adoretini	**2**	**633**	**64.23**	**0.0436**
Anomalini	**2**	**2461**	**68.9**	**0.0492**
Aphodiinae	1	3300	108.78	0.0323
Bolboceratidae	3	623	134.91	0.0207
Cetoniinae	**2**	**3920**	**85.98**	**0.0418**
Diphycerini	**2**	**39**	**59.05**	**0.0269**
Diplotaxini	**2**	**928**	**59.05**	**0.0503**
Dynastinae	**2**	**1999**	**60.96**	**0.0541**
Euchirini	**2**	**16**	**83.33**	**0.0145**
Geotrupidae	1	462	167.81	0.0159
Glaphyridae	2	206	142.11	0.0163
Glaresidae	4	95	147.63	0.0134
Hopliini	**2**	**791**	**73.27**	**0.0396**
Hybosoridae[a]	1	645	162.58	0.0173
Liparetrinae[3]	**1&3**	**1110**	**108.9**	**0.0280**
Lucanidae[b]	1	1766	147.63	0.0220
Macrodactylini	**2**	**1125**	**59.05**	**0.0517**
Melolonthini	**2**	**793**	**48.67**	**0.0596**
Ochodaeidae	5	146	132.95	0.0163
Orphninae	5	214	62.11	0.0375
Pachypodini	**2**	**6**	**82.89**	**0.0094**
Passalidae	1	849	69.63	0.0421
Rhizotrogini	**2**	**1950**	**67.12**	**0.0490**
Rutelini	**2**	**1195**	**74.02**	**0.0416**
Scarabaeinae	1	6741	108.78	0.0352
Schizonychini	**2**	**371**	**62.08**	**0.0414**
Sericini (Old World)	**2**	**3785***	**93.35**	**0.0383**
Sericini (Neotropics)	**2**	**200***	**93.35**	**0.0246**
Tanyproctini	**2**	**641**	**82.79**	**0.0339**
Trogidae	1	333	142.21	0.0177
Valginae	**2**	**357**	**42.73**	**0.0597**

Lines with Pleurostict lineages feeding on angiosperms are in bold

*(1) sensilla trichodea (covering almost 100% of the lamellar surface), (2) placoid sensilla (covering almost 100% of the lamellar surface); (3) both types (covering in relation of ca 50–50% of the lamellar surface); (4) polyedric sensilla; (5) undefined/polyauriculate (homology uncertain).)

[1]Stem lineage age based on Ahrens et al. (2014)

[2]After Magallón and Sanderson (2001)

[3]Here formally reestablished for the lineage so far termed as "Southern World Melolonthinae" (Ahrens and Vogler 2008) or "Liparetrini sensu lato" (Ahrens et al. 2014). It includes a number of Australian and South American tribes so far referred to Melolonthinae, such as Automoliini, Colymbomorphiniv, Comophorinini[$], Diphucephalini, Heteronycini, Liparetrini, Maechidiini, Pachytrichini[$], Phyllotocini, Phyllotocidiini[$], Scitalini, Sericoidini, Systellopini[$]. Some of these ([$]) were not so far included in molecular phylogenies, their assignment is based on a number of morphological diagnostic characters, such as the widely separated, preapical metatibial spines

[a]Including Ceratocanthinae; the actual species number has risen at time of submission to 719 species
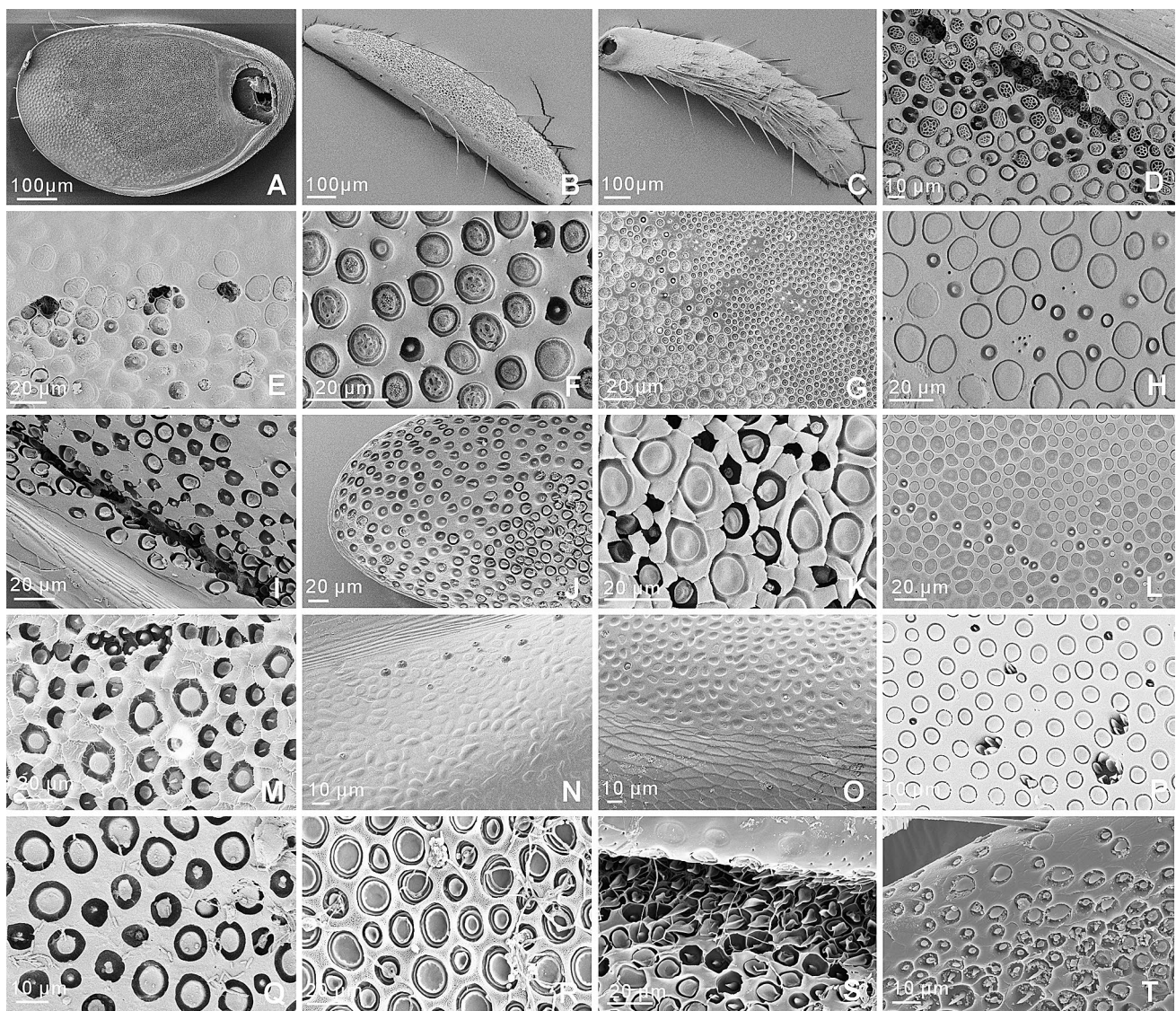
[b]Including Diphyllostomatidae

Fig. 2 **A–G** *Valgus hemipterus*; **B–D** *Prodoretus rhodesianus*; **E** *Anomala dubia*; **F** *Pentodon punctatus*; **H** *Osmoderma* sp.; **I** *Hoplia philanthus*; **J** *Schizonycha ruficollis*; **K** *Amphimallon assimile*; **L** *Cetonia aurata*; **M** *Melolontha melolontha*; **N** *Trichius* sp.; **O** *Gnorimus variabilis*; **P** *Pachypus* sp; **Q** *Photyna ornata*; **R** *Euchirus longimanus*; **S** *Pachytrichus* sp.; **T** *Aclopus* sp. **A** second lamella, distal view; **B** third lamella, distal view; **C** first lamella, basal view; **D–T**—antennal club surface showing sensilla morphology

(e.g., in *Codocera ferruginea*; Suppl. Fig. 1A–F.). External faces of the club or also the basal face of a lamella may have a reduced coverage with sensilla or an entire lack of them (Fig. 2B, C), a pattern that is found in almost all lineages (with numerous exceptions, however). Among Pleurosticts, in Liparetrinae (see Table 1) and Aclopinae we observed a transition from sensilla trichodea to placoid sensilla in which very different shapes of trichoid to auriculate and round placoid sensilla can be encountered in some taxa on the same lamella. In these cases, placoid and trichoid sensilla, or their intermediate forms, have often similar frequencies (almost 50:50; see Table 1). Other Liparetrinae have entirely sensilla trichodea only (e.g., Colymbomorphini, Sercoidini;

Bohacz et al. 2020), a state which is not encountered anymore in the sister clade (Melolonthinae + Rutelinae + Cetoniinae + Dynastinae). For this reason, we assume that this transformation series is likely: sensilla trichodea > scale-like sensilla > auriculate sensilla > placoid sensilla (Fig. 1I–M). In some lineages, such as Hopliini and Macrodactylini, these auriculate sensilla are preserved in mixture with normal placoid sensilla (Figs. 2I, 3T; see also Romero-López et al. 2013, 2017).

In most Pleurostict lineages can be found different types of placoid sensilla which are either mixed with each other (Fig. 2D–F, H) or sorted in different zones on each lamella (Fig. 2A, G). However, often transitions between different

Fig. 3 Antennal and sensilla morphology mapped onto the phylogeny of the Scarabaeoidea (Neita et al. 2019), with reference to the number of known species per lineage shown (simplified). Several nodes are simplified by a cartoon-like triangle, single branches were represented in that analysis only by a single species. Colored dots with letters refer to prevailing general sensilla type and illustrated species: **A** *Glarsis handlirschi*; **B** *Trox hispidus;* **C** *Sinodendron cylindricum*; **D** *Typhaeus typhoeus*; **E** *Passalus striolatus*; **F** *Odontaeus armiger*; **G** *Onthophagus fracticornis*; **H** *Aphodius fossor*; **I** *Codocera ferruginea*; **J** *Amphicoma bezdekorum*; **K** *Hybosorus illigeri*; **L** *Orphnus* sp.; **M** *Arctodium vulpinum*; **N** *Neophaenognata jenseni*; **O** *Liparetrus obscurus*; **P** *Camenta innocua*; **Q** *Astaena tridentata*; **R** *Maladera holosericea*; **S** *Euchirus longimanus*; **T** *Hoplia philanthus*; **U** *Photyna ornata*; **V** *Schizonycha ruficollis*; **W** *Melolontha melolontha*; **X** *Amphimallon assimile*; **Y** *Tanyproctus* sp.; **Z** *Valgus hemipterus*; **AA** *Pachypus* sp.; **BB** *Prodoretus rhodesianus*; **CC** *Anomala dubia*; **DD** *Pentodon idiota*
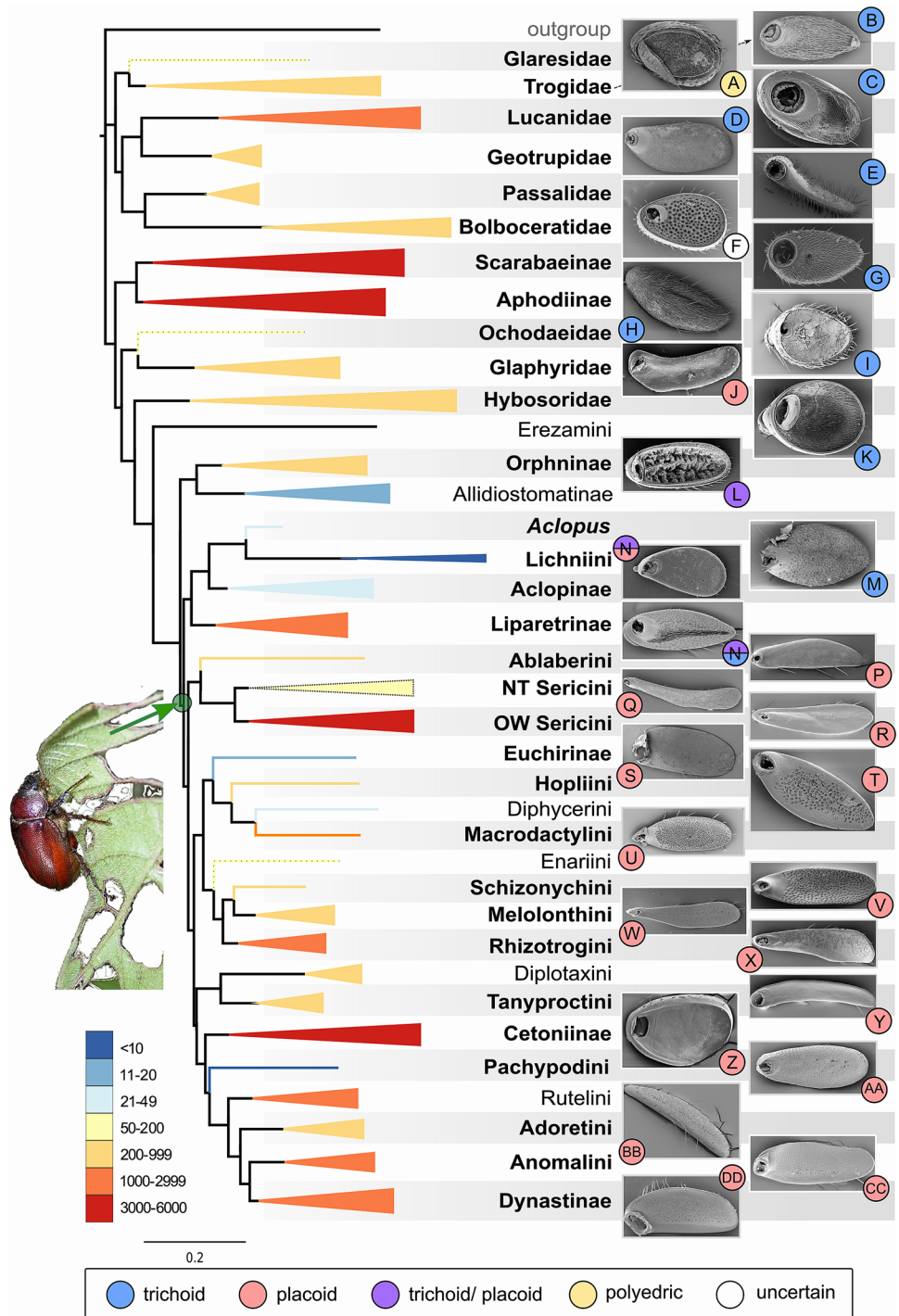
sensilla "types" can be rather fluent in one and the same lamella (see also Meinecke 1975). In several lineages, particularly in the clade Cetoniinae + Rutelinae, sensilla on the distal face of the lamella are situated in deepened pockets or sulci, which are encountered also in Hopliini, Melolonthinae or Pachytrichini. The dimension and number of these pockets can vary from group to group (see Suppl. Table 3). The round placoid sensilla can transform into elongate placoid sensilla which can be 20 times as long as wide, independently from the length of the antennal lamella (Fig. 1N–S). Such elongate placoid sensilla originated at least twice independently from each other, one time in Sericini + Ablaberini, and one time in Tanyproctini (and *Acoma* whose systematic placement is considered somewhat uncertain; Evans and Smith 2020). Interestingly, similar elongate placoid sensilla occur also in Glaphyridae (Fig. 1Y) which show

no transitional steps from the sister group Ochodaeidae. In these cases, no other sensilla types are mixed with these elongate placoid sensilla, except in the lineage of Neotropical Sericini (Pacheco et al. 2022) in which we find, as if complexity was not enough, a stepwise reversal from elongate placoid to almost round placoid sensilla (Fig. 1T). In Ablaberini and Old World Sericini, no such reversals have been found. Furthermore, in the Neotropical Sericini, placoid sensilla can be mixed with other placoid sensilla which have pores, similar to those found in Dynastinae.

While adopting Meinecke's (1975) typification of sensilla (Suppl. Tables 2, 3, Bohacz et al. 2020) and studying more species in particular several species of Liparetrinae and Aclopinae, we realized that incorporating the immense amount of infraspecific sensilla variation into a character matrix with multistate character coding would be rather complicate (e.g., Figs. 1I, K, 2D, S). Therefore, the correlation analysis was based on simpler assumptions (see methods). Nevertheless, neither the correlation analysis of pure species diversity ($r = -0.329$; $p = 0.066$) nor the diversification rate ($r = 0.048$; $p = 0.794$) could reveal a significant relation between the morphological constitution between sensilla type and evolutionary success of the lineages. Even if we excluded the evolutionary very successful dung beetles, which had also an indirect angiosperm follow-up associated with the mammals (Ahrens et al. 2014) and which possess antenna uniformly covered with sensilla trichodea (Fig. 1C, D), there was no change in levels of significance (species diversity: $r = -0.149$; $p = 0.431$/ diversification rate: $r = 0.054$; $p = 0.778$).

Discussion

While comparative studies on antennal sensilla morphology in insects are rare (Hallberg and Hansson 1999), we attempt here to relate the comparative morphology of antennal sensilla, as a proxy for the evolutionary impact of underlying chemical communication, with species diversity and diversification rates. General morphology of antennal sensilla is rather uniform in most insects, mainly composed of sensilla trichodea (e.g., Hallberg and Hansson 1999; Nowińska and Brożek 2017; Yuvaraj et al. 2018); this could be one cause for the lack of overarching studies in beetles and other insect groups. This, however, is not the case in scarabaeiform beetles in which at least 44 different sensilla types have been reported so far (Meinecke 1975; Scholtz 1990; Bohacz et al. 2020).

The general patterns of antennal sensilla morphology reported here suggest that there is an apparent link between the evolution of placoid sensilla and the association with angiosperms (including phytophagous feeding) (Fig. 3). This trend is possibly even parallel, as many taxa in the

Liparetrinae + Aclopinae, sister to the remainder Pleurosticti have mostly sensilla trichodea (Fig. 3; Suppl. Figs. 6, 8; Bohacz et al. 2020), while ancestral state of the latter remains uncertain yet since in all ancestral lineages already prevail placoid sensilla, either round or elongate ones.

No direct relation between species diversity or rate of diversification and general sensilla morphology was found, instead. This could be explained by the fact that some lineages with placoid sensilla include only few species belonging often phylogenetically isolated rogue lineages of uncertain systematic placement (e.g., Phaenomeridinae, Euchirini, Pachypodini), which were addressed in this study too. Many more were not yet included or just poorly sampled due to either insufficient available specimens (e.g., the polyphyletic Tanyproctini; Eberle et al. 2019) or the lack of DNA data for certain lineages, thus being not represented yet in the available molecular phylogenies (Hunt et al. 2007; Bocak et al. 2014; Ahrens et al. 2014; Timmermanns et al. 2016; Zhang et al. 2018; Song and Zhang 2018; McKenna et al. 2019; Neito Moreno et al. 2019; Ayivi et al. 2021; Cai et al. 2022). Moreover, there are also several lineages with exclusively trichoid sensilla that are quite diverse such as scarab dung beetles (Scarabaeinae, Aphodiinae) or stag beetles (Lucanidae) (Table 1).

We refrained here from a sister group comparison (e.g., Slowinski and Guyer 1993) due to two reasons: (1) existence of only one clear major shift from sensilla trichodea to placoid-like sensilla at the root of the clade (Melolonthinae + Rutelinae + Cetoniinae + Dynastinae) Pleurostict chafers (green dot, Fig. 3), while (2) the shift among Liparetrinae and Aclopinae is rather patchily known yet and in the known cases often quite gradual which requires a much denser lineage sampling for being able to link the sensilla morphology with exact number of species diversity or the rate of evolution. Yet, Pleurostict chafers, particularly the clade (Melolonthinae + Rutelinae + Cetoniinae + Dynastinae), are the most diverse lineage of Scarabaeoidea. Thus, the connection of chemical communication/sensilla morphology (i.e., a tendency to placoid sensilla) and diversification success continues being needed to be explored further in detail, particularly also because Zhang et al. (2018) reported, in comparison to all other scarabaeiform beetles, significantly increased diversification rates for Scarabaeidae, which in their tree included dung beetle lineages (Scarabaeinae + Aphodiinae) and phytophagous Pleurosticts.

We expect that chemical communication is rather more complex than it would become apparent from the simple examination of sensilla morphology. Sensilla trichodea prevail also in all hyperdiverse phytophagous beetle lineages of Phytophaga with no increased antennal surface (e.g., Ritcey and McIver 1990; Ranger et al. 2017; Vera and Bergmann 2018; Di Palma et al. 2019; Dong et al. 2020), thus high performance in chemical sensing is not necessarily

linked with complicated or highly modified sensilla structure (e.g., Symonds et al. 2011). For instance, Rutelinae and Melolonthinae, although having in many lineages quite similar placoid sensilla, differ completely in their pheromone compounds (Melolonthinae: amino acid derivatives and terpenoids; Rutelinae: fatty acid derivatives; Leal 1998). Since "Melolonthinae" referred in Leal (1998) to unrelated lineages of Liparetrini, Rhizotrogini and Melolonthini, it is uncertain, whether these compounds could be part of an "ancestral" phylogenetical pattern or if they do represent lineage specific components. Chapman (1982) suggested that insects with a generalist diet require a greater number of sensilla than species with a more specialized diet, a hypothesis which is, however, only moderately supported by more recent studies (Lopez et al. 2014).

Last but not least, the high plasticity of sensillar morphology, which was already noticed by Meinecke (1975) based on histological analyses, makes the extraction of categorical data and discrete character states difficult in a comparative context. However, such plasticity is less common in the much less diverse early lineages of Scarabaeoidea (besides prominent exceptions such as in Glaresidae, Bolboceratidae, Glaphyridae, or Ochodaeidae; Fig. 3). This might be a starting point for further hypotheses to test, particularly when exploring sensilla variation in the context of pheromone diversity which is so far rather fragmentarily known.

Interestingly, placoid sensilla (although with much inferior morphological diversity) are also known in other, less diverse beetle lineages with antenna that have an increased antennomere surface (Ramsey et al. 2015). However, increased antennomere surface is not generally linked to the presence of placoid sensilla, as e.g., in Drilidae with pectinate antenna yet only sensilla trichodea occur (Faucheux and Kundrata 2017). Nevertheless, Ramsey et al. (2015) argue that the elaborate lamellate antennae, such as in male *Rhipicera* beetles, increases the surface area, which changes the airflow across the antennae, and thus the likelihood of odorant–receptor interactions (Jaffar-Bandjee et al. 2020). The latter is supposed to have an impact, in consequence, to the sensilla morphology and their efficacy.

Conclusions

While there seem to be a clear connection between the sensillar morphology and the polyphagous–phytophagous feeding traits of Pleurostict chafers, simple linear statistics do not confirm this hypothesis. Simple evolutionary connections appear to be obscured by other additional patterns such as evolutionary opportunities and competition, in combination with dispersal chances. Some lineages of the diverse

Pleurosticts include only a relatively low number of species (e.g., Liparetrinae + Aclopinae) and are obviously restricted to the southern world (exception Podolasiini?). Also, among other Pleurosticts (i.e., the paraphyletic "Melolonthinae", Cetoniinae, and Rutelinae), there are some quite species-poor lineages and slowly diverging lineages with "modern" sensilla (Table 1). Hence, we see the here presented hypotheses and results as a starting point to refine our phylogenetical understanding of the Scarabaeoidea but most of all to open up for a molecular characterization of antennal sensilla functionality, regarding pheromones and odor binding proteins of sensilla (e.g., Leal 2001; Nikonov et al. 2002; González-González et al. 2019) to improve our understanding of their chemical communication, ecology and evolution.

Declarations

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