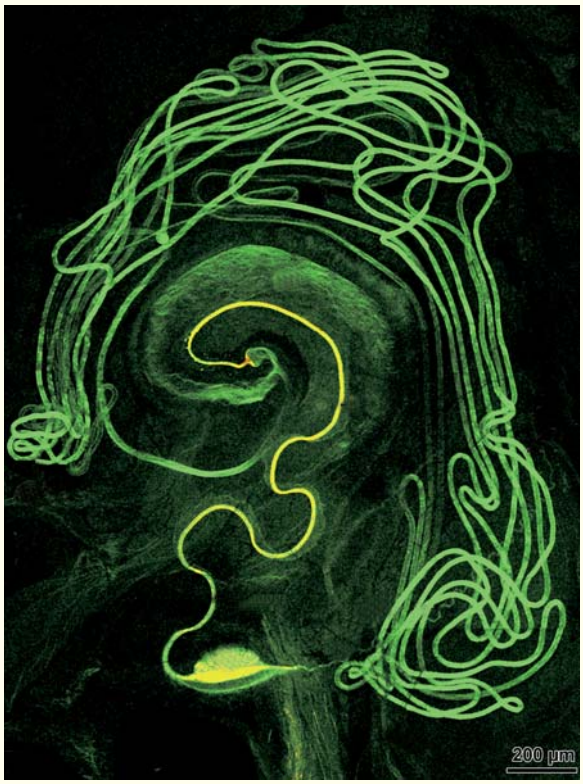
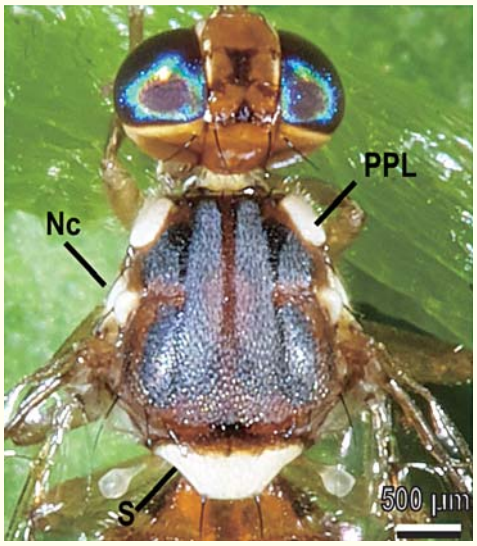
Tavole Rotonde sui maggiori problemi
riguardanti l'Entomologia Agraria in Italia
Sotto gli auspici del MIPAAF

XLI.
NOVEL FINDINGS IN ARTHROPODS FUNCTIONAL MORPHOLOGY AND ANATOMY





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Coordinatori:
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INSECT SYSTEMATICS FROM HENNIG TO TRANSCRIPTOMES - ANATOMY AND PHYLOGENOMICS CONVERGE UPON A ROBUST PHYLOGENY

ROLF GEORG BEUTEL - BENJAMIN WIPFLER - KAREN MEUSEMANN - HANS POHL

Corresponding Author: Rolf Georg Beutel, E-Mail: rolf.beutel@uni-jena.de
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Insect systematics from Hennig to transcriptomes - anatomy and phylogenomics converge upon a robust phylogeny

Insect systematics underwent an impressive "evolution" in the last 20 years, especially in terms of methodology, including analyses of very large molecular data sets, but also refined anatomical techniques. Nevertheless, some older phylogenetic concepts were largely confirmed in the "age of phylogenomics". A remarkable tree of insects was already presented by Carl Börner in 1904, very close to recent phylogenetic patterns. In the mid-20th century Willi Hennig revolutionized systematics, and his "Stammesgeschichte der Insekten" was a major breakthrough. The first cladistic analysis of morphological characters covering the entire Hexapoda, published in 2001, largely confirmed Hennig's hypotheses. In contrast, earlier molecular studies based on ribosomal genes yielded partly unorthodox results. Recent phylogenomic studies (1KITE) again largely confirm Hennig's views, with very few exceptions. The interordinal relationships are now largely resolved. Persistent problems are the relationships of the entognathous orders, the "Palaeoptera problem" (Odonata, Ephemeroptera, Neoptera), and the monophyly of Paraneoptera and Mecoptera. Future research perspectives are exploring insect evolution in the dimension of time and linking transformations on the phenotypic and genotypic levels. A major challenge is linking evolutionary insights with efforts to handle the rapidly declining diversity and biomass. These aims should be tackled in a close cooperation between ecologists, taxonomists, morphologists, palaeontologists, molecular systematists and geneticists.

KEY WORDS: Hexapoda, systematics, Hennig, evolution

INTRODUCTION

Entomology was often considered as a somewhat amateurish discipline, as collecting insects was popular among non-scientists for centuries (e.g. BEUTEL *et al.,* 2009; SANTAOJA, 2021). However, this is by no means a negative point. In contrast, amateurs have made a tremendous contribution, of course to taxonomy, but also by providing data which are now very important in the context of climatic change and the dramatically declining diversity and biomass of insects (HALLMANN *et al.,* 2017). Aside from this, entomology today is a strong and modern discipline, with a tremendous progress in anatomical and phylogenomic investigations (e.g. BEUTEL *et al.,* 2011; MISOF *et al.,* 2014).

There are many reasons why insects can be fascinating. One of them is the unparalleled diversity. With more than a million described species they comprise more than 50% of all known organisms. This immense diversification is a fascinating phenomenon from an evolutionary perspective. Methods and problems of phylogenetic reconstruction are more or less the same as in all other groups of animals. However, due to the enormous diversity and complexity of the group, collecting well-documented sufficient data can be challenging, and a lot of routine work is often required.

One of the main aims of the Entomology Group at the Phyletisches Museum (Institut für Zoology und Evolutionsforschung, FSU Jena) is to improve anatomical methods. An optimized combination of different techniques (e.g. WIPFLER *et al.,* 2016) has greatly accelerated the acquisition of high quality anatomical data. Within few years, matrices with several hundred well documented characters could be compiled in projects on the three major lineages of pterygote insects, Polyneoptera, Paraneoptera (in the strict sense, i.e. excluding Zoraptera, = Acercaria), and Holometabola (e.g. BEUTEL *et al.,* 2011; WIPFLER *et al.,* 2011, 2019; FRIEDEMANN *et al.,* 2013).

The value of morphology is undisputed today, for "plausibility checks" of molecular phylogenies, but especially for reconstructing evolution on the phenotypic level, for ancestral state reconstruction, and for placing fossils (e.g. KJER *et al.,* 2016). However, the tremendous progress of molecular systematics, especially in the last decade, was doubtlessly crucial in insect systematics. The international 1KITE (=1K insect transcriptome evolution) project plays an out-

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standing role in this context. Its primary aim is to reconstruct the phylogeny of the entire Hexapoda using transcriptomes of 1000 selected species. A first major study was Misof *et al.* (2014) and many others followed, either on specific evolutionary topics (e.g. McKenna *et al.,* 2019), or on large insect subgroups such as Polyneoptera (Wipfler *et al.,* 2019) or Paraneoptera (Johnson *et al.,* 2018).

Earlier Reconstructions of Insect phylogeny and Willy Hennig

Even though Hexapoda did not belong to the favourite groups of Ernst Haeckel, arguably the most famous German zoologist and founder of the Phyletisches Museum, he presented an insect phylogeny in one of his major works covering all animal phyla (Haeckel, 1896). Even by the standard of his time he chose a problematic approach. His tree was largely based on the mode of food uptake and related features of the mouthparts. It is not surprising that he displayed unorthodox groupings, like for instance the holometabolous Diptera with the hemimetabolous Hemiptera, or the holometabolous Coleoptera with the hemimetabolous Orthoptera. Not only from today's perspective his phylogenetic tree and classification are not convincing.

In contrast to Haeckel's flawed concept, a remarkable insect phylogeny was presented by Börner (1904), a specialist of grape phylloxera ("Reblaus"). His tree is very close to modern insect phylogenies, even though he still lacked a consistent systematic methodology (Fig. 1A). In his classification he combined the morphologically similar Archaeognatha and Zygentoma in a group Thysanura. However, interestingly, the silverfish were correctly placed as sistergroup of the pterygote insects in his tree, a concept commonly accepted today (e.g. Hennig, 1969; Misof *et al.,* 2014).

In the mid-20th century the dipterist Willi Hennig revolutionized systematics. His theoretical work had a tremendous impact on phylogenetics, and cladistics goes back to his innovations (e.g. Kjer *et al.,* 2016). His "Stammesgeschichte der Insekten" (Hennig, 1969) was a major breakthrough. It was based on an informal evaluation of morphological characters with relationships based on synapomorphies, and on a profound evaluation of the literature on extinct and extant insects (Fig. 1B).

Cladistic approaches

The first two cladistic analysis covering the entire Hexapoda were Beutel and Gorb (2001) and Wheeler *et al.* (2001). The former study was mainly focused on the evolution of attachment structures. The data were mostly taken from Hennig (1969) and from review studies published by N.P. Kristensen (e.g. 1975, 1995). The results were largely consistent with the ideas of these two eminent entomologists. The study provided new insights on the evolution of adhesive devices, but was not a breakthrough in terms of phylogenetic results (Fig. 1C).

Wheeler *et al.* (2001) was primarily based on 18S and 28S rRNA and an analytical approach called POY, simultaneous sequence alignment and parsimony analysis in a single step. Additionally, the study contained a large morphological data set, extracted from Hennig (1969) and other sources. The published cladograms based on the two genes or on either of them show a very unorthodox pattern, with most orders not monophyletic and Strepsiptera placed outside of insects. After combining molecular and morphological data, important groups were held together by morphological apomorphies. The final tree was "based on the discussion *and* the data" (Wheeler *et al.,* 2001: fig. 20). A result presented with great confidence was a clade "Halteria" comprising Diptera and the endoparasitic Strepsiptera. This was in stark contrast to earlier hypotheses (e.g. Hennig, 1969), were strepsipterans were tentatively placed as sistergroup of Coleoptera, or even as a subordinate group of polyphagan beetles (Crowson, 1981).

The 1KITE Project

Several single gene analyses followed in the next years, for instance Kjer (2004), who advocated a homology-based manual alignment of sequence data. A major early study based on transcriptomes was Meusemann *et al.* (2010), addressing the phylogeny of the entire Arthropoda. The first study published in the 1KITE project, Misof *et al.* (2014), was based on 1478 orthologues genes, with a sampling covering all insect orders, and also including rare and controversial taxa such as for instance *Tricholepidion* and *Nannochorista*, the former traditionally assigned to Zygentoma and the latter to Mecoptera. Analytical procedures were refined and a new working pipeline for processing such enormous amounts of data was developed.

An interesting outcome of Misof *et al.* (2014) was that Willy Hennig's ideas were confirmed in most cases, and issues which were considered difficult before remained obscure (Fig. 1D). A crucial result, already proposed by Meusemann *et al.* (2010), was that Hexapoda are not the closest relatives of myriapods but that they are nested within a large clade Pancrustacea, combining insects in the widest sense with paraphyletic crustaceans. This was never suggested based on morphology (e.g. Hennig, 1969; Kraus and Kraus, 1994; see Richter 2002), but

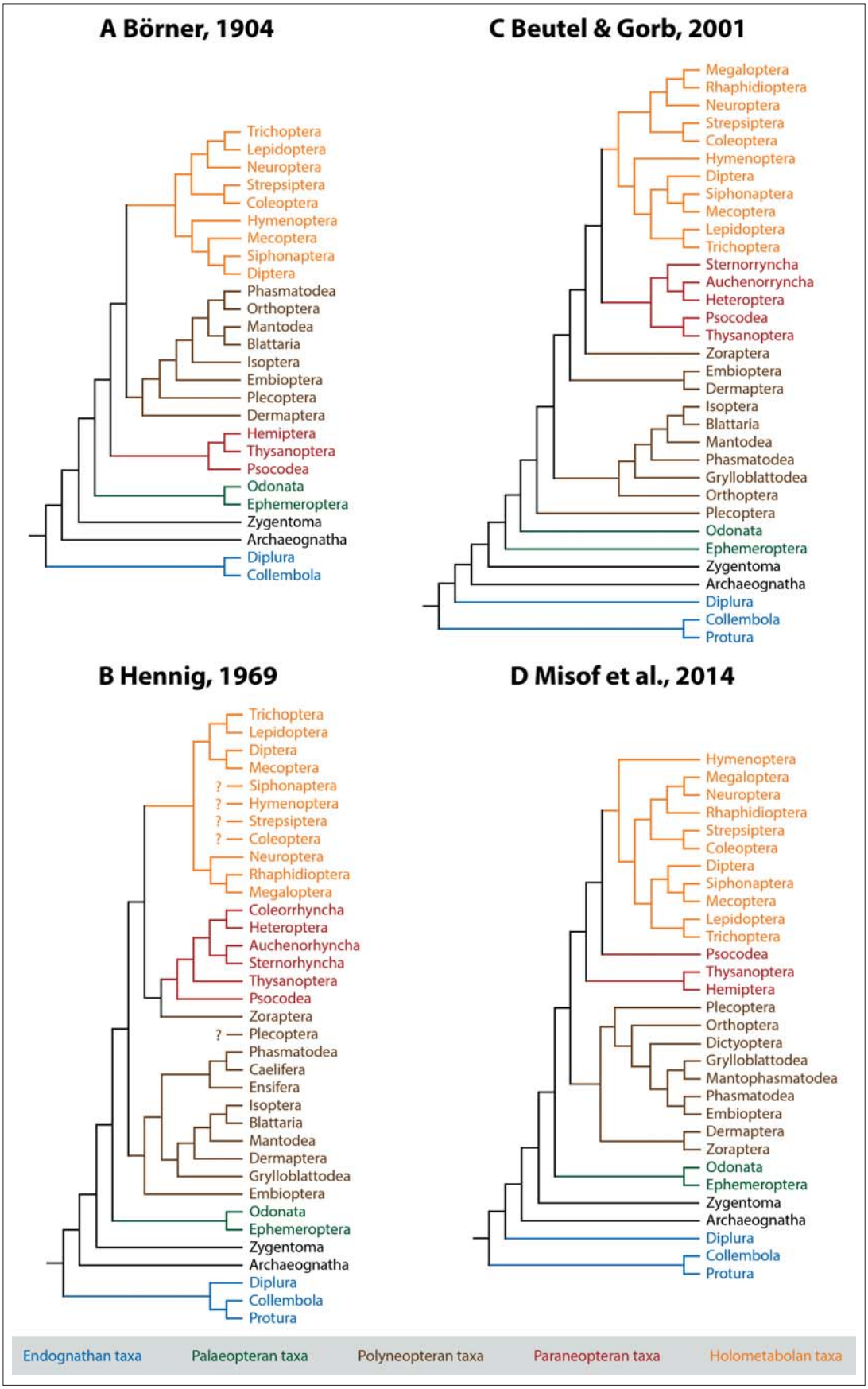

Fig. 1 – Cladograms showing different insect phylogenies. A) Börner (1904), based on an informal evaluation of morphological characters. B) Hennig (1969), based on an evaluation of morphological characters following the principles of phylogenetic systematics. C) Beutel and Gorb (2001), cladistic (numerical) evaluation of 115 morphological characters of adults and immature stages, including 10 character of attachment structures. D) Misof *et al.* (2014), based on 1478 orthologous protein-coding genes of representatives of all hexapod orders + outgroup taxa.

was already supported by early molecular studies with only few hundred base pairs (FRIEDRICH and TAUTZ, 1995).

In contrast to the robustly supported Pancrustacea (or Tetraconata), the precise placement of insects among crustacean taxa is not fully clarified. However, it is getting more and more likely that the highly specialized cave-dwelling Remipedia are the sister group. This is suggested by transcriptomic data (MISOF *et al.,* 2014) and a close relationship was previously postulated by FANENBRUCK *et al.* (2004), based on specific apomorphies of the brain. Unfortunately, morphological comparisons between crustaceans and hexapods are notoriously difficult. The fundamentally changed life style apparently resulted in similarly fundamental changes on the phenotypic level. Unclear homology impedes the phylogenetic evaluation, and in many cases phylogenetic signal is obviously completely eroded.

The Pancrustacea concept implies that hexapods and myriapods have invaded terrestrial habitats independently. What remains completely in the dark is the early evolution of stem group hexapods in the aquatic environment. A single impression fossil of the Lower Devonian Hunsrück slate was described as *Devonohexapodus bocksbergiensis* and as a marine stem group hexapod (HAAS *et al.*, 2003). This placement would have had far-reaching evolutionary implications and closed a major gap in the arthropod fossil record. However, an evaluation of additional specimens showed that this species was already described and is not even closely related to insects (KÜHL and RUST, 2009). This shows that fossils, especially single specimens or poorly preserved ones, should be investigated very critically, before they are used as a basis for phylogenetic or evolutionary interpretations (e.g. BAO *et al.,* 2021).

The monophyly of Hexapoda was never questioned in the morphological era of phylogenetics. It was briefly challenged by analyses of mitochondrial genomes (NARDI *et al.,* 2003), but it is now known that these data are not suitable for reconstructing very old splitting events. All recent transcriptomic and morphological characters clearly support the monophyly of Hexapoda (e.g. MISOF *et al.,* 2014). Moreover, the placement in Pancrustacea adds new strong support, like the tracheal system, Malpighian tubules, the loss of the mid gut glands, the unpaired labium, and other features. Most of these characters were formerly considered as autapomorphies of Tracheata (e.g. KRAUS and KRAUS, 1994).

A seemingly intractable problem is the basal branching pattern in Hexapoda, the relationships of the entognathous orders, Collembola, Protura and Diplura. Hennig postulated monophyletic Entognatha with Diplura as sister taxon of a clade comprising the other two orders, combined as Ellipura. The main potential apomorphy of Entognatha is the partial internalization of the mouthparts. KUKALOVÁ-PECK (1991) suggested a sistergroup relationship between Diplura and all the remaining insects, Insecta s.str. or Ectognatha, with good morphological arguments, the presence of cerci, paired claws, and a specifically modified sperm axoneme with a 9+9+2 pattern of microtubules. Ongoing in depth analyses of transcriptomes remain ambiguous, with phylogenetic signal for both alternatives.

A similar issue is the "Palaeoptera-problem", the basal splits in the pterygote insects, i.e. the relationships between Odonata, Ephemeroptera and the neopteran orders. HENNIG (1969) and more recent studies (BLANKE *et al.,* 2012a, b) suggested monophyletic Palaeoptera, i.e. Odonata + Ephemeroptera. Potential synapomorphies are aquatic immature stages and short and thin antennae of adults. BOUDREAUX (1979) combined Ephemeroptera and Neoptera as Chiastomyaria, with indirect flight muscles and direct insemination with a postabdominal aedeagus as potential synapomorphies. STANICZEK (2000) suggested Odonata + Neoptera as a clade Metapterygota, based on characters of the mandibles and associated structures. The loss of the subimago is an additional potential synapomorphy (e.g. KRISTENSEN, 1975). Again transcriptome analyses remain ambivalent, even though this problem was recently re-analysed in depth by Simon *et al.* (2018). Metapterygota can be excluded, but there is phylogenetic signal for both Palaeoptera and Chiastomyaria.

The Polyneoptera or more neutral the "lower neopteran insects" were a strongly disputed, with numerous conflicting hypotheses (e.g. KRISTENSEN, 1975, 1995; see WIPFLER *et al.,* 2019). The monophyly of the group including the notoriously controversial Zoraptera (MASHIMO *et al.*, 2014) was supported for the first time by YOSHIZAWA (2011) based on characters of the wing articulation, and this was confirmed by developmental features investigated by MASHIMO *et al.* (2013). Zoraptera was tentatively placed as sister to Paraneoptera by HENNIG (1969). However, its inclusion in monophyletic Polyneoptera was also confirmed by transcriptome analyses (MISOF *et al.,* 2014; JOHNSON *et al.,* 2018). MISOF *et al.* (2014) suggested a pattern with Zoraptera + Dermaptera basal within Polyneoptera, Plecoptera as the following branch, and then Orthoptera as sister to a clade comprising Mantophasmatodea + Grylloblattodea (Xenonomia), Embioptera + Phasmatodea (Eukinolabia), and also Dictyoptera (Mantodea + Blattodea including termites). Even though the backbone of this lineage was still relatively weak, exactly the same pattern was obtained in new analyses with a much denser polyneopteran sampling (WIPFLER *et al.,* 2019). An important point, aside from the inclusion of Zoraptera, is that Phasmatodea are not the closest relatives of

Orthoptera, as favoured by HENNIG (1969) and others, but form the sister group of the webspinners (Embioptera).

One of the most surprising results of MISOF *et al.* (2014) was the potential paraphyly of Paraneoptera. Previously nobody questioned the monophyly of this lineage (e.g. HENNIG, 1969; KRISTENSEN, 1975; BEUTEL and GORB, 2001). The analyses of MISOF *et al.* (2014) suggested a possible placement of Psocodea as sister taxon of Holometabola, thus rendering Paraneoptera paraphyletic. The same ambivalent result was obtained by JOHNSON *et al.* (2019) with a much denser paraneopteran taxon sampling. The paraphyly of Paraneoptera and a clade Psocodea + Holometabola is not plausible from a morphological perspective. Obviously this issue will require further scrutiny in the future.

The interordinal phylogenetic relationships of the megadiverse Holometabola are almost completely solved (WIEGMANN *et al.,* 2009; MISOF *et al.,* 2014; PETERS *et al.,* 2014). In contrast to HENNIG (1969) and others (e.g. BEUTEL and GORB, 2001), Hymenoptera is now placed as sister to all other groups, which are now addressed as Aparaglossata. This was already suggested based on a large morphological data set in BEUTEL *et al.* (2011) and earlier in RASNITSYN and QUICKE (2002). The remaining Holometabola comprise two large clades as suggested by HENNIG (1969). One of them comprises the three neuropterid orders, the megadiverse Coleoptera, and the controversial endoparasitic Strepsiptera. Again in agreement with HENNIG (1969), the other lineage is also subdivided into two subunits, Amphiesmenoptera (Trichoptera + Lepidoptera) and Antliophora (Diptera, Siphonaptera and Mecoptera). A problem that remains to be solved is the placement of the obscure Gondwanan genus *Nannochorista*, traditionally either placed in Mecoptera or as a separate order Nannomecoptera. Morphological features suggest a placement in monophyletic Mecoptera. In contrast, transcriptome analyses tentatively place it as sistergroup of fleas. Like few other issues, *Nannochorista* is obviously a persistent problem.

CONCLUSIONS AND OUTLOOK

It is safe to say, that with a theoretically sound concept of phylogenetic reconstruction W. Hennig already solved most problems in insect systematics (KJER *et al.,* 2016). After some deviations resulting from inadequate markers or analytical methods, molecular systematics now largely confirm Hennig's hypotheses. If we compare Hennig's phylogeny with 1KITE (MISOF *et al.,* 2014; see also KJER *et al.,* 2016) one may get the impression that the amount of new insights is limited. However, this interpretation would be superficial and misleading. First of all, the methodological progress owed to 1KITE is enormous. The handling of huge molecular data sets and the refinement of analytical methods are remarkable achievements. Moreover, 1KITE yielded a robust time frame for insect evolution for the first time, with estimations of the time of origin for all major lineages. Polyneopteran relationships, considered as highly problematic for a long time, appear to be nearly solved (WIPFLER *et al.,* 2019), and robust phylogenies were presented for various other groups (e.g. JOHNSON *et al.,* 2018; MCKENNA *et al.,* 2019). Persisting problems, as for instance the monophyly of Paraneoptera, were identified and will be addressed in future studies.

There is no simple or single answer how the remaining problems can be solved. Better taxon sampling may help in some cases, reducing gaps between taxa with long branches. Further refinement of analytical methods is also a perspective. And finally, what is likely essential, is an intensive cooperation between molecular systematists, geneticists, bioinformaticians, morphologists, developmental biologists and last but not least palaeontologists. Such a complex approach – including Hennig's principle of reciprocal enlightenment – will likely yield new and deep insights into the evolution of insects and other groups of organisms.

Exploring the genetic background of evolutionary changes is certainly an important future research perspective, and also investigating the evolution in the dimension of time, studying and analysing impression and amber fossils to illuminate past periods of insect evolution. An alarming scenario unfolding presently is the dramatic decline of insect diversity and biomass (e.g. HALLMANN *et al.*, 2017), caused by large scale environmental destruction, intensive agriculture and other factors. To link insights in insect evolution with these developments should have maximum priority in the future.

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AT THE MINIATURIZATION LIMIT - RESULTS AND PROSPECTS OF STUDYING THE SMALLEST INSECTS

ALEXEY A. POLILOV[a]

[a] *Lomonosov Moscow State University, Russia;* E-mail:polilov@gmail.com
Lettura tenuta durante la Tavola Rotonda "Novel Findings in Arthropods Functional Morphology and Anatomy". Seduta pubblica dell'Accademia, tenuta da remoto, 19 novembre 2021

Abstract

Size is one of the most important parameters of living and non-living things. Our age is an age of miniaturization. Miniaturization in electronics has made devices that used to occupy a whole room or even a building small enough to be carried in our pockets. But miniaturization is not only a trend in technology: it is also one of the trends in the evolution of life. Microinsects – the extremely diverse miniature insects less than a millimeter long – are one of the most intriguing components of this microworld. Having evolved to the size of unicellular organisms, the smallest insects managed not only to preserve structural complexity, but also to invent some novel features not found in larger insects. This talk is about comprehensive study of microinsects: from morphology to connectomics, from locomotion to genomics, and also about the potential benefits of the study of microinsects for solving fundamental scientific and biotechnological problems. Because the smallest insects are among the smallest metazoans and have the most complex organization among organisms of the same size, their peculiar structural features and the factors that limit their miniaturization are of considerable theoretical interest to general biology.

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COEVOLUTION BETWEEN SPERM AND SPERM STORAGE-ORGANS IN INSECTS

ROMANO DALLAI[a]

[a] *Department of Life Sciences-University of Siena-Via A. Moro 2 -5300 Siena;*E-mail: romano.dallai@unisi.it
Lettura tenuta durante la Tavola Rotonda "Novel Findings in Arthropods Functional Morphology and Anatomy". Seduta pubblica dell'Accademia, Firenze, 19 novembre 2021

Coevolution between sperm and sperm storage-organs in insects

As a general finding, whether giant sperm are evolved in a species, well developed seminal receptacles in the same species are expected. Experimental and comparative evidences indicate that natural selection acts on female genital districts driving the evolution of male genitalia. In the same way female reproductive tracts drive the evolution of sperm form. Examples of a coevolution between the sperm and the female spermatheca or the spermathecal duct are described in a few heteropteran insects and in a zorapteran species.

KEY WORDS: Insect reproduction, male and female reproductive systems, insect ultrastructure.

In his fundamental book "The descent of Man and Selection in Relation to Sex", Darwin (1871) wrote:

> "The sexual struggle is of two kinds; in one it is between individual of the same sex, generally the males, in order to drive away or kill their rivals…; whilst in the other, the struggle is likewise between the individual of the same sex, generally the females, in order to excite or charm those of the opposite sex, which… select the more agreeable partner…"

Thus, according to Darwin, during the "pre-mating sexual selection" two mechanisms are involved: the intrasexual selection (typically of males) and the intersexual selection (typically of females). The Author hypothesized that the evolution of the numerous and diversified structures exhibited by males are the result of a selection by the female giving success to them at mating.

Contrary to what was always thought about the main role played by the male at the reproduction, it is now well established that such a role is proper to the female. The female choice is thus the main purpose of the sex-selection and it is at the base of the coevolution between the female preferences and the extraordinary development of male characteristics. Several studies have established that reproductive traits evolve more rapidly than other types of characteristics and, remarkably, the female genital shape diverges much more rapidly than male genital shape (SWANSON and VACQUIER, 2002; SIMMONS and FITZPATRICK, 2019). Experiments on *Drosophila* indicate that natural selection acting on female genital traits might drive the evolution of male genitalia in much the same way as female reproductive tracts are thought to drive the evolution of sperm form (HIGGINSON *et al.,* 2012).

Sperm and female tract morphology interact such that the fitness advantage to males of producing relatively long sperm increases with increasing length of narrow sperm- storage organs (MILLER and PITNICK, 2002). Thus, the evolution of longer sperm-storage organs of females drives the evolution of longer sperm (MILLER and PITNICK, 2002; HIGGINSON *et al.,* 2012). On the other end, it has been well established that the longer sperm are better at displacing and resisting being displaced by shorter sperm from the proximal end of the organ (PATTARINI *et al.,* 2006).

Because the female reproductive tract is the selective environment for sperm, one taxonomically wide spread example of this pattern is the co-diversification of sperm length and female sperm - storage organ dimension (SYED *et al.,* 2021).

The best example of longest sperm and the occurrence of a coevolution between the male and the female genital systems is that found in *Drosophila bifurca* (PITNICK *et al.,* 1999). This species has a sperm, about 6 cm long (58.28 mm) and a spermathecal duct, about 8 cm long (81.67 cm) (Fig. 1).

A similar coevolution between the male and the female genital apparatuses has been described in several insects (ARNQVIST, 1988; EBERHARD, 2010; SIMMONS, 2014), including the coleopteran Dytiscidae (HIGGINSON *et al.,* 2012), Ptiliidae (DYBAS and DYBAS, 1981), and some other beetles

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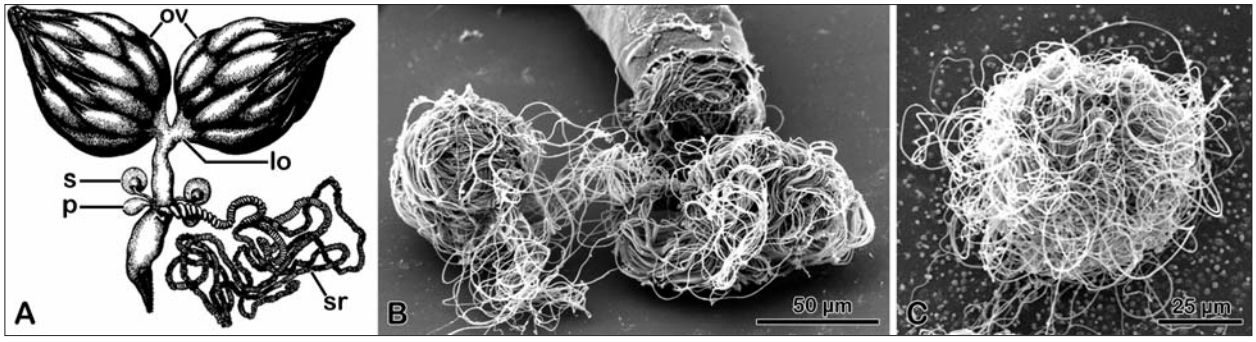

Fig. 1 – *Drosophila bifurca*. **A** - female reproductive system with ovaries (ov), lateral oviducts (lo), spermatheca (s), seminal receptacle (sr), parovarian (p) (from Pitnick et al., 1999). **B** - Scanning electron microscopy (SEM) of a deferent duct with some rolled sperm. **C** - SEM micrograph of a single rolled sperm. The rolled sperm are organized in a special district at the end of the deferent duct (JOLY and BRESSAC, 1994).

(GARCÍA-GONZÁLEZ and SIMMONS, 2007; SIMMONS and GARCÍA-GONZÁLEZ, 2011; SIMMONS and FITZPATRICK, 2016), moth (MORROW and GAGE, 2000), damselflies (MCPEEK *et al.,* 2009).

The sexes are known to have conflicting interests within nearly all sexually reproducing species (ARNQVIST and ROWE, 2005). This conflict is expected to give rise to sexually antagonistic coevolution, which may produce extremely developed male and female resistance traits (PARKER, 1979; HOLLAND and RICE, 1998; ROWE *et al.,* 2005). In other words, sexual conflict is an important force driving genital divergence with male and female genitalia evolving antagonistically (ARNQVIST and ROWE, 2002; HOSKEN and STOCKLEY, 2004; SIMMONS, 2014). Several works have demonstrated that a sexually antagonistic coevolution is present in the water-striders, with correlated evolution of armaments in the two sexes (ARNQVIST and ROWE, 2002; PERRY and ROWE, 2012). These works on water-striders showed that mating is costly for females and that the coevolution of male grasping genitalia and female anti-grasping spines is most likely the consequence of antagonistic coevolution. In detail, while the male of the water-strider has a prolonged genital and pregenital segments for grasping adaptation, the female evolved erected abdominal spines obstructing the male grip during premating struggles.

To give support to the above evidences of coevolution, we have examined the inner structure of male and female reproductive systems of some heteropterans and a coevolution of these apparatuses was established (DALLAI *et al.,* 2021a, b):

A) Gerris palustris L. has a sperm 3.2 mm long and an extraordinary long seminal receptacle of 30 mm. The sperm of the species can be considered as a giant sperm and it consists of a quite long acrosome which has a brilliant auto-fluorescence due to the presence of the flavin-adenine-dinucleotide (FAD) (MIYATA *et al.,* 2011). On the contrary, the nucleus is very short. A long flagellum has the classic axoneme structure 9+9+2, with the two connecting bridges between the axoneme and the large mitochondrial derivatives, typical of all Heteroptera (MERCATI *et al.,* 2009). Due to the peculiar fluorescence of the sperm acrosome, the sperm can be followed within the extremely long seminal receptacle of the female (DALLAI *et al.,* 2021a) (Fig. 2).

B) The same features described for the water-strider *G. lacustris* are also valid for the water- measurer *Hydrometra stagnorum* L. The sperm of this species is 1.5 mm long and the seminal receptacle is about 6.5 mm long. Several characteristics described for the previous species are also present in *H. stagnorum*: a long and fluorescent acrosome, a short nucleus and a long flagellum with the same structures above mentioned (DALLAI *et al.,* 2021b) (Fig. 3).

C) Notonecta glauca L., the backswimmer, is the third heteropteran studied. The species has a sperm extremely long, a giant sperm, 16.5 mm long, characterized by giant mitochondrial derivatives (AFZELIUS *et al.,* 1976) rich of the protein "crystallomitin" (BACCETTI *et al.,* 1977). The female storage organ is not yet known in detail, and a simple report of the organ shape is found in a related species described from Peru, *N. inca*, by S. MAZZUCCONI (2000). *N. glauca* has a long spermatheca with the proximal part of the duct, about 20 mm long, with 5 turns round a central axis, and an apical bulb (Fig. 4).

In addition to the three heteropteran species above mentioned, a further example of coevolution between sperm and female seminal receptacle has been described in the angel insect, the zorapteran *Zorotypus impolitus* Mashimo *et al.*. In this species the sperm is 3 mm long and the seminal receptacle is about 2.9 mm (DALLAI *et al.,* 2013; 2014). Different from several other congeneric species, *Z. impolitus* has a male genital system with large seminal vesicles, a characteristic shared only with *Z. hubbardi* (Fig. 5). The sperm has a helicoidal

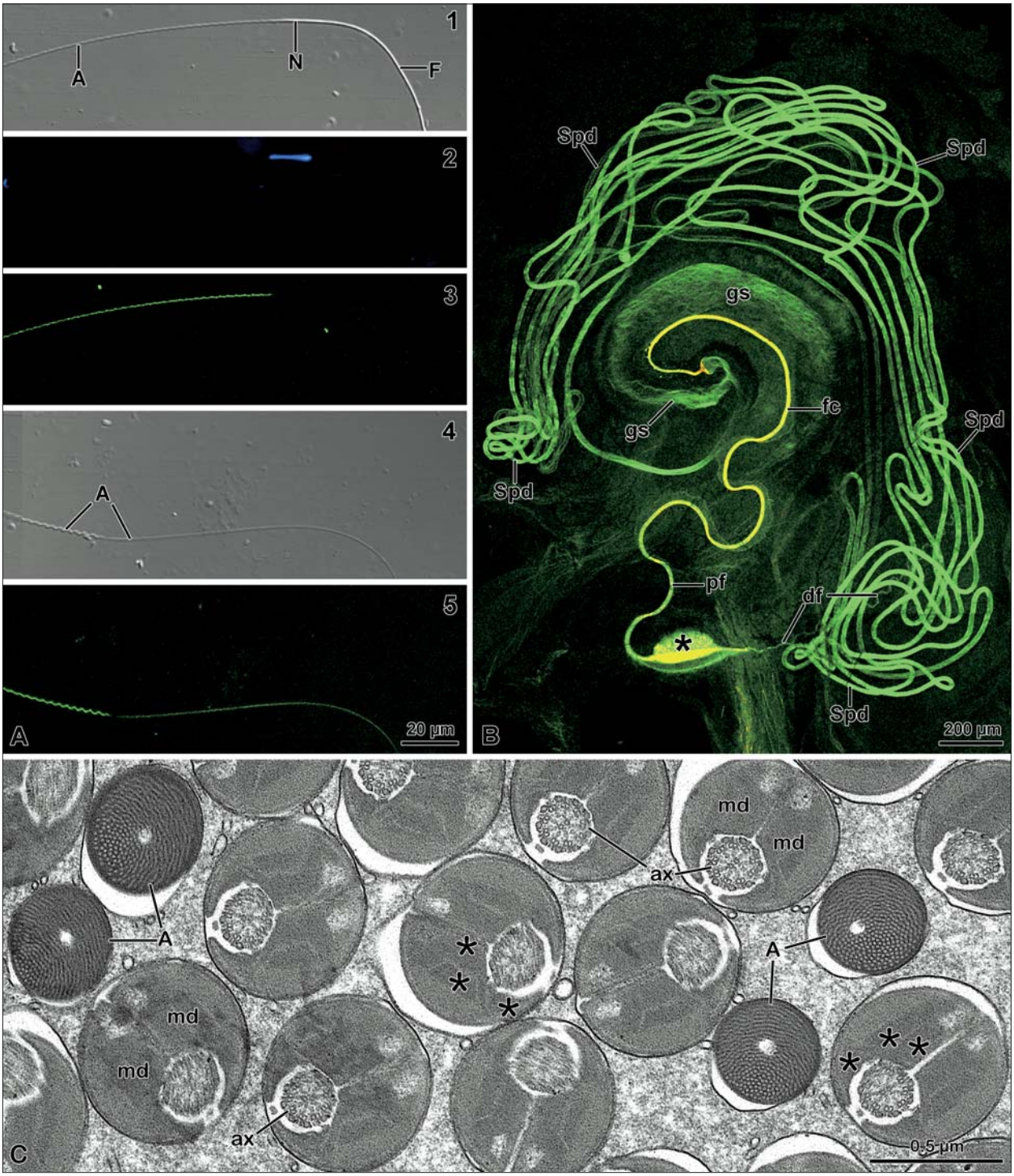

Fig. 2 – *Gerris lacustris*. A - 1-5 free sperm with the long acrosome (A), the short nucleus (N) and the long flagellum (F) (1-4). Note the autofluorescence of the acrosome (3-5) and the Hoechst staining of nucleus (2). **B** - Cross section of the sperm showing the acrosome, the axoneme (ax) and the mitochondrial derivatives (md) with areas of matrix crystallized (asterisks). **C** - Whole spermathecal structure with the extremely long spermathecal duct (spd). The asterisk indicates the region provided with muscles close to the end of the spermathecal duct. Note the fluorescence in the duct lumen due to the presence of the sperm acrosome.

acrosome, a cylindrical nucleus and a flagellum with axoneme, accessory bodies and the huge mitochondrial derivatives in a tightly helicoidal pattern (Fig. 6 A-C). The female has a genital apparatus with a very large spermatheca provided with a long helical spermathecal duct (Fig. 6 F). The greater size of the sperm and the very large spermatheca suggest a coevolution between the two reproductive apparatuses (DALLAI *et al*., 2014).

As known *Z. impolitus* is the only species, among Hexapoda, to perform a reproduction by an "indirect sperm transfer" similar to what has been described in some apterygotan insects. The male produce, after a peculiar courtship, a small spermatophore, about

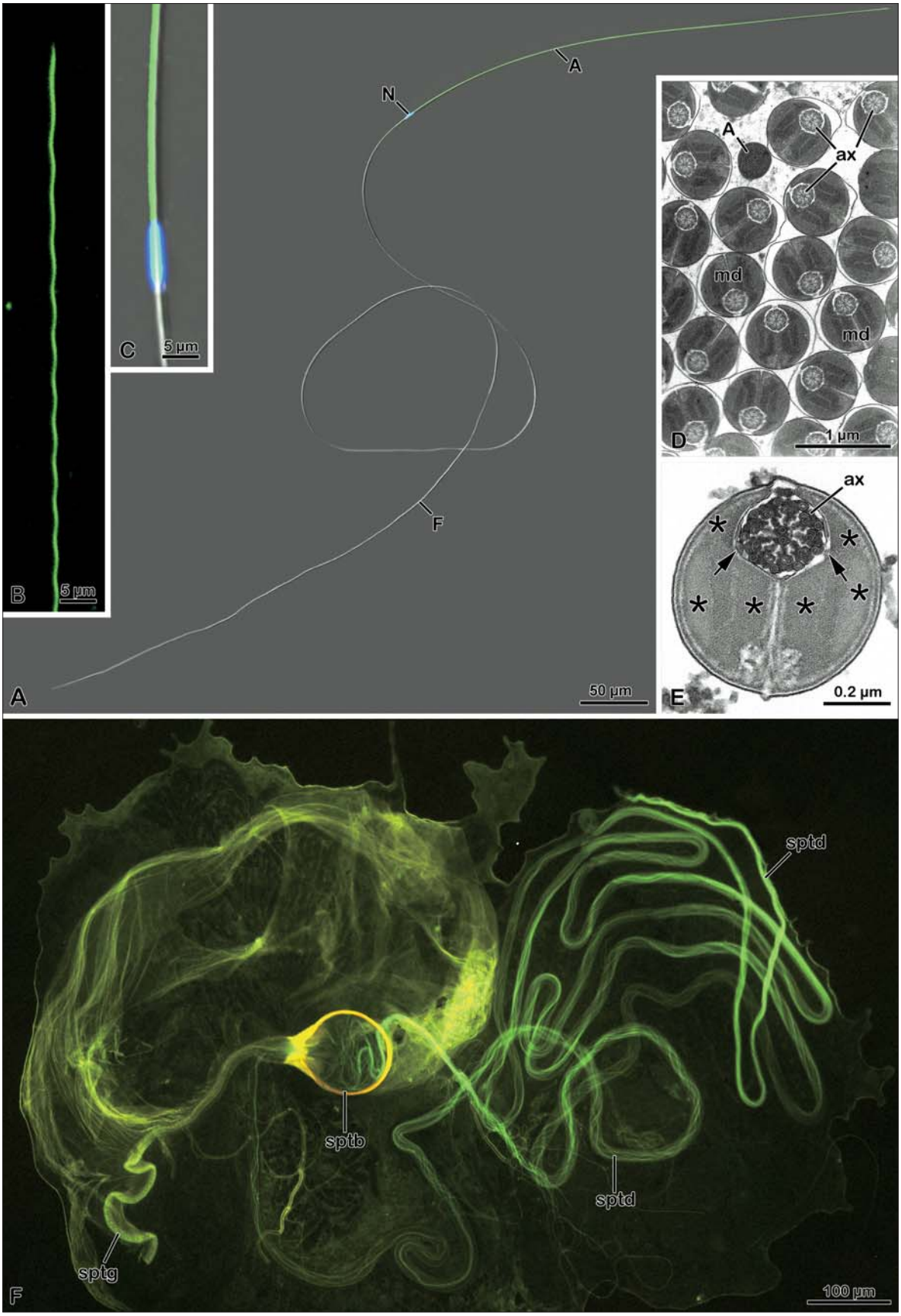

Fig. 3 – *Hydrometra stagnorum*. **A** - The whole sperm to show the autofluorescent acrosome (A), the short nucleus (N) after Hoechst staining and the long flagellum (F). **B** - Detail of the acrosome autofluorescence. **C** - Detail of the nucleus after Hoechst staining. **D**, **E** - Cross section through a bundle of sperm showing the acrosome (A), the axoneme (ax), and the two mitochondrial derivatives (md). Note the crystallized areas of the matrix (asterisks) and the connecting bridges between the axoneme and the mitochondrial derivatives (arrows). **F** - Whole preparation of the spermatheca with the long spermathecal duct (sptd), the spermathecal bulb (sptb) and the spermathecal gland (sptg). Note the fluorescence in the duct lumen due to the presence of the sperm acrosome.

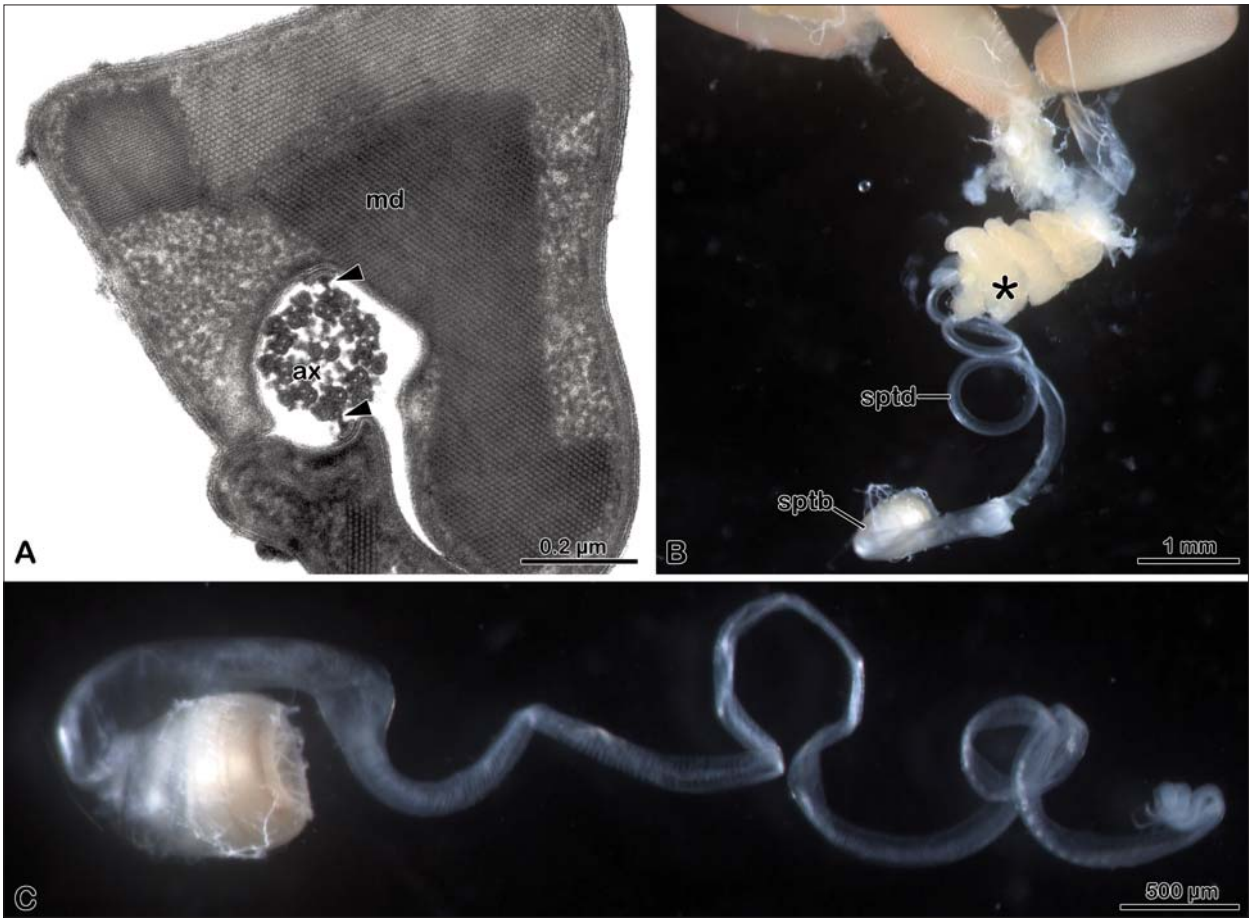

Fig. 4 – *Notonecta glauca*. **A** - Cross section of the sperm flagellum with the axoneme (ax), the giant crystallized mitochondrial derivatives (md) and the connecting bridges between the axoneme and the mitochondrial derivatives (arrowheads). **B** - Spermatheca with the long spermathecal duct (sptd) and the apical bulb (sptb). Note the helical array of the proximal region of the spermathecal duct (asterisk). **C** - Spermathecal duct and apical bulb after stretching.

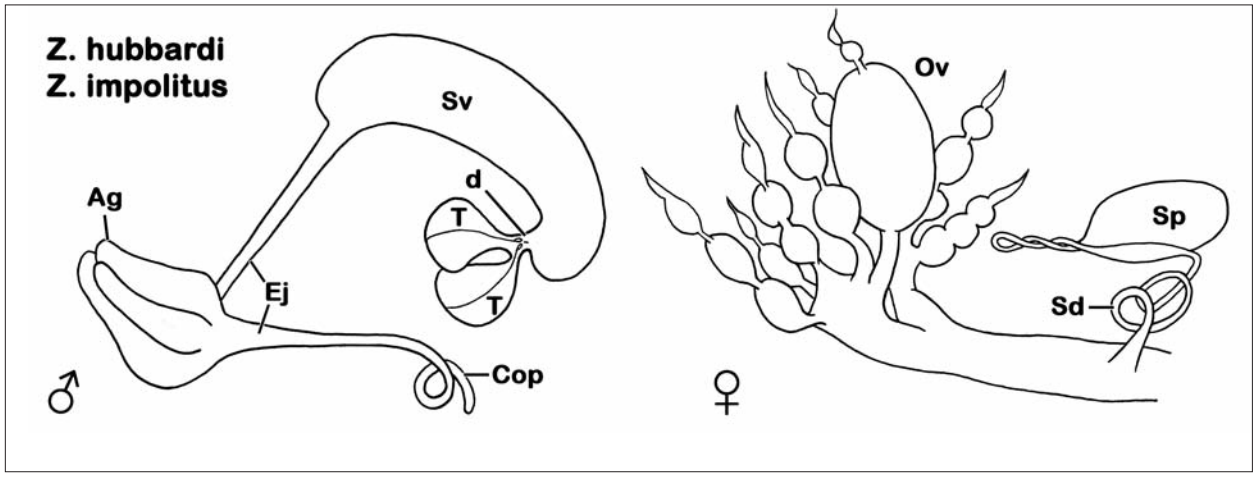

Fig. 5 – *Zorotypus impolitus*. Schematic drawing of the male and female genital apparatuses of the species similar to those of *Z. hubbardi.* Ag, accessory glands; Ej, ejaculatory duct; Cop, copulatory organ; sv, seminal vesicle; d, deferent duct; T, testes; Ov, ovaries; Sp, spermatheca; Sd, spermathecal duct.

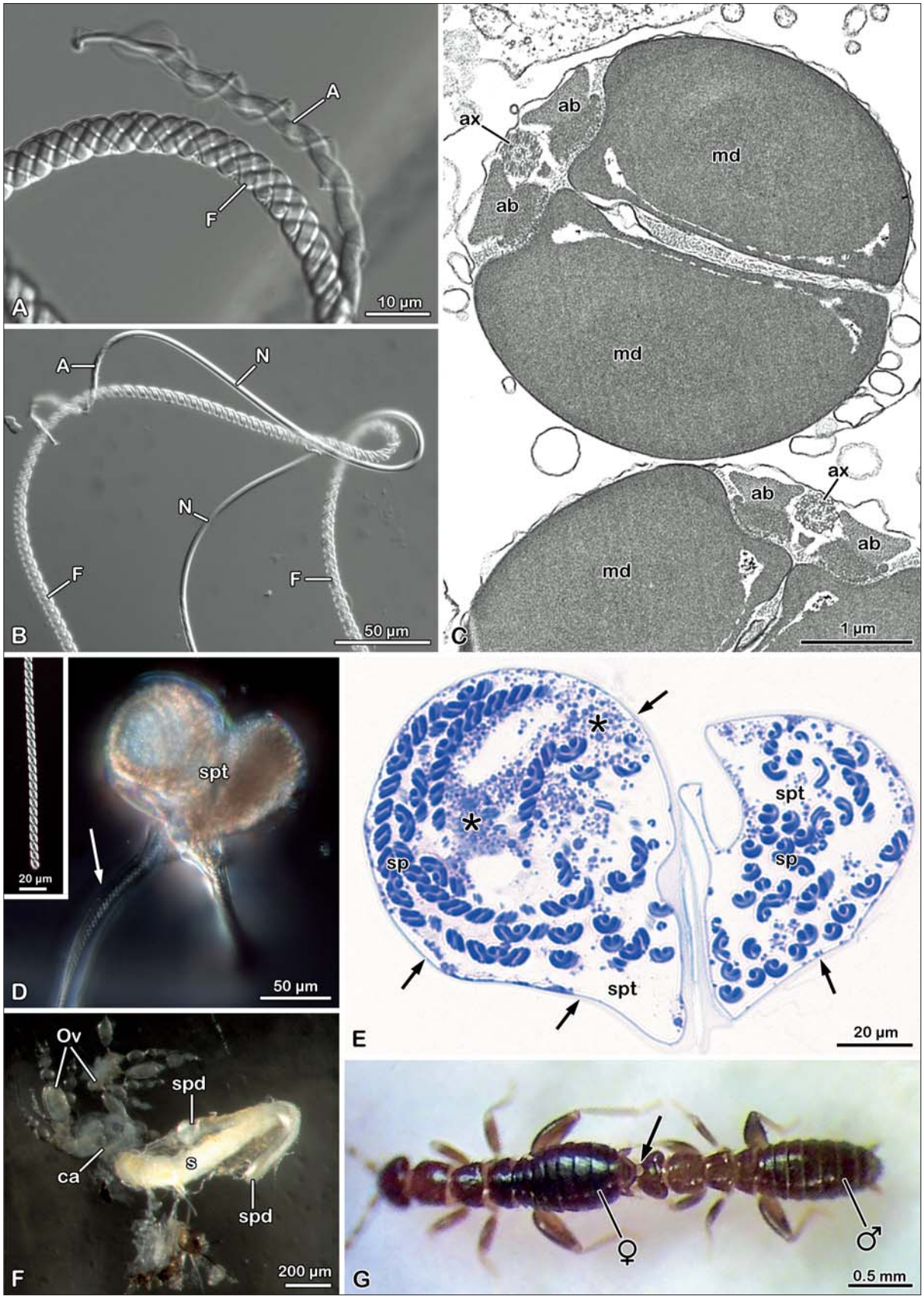

Fig. 6 – *Z. impolitus.* **A**, **B** - light microscopic preparations of the whole sperm with the acrosome (A), the nucleus (N) and the flagellum (F). **C** - Cross section of a couple of giant sperm with the axoneme (ax), the accessory bodies (ab) and the two giant mitochondrial derivatives (md). **D** -Two spermatophores (spt) recovered from the female abdomen. In the inset, a detail of the flagellum (arrow). **E** - Cross section through the spermatophores (spt) of the previous figure to show the single sperm (sp) present in each of them. Arrow indicates the thin walls surrounding the structures and asterisks the material contained within the structures. **F** - Female genital system with ovaries (Ov), calices (ca), the large spermatheca (s) with the long spermathecal duct (spd). **G -** *Z. impolitus*. Two specimens, a female (anterior) and a male (posterior), after a spermatophore was deposited on the female abdominal end (arrow).

100 µm in diameter (Fig. 6 D), that further is deposited on the female abdominal end (Fig. 6 G). The female takes this spermatophore with her mouth parts and, bending her body, inserts the spermatophore into her vagina. The female refuses a second courtship by the male, as far as she has not recovered the former spermatophore. Each spermatophore contains only one spermatozoon (Fig. 6 E). During several hours of continuous observations with light microscope (8 hr.), about 20 spermatophores were produced by the male and after taken by the female.

Conclusions

From the examples above described, corroborated by previous data from different insect species, we can suggest that the evolution of the sperm length is the result of selection driven by the female reproductive system. As the sperm structure is well known in many insect groups (Jamieson et al., 1999; Pitnick *et al.,* 2009; Dallai, 2014), it would be interesting to extend the study to the female reproductive apparatus of those species provided with a long sperm to verify whether a large spermatheca and a long spermathecal duct are also present.

As a final remarque it could be of interest the nice sentence by Miller and Pitnick (2002) who have written: "giant sperm tails are the cell equivalent of the peacock's tail, having evolved reproductive traits that selectively bias paternity in favor of males with longer sperm".

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RIASSUNTO

Molti studi condotti su specie diverse hanno dimostrato che se una specie ha evoluto uno spermio molto lungo anche il ricettacolo seminale della femmina avrà un grande sviluppo. La selezione naturale agisce sull'apparato genitale femminile guidando l'evoluzione di quello maschile determinando la produzione di spermi molto lunghi. Vengono descritti esempi di coevoluzione fra spermi e spermateche in tre Eterotteri (*Gerris lacustris* L.; *Hydrometra stagnorum* L. e *Notonecta glauca* L.) ed in uno Zorattero (*Zorotypus impolitus* Mashimo *et al.)*

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MECHANO- AND CHEMO RECEPTORS IN MITES AND THEIR INVOLVEMENT IN SECRETORY ACTIVITIES

ANTONELLA DI PALMA[a]

[a] *Department of Agriculture, Food, Natural Science and Engineering, University of Foggia, Via Napoli 25, 71122 Foggia*
E-mail:antonella.dipalma@unifg.it
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Mechano- and chemo receptors in mites and their involvement in secretory activities

Setiform sensilla (=sensory hair) are part of the sensory system of Acari and represent sensory organs that possess a cuticular shaft protruding externally over the surface of the integument.

Based on the similar ultrastructural features that setiform sensilla generally show among arthropods, they can be divided into three categories: terminal pore sensilla, wall pore sensilla, and no pore sensilla.

No pore sensilla in mites perhaps show the most diverse shapes; moreover, in many taxa, the number of these setae has increased considerably and sometimes may cover the whole body. A peculiar type of these sensilla is represented by the trichobothria. They are setae set in a deep cup-like socket (bothridium) and occur in restricted numbers on the body as well as on the legs and may have different shapes. They are considered to act as highly specialized mechanoreceptors, likely reacting to airborne stimuli.

Regarding wall pore sensilla in mites, detailed knowledge about putative chemoreceptor sensilla is limited or even lacking in several groups. Solenidia are unusual setae that have wall pores and contain branching dendrites; thus, their ultrastructure supports the assumption that they are olfactory receptor organs. However, the variation in their structural design is by no means understood and other functions are supported by a different ultrastructural organization observed in some taxa.

A so far unique example of sensilla involved in secretory activities in mites is represented by the dorsal setae in the genus *Raoiella* (Acari: Actinotrichida: Tenuipalpidae). Recent biological and ecological observations revealed that each *Raoiella* developmental stage presents droplets of fluid associated with their dorsal setae. Ultrastructural characters indicate that these setae should be terminal pore sensilla and might act as both mechanoreceptors and contact chemoreceptors. Moreover, epidermal cells underling these setae have an ultrastructure that suggests they have a glandular function representing the likely source of the droplets visible on the setal tips. The connection of these epidermal cells to pore systems permits the transport of the secretion through the cuticle and its eventual accumulation on the setal shaft and tip. Based on similar arrangements in some insect taxa, the organization of the structures observed in *Raoiella* suggests the passage of a non-polar, waterproofing, lipoid fluid through the cuticle. Such pore tubule-like systems and their association with setae represent a new finding in mites since setae of mites have never been regarded as secretory structures.

Key Words: Sensilla, sensory hair, ultrastructure, terminal pore sensilla, wall pore sensilla, no pore sensilla

This note focuses on some aspects related to sensory organs in mites. The sensory system of Acari includes setiform sensilla (=sensory hair) beside non-setal sensilla, photosensitive areas, and photoreceptor organs (Alberti and Coons, 1999). Anyway, setiform sensilla represent a conspicuous feature of their integument. In fact, setation is one of the most important diagnostic characteristics examined in taxonomic studies. Regarding their functional morphology, setiform sensilla represent sensory organs that possess a cuticular shaft protruding externally over the surface of the integument. Arthropod sensilla generally present similar ultrastructural features, thus, based on these similarities, three categories of setiform sensilla are distinguished in mites as well as other arthropods: no pore sensilla, wall pore sensilla, and terminal pore sensilla (Alberti and Coons, 1999 and references therein).

No pore sensilla usually have a movable base (socket) with an articulating membrane provided with radiant fibers; according to their name, they lack openings to the outside and dendrites in the setal lumen. In mites, they are usually innervated by two cells while in other arthropods the number of sensory cells might be more (e.g., spiders or scorpions have three to four or up to seven). Their dendrites terminate at the base of the setal shaft in a bundle of densely arranged microtubules embedded in an electron-dense material (i.e., tubular bodies) and contacting the flexible setal socket. Tubular bodies are considered to represent the site of stimulus transduction in that the stimulating forces are transmitted by cuticular lever structures

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to the dendrite sheath and via small bridge structures to the dendritic membrane, the membrane-integrated cones, and then backed by the microtubules and an electron-dense substance. Thus, no pore sensilla are considered to be mechanoreceptors perceiving mechanical distortions of the exoskeleton or of the seta itself (ALBERTI and COONS, 1999 and references therein).

No pore setiform sensilla (=sensory hair) in mites are usually arranged in distinct patterns on the body as well as on the appendages and perhaps show the most diverse shapes. Moreover, in many taxa, the number of setae has increased considerably and sometimes may cover the whole body (polytrichous mites). Among tarsonemid mites, the genera *Daidalotarsonemus* De Leon and *Excelsotarsonemus* Ochoa & Naskrecki offer a peculiar example of how much modified these setae might be. In fact, females of these two genera are mainly characterized by the presence of sculpturing on the dorsal shields and by highly modified dorsal setae, greatly enlarged, laminar, or sail-shaped (DI PALMA *et al.*, 2021a). The peculiar morphology of their dorsal setae has even suggested they might have other functions beside the sensory one. Anyway, according to their ultrastructure, these setae, albeit extravagantly modified, should act as mechanoreceptors in agreement with most of the previous observations in mites (DI PALMA *et al.*, 2021a). Nevertheless, their morphological modifications (pronounced cup shape, enlarged shaft with concave longitudinal strips) suggest they play, in addition to the tactile function, storage and dispersive role for fungal spores collected by the mites while moving in the humid environment of the tropical forests where they live. In addition, these modified setae, inserted on elevated sockets, are probably movable by the action of dorsoventral muscles observed inserting close to the elevated setal sockets (DI PALMA *et al.*, 2021a). Thus, the mite might lift these cup-like setae to spread the fungal particles on the body or over adjacent vegetation as well. Biological and feeding studies are obviously necessary to better understand the role such fungi might play in the mite life cycle. In addition, mites might use their sail-shaped setae to become airborne as suggested by REZENDE *et al.* (2015). In fact, in all tarsonemids it is the adult female only to be the dispersal instar (LINDQUIST, 1986) and, thus, some of its morphological structures might be correlated to this function.

A very remarkable type of mechanoreceptive setiform sensilla in mites is represented by the trichobothria. They are variously shaped setae inserted in a deep cavity (bothridium) and are known in several terrestrial arthropods (e.g., myriapods, some insects, most arachnids). In mites, the trichobothria are only exceptionally present in Anactinotrichida, while they are quite common in Actinotrichida (one of the two major groups mites are divided into). Moreover, they always occur in restricted numbers on the body as well as on the legs. In their most simple form, they are long, thin hair; but they can be globose, clavate, or capitate. The exact functional significance of these differences in shapes is not known (ALBERTI and COONS, 1999 and references therein). In spite of their common presence in actinotrichid mites, trichobothria have been ultrastructurally studied in few species only (ALBERTI and COONS, 1999 and references therein). Trichobothria present a solid setal shaft without pores, they are innervated by two dendrites terminating into two tubular bodies while their setal base is attached to an articulating membrane provided with suspension fibers. Thus, they represent no pore sensilla and are considered to act as highly specialized mechanoreceptors, likely reacting to airborne stimuli (ALBERTI, 1998).

In particular, in the siteroptid mite *Pediculaster mesembrinae* (Canestrini) (DE LILLO and ALDINI, 2002) the trichobothrium has a shaft that stands straight in its socket. Internally, the socket cavity (bothridium) is provided with projections that divide the cavity into chambers. The shaft passes straight through these chambers and connects proximally to an articulating membrane provided with radiating fibers. Two receptor cells are located under the setal base, each terminating with a tubular body. Thus, the peculiarly arranged bothridium forms a sort of bell in which the bothridial seta works as a clapper. The shaft might press on the chamber projections during its movement and in this way induce stress on the tubular bodies and the socket. The ultrastructural organization of *P. mesembrinae* trichobothrium confirms a vibro- and anemoreceptive function for siteroptid mites, as in other arthropods, where the trichobothrium should be able to detect slight air currents. Since siteroptids are typically phoretic, they might utilize the trichobothrium to detect the specific frequency of the wing vibrations produced by the flies they use as carriers. In fact, the socket chambers look like a sound box and their projections might vibrate for resonance to a particular sound frequency while the cuticular frame of the socket might transmit the stimulus to the tubular bodies connected to the bothridium. Of course, the mite could combine vibroreception with info-chemical perception to identify the vector.

Although trichobothria have mostly a filiform shape, this is not the case in oribatid mites, where a great diversity of form (e.g., filiform to globose or pectinate shapes) occurs (ALBERTI and COONS, 1999). Moreover, Oribatida are provided with a setal basis (bothridium) of very high complexity not known from other arthropods. Finally, in oribatid mites, the trichobothria are

represented by only one pair on the prodorsum with no presence on the legs (ALBERTI and COONS, 1999). In the early derivative Oribatida the bothridial seta is mostly placed in a straight and upright manner within the bothridium while in most oribatid mites the base of the bothridial seta is S-shaped, corresponding to a similarly formed bothridium. This straight shape of seta and bothridium is considered to be plesiomorphic since it occurs also in other actinotrichid mites as well as in other Arachnida (ALBERTI *et al.*, 2016). It has been suggested that the particular arrangement of bothridial setae in the more derivative oribatid mites increases the protection of the sensillum and may also improve its sensitivity to perceive vibrations (ALBERTI and MORENO TWOSE, 2016). Intermediate conditions have been observed in different species of Oribatida with the bothridial seta and the bothridium sharply bent proximally, but only once, compared with the straight trichobothrium of early derivative oribatid mites and the double-curved, S-shaped base, found in more evolved taxa (ALBERTI *et al.*, 1994; ALBERTI and COONS, 1999; ALBERTI and MORENO TWOSE, 2016).

Wall pore sensilla, may or may not be provided with a movable base while, as suggested by their name, have walls with multiple pores along their hair shafts. Moreover, they contain branching or not branching dendrites of a variable number of cells in the shaft itself. All are considered to be olfactory chemoreceptors (=pore hairs).

Regarding wall pore sensilla in mites, detailed knowledge about putative chemoreceptor sensilla is limited or even lacking in several groups (ALBERTI and COONS, 1999 and references therein). A peculiar example of these sensilla is represented by the solenidia. They are setae of variable shape occurring on some leg segments (usually genua, tibiae, and tarsi) of actinotrichid mites. In some taxa, they may represent long, erect, bacilliform seta-like organs, or they can be whip-like forms or peg-like. They usually insert into the cuticle with a broad, immovable basis. They have been studied in two systematic distant species only: one siteroptid and one phytoptid mite. In siteroptids, the solenidial shaft consists of a multiporous wall enclosing several dendritic branches while no tubular bodies are associated with this sensory structure. According to these ultrastructural features, the siteroptid solenidion represents a wall pore sensillum playing an olfactory role (DE LILLO and ALDINI 2001). On the other hand, in phytoptids the solenidion shaft has very small apical pores and an aporous outer surface along its length while dendrites segments are unbranched and run-up to the apex that is blunt and developed into a small knob. As with the siteroptid, no tubular bodies are associated with this structure. Thus, this solenidion belongs to the terminal pore sensilla and is assumed to be gustatory (DE LILLO and ALDINI 2001). Unfortunately, the ultrastructure of solenidia in other mite taxa is still poorly known, thus a comparison can't be realized and the enormous variation in their structural designs is by no means understood.

Terminal pore sensilla, obviously, have an opening at or near the apical end of the shaft and an innervated core with dendrite branches reaching deep inside the setal shaft. They have usually an additional mechanoreceptive termination (i.e., tubular body) contacting the base of the seta (which is movable). They are considered to be contact chemoreceptors (=gustatory receptors, taste hairs). The apical single pore (or the multiple pores or slits) presumably allows or mediates the entrance of molecules into the shaft where they diffuse along pore tubules and reach the membrane receptors in the dendritic membranes, thereby provoking stimulus transduction (ALBERTI and COONS, 1999 and references therein).

Among terminal pore sensory hair in mites, an interesting example was recently described in the flat mite genus *Raoiella* Hirst (DI PALMA *et al.,* 2021b). The setae of mites have never been regarded as secretory structures, yet biological and ecological observations on this genus revealed that each developmental stage presents droplets of fluid associated with the tips of their dorsal setae. Ultrastructurally, these setae present the typical features of mechanoreceptors (flexible socket, innervation by dendrites ending with two tubular bodies), but have a "hollow" axis represented by a protoplasmatic core containing dendritic branches. This combination of ultrastructural characters indicates that the setae might be multimodal receptors: acting as both mechanoreceptors and contact chemoreceptors. The epidermal cells that underlie the setal sockets have an ultrastructure that suggests they have a glandular function representing the likely source of the droplets visible on the setal tips. Moreover, the epidermal cells present apical microvilli and form extracellular cuticular canals, containing epicuticular filaments, that are proximally connected with the microvilli and distally open via pores onto the surface of the setal base. Based on similar arrangements in some insect taxa, the organization of these structures suggests the passage of a non-polar, waterproofing, lipoid fluid through the cuticle. The secretion would pass from the microvilli into the tubular cuticular structures and, by means of the epicuticle filaments, reaches the pores at the base of the setal socket. Here the secretion accumulates in small droplets, and gradually moves up the shaft also assisted by natural vibrations and movements of the setae as the mite walks around. Preliminary chemical analysis of the fluid in the droplets, suggests that a non-polar, long-chain hydrocarbon molecule is involved. Thus, considering the suspected waterproofing nature

of these droplets, their presence in all developmental stages, and that the female deliberately deposits a droplet on the tip of the eggs, it might be that such a secretion has some action in preventing dehydration. Alternatively, a role in pheromone secretion and dissemination may also be considered. In fact, at least some insect pheromones have apparently evolved as a special branch from the general biosynthesis of cuticular hydrocarbons. Moreover, the variable color of the secreted droplets in different species of *Raoiella*, may represent species-specific differences in the composition of the secretion, which may, in turn, relate to species-specific pheromones. Since the same fluid is present in all stages it is unlikely that it might be related to finding a mate. On the other hand, it might be an aggregation pheromone since *Raoiella* forms aggregations and putting droplets on eggs might also help groups stay together around the "nursery". In this respect, the dorsal setae, in addition to having a mechanoreceptor function, might act as contact chemoreceptors helping the mite to perceive the pheromone deposited on the eggs, or the setae and body of conspecific individuals, or to simply perceive the substance accumulating on the tips of its setae.

In this short note, some peculiarities regarding setiform sensilla in mites have been reported to give an idea of how variable these structures can be and show how they are adapted to solve different problems and play different roles. On the other hand, considering that Acari represent one of the most diverse group among arthropods, it is striking how little we still know about most of their taxa.

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A SYNOPSIS OF HAEMOCYTE MORPHOLOGY IN INSECTS

ANITA GIGLIO[a]

[a]*Department of Biology, Ecology and Earth Science, University of Calabria, Italy*
email: anita.giglio@unical.it
Lettura tenuta durante la Tavola Rotonda "Novel Findings in Arthropods Functional Morphology and Anatomy". Seduta pubblica dell'Accademia, tenuta da remoto, 19 novembre 2021

A synopsis of haemocyte morphology in Insects

Haemocytes have been characterised mainly on the basis of their morphological, cytochemical and functional features or by monoclonal antibodies and genetic markers. The most common types of haemocytes described in species belonging to different orders such as Lepidoptera, Hymenoptera and Coleoptera are named prohaemocytes, granular cells, plasmatocytes, spherule cells and oenocytoids. However, the classification of haemocytes is the subject of much controversy, in part due to differences in study methods as well as to species-specific variability. Indeed, the differences between species are related to the methods used to stain cells without clear dye affinities in light microscopy. Furthermore, it is difficult to compare cell types described by electron microscopy with those described by optical and confocal microscopy. In addition, there is intrinsic phenotypic variability from cell to cell related to circulating haemocyte function, which is mistakenly considered as an indicator of morphological diversity.

KEY WORDS: haemocytes, microscopy, phagocytosis.

The immune response in insects is based on both humoral and cellular components (OTTAVIANI, 2005). Haemocytes are responsible for the cell-mediated immune response and have a key role in the pathogen clearance from the haemocoel (GILLESPIE *et al.,* 1997). They are the main players in cell-mediated responses such as wound repairing, melanisation, encapsulation, phagocytosis, nodulation and clotting (LAVINE & STRAND, 2002; STRAND, 2008; ROSALES, 2011; DUBOVSKIY *et al.*, 2016; MELCARNE *et al.*, 2019). In addition, they secrete effectors involved in humoral immune defence, such as antimicrobial peptides, reactive oxygen and nitrogen intermediates and complex enzymatic cascades that regulate coagulation or melanisation against bacteria and parasites (TSAKAS & MARMARAS, 2010; ALI MOHAMMADIE KOJOUR *et al.*, 2020; ELEFTHERIANOS *et al.*, 2021). Maintenance of circulating haemocytes has been attributed to the mitosis of haemocytes already in circulation as well as to their release from hematopoietic organs (GUPTA, 1979; GILLESPIE *et al.*, 1997; HOLZ *et al.*, 2003; TAN *et al.*, 2013). The hematopoietic origin of haemocytes has well studied in model species such as *Drosophila melanogaster* Meigen, 1830 (GRIGORIAN & HARTENSTEIN, 2013; HONTI *et al.*, 2014; HILLYER, 2016). In the larval stage of *D. melanogaster*, the hematopoietic organ is a lymph gland consisting of paired primary and secondary lobes located along the anterior end of the dorsal vessel (LANOT *et al.*, 2001). At the posterior base, a dedicated group of cells called Posterior Signaling Center is responsible for the maintenance of lymph gland haematopoiesis. The medullary zone contains precursor cells of mature plasmatocytes and crystal cells. In the larval stage, there is also a functional set of haemocytes located in the subepidermal layer of the body cavity, forming the sessile hematopoietic tissue. In the adults, haemocytes occupy only two blood cell compartments, the circulation and the sessile tissue. In response to infection or injury, lymph gland and sessile hematopoietic tissue release haemocytes into the circulation. The peripheral nervous system is involved in the lation of haemocyte homing and anchoring; indeed haemocytes are located near the projections of neurons, and their attachment is dependent on neuronal signalling events (HILLYER, 2016).

The classification of insect haemocytes is the subject of numerous controversies related to the terminology used by researchers, methods of investigations, the variability of haemocyte physiology in the immune response. Cell morphology is the basic method used to describe haemocytes, performed using light, electron, fluorescence, confocal and differential interference contrast (DIC) microscopy (GUPTA *et al.*, 2010; BRYANT & MICHEL, 2014). Here, haemocyte types will discussed referring to their morphological, histochemical and functional features or based on molecular markers.

A large number of haemocytes have been

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characterized under light microscopy and transmission and scanning electron microscopy (Tab. 1). Previous studies on morphological types have been revealed many difference among orders and across species within the same order (Gupta, 1979; Siddiqui & Al-Khalifa, 2012; Ghoneim, 2019). In *D. melanogaster*, three types of haemocytes have been described: crystal cells, plasmatocytes, and lamellocytes (Parsons & Foley, 2016). Five types of circulating haemocytes that are classified as prohemocytes, granulocytes, plasmatocytes, spherulocytes and oenocytoids have been described in *Bombyx mori* (Linnaeus, 1758) (Liu *et al.*, 2013). Nine morphological types have been commonly described in species belonging to Diptera, Coleoptera and Lepidoptera, named prohaemocytes (PRs), plasmatocytes (PLs), oenocytoids (OEs), spherule cells (SPs), thrombocytoids (THs) and four types of granular haemocytes (GR) (Brehélin & Zachary, 1986; Ribeiro & Brehélin, 2006). The comparative analyses of the structure and function have been highlighted four main circulating haemocytes i.e. PRs, PLs, GRs and OEs, while the other types have been indicated to be precursors of differentiated haemocyte types (Ribeiro & Brehélin, 2006). However, it is difficult to define whether the variability for the other listed types is real or only due to the difference in the used methodology.

The characteristics provided by *in vitro* or *in vivo* behaviour of haemocytes, added to their morphological features, also showed many differences among species even within the same order. For instance, the phagocytosis is performed by PLs in adults of Orthoptera, Diptera and Hemiptera and GRs in Lepidoptera such as *B. mori* (Gupta, 1979). In some cases, other types of haemocytes can also perform this function, such as prohemocytes (Ling *et al.,* 2005) and oenocytoids (Giulianini *et al.,* 2003; Giglio *et al.,* 2008). In Coleoptera, functional differences are found between adults and larvae in species so far described (Tab. 2) likely related to the difference between adults and larvae in the costs of immune response, resource allocation and range of pathogens (Sadd & Schmid-Hempel, 2009; Schulenburg *et al.,* 2009). However, information is not sufficient to make an suitable comparison or a phylogenetic analyses and the involvement of different haemocyte types in phagocytosis need further studies.

To rule out the controversy of terminology to designate haemocytes, fluorescent probes are also largely used as markers of cellular elements as well as antibodies (Gillespie *et al.,* 1997). Fluorescent stains have been used to characterize haemocyte subpopulations by flow cytometry (Ling *et al.,* 2003; Castillo *et al.,* 2006; Marringa *et al.,* 2014). Molecular markers have been used to characterize subpopulations of haemocyte in the dipterans *Aedes aegypti* Linnaeus, 1762 and *Anopheles gambiae* (Giles, 1902) (Castillo *et al.,* 2006), the moth *Pseudoplusia includens* (Walker, 1858) (Gardiner & Strand, 1999), *Manduca sexta* (Linnaeus, 1763) (Willott *et al.,* 1994; Beetz *et al.,* 2004) and *B. mori* (Tan *et al.,* 2013), the honey bee *Apis mellifera* Linnaeus, 1758 (Gábor *et al.,* 2020), the American cockroach *Periplaneta americana* (Linnaeus, 1758) (Chain *et al.,* 1992). Some of the tested antigens have been characteristic of different lineages or stages of haemocyte maturation. However, these methods have also shown limitations due to nonspecific responses that cause different morphological types to have the same degree of affinity for the same marker.

Final remarks

All methods cited before are suitable method used for the haemocyte identification but at the same time have limitations and their application depends on the purpose of the investigation. The controversy on the number of cellular subpopulation in the haemolymph can be resolved according with the single-cell theory which states that various haemocyte types are merely stages, with separate functions. Each morphological type derives from a unique germinal cell type named prohaemocyte, despite they perform separate functions (Ottaviani, 2005; Manfredini *et al.,* 2008; Strand, 2008; Hillyer, 2016). Indeed, the conversion of already differentiated circulating haemocytes into another cell type has been observed *in vitro* in PLs of T*enebrio molitor* Linnaeus, 1758*, P. americana*, *Galleria mellonella* (Linnaeus, 1758) (Gupta & Sutherland, 1966), *D. melanogaster* (Csordás *et al.,* 2021) and in prohemocytes of *B. mori* (Yamashita & Iwabuchi, 2001). Transdifferentiation is a special differentiation process, in which a mature cell type transforms into another mature cell type. This process does not require the involvement of stem cells for the formation of a fully functional cell. Experiments on the induction of the larval cellular immune response in *D. melanogaster* with a parasitic wasp highlighted the potential pluripotency of plasmatocytes which transdifferentiate into lamellocytes through intermediate forms (Anderl *et al.,* 2016; Csordás *et al.,* 2021). Circulating haemocytes in *T. molitor* have been shown wide phenotypic variability in the four populations of PRs, PLs, GRs and OEs with intermediate features under both light and electron microscopy (Vommaro *et al.,* 2021). Moreover, some morpho-

Table 1 – List of the most common haemocytes in Insects as reported in (Gupta 1979; Brehélin & Zachary 1986; Siddiqui & Al-Khalifa 2012).

Cell types	acronyms	Morphological features
Prohaemocytes	PRs	small rounded cells, high nuclear-cytoplasmic ratio, numerous free ribosomes, poorly developed RER
Plasmatocytes	PLs	spindle-shaped cells, RER well developed, small electron-dense vesicles
Granular Cells	GRs	polymorphic cells, cytoplasmic digitations, large electron-dense vesicles, pinocytotic vesicles
Coagulocytes	COs	
Spherule cells	SPs	large and numerous electron dense inclusions
Adipohaemocytes	ADs	
Lamellocytes	LAs	intricate invaginations of plasma membrane
Oenocytoids	OEs	large cells, low nuclear-cytoplasmic ratio, little developed cytoplasmic organelles, phenol-oxidase activity in the cytoplasm
Vermicytes	VEs	are very large, extremely flattened plasmatocyte-like cells
Podocytes	POs	
Thrombocytes	THs	
Spinocytes	SNs	

Table 2 – Phagocyting haemocytes in Coleoptera

	PLs	GRs	OEs	References
Cetonischema aeruginosa (Drury, 1770)		larva	larva	(Giulianini *et al.* 2003)
Rhynchophorus ferrugineus Olivier, 1790	larva	larva		(Manachini *et al.* 2011)
Melolontha melolontha Linnaeus, 1758	adult			(Brehélin & Zachary 1986)
Allomyrina dichotoma (Linnaeus, 1771)		larva		(Hwang *et al.* 2015)
Harmonia axyridis (Pallas, 1773)		adult		(Firlej *et al.* 2012)
Carabus lefebvrei Dejean, 1826	adult larva	larva		(Giglio *et al.* 2008; Giglio & Giulianini 2013)
Pterostichus melas italicus (Dejean, 1828)	adult			(Giglio *et al.* 2015)
Harpalus rufipes (De Geer, 1774)		adult		(Cavaliere *et al.* 2019)

logical evidences - such as 1) high autophagic activity in the cytoplasmatic compartment involved to maintain the regular homeostatic turnover of organelles and 2) mitotic circulating cells involved in replace apoptotic and necrotic cells which are removed by phagocytosis - suggest that proliferation, turnover and transdifferentiation are constantly active processes in the haemolymph.

Finally, these evidences indicate that the large numerous of morphotypes reported in the species described so far are only intermediate forms of the same cell type that perform different functions. Thus, transdifferentiation acts as an mechanism adapted by phylogenetically distant organisms to optimize available resources to environmental challenges.

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IL COLORE BIANCO STRUTTURALE DELLA MOSCA DELLE OLIVE *BACTROCERA OLEAE* (DIPTERA, TEPHRITIDAE)

MANUELA REBORA[a]

[a]*Dipartimento di Chimica, Biologia e Biotecnologie, Università di Perugia Via Elce di Sotto, 8 06121 Perugia, Italy*.
Email: manuela.rebora@unipg.it
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Structural white coloration in the olive fruit fly Bactrocera oleae *(Diptera, Tephritidae)*

The presented study focuses on the white patches on the thorax and head of the olive fruit fly *Bactrocera oleae*. Ultrastructure and development of these white patches on thorax and head are analysed using scanning electron microscopy, transmission electron microscopy, and fluorescence microscopy. Modified air sacs with arborisations rich of beads in an empty space, constitute a three-dimensional photonic solid responsible for light scattering. Structural white and associated autofluorescence are described for the first time in Diptera. Moreover, a structural colour not produced by external cuticle but by an internal structure located under transparent cuticle is described in Insects. In particular, portions of the air sacs with their modified internal structure assume a function that is so far undescribed in insects. The identification of these complex structures producing structural white lays the foundation for further investigations aiming to understand the biological role of the white patches on the body of *B. oleae* and their possible use as visual cues in sex recognition or predatory avoidance. These investigations, adding information on the biology of this dangerous species, could help to develop methods for its biological control.

KEY WORDS**:** air sacs, scattering, fluorescence, Diptera**,** ultrastructure

INTRODUZIONE

Gli insetti mostrano una notevole diversità di colori che rappresentano importanti stimoli visivi per la comunicazione intra ed inter-specifica, per esempio nel riconoscimento dei sessi o nella protezione dai predatori (colorazioni criptiche o aposematiche) o che possono avere un ruolo nella termoregolazione o nella protezione nei confronti dei raggi ultravioletti (i.e. THÉRY e GOMEZ, 2010; CUTHILL *et al.*, 2017). Tali colorazioni, come noto, possono essere dovute a pigmenti (melanine, carotenoidi, ommocromi, pteridine, etc.) collocati nella cuticola o al di sotto di cuticola trasparente (SHAMIM *et al*., 2014), che assorbono la radiazione elettromagnetica visibile in maniera selettiva, o a fenomeni fisici strutturali come interferenza, diffrazione e diffusione, che riflettono la luce in maniera selettiva (BURG e PARNELL, 2018). Questa seconda tipologia di colorazioni è piuttosto diffusa negli insetti in relazione all'organizzazione multi-stratificata della cuticola e alle nanostrutture che la caratterizzano. I colori strutturali, talvolta associati a pigmenti, sono stati ampiamente studiati, soprattutto in alcuni ordini di insetti come Lepidotteri (GHIRARDELLA, 1991; VUKUSIC, 2006; TRZECIAK *et al.*, 2012), Coleotteri (SEAGO *et al.*, 2008) e Odonati (PRUM *et al*., 2004; SCHULTZ, e FINCKE, 2009; GUILLERMO-FERREIRA *et al.,* 2015; HENZE *et al.,* 2019) che offrono notevoli esempi in questo ambito.

Il bianco strutturale negli insetti è meno comune dei colori e richiede processi di diffusione per tutte le lunghezze d'onda visibili (VUKUSIC *et al.,* 2007). La luce bianca può essere diffusa dalla cuticola, da setole, da squame e cere superficiali non pigmentate con forma e dimensione specifiche, producendo così un bianco strutturale. Casi esemplari a questo riguardo sono le ali delle farfalle Pieridae, bianche a causa di una serie di microsfere sospese all'interno delle squame alari (STAVENGA *et al.,* 2004; LUKE *et al*., 2009) e gli Odonati, per la presenza di cristalli di cera sull'epicuticola (GORB *et al*., 2000; NIXON *et al*., 2017). Inoltre sono stati ampiamente studiate alcune specie di coleotteri del genere *Cyphochilus* che possiedono cuticola dotata di squame con strutture interne ottimizzate per produrre dispersione ottica e un colore bianco estremamente intenso con uno spessore estremamente sottile (VUKUSIC *et al*., 2007; LUKE *et al*., 2010; BURRESI *et al*., 2014; BURG *et al*., 2019).

Nella presente trattazione verrà descritta la morfologia funzionale delle aree bianche chiaramente visibili sul torace e sul capo della mosca delle olive, *Bactrocera oleae* (Diptera, Tephritidae). Attraverso indagini di microscopia ottica, microscopio a fluorescenza e microscopia elettronica (microscopio elettronico a scansione e a trasmissione) viene descritta ed analizzata la particolare ultrastruttura dei sacchi aerei della mosca delle olive che solo in corrispondenza di tali aree si

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modificano per generare strutture fotoniche capaci di diffondere la radiazione luminosa e generare colore bianco.

La particolare morfologia dei sacchi aerei in corrispondenza delle aree bianche

La mosca delle olive presenta diverse aree bianche in corrispondenza del torace e del capo (Fig. 1 a-c). Tra esse di esse è presente lo scutello costituito, come le altre parti bianche, da cuticola trasparente al di sotto della quale sono visibili i sacchi aerei (Fig. 1 b,d,e). Osservando l'adulto nelle prime ore di vita è chiaramente visibile come la colorazione bianca dello scutello sia direttamente correlata allo sviluppo dei sacchi aerei che si gonfiano nell'arco di 24 ore a partire dallo sfarfallamento (Fig. 1 d,e). Infatti, nelle prime ore dopo lo sfarfallamento, lo scutello appare bianco sono in piccola parte, esattamente in corrispondenza di tali strutture, mentre è chiaramente visibile la cuticola trasparente (Fig. 1d). Osservazioni al microscopio elettronico a scansione rivelano che solo in corrispondenza delle aree bianche, al di sotto della cuticola trasparente multistratificata, i sacchi aerei presentano particolari caratteristiche (Fig. 2 a,b). Infatti solo in tali aree le cellule rivestite di cuticola che costituiscono il sacco aereo formano strutture arborescenti altamente ramificate (Fig. 2b). Tali arborizzazioni corrono perpendicolarmente alla cuticola e misurano circa 15 µm di lunghezza (Fig. 2 a,b). Ciascuna arborizzazione è caratterizzata dalla presenza di numerose strutture sferoidali spinose con un diametro di circa 0,35 µm (Fig. 2 c,d). Sezioni ultrafini dello scutello osservate al microscopio elettronico a trasmissione rivelano la struttura multistratificata della cuticola che sovrasta uno strato di vescicole piuttosto sviluppato, al di sotto del quale sono visibili l'emolinfa e il sacco aereo (Fig. 3a). Il sottile strato di cellule che costituisce il sacco aereo mostra uno spesso strato di arborizzazioni rivestite da epicuticola (Fig. 3 a-c). Sono chiaramente visibili le strutture sferoidali spinose molto elettrondense che bordano le arborizzazioni (Fig. 3 a-c).

Lo scutello e le altre aree bianche sia sul torace che sul capo di *B. oleae*, mostrano fluorescenza indotta dai raggi UV (Fig. 4 a,b). L'eccitazione avviene a 365 nm (luce UV) e l'emissione da 397 nm (luce blu). Tale fluorescenza è molto evidente 24 h dopo l'emergenza, quando i sacchi aerei sono completamente sviluppati. È più debole negli adulti appena emersi, dove i sacchi aerei sono ancora di dimensioni ridotte e non pieni d'aria. Fluorescenza più debole (verde o rossa) è stata osservata con altri filtri (eccitazione a 450–490 nm ed emissione da 520 nm ed eccitazione a 546 nm ed emissione da 590 nm).

Anche in altre specie di Tephritidae, come per es.

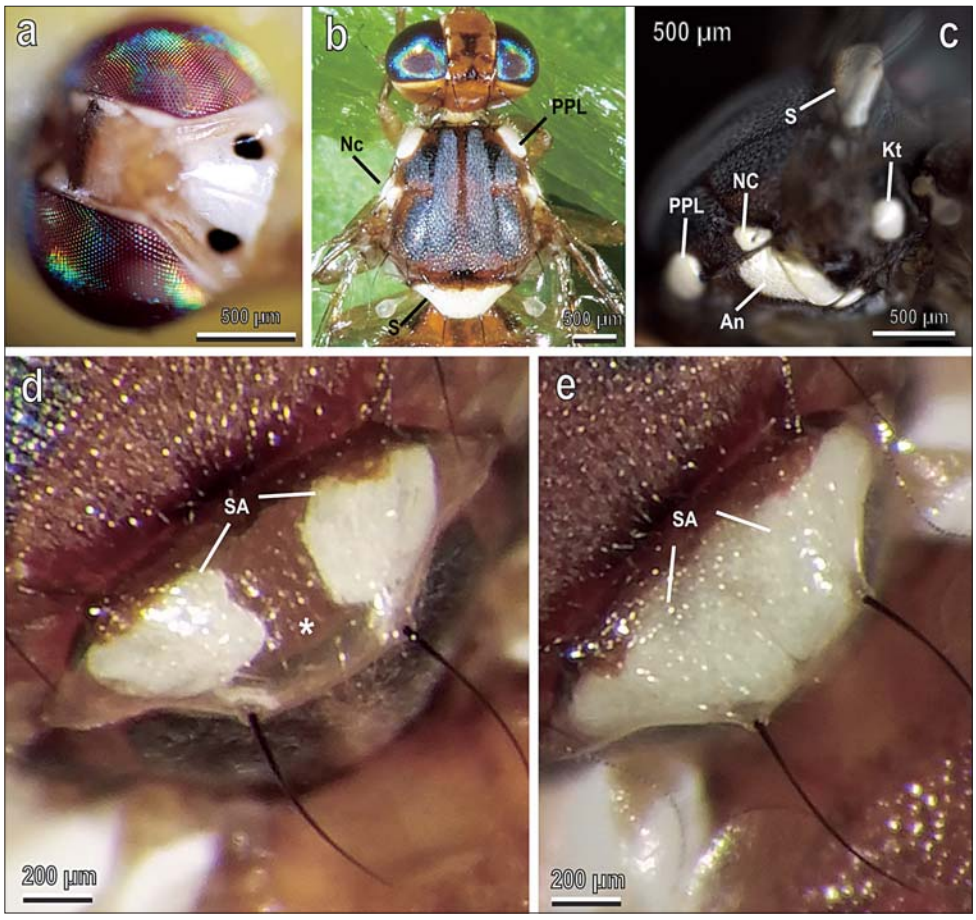

Fig. 1 – Aree bianche in corrispondenza del capo (a) e del torace (b,c) dell'adulto di *Bactrocera oleae* allo stereomicroscopio. S, scutellum; PPL, post pronotal lobe; AN, anepisternal area; NC, notopleural callus; KT, katatergite. Notare lo sviluppo dei sacchi aerei (SA) e la concomitante comparsa delle aree bianche nello scutello al di sotto della cuticola trasparente (asterisco) a partire dalle prime ore dopo lo sfarfallamento (d) fino a 24 ore dopo lo sfarfallamento (e).

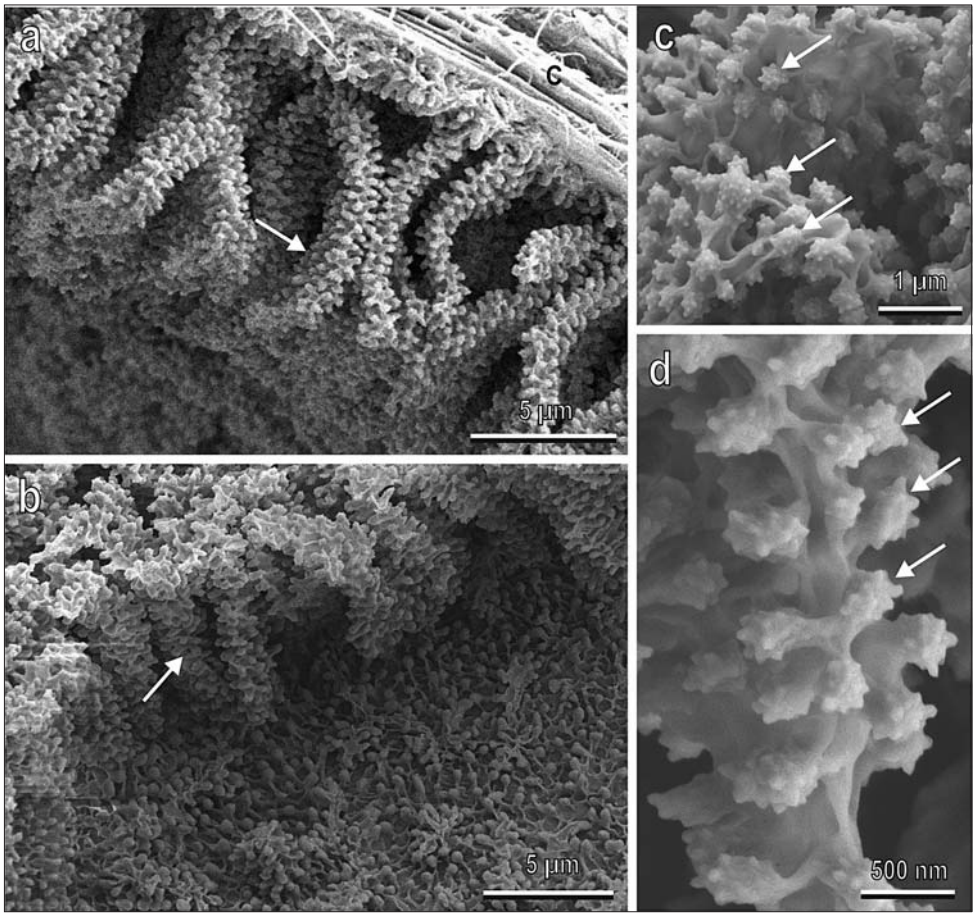

Fig. 2 – Parte interna di una delle aree bianche del torace di *Bactrocera oleae* osservata al microscopio elettronico a scansione**.** a, Cuticola trasparente multistratificata (C) al di sotto della quale i sacchi aerei presentano strutture arborescenti altamente ramificate (freccia) che corrono perpendicolarmente alla cuticola; b, Area di transizione tra la parte bianca e la parte pigmentata. Notare che le arborizzazioni (freccia) sono presenti solo al di sotto della parte bianca; c,d, Dettagli delle arborizzazioni bordate da strutture sferoidali spinose (frecce).

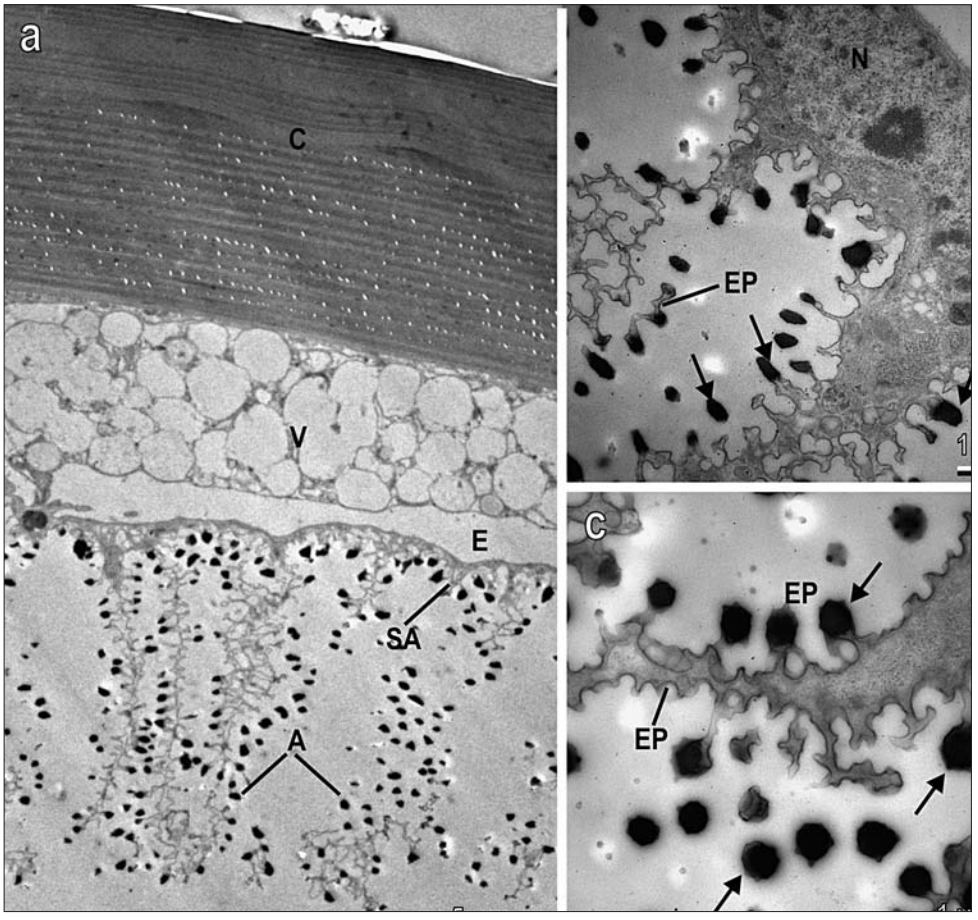

Fig. 3 – Sezioni ultrafini dello scutello di *Bactrocera oleae* osservate al microscopio elettronico a trasmissione. a, Struttura multistratificata della cuticola (C) che sovrasta uno strato di vescicole piuttosto sviluppato (V), al di sotto del quale sono visibili l'emolinfa (E) e il sacco aereo (SA) con il lume (L) contenente aria caratterizzato da arborizzazioni (A); b,c, Dettagli delle arborizzazioni. Notare la cellula del sacco aereo con il suo nucleo (N), e le arborizzazioni rivestite da epicuticola (EP). Sono chiaramente visibili le strutture sferoidali spinose molto elettrondense (frecce) che bordano le arborizzazioni.

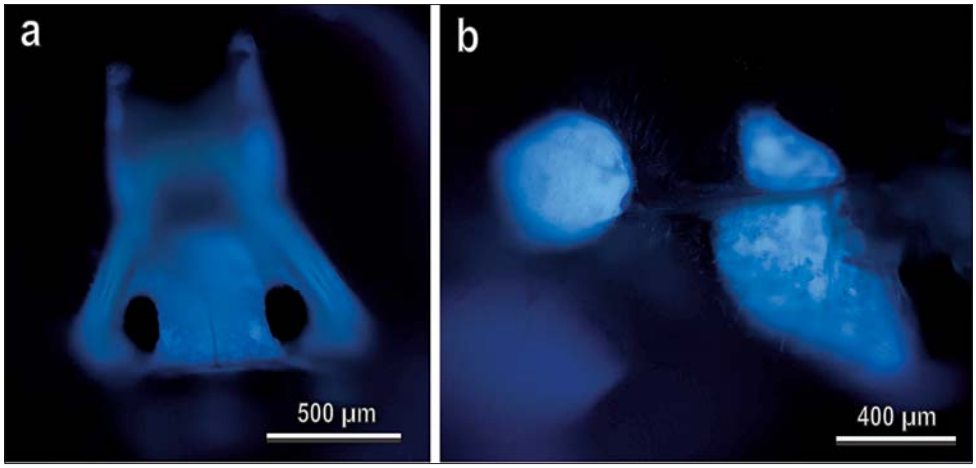

Fig. 4 – Aree bianche sul capo (a) e sul torace (b) di *Bactrocera oleae* osservate al microscopio a fluorescenza (excitation filter 365 nm, chromatic beam splitter FT 395 nm, emission 397 nm). Notare la fluorescenza blu indotta dai raggi UV.

in *Ceratitis capitata* (Fig. 5 a-d), i sacchi aerei, in corrispondenza di aree del corpo bianche, presentano internamente morfologia simile a quella osservata nelle aree bianche di *B. oleae*.

I sacchi aerei sono responsabili del bianco strutturale di *Bactrocera oleae*

Le peculiari arborizzazioni dei sacchi aerei in corrispondenza delle aree bianche, con numerose strutture sferoidali spinose distribuite e orientate casualmente in uno spazio con aria, hanno caratteristiche morfologiche altamente compatibili con la produzione di bianco strutturale. Infatti la percezione del bianco è dovuta alla diffusione della luce da parte di un materiale contenente superfici disordinate capaci di riflettere tutte le lunghezze d'onda (Mason, 1926). Il bianco strutturale delle piume degli uccelli o degli insetti è solitamente prodotto da nanostrutture di un materiale solido come la chitina o la beta-cheratina collocate in uno spazio vuoto contenente aria (Burg e Parnell, 2018). Nell'ambito dei Lepidotteri, il bianco delle ali delle cavolaie (Pieridae) è prodotto da microscopici granuli sospesi all'interno delle squame alari (Stavenga *et al.*, 2004; Luke et al., 2009), mentre nell'ambito dei

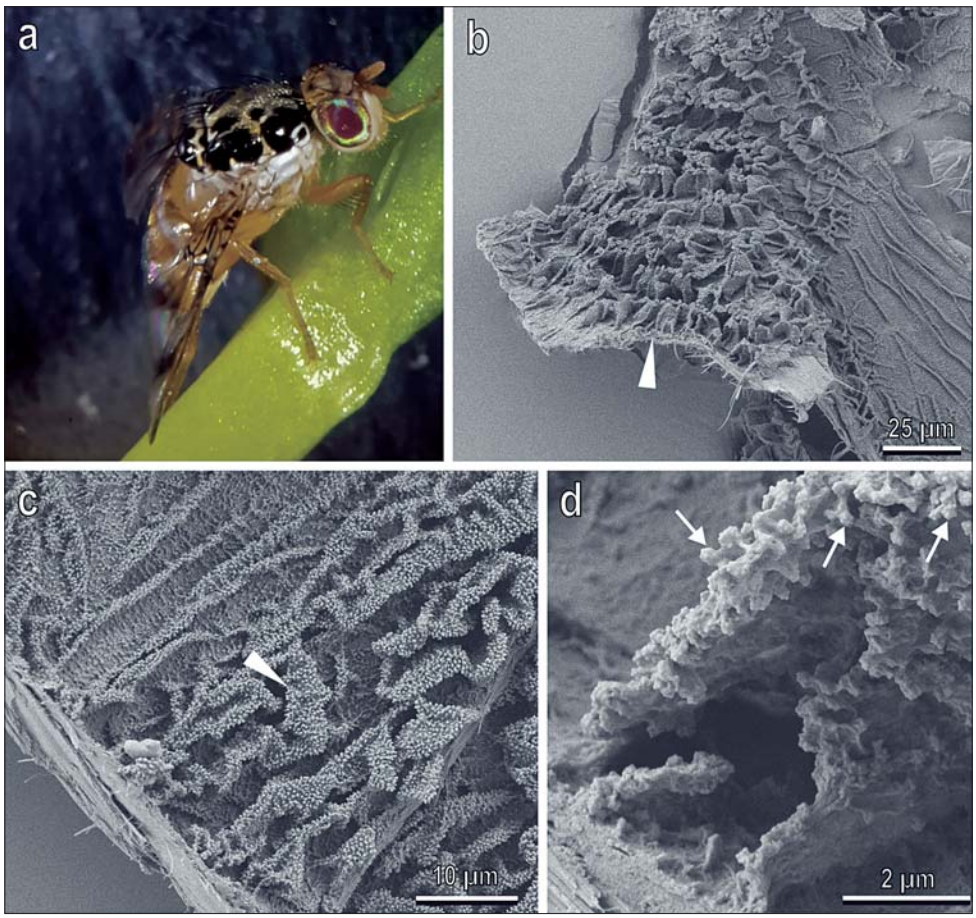

Fig. 5 – Adulto di *Ceratitis capitata* osservato allo stereomicroscopio (a) e struttura interna dei sacchi aerei del torace in corrispondenza delle aree bianche (b-d) al microscopio elettronico a scansione. a, Notare le aree bianche sul torace; b Struttura interna dei sacchi aerei che, solo in corrispondenza delle aree bianche, presentano strutture arborescenti altamente ramificate (testa di freccia) che corrono perpendicolarmente alla cuticola; c, Area di transizione tra la parte bianca e la parte pigmentata. Notare che le arborizzazioni (testa di freccia) sono presenti solo al di sotto della parte bianca; d, Dettaglio delle arborizzazioni bordate da strutture sferoidali spinose (frecce).

Coleotteri il bianco del corpo degli scarabei del genere *Cyphochilus* è prodotto da una rete di filamenti cuticolari interconnessi all'interno di squame piuttosto sottili che mostrano un'elevata efficienza di scattering (Vukusic *et al*., 2007; Luke *et al*., 2010; Wilts *et al*., 2017). Di conseguenza, le arborizzazioni dei sacchi aerei presenti sotto la cuticola trasparente, collocate a livello delle aree bianche del torace e del capo di *B. oleae*, con il loro strato epicuticolare e le strutture sferoidali spinose orientate casualmente, possono costituire un cristallo fotonico tridimensionale circondato da aria in grado di riflettere tutte le lunghezze d'onda e generare così bianco strutturale.

Le tipiche funzioni dei sacchi aerei negli insetti sono l'aumento dell'efficienza respiratoria tracheale, l'assistenza al volo riducendo il peso specifico, la spinta idrostatica negli insetti acquatici, la termoregolazione, l'amplificazione e la risonanza per la produzione/ricezione dei suoni (Wigglesworth, 1963). In tale contesto, la capacità di produrre colorazioni strutturali rappresenta un'ulteriore funzione dei sacchi aerei, evidenziando così ancora una volta la stupefacente plasticità adattativa degli insetti in grado di modificare strutture preesistenti per nuovi scopi. Questa caratteristica, come sopra riportato, comune anche ad altri Tephritidae, potrebbe essere presente anche in altri artropodi poiché nella specie *Scutigera coleoptrata* L. (Scutigeromorpha: Scutigeridae) fasci di trachee appaiono come macchie bianche sotto la cuticola trasparente (Hilken *et al*., 2021). Il bianco prodotto non è così pronunciato come in *B. oleae*, ma potrebbe rappresentare un possibile stadio preliminare per un'ulteriore specializzazione osservabile negli insetti. In *B. oleae* le strutture sferoidali spinose poste lungo le arborizzazioni dei sacchi aerei sotto le aree bianche potrebbero derivare dalle papille descritte lungo la superficie interna delle trachee e dei sacchi aerei degli insetti (Edgecomb *et al.,* 1995; Appel *et al*., 2015; Webster *et al*., 2015). La cuticola tracheale negli insetti è costituita da una sottile epicuticola e da una cospicua procuticola e segue regolari sporgenze della membrana plasmatica apicale delle cellule epiteliali tracheali da cui derivano i taenidia (Moussian, 2013). Molto probabilmente, taenidia, papille e strutture sferoidali spinose spinose hanno la stessa origine evolutiva e la stessa composizione chimica di procuticola ed epicuticola, come suggerito anche dalle nostre osservazioni ultrastrutturali dove tali strutture appaiono molto elettrondense.

Il possibile ruolo biologico delle aree bianche su capo e torace di *Bactrocera oleae*

Le aree bianche sul corpo di *B. oleae* emettono fluorescenza. In particolare, l'eccitazione più forte avviene a 365 nm (luce UV) e l'emissione a partire da 397 nm (luce blu). Una spiegazione dell'origine dell'autofluorescenza delle aree bianche di *B. oleae* potrebbe essere la presenza di resilina (una proteina autofluorescente con un'emissione nella regione blu) nella cuticola dei sacchi aerei. Nelle trachee (e di conseguenza nei sacchi aerei) è stata infatti dimostrata la presenza di di- e tritirosina, che fungono da indicatori della resilina (Andersen, 2004). La resilina è presumibilmente responsabile dell'elasticità/resilienza dei tubi tracheali e delle sacche d'aria. È interessante notare che indagini elettrofisiologiche sulla sensibilità visiva dell'adulto di *B. oleae* hanno rivelato un picco di sensibilità maggiore a 485-500 nm (luce blu) (Agee *et al.,* 1982), supportando così l'ipotesi di un possibile ruolo biologico delle aree bianche sul torace e sul capo di *B. oleae*. D'altra parte la sensibilità visiva alla luce blu è diffusa negli insetti e la capacità di assorbire la radiazione ultravioletta della luce solare e riemetterla come luce visibile nel blu è sfruttata da diversi fiori che attraggono gli insetti impollinatori attraverso la fluorescenza del polline e delle antere (Mori *et al.,* 2018) o dalle piante carnivore come *Nepenthes* che presentano il bordo dell'ascidio fluorescente per attirare gli insetti al loro interno (Kurup *et al*., 2012).

Fino ad oggi nessuna attenzione è stata prestata al possibile ruolo biologico delle aree bianche sul corpo di *B. oleae*. Un'ipotesi potrebbe essere un loro ruolo come segnali visivi nel riconoscimento intra e intersessuale durante l'accoppiamento. A tal proposito, è importante ricordare che le indagini sul riconoscimento di potenziali rivali/partner durante il comportamento di accoppiamento nella mosca delle olive (e nei Tephritidae in generale) si sono concentrate principalmente sui segnali chimici (Benelli *et al*., 2014), mentre nessuna indagine è stata effettuata sul ruolo comportamentale dei segnali visivi nella difesa della lek, nel corteggiamento e nel comportamento di accoppiamento e sono necessarie ulteriori studi per chiarire questi aspetti. Ciò, anche in considerazione che in altri Ditteri (i.e. *Lispe consanguinea* Loew (Diptera: Muscidae) e *L. tentaculata* DeGeer (Diptera: Muscidae) segnali visivi costituiti da squame argentate concave sul capo hanno grande importanza nel riconoscimento intra e intersessuale (Frantsevich e Gorb, 2006).

Un'altra possibile funzione delle macchie bianche potrebbe essere collegata alla difesa nei confronti dei predatori. Infatti, in altre specie di Tephritidae appartenenti al genere *Rhagoletis* (Eisner, 1984) e *Zonosemata* (Greene *et al*., 1987; Whitman *et al*., 1988) è stato riportato il mimetismo morfologico e comportamentale aposematico con i ragni saltatori Salticidae. È interessante notare che le femmine dei Salticidae hanno palpi con una fluorescenza indotta dai raggi UV (Lim *et al*., 2007) simile a quella osservata nelle aree bianche di *B. oleae*. L'imitazione dei ragni saltatori potrebbe essere di beneficio contro ragni, insetti e vertebrati

poiché i ragni saltatori sono difficili da catturare e velenosi.

Infine, le aree bianche di *B. oleae* potrebbero potenzialmente servire ad altre funzioni non legate a stimoli visivi, come la termoregolazione. Nei coleotteri della specie *Neocicindela perhispida* (Broun) (Coleoptera: Cicindelidae), gli esemplari chiari, quando trasferiti dal loro habitat naturale di sabbia bianca a un terreno di sabbia nera sono in grado di cercare cibo per un tempo più lungo senza surriscaldarsi rispetto alle morfe scure, che sfuggono al caldo scavando nella sabbia (Hadley *et al*., 1992).

In conclusione, i dati qui presentati descrivono in dettaglio l'ultrastruttura delle macchie bianche sul corpo della mosca dell'olivo *B. oleae*. Le proprietà ottiche di tali aree ed un maggiore approfondimento della loro ultrastruttura sono riportati in Rebora *et al*., (2021). Tali indagini hanno permesso di individuare per la prima volta nei Ditteri bianco strutturale e autofluorescenza associata. Inoltre, è stata descritta per la prima volta negli Insetti una nuova funzione associata ai sacchi aerei ed un colore strutturale non prodotto dalla cuticola esterna ma da una struttura interna situata sotto la cuticola trasparente. Infine, l'identificazione di queste complesse strutture che producono bianco strutturale pone le basi per ulteriori indagini volte a comprendere il ruolo biologico delle aree bianche sul corpo di *B. oleae* e il loro possibile utilizzo come segnali visivi nel riconoscimento del sesso o nella difesa dai predatori. Ulteriori conoscenze sulla biologia di questa specie di notevolissima importanza dal punto di vista fitosanitario, potrebbero potenzialmente aiutare a sviluppare metodi per il suo controllo biologico.

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