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Research

'Crystal Macrosetae': Novel Scales and Bristles in Male Arctiine Moths (Lepidoptera: Erebidae: Arctiinae) Filled with Crystallizing Material

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Abstract

Scales, exoskeletal features characteristic of the Lepidoptera, occur in enormous structural and functional diversity. They cover the wing membranes and other body parts and give butterflies and moths their often stunning appearance. Generally, the patterns made by scales are visual signals for intra- and interspecific communication. In males, scales and/or bristles also make up the androconial organs, which emit volatile signals during courtship. Here, a structurally and putative functionally novel type of scales and bristles is reported: 'crystal macrosetae'. These lack trabeculae and windows, are made up by a very thin and flexible envelope only and contain crystallizing material. In 'crystal scales', there is a flat surface ornamentation of modified ridges, while 'crystal bristles' often show large protrusions. Crystal macrosetae usually cannot be reliably recognized without destruction. Apparently, they serve as containers for large amounts of material that is viscous in living moths, highly hygroscopic, crystallizes when specimens dry up, and can be visualized by scanning electron microscopy. Crystal macrosetae occur in males only, always associated with or making up androconial organs located on various parts of the body, and have numerous forms with diverse surface ornamentation across many species and genera. The newly identified structures and the discovery of crystallizing material in scales and bristles raise many questions and could shed new light on ontogenetic development of macrosetae, and on the biology and physiology as well as the evolution and systematics of Arctiinae. There is evidence that crystal macrosetae occur in other moths too.

Key words: androconial organ, scale, fine structure, crystallizing material

Scales, adult cuticular structures found throughout the entire insect order Lepidoptera, have been the subject of many investigations since invention of the microscope. An enormous diversity of scale morphology has been revealed (e.g., Scoble 1992, Kristensen and Simonsen 2003, Ghiradella and Butler 2009, Zhang et al. 2015). Their functional diversity reflects and probably explains much of this structural heterogeneity—although their main uses appear to be in coloration and patterning (e.g., Vane-Wright and Boppré 1993, Stavenga 2009), and the storage and deployment of male courtship pheromones (e.g., Boppré 1984, Ômura and Yotsuzuka 2015, Darragh et al. 2017). Scales thus play critical roles in the intra- and interspecific visual and chemical communication of Lepidoptera. Additional functions include thermoregulation (e.g., Miaoulis and Heilman 1998), 'waterproofing' (Wagner et al. 1996), and predator escape (e.g., Dodd 1902, Eisner et al. 1964).

Typical scales and bristles exhibit a universal common structure: a socket, a petiole, and a basal surface from which arise pillar-like trabeculae. The trabeculae support a more complex upper surface, which has longitudinal ridges made up of microribs with lamellae. The ridges are connected by cross ribs and fenestrated by more or less regular openings called 'windows' (Downey and Allyn 1975, Ghiradella 1998, 2005, 2010). All previously investigated scales can be recognized as modifications of this structure, or *bauplan*.

In the course of comparative studies undertaken in Costa Rica and Peru on the diversity of androconial organs found within communities of Arctiinae (Erebidae), in numerous species, genera and subtribes of the Arctiini (also in some Lithosiini and in a notodontid moth) we unexpectedly discovered scales that exhibit a previously unencountered degree of reduction of the *bauplan*. These scales have neither trabeculae nor windows, the ridges and microribs are modified, being very flat, while the entire scale lumen (in most cases the basal and upper surface can no longer be differentiated) is filled with a more or less hygroscopic liquid or gel which—when dried (as in dead specimens)—crystallizes. These special, sac-like scales always form part of or are closely associated with androconial organs, be they 'coremata' ('hairy' tubes of varying size, located within the abdomen between segments 7 and 8, that are pneumatically expanded during courtship behavior), or patches or pockets on other parts of

the body, also likely displayed during close-range intraspecific communication. Even more surprisingly, in many species, the bristles or 'hairs' that make up the coremata are also filled with crystallizing material.

In this paper, we illustrate, describe, and characterize these 'crystal scales' and 'crystal bristles' in general, including the great diversity of their appearance, and discuss the potential biological significance of this new type of macrosetae. Although we can provide many precise observations and data, unfortunately these are compiled from the study of field-collected moths only; a systematic, functional study cannot yet be undertaken because culturing 'model species' of these moths is not yet possible (see Discussion).

Materials and Methods

In the context of a project on functional diversity of tiger and wasp moths (Lepidoptera: Erebidae: Arctiinae; cf. Boppré 2015), specimens were either collected at artificial light or baited with pyrrolizidine alkaloids mainly at 'El Bosque Nuevo', nr Santa Cecilia, Guanacaste, Costa Rica (11°03′N, 85°21′W) (Boppré 2011), with additional sampling at 'Panguana', nr Yuyapichis, Huánuco, Peru (9°37′S, 74°56′W), during several visits. They were provisionally sorted according to an unpublished illustrated catalogue of parataxonomic units (morphotypes: Krell 2004), prepared by the FZE team (Freiburg). In this catalogue, each parataxonomic unit is numbered; specimens collected were labeled with this number and given an individual code in addition. In the laboratory, specimens were set and finally identified.

More than 200 species of all Arctiini tribes were checked for the presence of crystal macrosetae, however, the choice of species was purely opportunistic and only specimens not needed for our core project were used for inspection for crystal macrosetae. Thus, the findings reported here deal with an unexpected side aspect, and were therefore not gathered systematically and cannot be used for taxonomic conclusions (see Discussion). Accordingly, the names of species possessing crystal macrosetae are provided in Table 1 (with reference to Figures) and intentionally not given in figure legends—the names are not meaningful for the general characterization of crystal macrosetae communicated here.

Specimens that had to be partly destroyed for preparations were photographed (usually with a Nikon Coolpix P300) beforehand, and the remains kept as vouchers for eventual taxonomic identification. The catches were evaluated for various studies (Boppré 2015, Boppré et al., unpublished data), one being a comparative account of male scent (androconial) organs which occur in multifold architecture and are found on almost every part of the body (Fischer et al., unpublished data). For this work, preparations of abdomens, wings, legs, and thoraces were made by carefully 'setting' the organs with minuten pins on styrofoam boards and air-drying them for microscopical investigation; several preparations were dried in a simple drying chamber with a light bulb. Coremata were artificially protruded and dried, usually in boxes with silica gel. In some cases, organs or parts were directly glued on aluminium pin stub specimen mounts (Plano GmbH, Wetzlar, Germany) using an adhesive disc (Leit-Tab; Plano GmbH, Wetzlar, Germany) for subsequent scanning electron microscopy (SEM), while others were mechanically disrupted, accidentally but also intentionally, then dried under different humidity conditions.

In the laboratory, images of air-dried preparations of androconial organs from the field were taken with a KEYENCE VHX-700FD digital microscope equipped with a VH-Z20R/VH-Z20W zoom lens 20–200× and a polarization filter OP-87429. With or without further manipulations, parts of organs were glued on stubs with an adhesive disc (see above), gold-coated (sputtered) and studied with a ZEISS DMS 940A SEM equipped with a DISS5 unit (point electronic GmbH, Halle, Germany). Digital images were adjusted for brightness and contrast with Adobe Photoshop CS6 and compiled with Adobe InDesign CS6 on a MacBook Pro.

Samples of fresh crystal scales were mechanically fractured with pins or razor blades and later studied with the SEM. Also, air-dried preparations with intact and/or damaged crystal scales were put into a humid chamber up to 12 h, then air-dried again and studied microscopically.

For numerous exemplar taxa, scales were treated with various solvents (hexane, acetone, ethanol, methanol, and water) under a microscope to see if the crystalline contents (as seen with SEM) were soluble.

Results

Recognition and Location of Crystal Scales

In most species possessing expandable abdominal coremata between the seventh and eighth abdominal sternites, a few or many scales of different appearance are very conspicuous at the bases of the bristle-covered tubes (Fig. 1A–D), even when the organs are only partially expanded (Fig. 1E–H). These 'crystal scales' (see below) differ in shape (slender to broad, not necessarily lamellar but rather thickened), size, color (white, red), and number (few to hundreds), unrelated to the size of the androconial organs. Some are large and glossy (Figs 1H and 7A–C) and/or appear stuck together (Fig. 7), others form dense 'furs' or 'piles' (Fig. 1F–H), while some occur in small groups of tiny scales only (Fig. 1C and N).

Among noncorematal androconial organs, such as patches on wings (Fig. 1I and K), or ventral abdominal pouches (Fig. 1L and M), or grooves on the legs, such peculiar scales are also found—but these can mostly be recognized under high magnification only. Again, they are associated with bristles and scales which likely disseminate pheromones during courtship.

All these crystal scales, which are mostly hidden when not in use, occur without exception in males only, and always as part of or in very close proximity to androconial organs. However, crystal scales are not obligatory parts of androconial systems—many species have comparable organs but lack crystal scales completely.

Bases of crystal scales taper to stalks (petioles) and insert into sockets (Fig. 2A–D). Thus they are proper macrosetae (Ghiradella 2010)—as are the crystal bristles (Fig. 10A and B; see below). Notably, crystal scales are often much (up to 10 times) larger than wing scales.

In several species crystal scales can be distinguished not only in fresh specimens but also in dry abdomens boiled in KOH for genitalic preparations (Fig. 3); however, they lose their filling during the process.

Surfaces of Crystal Scales

The enormous macroscopic diversity in expression of crystal scales indicated above (Fig. 1) is paralleled by the variety of their surface microstructure under SEM (Fig. 4B–I). Between species there is great variation yet great similarity at the same time. Surface ornamentation of crystal scales is distinctly different from covering scales (Fig. 4A), most notably the absence of windows. Overall, in most cases variations of a herringbone pattern are seen but the elevation is minimal. It looks as if the surfaces are not rigid but rather flexible or even elastic; scales can appear 'stretched' (Fig. 4K–M) or more or less shrunken (Fig. 4F and G; apparently depending on their filling), causing additional variation. Further, most crystal scales are similar

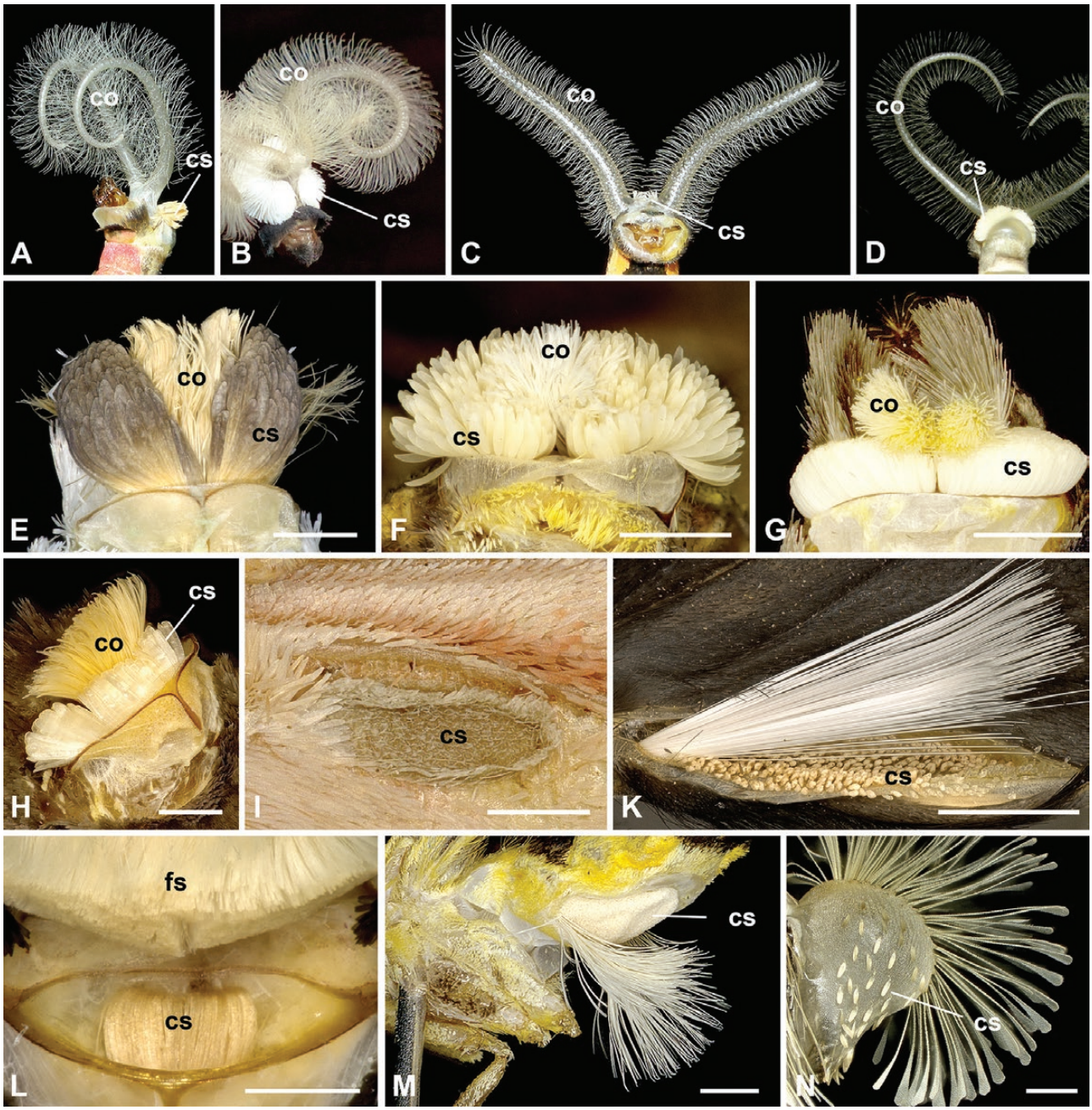

Fig. 1. Macrographs of androconial organs, artificially fully or partly everted, showing more or less prominent groups of crystal scales (**cs**) at the bases of abdominal coremata (**co**)(A–H), forewing patches (I), hindwing costal folds (K), abdominal pouches (L, M) and on eversible bladders at valves (N). **fs** = flocculent scales. Scale bars (approx.): E, L, N = 0.5 mm; F, G, K, M = 1 mm; H, I = 0.5 mm. For names, see Table 1.

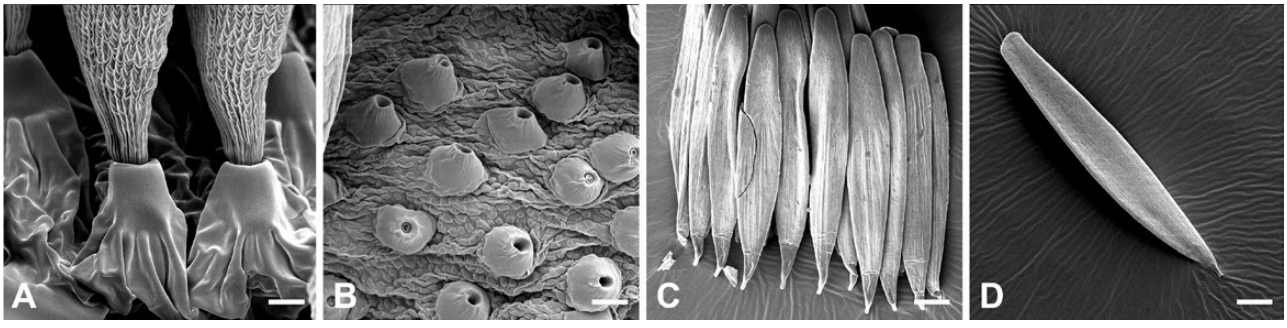

Fig. 2. Crysal scales are macrosetae, exhibiting sockets. Scale bars: A = 10 µm; B = 20 µm; C, D = 50 µm. For names, see Table 1.

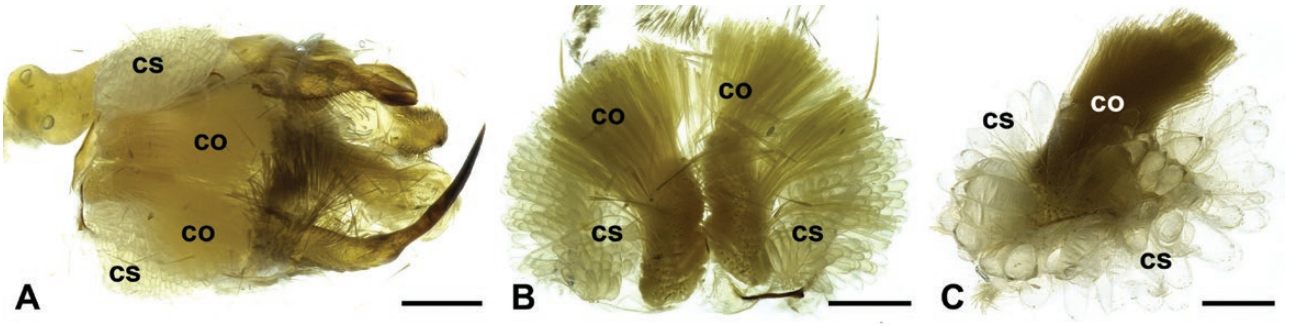

Fig. 3. Macrographs of (partly everted) dense clusters of scales–representing crystal scales (**cs**)–at the base of coremata (**co**) as seen in KOH preparations of dry abdomens. Scale bars: 1 mm. For names, see Table 1.

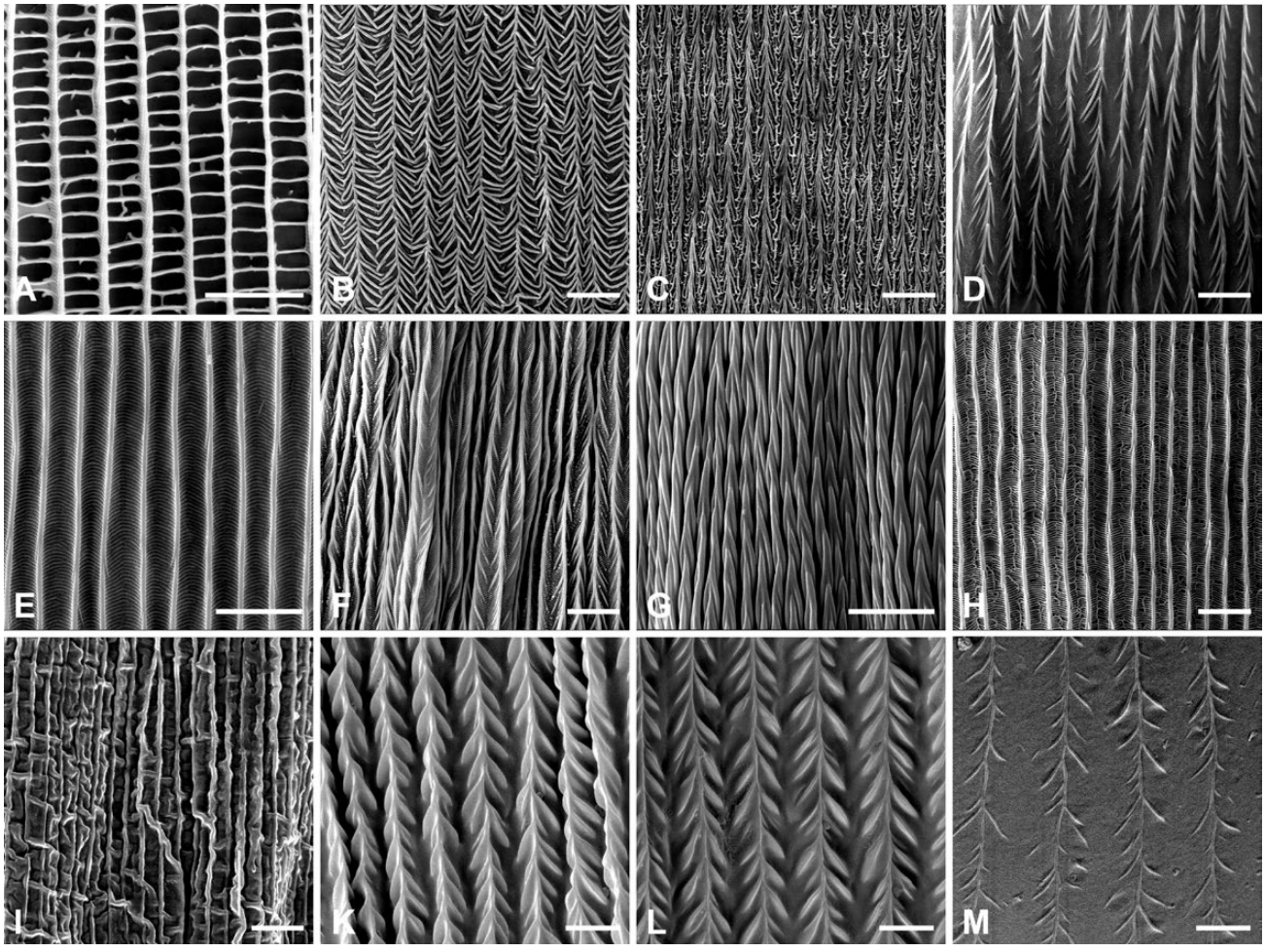

Fig. 4. Scanning electron micrographs of upper surface of a wing scale with ridges, cross ribs and windows (A; *Belemnia eryx* [Fabricius, 1775]) and crystal scales (B–I) of a selection of species showing a high diversity in surface ornamentation which is not rigid; within a species due to flexibility it can vary significantly (K–M). Scale bars: 5 µm. For names, see Table 1.

on all sides, not different on upper and lower surface as in typical lepidopteran wing scales.

Inner Structure of Crystal Scales

In contrast to normal scales, upon mechanical disruption, crystal scales neither show the typical spongy structure with trabeculae (Fig. 5A) nor irregular disintegration. Rather, when they are dry and break, the fracture surfaces reveal a stunning view: there is a surprisingly thin envelope which encloses a huge mass of homogenous material which shears absolutely smoothly and straight (Fig. 5B–H). These fracture surfaces can have a 'wavy' appearance (Fig. 5D). Transmission electron micrographs (Fig. 6) also show the lack of substructures and demonstrate the chitinous envelope to be no more than 60 nm thick.

Thus, crystal scales lack typical *bauplan* structures, exhibit a homogenous 'filling' which appears as a crystal, and have a strikingly thin envelope which is not rigid but appears flexible. Comparing crystal scales with a candy wrapped in cellophane foil sounds odd but makes the point—and gives an idea of the relative dimensions.

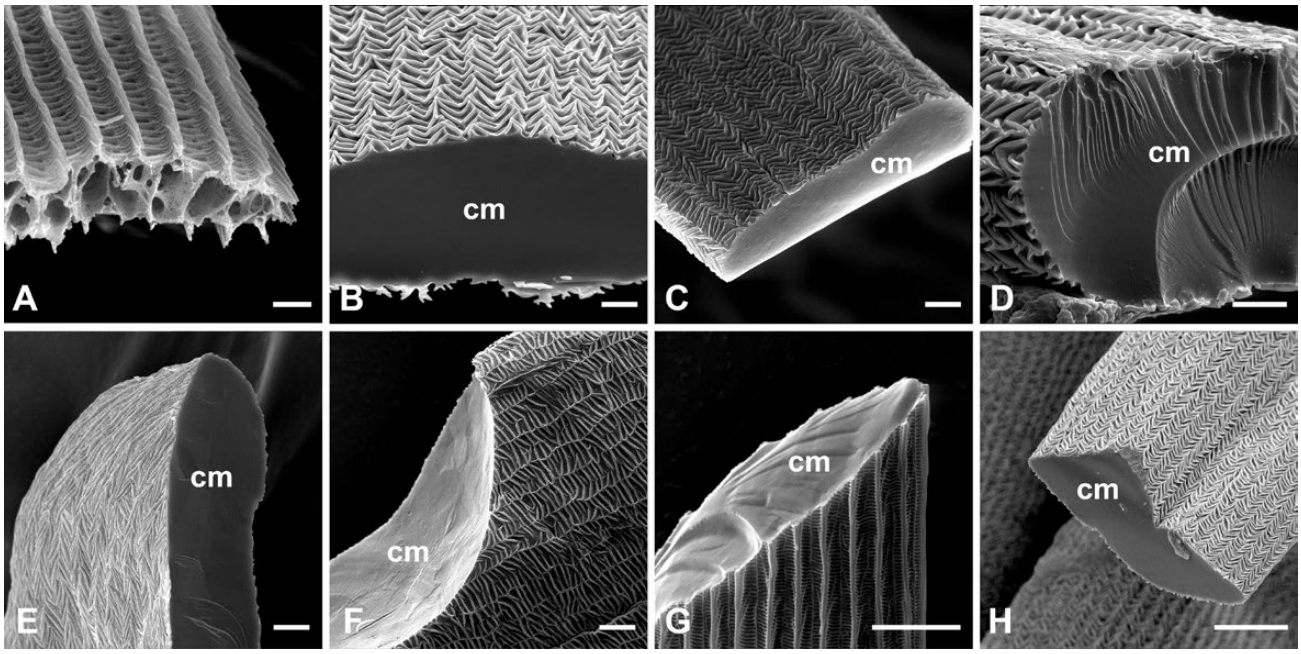

Fig. 5. Scanning electron micrographs of a disrupted wing scale showing hollow lumen with trabeculae (A) and crystal scales (B–H) exhibiting homogenous crystalline material which breaks like security glass, completely smooth or in waves. **cm** = crystallized material. Scale bars: A = 2 µm; B–G = 5 µm; H = 10 µm. For names, see Table 1.

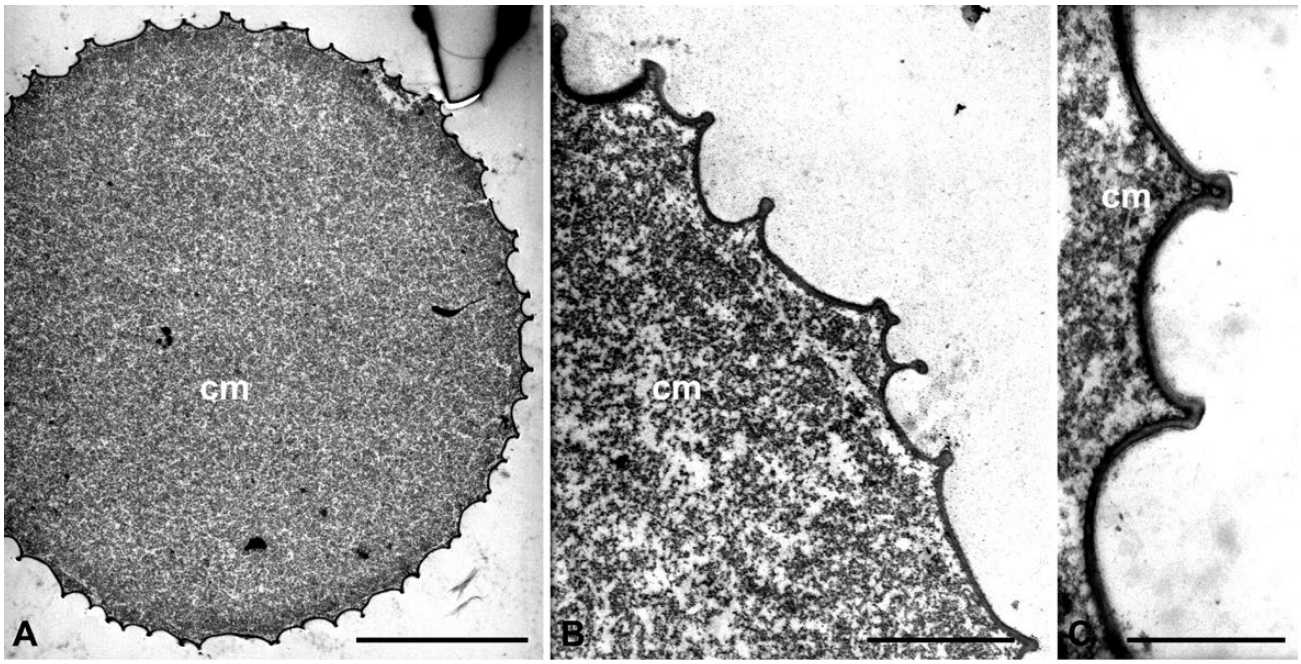

Fig. 6. Transmission electron micrographs of sections of a crystal scale showing a uniform granular substance enveloped within a thin cuticular 'skin' of about 60 nm. **cm** = crystallized material. Scale bars: A = 10 µm; B = 2 µm; C = 0.5 µm. For names, see Table 1.

Crystal Scales: Dry Versus In Vivo

The description of the microstructure given above is, necessarily, made from completely dry scales (because in the SEM the samples are under vacuum and because of the elapse of time between preparation of the organs in the field and their study in the laboratory). Thus, what is seen as a crystallized material might be liquid or viscous in the living insect. Cases of crystal scales appearing glued together support the idea that in living animals there are no crystals but rather sticky gels which—in some but not in all cases/species—can pass through the chitinous envelope and glue together individual scales (Fig. 7A–G).

Damaging crystal scales of freshly killed moths resulted either in picturesque crystal formations of various forms (Fig. 8A–D), or a smeared appearance, like a cut tube of toothpaste (Fig. 8E–G). This very strongly suggests that in a living moth crystal scales do not contain crystallized material, but a viscous substance or gel. Surprisingly, the little surface sculpturing is sometimes smoothened (Fig. 8H).

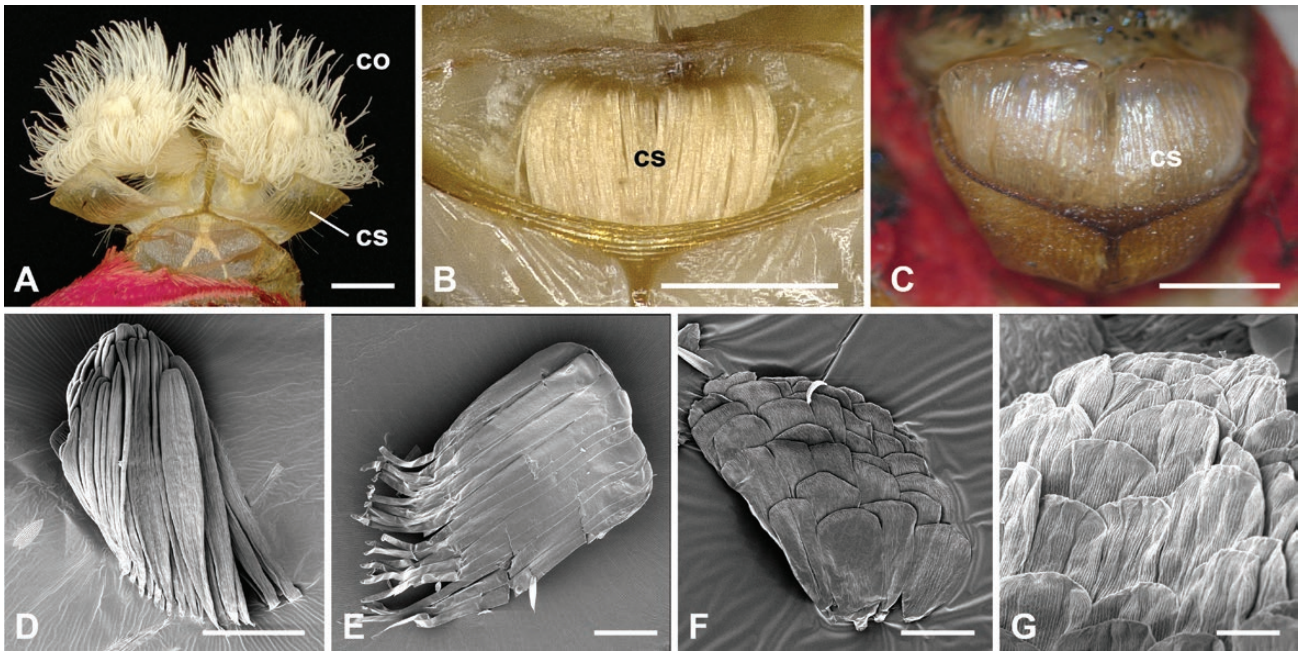

Fig. 7. Crystal scales (**cs**), in certain species, may be clumped, appearing like shields. **co** = coremata. Scale bars: A–C (approximate only) A, C = 1 mm; B = 0.5 mm; D, F = 200 μm; E = 400 μm; G = 50 μm. For names, see Table 1.

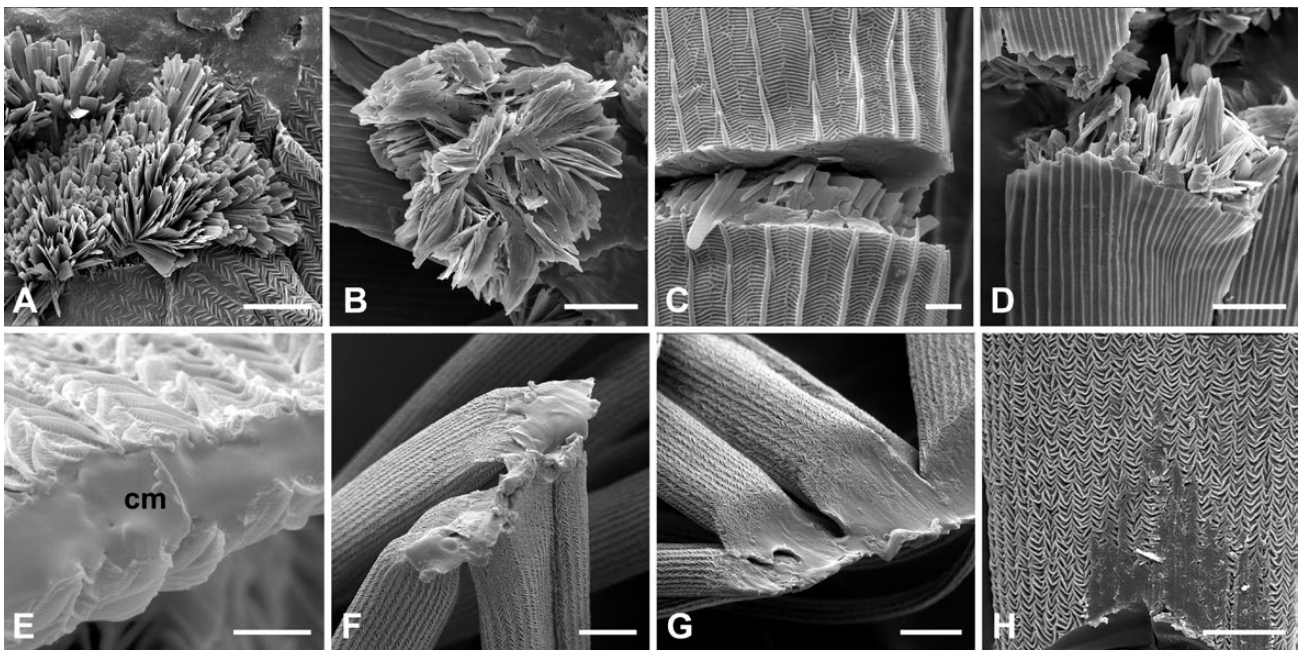

Fig. 8. Scanning electron micrographs of crystal formations observed when scales of freshly killed specimens are artificially desintegrated (A–D) or cut (E–G) and allowed to dry under different (uncontrolled) humidity conditions; crystal formations of various kinds (A–D) and 'smears' (E–G), respectively, are seen indicating that in living moths the content of crystal scales is viscous, if not liquid; H smoothed surface of crystal scale. **cm** = crystallizing material. Scale bars: A, F–G = 10 μm; B, D = 5 μm; C = 2 μm; E = 3 μm; H = 100 μm. For names, see Table 1.

Solubility and Hygroscopicity of the Content of Crystal Scales

Contact of crystal scales with different potential solvents (water, methanol, hexane, dichloromethane and others) under microscopic observation demonstrates that the filling is water-soluble only. Evaporation of the water makes the material solidify, forming a crust of crystals (Fig. 9A–D).

When artificially damaged dry crystal scales are kept in a moist atmosphere for some hours (Fig. 9E–L), droplets form at points of damage (Fig. 9F,H,I) and become homogenous smooth plates when dry (Fig. 9H and L), while the surface structure of the envelope remains. The high hygroscopicity can even be obvious in entire coremata kept in a humid chamber (Fig. 9M). Thus the filling of crystal scales is highly hygroscopic and can liquify. How the liquified content of the scales behaves when drying and how it then shows up

Table 1. Examples of Arctiini showing crystal macrosetae

Subtribe*	Species	Crystal macrosetae		Figure(s)	Origin
		Type	Location		
Callimorphina	*Utetheisa ornatrix* (Linnaeus, 1758)	cs	bladder	1N, 4G	CR
Spilosomina	*Virbia* spp.	cs	base of coremata	1C	CR, PP
Phaegopterina	*Agaraea semivitrea* Rothschild, 1909	cs		7F	CR
	Bertholdia detracta Seitz, 1921	cs	forewing us patch	—	CR
	Melese flavimaculata Dognin, 1899	cs		—	CR
	M. incertus (Walker, 1855)	cs		1I	CR
	Pseudapistosia umber (Cramer, 1775)	cs	base of coremata	—	PP
Ctenuchina	*Aclytia albistriga* Schaus, 1911	cs		1G, 7G, 8E	CR
	A. heber (Cramer, 1780)	cs		—	PP
	A. bractea Cerda, 2017	cs		—	PP
	A. halys (Stoll, 1781)	cs		3A, B	CR
	A. punctata Butler, 1876	cs		4C	CR
	A. sp.	cs		5E	CR
	Antichloris viridis Druce, 1884	cs, cb		4I, 10G,H	CR
	A. scudderi Butler, 1876	cs		—	PP
	Argyroeides menephron Druce, 1884	cs		—	CR
	Belemnia eryx (Fabricius, 1775)	cs, cb		7A	PP
	B. inaurata (Sulzer, 1776)	cs, cb		7C, E, 10B–D	CR
	Cercopimorpha sylva Schaus, 1920	cs, cb		8B	CR
	Corematura chrysogastra (Perty, 1833)	cs		—	PP
	Aethria sp.	cs		—	PP
	Coreura albicosta Draudt, 1915	cs		—	CR
	Correbidia testacea (Druce, 1884)	cs, cb		4D	CR
	C. sp. 1	cs		1H, 5G	CR
	C. sp. 2	cs		2B	CR
	Delphyre testacea (Druce, 1884)	cs	hindwing costal fold	— (5A)	CR
	Dinia eagrus (Cramer, [1779])	cs	base of coremata	4E	CR
	Diospage rhebus Cramer, 1779	cs		—	PP
	Ecdemus obscuratum Schaus, 1911	cs		—	CR
	Epidesma oceola (Dyar, 1910)	cs, cb	bladder	5H, 6A–C	CR
	E. klagesi (Rothschild, 1912)	cs	base of coremata		PP
	E. sp. nov. 2	cs, cb	bladder	1F, 2A, 5B, D, 8F, G, 10L, M	CR
	Episcepsis demonis (Druce, 1896)	cs	base of coremata	4B, 5F, 9E–M	CR
	E. redunda (Schaus, 1910)	cs	hindwing costal fold	1K	CR
	Eriphioides tractipennis (Butler, 1876)	cs	base of coremata	—	CR
	Eucereon aroa Schaus, 1894	cs		2D, 4H, 8C	CR
	E. dentatum Schaus, 1894	cs		1D	R
	E. leria Druce, 1884	cs		—	PP
	E. punctatum (Guérin-Méneville, [1844])	cs, cb		10E, F	CR
	E. obscurum (Möschler, 1872)	cs		2C	CR, PP
	E. varia (Walker, 1854)	cs, cb		1B, 5C	CR
	Mydromera isthmia (Felder, 1868)	cs		—	CR
	Napata alterata (Walker, [1865])	cs		—	PP
	Nelphe relegatum (Schaus, 1911)	cs, cb		—	CR
	Pionia sp. 1	cs		4F	CR
	Pionia sp. 2	cb, cb		10A, I, K	CR
	Syntrichura virens Butler, 1876	cs		—	CR
	Trichura cerberus (Pallas, 1772)	cs		—	PP
	T. latifascia (Walker, 1854)	cs		—	PP
	Uranophora flaviceps (Hampson, 1901)	cs		8D	CR
	U. leucotelus (Butler, 1876)	cs		—	CR, PP
	U. walkeri (Druce, 1889)	cs		3C	CR
	Xanthopleura persspicua (Walker, 1856)	cs		—	PP
Euchromiina	*Loxophlebia flavipicta* Schaus, 1912	cs	abdominal pouch	1M	CR
	Myrmecopsis strigosa (Druce, 1884)	cs	base of coremata	—	CR
	Pheia albisigna (Walker, 1854)	cs	tibia of 2nd leg	—	CR
	Pleurosoma nigrifer (Dyar, 1910)	cs	base of coremata	7D	CR
	Pseudomya sanguinea (Druce, 1884)	cs	abdominal pouch	—	CR
	Sphecosoma aliena (Walker, 1854)	cs		1L, 7B	CR

Table 1. Continued

Subtribe*	Species	Crystal macrosetae		Figure(s)	Origin
		Type	Location		
	Sphecosoma sp.	cs	base of coremata	—	PP
Arctiinae: Lithosiini	*Lycomorphodes correbioides* Schaus, 1911	cs	base of coremata	11A–E	CR
Notodontidae: Dioptinae: Josiini	*Polyptychia hermieri* Miller, 2008	cs	hindwing us patch	11F–H	French Guiana

Note that because arctiine systematics is in a very unsatisfactory state (e.g., Weller et al. 2009) many current names are subject to change, also that the selection of species for study is unsystematic. **cs** crystal scales, **cb** crystal bristles, **us** underside; **CR** Costa Rica, **PP** Panguana/Peru.

*According to Zenker et al. (2017).

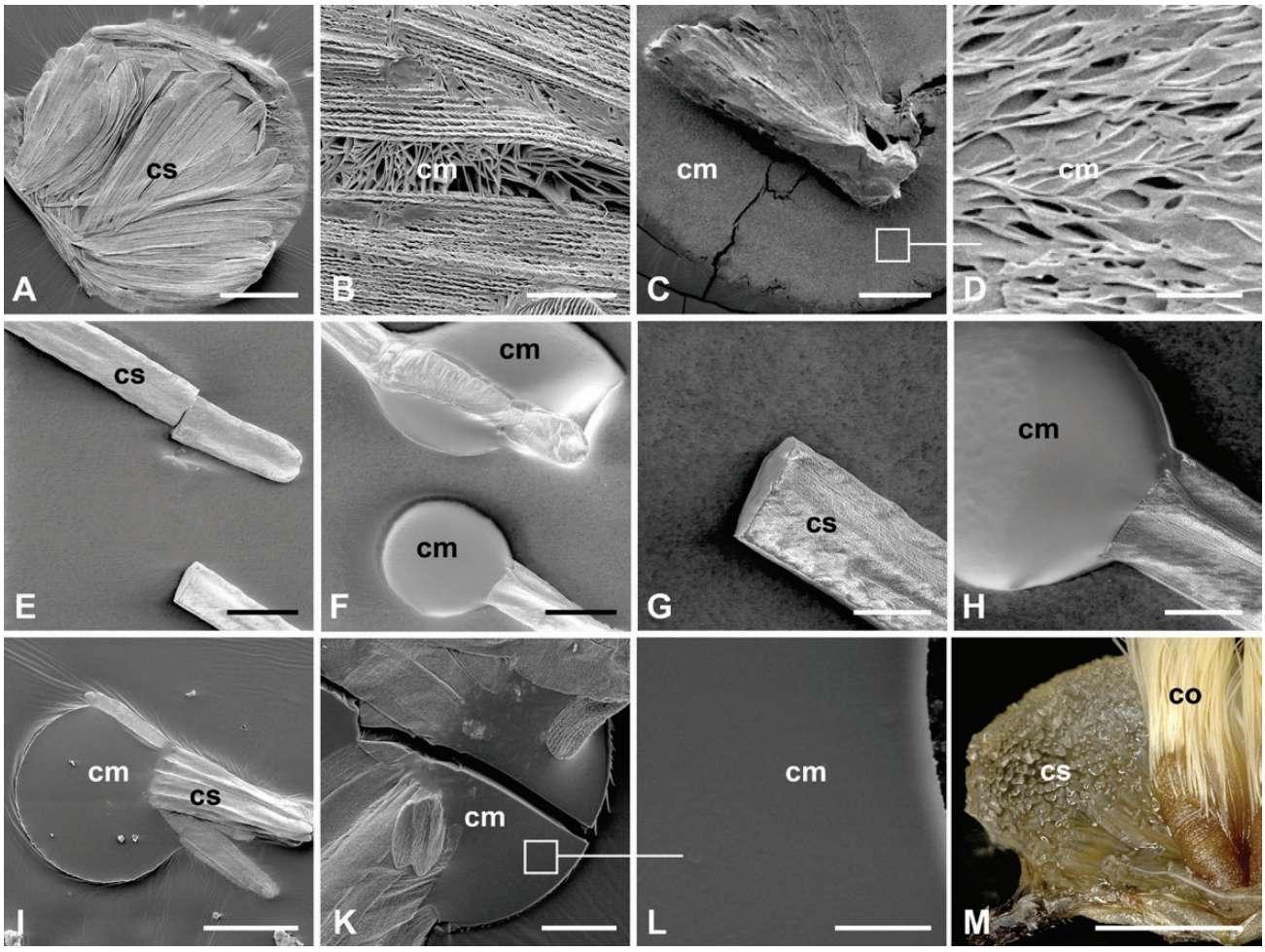

Fig. 9. Scanning electron micrographs (A–L; E–L of unsputtered preparations) of crystal scales treated with a drop of water and dried again (A–D) or cut (E, G) and then kept in a humid chamber (F, H–L) causing appearance of droplets (F, H, I). Drying produces plates with crystals (B–D) or solid and very smooth plates of material (cm) (F, H–L), depending on the species. M Macrograph of an androconial organ kept in a humid chamber showing wettening/hygroscopicity of crystal scales (cs) while coremata hair (co) remain dry. Scale bars: A = 200 µm; B, C, F = 50 µm; D, H = 5 µm; E, G = 400 µm; I, K = 90 µm; L = 40 µm; M = 1 mm. For names, see Table 1.

seems to depend on the temperature and/or humidity conditions, or differs because of varying chemical composition.

Crystal Bristles

Bristles and scales have basically the very same general *bauplan*; thus, scales can be seen as flattened bristles—their differentiation is generally difficult and not uniformly applied in the literature.

In many species, bristles on the expandable coremata tubes also lack windows—as do crystal scales—and some have a similar surface as crystal scales (Fig. 10L and M). Numerous others have prominent surface ornamentation, some with protuberances and extensive surface sculpturing (Fig. 10B–K). Bristles with spine-like structures and/or with differences of 'upper' and 'under' sides occur (Fig. 10C). Checking interiors of broken bristles for selected species with SEM also revealed a crystalline content (Fig. 10D,F,H,K,M)—but not in

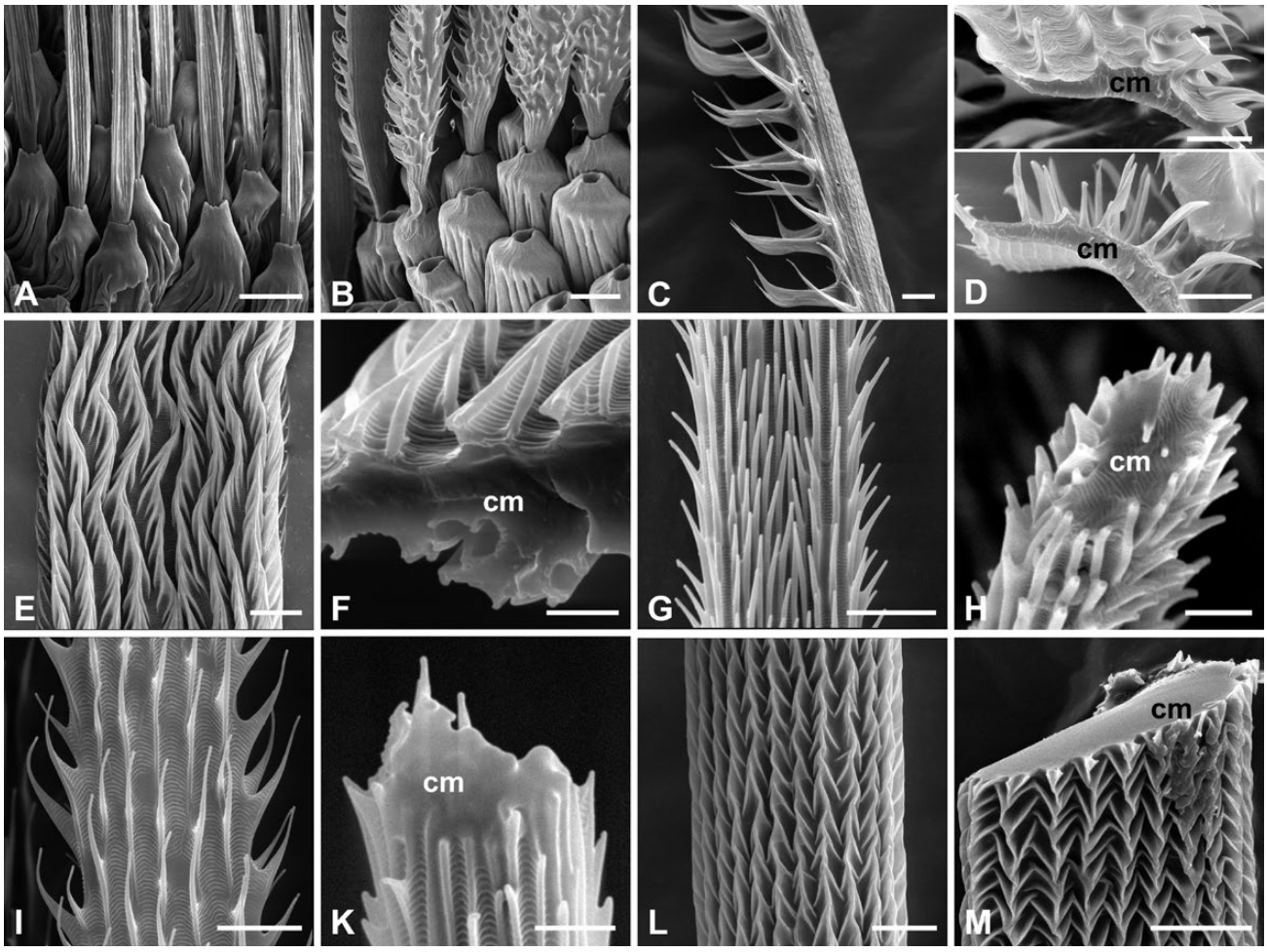

Fig. 10. Scanning electron micrographs of androconial bristles showing bases (A, B) and diverse surfaces which are different from crystal scales (C, E, G, I; cf. Fig. 4B–M) or in other cases very similar (L; cf. Fig. 4K) but always filled with crystallized material (cm; D, F, H, K, M) and never showing windows. Scale bars: A, B = 20 μm; C, E, L = 5 μm; D, M = 10 μm; F = 2 μm; G, I = 5 μm; H, K = 2 μm. For names, see Table 1.

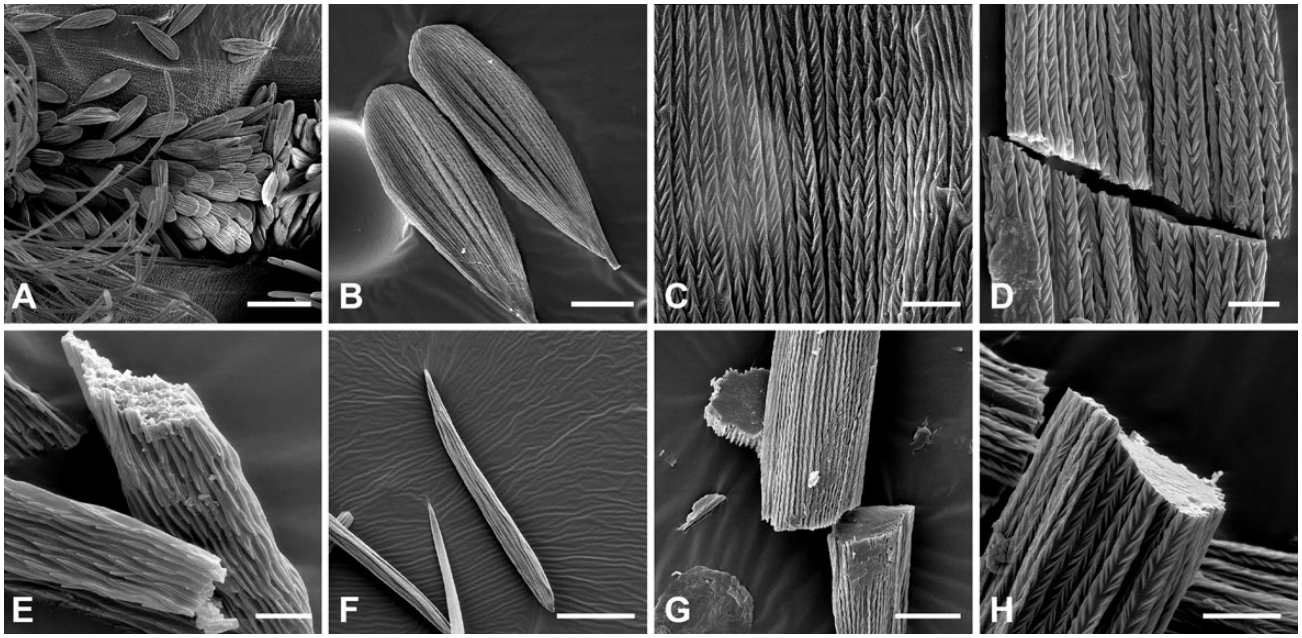

Fig. 11. Examples of crystal scales from non-Arctiini, A–E at the base of coremata of a Lithosiini, F–H in a hindwing fold of a Notodontidae (Dioptinae: Josiini). Scale bars: A, F = 100 μm; B, E–G = 20 μm; C, D, E, K = 5 μm; H = 10 μm. For names, see Table 1.

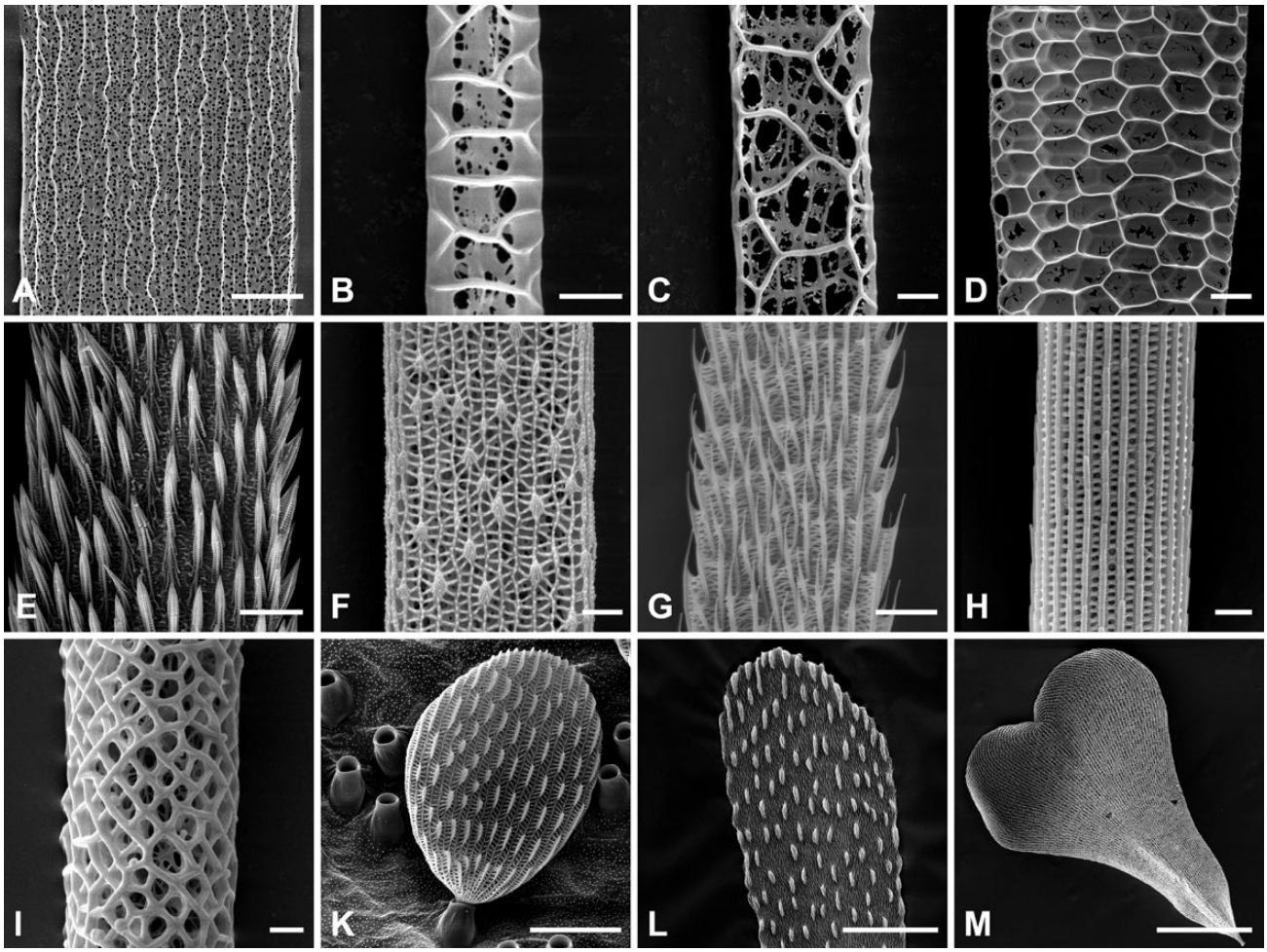

Fig. 12. Examples of the diversity of structures of scales and bristles in androconial organs of Arctiini, all being modifications of typical scales and bristles but not representing crystal scales. Scale bars: A, K = 20 µm; B, C, F, I = 2 µm; D, E, G, H = 5 µm; L, M = 50 µm. For names, see Table 1.

coremata hair with windows. Like crystal scales, crystal bristles do not show trabeculae and are highly hygroscopic.

Occurrence of Crystal Scales and Bristles Within the Lepidoptera

To date, we have found crystal scales and crystal bristles in 60+ species in 30+ genera within the Arctiini subtribes Ctenuchina, Euchromiina, Callimorphina, Spilosomina, and Phaegopterina (Table 1), but never in Pericopina (which seem generally to lack androconial organs). It needs to be emphasized that not all Arctiinae possess androconial organs, and crystal macrosetae are not found in all arctiines that do have androconial organs.

Lithosiini (Erebidae: Arctiinae) and Josiini (Notodontidae: Dioptinae) were not the focus of our study and only a few species were investigated; however, associated with androconia, crystal scales identical to some of those described here for Arctiini were found (Fig. 11A–H).

Discussion

The crystal scales and bristles characterized above and unreported previously represent a peculiar type of macrosetae—both in morphological and, obviously, chemical terms. Although in the Arctiinae an overwhelming diversity of structural modifications of scales and bristles is found in which one does not always recognize the basic *bauplan* structures at first glance (for examples, see Fig. 12), crystal scales and bristles—although very diverse in appearance—form a distinct type of macrosetae characterized by 1) lack of trabeculae and windows, with ridges highly modified, 2) possession of a very thin outer envelope only, and 3) being filled with viscous or gelled material that can crystallize.

What we describe here as crystal macrosetae other researchers seem to have seen without realizing their peculiar nature. DaCosta and Weller (2005: fig. 35 ss, fig. 78 EAP) published sketches of 'scent scales' and an 'eversible androconia pouch' at the base of the coremata of *Euchaetes zella* (Phaegopterina) and *Sebastia argus* (Callimorphina), respectively. Due to their position, we imagine these could be crystal scales. Miller (2009: fig. 300C–F, fig. 301A) provided SEM micrographs of the surface of the 'deciduous androconium' of *Polyptychia hermieri* (Notodontidae: Dioptinae: Josiini) which greatly resemble those we present in Fig. 4G; these scales lie within an androconial organ in the center of the hindwing. We had the chance to check one specimen and, indeed, found Miller's 'thickened, fleshy-looking deciduous scales' to be filled with crystallized material (Fig. 11F–H)—but we wonder if they really are deciduous. Obviously, this finding extends the significance of finding crystal macrosetae and shows that this new scale type is not restricted to a single group but occurs in quite unrelated taxa; it does not, however, help to clarify their functional role(s) (see below).

Within Arctiinae crystal macrosetae are frequently encountered and occur very widely over the entire subfamily. Since our study largely concentrates on one habitat in Costa Rica, 1) only a comparatively small range of species is covered and 2) of these not all species can be studied because they are rare—a detailed and systematic overview on the occurrence of crystal macrosetae thus cannot yet be given. In phylogenetic schemes (e.g., Zenker et al. 2017) most of the genera we have studied are not included.

Functional Aspects

Scales typically are pure chitin, some carrying pigments or signal compounds. Generally, it is assumed (but demonstrated only in few cases) that androconial bristles and scales are made for release and dissemination of volatile secretions (pheromones). Their typical spongy structure appears ideal to hold as well as release such chemicals. Our finding of scales which functionally appear like flexible envelopes filled with a likely nonvolatile, amorphous or gel-like substance, and, in particular, corematal hairs with similar characteristics, is a novel finding that, ultimately, demands a functional interpretation. Since crystal macrosetae were only found in males and always as part of or in association with androconial organs, this suggests a function in a sexual chemical communication context. If there were only groups of such structures associated with androconial organs (crystal scales, above) one might imagine that they store (as a reservoir) chemicals relevant for pheromone biosynthesis. Finding coremata bristles also filled with crystallizing material makes a functional interpretation more challenging. Unfortunately, until their chemical composition has been finally elucidated, no plausible speculation on the role of crystal macrosetae seems possible. Their hygroscopicity and their causing of a sweet sensation on the human tongue might suggest that the crystalizing material is a kind of sugar. This appears to be confirmed by ongoing chemical analyses (S. Schulz et al. personal communication) to be published in due course.

Crystallizing material associated with lepidopteran scales has been described before. Barth (1950, 1952, 1953) reported crystals associated with androconial scales in *Parides* and *Battus* (as *Papilio*; Papilionidae) as well as in *Opsiphanes* and *Caligo* (Nymphalidae: Brassolini); however, the scales Barth investigated are typical in structure, quite unlike the scales investigated here.

The diversity in which the crystallizing material(s) appear in fractured preparations of fresh and dry macrosetae is remarkable. It might reflect different chemicals in different species. Another possible—and even for similar chemicals most likely—explanation is that the conditions for crystallization (humidity, temperature) cause differences in the visual appearance of the yet unidentified material, that is, in nature the chemicals probably behave more similarly than in our artificial conditions; crystal macrosetae are usually hidden, so that a steady interaction with environmental conditions seems unlikely.

Is the content of crystal macrosetae species-specific? Does it change (fill, become depleted) during the life of the moths? If they serve as reservoirs, in which circumstances is the material used, and what for? Although we found different filling states in our samples (see Figs. 4K–M), because only specimens from the field were available, such questions cannot be addressed (see below).

Macrosetae in insects fulfill numerous and diverse roles (Watson et al. 2017) to which crystal-filled ones—independent of structural peculiarities—add another option: a reservoir. Although the chemistry of the scale contents is not yet clear, definitely the structures hold large amounts of secretion in a kind of 'micro-bottle', something so far unknown for scales and bristles.

Many Arctiini are pharmacophagous with respect to pyrrolizidine alkaloids (PAs), that is, they actively search for and take up these secondary plant metabolites and sequester them for defense and, in some cases, as precursors for the biosynthesis of male pheromones (Boppré 2011 for overview). Although the vast majority of the species where crystal macrosetae have so far been found are PA-pharmacophagous, crystal macrosetae are not restricted to such species; thus, the hypothesis that crystal material plays a role in handling PAs is perhaps unlikely, but should not be excluded.

Perspectives

Unfortunately, the natural history of tropical tiger and wasp moths is poorly studied, in particular very little is known about their intra- and interspecific communication, which involves not only chemicals but also colors and sounds (e.g., Conner 2009, Boppré 2015). Thus, for all types of signaling, we have extensive knowledge about structural peculiarities but lack functional understanding. For androconial organs of tropical arctiines in-depth studies are scant (e.g., *Utetheisa ornatrix*, Conner 2009a; *Creatonotos* spp., Boppré and Schneider 1989; *Estigmene acrea*, Davenport and Conner 2003). However, these are insufficient for generalizations across the subfamily with its about 12,000, day- or night-active super-diverse species in which such organs occur in a stunning variety of architectures (Boppré 2015).

With respect to crystal macrosetae, some open questions seem comparatively easy to be addressed but, in fact, are currently impossible to explore because cultures are unavailable which would provide not only more material of a given species than can be found in the field, but also specimens with known individual history (e.g., age, mating status); and for the majority of arctiines larval hostplants remain unknown.

Cultures would not only help us gain understanding of the use and role of crystal macrosetae in the life of the moths, they would also permit more detailed chemical analyses as well as histological investigations into the ontogeny and formation of the structures and their mode of being filled. The latter would permit a more detailed comparison with other lepidopteran scales, bristles, hairs, and sensilla (Kristensen and Simonsen 2003, Ghiradella 2010). In particular, this could allow us to place crystal macrosetae into the general framework of current understanding on micro- and nanostructures of macrosetae (e.g., Galant et al. 1998, Ghiradella 1994, 2010, Ghiradella and Butler 2009, Dinwiddie et al. 2014, Day et al. 2019)—perhaps the specialization of crystal scales is incomplete development (with F-actin filaments but little chitin deposit only and no (?) Fascin) in order to retain softness and thus suitability to serve as a reservoir for secretion?

Despite all the many limitations of our report and the many open questions, recognizing crystal scales and bristles as a peculiar and—in Arctiinae at least—widely occurring type of macrosetae provides a basis for future targeted studies. Eventually these will hopefully provide information on their biological significance and help to understand better the biology and physiology of Arctiini, their evolution and systematics, their 'features' (sensu Schroeder et al. 2018) and the development of macrosetae in Lepidoptera. Also it will be worthwhile to look out for crystal macrosetae in other taxa, unrelated to Arctiinae, such as Notodontidae.

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