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Fatty acids as cuticular surface components in oribatid mites (Acari: Oribatida)

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Abstract: The cuticular surface chemistry of some oribatid mite species representing members of different phyletic groups – *Steganacarus carinatus, Collohmannia gigantea, Hermannia gibba* and *Zetorchestes falzonii* – was investigated by combined gas chromatography - mass spectrometry. Even though the species exhibited very different body surface structures as demonstrated by scanning electron microscopy, they generally showed large amounts of stearic-, oleic- and linoleic acid along with smaller amounts of palmitic acid as dominating body surface components. Further homologous fatty acids of 12, 13, 14, 15, 17, 19 and 20 carbon atoms could be detected in traces depending on species. Extraction procedures, although using a wide range of organic solvents, confirmed insolubility of the structures of the outer cerotegument. Nevertheless, cement layers began to detach from the surface of the cuticle after extraction, indicating that the connective lipid layer had been removed. These results confirm a chemically more complex and probably non-waxy nature of outer cerotegumental formations and recommend the extracted fatty acids as lipid layer constituents. Furthermore, fatty acids – their distribution and relative amounts – were used to construct fatty acid patterns for each species, resulting in very similar acid profiles. In contrast to other arthropods, fatty acids may generally constitute the main fraction of the extractable cuticular lipids in oribatid mites.

Introduction

Body surface components are known to play crucial roles in the biology of arthropods, not only building up protective layers on the cuticle surface to minimize water loss but also serving as barriers against microorganisms or even taking part in chemical communication (JACKSON & BAKER 1970; JACKSON & BLOMQUIST 1976; BLOMQUIST & DILLWITH 1985; LOCKEY 1988; DERENOBALES et al. 1991). Moreover, cuticular surface patterns have been shown to represent powerful tools for chemosystematic analysis (e.g. HUNT 1986; HAVERTY et al. 1988; ESTRADA-PEÑA et al. 1992a).

Consequently, the body surface has been the subject of chemical investigations in more than a hundred insect species (DERENOBALES et al. 1991), revealing a diversity of hydrocarbons, alkyl esters, sterols, alcohols, aldehydes, glycerides and fatty acids (LOCKEY 1988). These extractable free lipids build up a special layer which covers the outer epicuticle and partly permeates it, respectively. Moreover, this wax layer is usually covered by a further, protec-

tive cement layer which – comparable to free lipids – originates in glandular epidermal cells (FILSHIE 1982; BLOMQUIST & DILLWITH 1985; LOCKEY 1988).

In arachnids, our knowledge of body surface components is limited to a few groups, especially to scorpions (HADLEY & JACKSON 1977; TOOLSON & HADLEY 1977), to spiders (TRABALON et al. 1996; BAGNERES et al. 1997), to ticks (HUNT 1986; ESTRADA-PEÑA & DUSBABEK 1993; ESTRADA-PEÑA et al. 1992a, b, 1994, 1996) and to astigmatid mites (LEAL & KUWAHARA 1991; KUWAHARA et al. 1995). Nevertheless, chemical congruence between arachnid and insect body surface components has become obvious and these compounds are likely to be expected for other arachnid groups as well (e.g. ALBERTI et al. 1981).

In oribatid mites which constitute a large mite suborder comprising more than 6000 species (BALOGH & BALOGH 1992), very unusual and sometimes highly ornamented body surface structures have been developed, which are referred to as secretion layers (ALBERTI et al. 1981) or as cerotequments (GRANDJEAN 1951; EVANS 1992). Even though oribatid body surfaces are morphologically known in some detail, cerotegumental compositions – especially the chemistry of extractable cuticular lipids – have remained obscure yet. Oribatid cerotequments at least structurally resemble to the waxy or lipid coverings of other arthropods, for they may plaster the outermost epicuticula in two distinct layers (EVANS 1992). The outer cement layer – which is not always present – is said to consist of tanned proteins and lipids while the components of the inner lipid layer are completely unknown. However, both layers are believed to represent secretion products from glandular epidermal cells, possibly from cells underlaying the so-called porose areas (ALBERTI et al. 1981; EVANS 1992; NORTON et al. 1997).
As it has been stressed recently, body surface components of oribatid mites are not only essential barriers against environmental influences but obviously are also involved in intraspecific chemical communication (JONES 1954; ALBERTI et al. 1997; NORTON & ALBERTI 1997). An investigation on the chemistry and biological role of oribatid extractable surface components may be one important step to a new understanding of these mites' biology.

The great diversity and elaborated ornamentation of oribatid body surfaces with regard to functional biology and genesis has always been one special interest of Prof. Schuster. Therefore we dedicate to him the present paper.

Materials and methods

Mite collection and sample preparation

Mite species (*Steganacarus carinatus, Collohmannia gigantea, Hermannia gibba, Zetorchestes falzonii*) were collected in mixed forests north of Graz (Styria, Austria) and in the surroundings of Ferlach (Carinthia, Austria) by sieving samples of soil and litter and subsequently subjecting them to Berlese-Tullgren-apparatus analysis. Species separation as well as the separation of juvenile specimens from adults was carried out under a stereomicroscope. Fine brushes were used for mite collection. For extraction of body surface components, mite specimens (from 20 up to more than 200 individuals per sample, depending on the size of the specimens) were immediately transferred into glass vials containing 250µl of solvents like ethanol, acetone, ethyl acetate, chloroform-methanol (2:1), chloroform and hexane, respectively. Alternatively, mite specimens were placed into the vials and poured over with solvent. Specimens for preparation of different samples were collected at different times over several months in order to be able to draw more general conclusions (*S. carinatus*: 11 samples, in total containing about 1000 specimens; *C. gigantea*: 58 samples, about 3000 specimens; *H. gibba*: six samples, about 800 specimens; *Z. falzonii*: 11 samples, about 2000 individuals). Extraction procedures lasted from a few minutes up to several weeks. For scanning electron microscopy (SEM; using a Leitz AMR 1000) some of the extracted specimens were air-dried, mounted on Al-stubs with a double-sided sticky tape and finally sputtered with gold. SEM micrographs were edited electronically by image-processing software to letter the micrographs and to retouch the background of some images (Fig. 1a, 3a, 4a, 5a and 7a).

Analysis of extracts

Supernatants of mite extraction were subjected to gas chromatographic - mass spectrometric analysis, using a Fisons 8000 gas chromatograph (GC) coupled to a Fisons Trio 1000 mass spectrometer (MS). The GC was equipped with a DB-5MS fused silica capillary column (30m x 0.25mm i.d., 0.25µm film thickness) from Fisons. The column was directly connected to the ion source of the MS. The splitless Grob injector was kept at 260°C, helium was used as a carrier gas. All data in the text refer to the following temperature programs:

1. Initial temperature 70°C for 1 min, followed by an increase of 10°C/min to 310°C and an isothermal hold of 45 min.
2. 70°C for 1 min, followed by an increase of 10°C/min to 100°C, with 5°C/min to 150°C, with 7°C/min to 300°C and 5 min isothermal at 300°C.

3. 70°C for 1 min, followed by an increase of 10°C/min to 100°C, with 5°C/min to 150°C, with 10°C/min to 320°C and 10 min isothermal at 320°C.

Ion source of the mass spectrometer and transfer line were kept at 220°C and 320°C, respectively. Electron impact (EI) spectra were recorded at an electron energy of 70 eV; additionally, positive chemical ionisation (PCI) spectra were taken to confirm molecular mass of certain compounds.

Chemical derivatization was achieved as follows: Trimethylsilylated (TMS) products were formed with BSTFA/pyridine (2:1, v/v) for 30 min at 75°C. Methylation was accomplished with a saturated solution of diazomethane in diethyl ether/methanol (9:1, v/v) for 25 min at room temperature.

Bis-(N,O-trimethylsilyl)trifluoro acetamide (BSTFA) and silylation grade pyridine were bought from Pierce Chemical Co. (Rockford, IL, USA). Authentic fatty acids and their derivatives were purchased from Sigma (Vienna, Austria), all other reagents and solvents were obtained from Merck (Darmstadt, FRG).

Results

Structure of oribatid body surfaces

Steganacarus (*Tropacarus*) *carinatus* (C. L. KOCH, 1841)

The body surface of the phthiracaroid mite *S. carinatus* shows different structures in different body regions. The prodorsal region, the anterior part of the projecting knob of the notogaster as well as the genito-anal region appear to be reticulate-foveate. In contrast to this, the main part of the notogaster is studded with cuticular tubercles. Each region is covered by a thin cerotegumental layer.

Treatment with organic solvents resulted in multiple cracking of the outermost cerotegumental layer which partly detached from the cuticle. Beginning detachment of cerotegumental shreds from certain cuticle regions is demonstrated in Fig. 1 and 2. In some specimens, cerotegumental coverings could be removed from larger body areas which – after extraction – appeared extremely clean in SEM. All solvents seemed to have nearly the same effects on the cerotegument; they caused detachment but did not affect cerotegumental structure.

Collohmannia gigantea SELLNICK, 1922

C. gigantea possesses a very smooth cuticle as it can already be seen in the stereomicroscope. Poorly developed wrinkles are only detected in the lateral parts of the notogaster. Using higher magnifications, a reticulate to squamous pattern on the cuticle surface of the notogaster becomes apparent (Fig. 3). Living specimens show a shiny or oily surface in reflected light.

A definite and clearly separated cerotegumental layer was not found by SEM. The oily covering of the cuticle was lost after treatment with organic solvents while the structure of the cuticular surface remained unaffected.

Cuticular structures of juveniles are less sclerotised and also appear smooth by SEM.

Hermannia gibba (C. L. KOCH, 1840)

The cuticle of *H. gibba* is studded with little granules or verrucae which represent sclerotised cuticular structures covered by cerotegument (Fig. 4a). The cerotegument is present all over the body surface being developed as a thin layer which is easily damaged at the tops of the verrucae, especially when treated with hexane (Fig. 4b). Cuticle structures of juveniles are quite different in appearance (Fig. 5a). The juvenile cuticle of the prodorsum and the legs is reticulate-foveate while the hysterosoma looks very wrinkled. These wrinkles are massive and represent thickened parts of the cuticle. All ridges and wrinkles are covered by a simple, not elaborated cerotegument (Fig. 5b).

Zetorchestes falzonii COGGI, 1898

Adults and juveniles of *Z. falzonii* are completely covered with cerotegument (Fig. 6a, 7a). This cerotegument consists of a thin basic layer in which granules or blooms of different size are embedded. In general, the blooms show a reticulate surface structure (Fig. 6b). Most of the smaller blooms are situated on the lateral and posterior borders of the notogaster, around and in the bothridium and on the lateral sides of the podosoma. In some cases, also notogastral setae are covered with small blooms (Fig. 6b).

Nymphal blooms are distinctly different in structure from those of adults (Fig. 7a), but also are developed as blooms of different size. Big and small blooms on the ventral side and on the podosoma are spiny as shown in Fig. 7c. On the anterior border of the notogaster the blooms ap-

pear less structured, but still a little bit spiny (Fig. 7b). 'Old' blooms on the nymphal scalps have completely lost their surface structure and they look like being rubbed off or like being partly melted. Distribution of blooms on the surface of the notogaster exhibits a polygonal pattern (Fig. 7a), while blooms on the ventral side as well as blooms on the podosoma are distributed comparably to adults.

Ethanol had no obvious effect on cerotegumental structures. Multiple detachment of the cerotegumental basic layer in small areas all around the notogaster was achieved by acetone, hexane and ethyl acetate. After treatment with chloroform the basic cerotegumental layer appeared to be very corroded and had detached from large areas of the cuticle. In contrast to these findings, the structure of the blooms remained unaffected by any solvent used, including chloroform.

Separation, identification and classification of extract components

Chromatograms of ethanolic extracts of all investigated species irrespective of age constantly showed 4 major peaks at the following retention times (referring to temperature program No. 1): Peak 1: RT=19.87 min; Peak 4: 21.95 min, Peak 5: 22.05 min; Peak 6: 22.32 min as it is demonstrated in Fig. 8. Details of this main chromatographic pattern are exemplarily presented for *H. gibba* adults and juveniles in Fig. 9.

EI-mass spectra of the separated components revealed similar fragmentation patterns as shown in Fig. 10. Compound No. 1 and No. 6 exhibited base ions at m/z 88 along with diagnostic ions at m/z 101, indicating McLafferty-rearrangement of ethyl esters of fatty acids. Molecular ions at m/z 284 and m/z 312 pointed to ethyl esters of palmitic and stearic acid, respectively. Fatty acid ethyl ester structure was further confirmed by ions from α-cleavage at m/z 45 in both structures and at m/z 239 and m/z 267 in palmitic acid and stearic acid ethyl ester, respectively. Compounds No. 4 and No. 5 mainly showed unsaturated hydrocarbon ions in the lower mass range along with fragments at m/z 88 and m/z 101, at least being moderatly intense in the spectrum of compound No. 5. Molecular ions at m/z 308 (compound No. 4) and m/z 310 (compound No. 5) along with α-cleavage fragments at m/z 263 and m/z 265 strongly indicated the double- and mono-unsaturated pendants to stearic acid ethyl ester, linoleic- and oleic acid ethyl ester, respectively. All four components were finally identified by comparison of retention times to authentic standards (Fig. 9C).

Remarkably, in methanolic extracts no trace of fatty acid ethyl esters could be detected, although these extracts revealed comparable chromatographic patterns at shortened retention times instead (Fig. 11A, B). The

compounds clearly exhibited mass spectra of the corresponding fatty acid methyl esters as indicated by McLafferty fragments at m/z 74 and McLafferty plus 13-ions at m/z 87 along with molecular ions at m/z 270, m/z 294, m/z 296 and m/z 298, respectively.

Chromatograms of hexane and chloroform did not show any peaks in this chromatographic region (Fig. 11D). Only in case of injection of very concentrated extracts one broad and untailed peak occurred at the same retention time as a peak just in front of the C18-acid ethyl ester cluster of ethanolic extracts (Peak No. 3 in Fig. 9A), exhibiting no single component mass spectrum. This peak obviously corresponded to free C18-fatty acids which were not readily soluble in hexane and could not be separated due to our chromatographic conditions.

From these results we concluded that originally only free fatty acids were present in our extracts, but they partly auto-esterified with solvents like ethanol and methanol. To confirm this, we derivatized mite extracts and tried to detect still unesterified C18-fatty acids as TMS- or methyl esters. Actually, trimethylsilylation of ethanolic extracts resulted in the appearance of 3 new peaks in the chromatograms which corresponded to fatty acid TMS-esters in addition to the already present ethyl esters and showed identical relative intensities. Stearic acid TMS-ester was easily identified by a base ion at m/z 341 and an additional ion at m/z 356, representing the M-15 fragment and the molecular ion itself, respectively. Further fragments could be observed at m/z 117 (α-cleavage), at m/z 129, at m/z 132 (McLafferty-product) along with intense ions at m/z 73 (trimethylsilylion) and at m/z 75 (dimethylsilanol). The unsaturated TMS-esters also showed fragments corresponding to their molecular ions (linoleic acid TMS-ester: m/z 352; oleic acid TMS-ester: m/z 354) and ions at 15 mass units lower along with the already known fragmentation pattern in the lower mass range. Comparably, methylation of ethanolic extracts resulted in the appearance of new peaks representing the methyl esters of our fatty acids in addition to ethyl esters (Fig. 11C).

A search for further fatty acids revealed a homologous fatty acid series in trace amounts, from 12 to 20 carbon atoms. They all were best detected as ethyl esters in ethanolic extracts and subsequently were identified by their typical fatty acid ethyl ester mass spectra along with the always present molecular ions (see Table 1). Some of the fatty acids (myristic- and palmitic acid and the acids of the C18-cluster) obviously partly matched to pass the chromatographic column and could be detected in their free form, as it was often the case in ethanolic extracts (see Table 1). Free fatty acids were tentatively identified by their mass spectra which revealed diagnostic

ions of carboxylic acids originating from McLafferty-rearrangement (at m/z 60) and ions belonging to the $(CH_2)_nCOOH$-series with n = 2, 6, 10, ... [at m/z 73, m/z 129, m/z 185 and m/z 213 (only palmitic acid)]. Very intense but less typical ions could be observed at m/z 41, m/z 43 (base ion), m/z 45, m/z 55 and m/z 57. Molecular ions were detected at m/z 228 (myristic acid) and m/z 256 (palmitic acid). Moreover, myristic and palmitic acid could be found in two iso-forms in certain mite extracts (see Fig. 12-15), indicated by additional peaks of different retention times but with identical mass spectra. Iso-forms of fatty acids were not further investigated, they are designated with Arabic numbers in brackets in Fig. 12-15. Moreover, they could not be found constantly, even not in extracts of one and the same species (compare Table 1 and Fig. 15).

To be able to class the detected fatty acids with certain body systems of the mites we shortened the extraction time for a model species (*C. gigantea*) in ethanol for some experiments to 5 min, resulting in unaffected extraction of the C18-fatty acid cluster in usual amounts.

Other peaks (not considered in this paper) which could be found in the chromatograms of extracts of certain mite species (first half of the chromatograms in Fig. 8) originated from oil gland secretions and will be presented elsewhere in detail.

Cuticular fatty acid patterns

To construct fatty acid patterns we quantified the fatty acid ethyl esters in ethanolic extracts, their amounts being proportional to the amounts of initially present free fatty acids. To prove this, we checked the amounts of fatty acid ethyl esters in ethanolic extracts plus the amounts of arising methyl esters after methylation which – all in all – semiquantitatively represented the whole fatty acid amount of the extracts. From this we calculated that about 25% of free fatty acids spontaneously esterified to ethyl esters: linoleic acid to 27.76% (Sd: 4.48), oleic acid to 22.11% (Sd. 1.83), stearic acid to 19.17% (Sd: 3.18); these results refer to 10 samples each with 20 individuals of *C. gigantea* (see also Fig. 11C).

Although some differences in fatty acid patterns – regarding both, qualitative and quantitative compositions – could be noted between different species and different stages of one species, these differences were not very significant, especially when slight variations which also occurred between different samples of one and the same species are considered. The full set of 15 fatty acids could not be detected in any species. Nearly complete acid patterns were frequently registered for *C. gigantea* (Fig. 15). A comparison of fatty acid patterns of all investigated adult species and some of the juveniles is

given in Fig. 12-15. In *C. gigantea* who exhibits distinct sexual dimorphism no differences between male and female fatty acid patterns were observed. Intraspecific variations of fatty acid patterns were investigated using different populations of *S. carinatus* who showed the lowest relative amount of palmitic acid along with a very high amount of linoleic acid which was the most abundant component in specimens from Carinthia (Fig. 13). In all other investigated species, oleic acid could be identified as the main component of the body surface extracts.

Peak atRT (min)*	M^+ at m/z	Identified as
18.38	228	C12 - FAEE
19.90	242	C13 - FAEE
20.80	228	C14 - FA
21.25	256	C14 - FAEE
22.46	270	C15 - FAEE
23.25	256	C16 - FA
23.38	282	C16:1 - FAEE
23.58	284	C16 - FAEE
24.42	296	C17:1 - FAEE
24.63	298	C17 - FAEE
25.25	not to determine	C18:2-, C18:1-, C18 - FA
25.38	308	C18:2 - FAEE
25.45	310	C18:1 - FAEE
25.60	312	C18 - FAEE
26.55	326	C19 - FAEE
27.44	340	C20 - FAEE

Table 1: Identification of fatty acid patterns in one extract of *Collohmannia gigantea* (data refer to another sample than data presented in Fig. 15; differences to Fig. 15 are due to deviations between samples); FA = fatty acid, FAEE = fatty acid ethyl esters. * Temperature program No. 3 used

In contrast to *S. carinatus* and most of the other species, *Z. falzonii* interestingly showed rather high proportions of palmitic acid, in adults comparable to the amounts of stearic acid (Fig. 12A). In *H. gibba* and in *C. gigantea* adults, stearic acid represented the second abundant component. Among the trace components, heptadecanoic acid (e.g. in *Z. falzonii* juveniles, *S. carinatus*, *H. gibba* and *C. gigantea*) seemed to be most abundant (Fig. 12-15).

Discussion

In our investigation we tried to compare the cuticular surface chemistry of oribatid species of different phyletic groups which moreover exhibit very different body surface structures. According to the classification of GRANDJEAN (1969) *S. carinatus* as well as *C. gigantea* belong to the Mixonomata which constitute one major subgroup of the lower Oribatida. *H. gibba* is often classed within the Desmonomata (syn. Nothronata) – and therefore belongs to the lower Oribatida (JOHNSTON 1982; BALOGH & BALOGH 1992) – or is already ranked within the Brachypylida (KRANTZ 1978) which represent the higher oribatids. *Z. falzonii* is a typical member of the higher Oribatida.

The results of the microscopic observations on body surface structures and cerotegumental layers which accompanied the chemical investigations raised many questions, especially those on cerotegumental formation and genesis. On one hand, we would not be able to answer these questions at this moment, on the other hand we believe this to be beyond the scope of this paper. Here, we mainly deal with the discovery of a certain class of compounds which are obviously widely – if not generally – distributed among oribatid mites.

Fatty acids as cuticular lipids of oribatid mites

In all probability, the detected fatty acids in the examined mite extracts have been extracted from the cuticular surface of the specimens and not from inner organ systems. This is stressed by fatty acid containing extracts even in case of very short extraction times which is a common technique for selective receipt of cuticular lipids of arthropods avoiding the extraction of other body components (JACKSON & ARMOLD 1977; LOCKEY 1988). However, short extraction times may be necessary to reduce the risk of extracting substances of inner body systems but are not always suited to remove all surface components. As it has been noted by WIGGLESWORTH (1985), the lipid layer of some arthropod species is honeycombed with proteins and tanning agents resulting in a hard lipid-sclerotin mixture which is not readily soluble and which needs more aggressive and longer extraction procedures (LOCKEY 1985 a, b). Moreover, in most arthropods the lipid layer is additionally covered by the cement layer which may constitute a barrier for lipid extraction.

Anyway, even if some other classes of cuticular lipids might have remained inaccessible to our investigation, free fatty acids occur in large amounts on oribatid body surfaces and generally may constitute the major

part of their extractable cuticular lipids. Free fatty acids are also common in other arthropods and have been established as cuticular components in a large number of species (LOCKEY 1988). However, their occurrence as the main components on the cuticular surface is rather unusual: In insects, they may account from 2.5% (BAKER et al. 1979) up to 50% (ARMOLD et al. 1969) of body surface lipids, with hydrocarbons, alkyl esters, sterols etc. being usually much more abundant (LOCKEY 1988). These other classes of cuticular lipids may also be present in oribatids but have not been detected in considerable amounts in our extracts. In arachnids, investigations concerning the cuticular chemistry hitherto mainly focused on hydrocarbons (HUNT 1986; ESTRADA-PEÑA & DUSBABEK 1993; ESTRADA-PEÑA et al. 1992a, b, 1994, 1996) but recently also revealed a set of other components like unusual alkyl esters (BAGNERES et al. 1997). Nevertheless, the presence of cuticular fatty acids is evident in certain groups: Fatty acids have been shown to occur on the epicuticle of some species of scorpions where – together with hydrocarbons, aliphatic alcohols and sterols – they are the dominating body surface components (TOOLSON & HADLEY 1977). To some extent, certain free fatty acids have also been detected in cuticular extracts of spiders (TRABALON et al. 1996; BAGNERES et al. 1997) and – in larger amounts – in genital extracts of ticks (ALLAN et al. 1988). More consistent with our findings, free fatty acids represent the predominant body surface components in the astigmatid mite *Tortonia* sp. (KUWAHARA et al. 1995). However, the surface wax chemistry of other astigmatid species has not revealed any sign of fatty acids yet and hydrocarbons, esters as well as cholesterol and squalen appear to be more common (LEAL & KUWAHARA 1991).

Free fatty acids on the insect cuticle generally occur in homologous series of even carbon numbers which can be short or extensive, in most cases being located in the range between 10 and 36 carbon atoms (LOCKEY 1988). Just as in oribatids, oleic acid is usually most abundant, being frequently accompanied by other C18-fatty acids of different saturation grade as well as by minor amounts of C16-, C14- and C12-fatty acids. This situation may also be true for many arachnids: In *Tortonia* sp. e.g. the cuticular fatty acid profile contains large quantities of C18:1-, C18:2-, C18- and C16-carboxylic acids along with minor amounts of C16:1-, C14- and C14:1- and C18:3-acids (KUWAHARA et al. 1995) and therefore exhibits a similar but less extensive fatty acid series if compared to oribatid mites. Correspondence of cuticular fatty acid profiles between oribatid and astigmatid mites may be of special interest, regarding the postulated near phylogenetic relationship between these two suborders (NORTON 1994). In scorpions, the existence of more extensive even carbon series of fatty ac-

ids from 10 up to 30 carbon atoms has been reported, with longer chain acids in the range from C24 to C30 being most prominent (TOOLSON & HADLEY 1977).

On the contrary, free fatty acids with odd carbon numbers are not known from the arachnid cuticle and seem to be rare in insects as well. Evidence for certain odd carbon numbered fatty acids on body surfaces is only given in some species of dragon flies (JACOB & HANSSEN 1979). In oribatids, minor or trace amounts of fatty acids with odd carbon numbers may generally occur and they even form homologous series, especially being worth to mention the presence of tri-, penta-, hepta- and nonadecanoic acid along with unsaturated pendants like heptadecenoic acid. To our knowledge, these acids – with the exception of pentadecanoic acid (JACOB & HANSSEN 1979) – are not known from body surfaces of other arthropods yet.

Fatty acid localization in oribatid ceroteguments

Regarding the chemical resistance of outer cerotegumental structures along with their detachment from cuticle due to extraction procedures, cuticular fatty acids in oribatids most likely have been extracted from the lipid (or wax-) layer below the cement layer and they obviously not emerge from ornamentated blooms as it has been shown in *Z. falzonii*. Traditionally the lipid layer of arthropods is regarded to be a monolayer which – in some areas – permeates the covering cement and forms crystalline waxy blooms which penetrate the cement layer completely and consequently reach the body surface (JACKSON & BAKER 1970). In transmission electron micrographs of oribatid cerotegument**s**, the lipid or wax layer appears as a thicker transparent structure below the cement layer but it does not permeate it. Actually, also cerotegumental excrescences of oribatids which strikingly resemble to waxy blooms of other arthropods have been shown to represent structures of the cement-, but not of the lipid layer (NORTON et al. 1997).

However, fatty acid location in a lipid layer clearly separate from the cement layer is indicated by the extraction results of *C. gigantea* which lacks a cement layer and possesses a smooth outermost cuticular surface structure with a smeary, shiny or oily appearance. In this species, a possibly liquid lipid coating covers the outermost body surface and can be readily removed from the surface by extraction with organic solvents. This is indicated by both, by detection of fatty acids in body extracts as well as by loss of the oily appearance after extraction. Actually, "smooth" oribatid taxa with shiny and clean

body surfaces – possibly due to outermost lipid layers – can frequently be found among higher oribatids (NORTON et al. 1997).

In species with well developed ceroteguments, the cement layer begins to burst and to detach from the cuticle in shreds when exposed to different solvents, especially to chloroform. This beginning detachment of the cement layer could be observed in some body areas of *H. gibba* and *S. carinatus*, both possessing a cement layer as outer part of the cerotegument. Detachment of the cement layer during the maceration of specimens is well known from literature (GRANDJEAN 1951) and might be due to the removal of the lipid layer below which possibly ties the cement layer to the epicuticle. The cement layer itself remains insoluble in a wide range of organic solvents and represents a chemically very resistant structure as it has already been reported (GRANDJEAN 1951; NORTON & BEHAN-PELLETIER 1991; ALBERTI et al. 1997). Cerotegumental structures, especially those of the cement layer, might be comparable to mucopolysaccharid layers which are thought to exist on body surfaces of certain insects (LAI-FOOK 1972) or they may constitute hardly soluble structures containing tanned proteins (WIGGLESWORTH 1985; EVANS 1992).

Ornamented parts of the cerotegument – as they are present in *Z. falzonii* – are said to represent paracrystalline formations (NORTON et al. 1997); in *Z. falzonii*, these blooms have been shown to be insoluble and therefore are not likely to be composed of simple epicuticular waxes as stated by other authors (e.g. WOOLLEY 1988). Actually, wax content in oribatid cerotegument may have generally been overestimated yet (e.g. BERNINI & AVANZATI 1989). Structural stability of cerotegument, however, may be required for certain biological functions, as it is true for plastron mechanisms (PUGH et al. 1987; MESSNER et al. 1992).

The origin of the fatty acids of all investigated oribatid species might be located in diverse single or compact dermal glands which are distributed throughout the hypodermis (ALBERTI et al. 1981; ALBERTI et al. 1997; NORTON & ALBERTI 1997; NORTON et al. 1997).

Biological roles of cuticular fatty acids

The biological significance of fatty acids as cuticular components of oribatids may be discussed under the following aspects:

First, in oribatid species with well developed cerotegumental layers, fatty acids may contribute to the adhesion of the outer cerotegument to the cuticle, indicating a structure-preserving role in the integument as it has been shown for *S. carinatus*, *H. gibba* and *Z. falzonii* in our experiments.

Second – and perhaps most important – fatty acids on oribatid body surfaces may serve as barriers against microorganisms as it is indicated by first experiments using *C. gigantea* as a model species: if *C. gigantea* individuals are washed in ethanol for a few seconds so that the oily surface layer is at least partially removed they become very prone to microbial infections and often die after some days of culture.

Third, fatty acids may also improve the qualities of the cuticle by impregnating it; they may act as anti-wetting agents and may form a protective shield against water loss as it is known to be important in insects (BLOMQUIST & DILLWITH 1985; LOCKEY 1988) as well as in arachnids (FROST 1997; YODER et al. 1997). WOODRING & COOK (1962) even suggested that oily surface layers in *Ceratozetes cisalpinus* are responsible for the cleanliness of the animals, preventing from picking up dirt or particles from soil, exhibiting an "anti-glue" effect (ALBERTI et al. 1997; see also HEYDEMANN & MÜLLER-KARCH 1976; WOODRING & GALBRAITH 1976).

Fourth, a communicative function of some components of the lipid layer may not be neglected, for components of dermal glands – which build up body surface lipid layers – are supposed to take part in sexual communication in certain oribatids (ALBERTI et al. 1997; NORTON & ALBERTI 1997) but it is questionable whether fatty acids are involved. Anyway, in ticks fatty acids constitute components of the genital sex pheromone (SONENSHINE et al. 1982; ALLAN et al. 1988) and in a variety of other arthropods, carboxylic acids are involved in many modes of chemical signalling, including defence (EISNER et al. 1961) as well as pheromonal communication (HAYASHI & KOMAE 1977; BATRA & HEFETZ 1979; FLETCHER & BELLAS 1988; WHEELER & DUFFIELD 1988). The only sex pheromone known among spiders is composed of a short chain carboxylic acid and its dimer (SCHULZ & TOFT 1993). On the other hand, fatty acids occur in both sexes of *C. gigantea* and therefore are not likely to represent sex pheromones and recognition cues, respectively. Moreover, fatty acids are obviously common components in oribatids and not species-specific. For that, a role in sexual communication would not be consistent with expected sex pheromonal specificity but it should be noted that also other mites are known to communicate with non-species specific sex pheromones as it is the case with 2,6-dichlorophenol, the sex pheromone of several tick species (SONENSHINE 1991). Anyway, common lipid layer constituents rather would be candidates for building up aggregation pheromones, regarding that aggregation is an important factor in oribatid biology. Aggregation pheromones originating in dermal glands have been suggested by JONES (1954) and are seriously discussed by NORTON &

ALBERTI (1997). As it has been shown recently, fatty acids and their methyl esters are able to act as attractive stimuli to form feeding aggregations in astigmatid mites (SATO et al. 1993).

Especially in *C. gigantea* where a pairing ceremony – probably mediated by certain body surface components – is known (SCHUSTER 1962) further research into the body surface chemistry would be desirable.

Fatty acid patterns

Fatty acids are obviously not well suited to construct species-specific patterns as it might be the case with cuticular hydrocarbons of ticks (HUNT 1986; ESTRADA-PEÑA et al. 1992a), even though some differences in fatty acid profiles between the species could be detected. Juvenile individuals exhibit fatty acid patterns very similar to adults which is in contrast to the situation in some other arthropods (e.g. BAKER et al. 1979). Moreover, all differences must be regarded under the aspect of a snapshot taken from populations of certain oribatid species, not representing patterns of one species as a whole. For this preliminary investigation, neither seasonal influences on acid patterns nor inter-population deviations could have sufficiently been considered yet. However, extracts of *S. carinatus* from the population in Styria and from the population in Carinthia exhibit nearly identical fatty acid patterns, clearly being more similar to each other than to profiles of other species.

Detection of components in trace amounts obviously highly depended on the quantity of mite material available for extraction, so the occurrence or non-occurrence of trace constituents in acid profiles should not be overvalued. In *C. gigantea*, more extensive investigations – including seasonal and nutritional aspects as well as aspects of age – have been carried out, revealing quite constant results concerning the 4 main extract components but simultaneously showing large variations in trace-constituent composition.

Further investigations on the cuticular chemistry of oribatid mites with regard to species-specificity of surface patterns are in progress.

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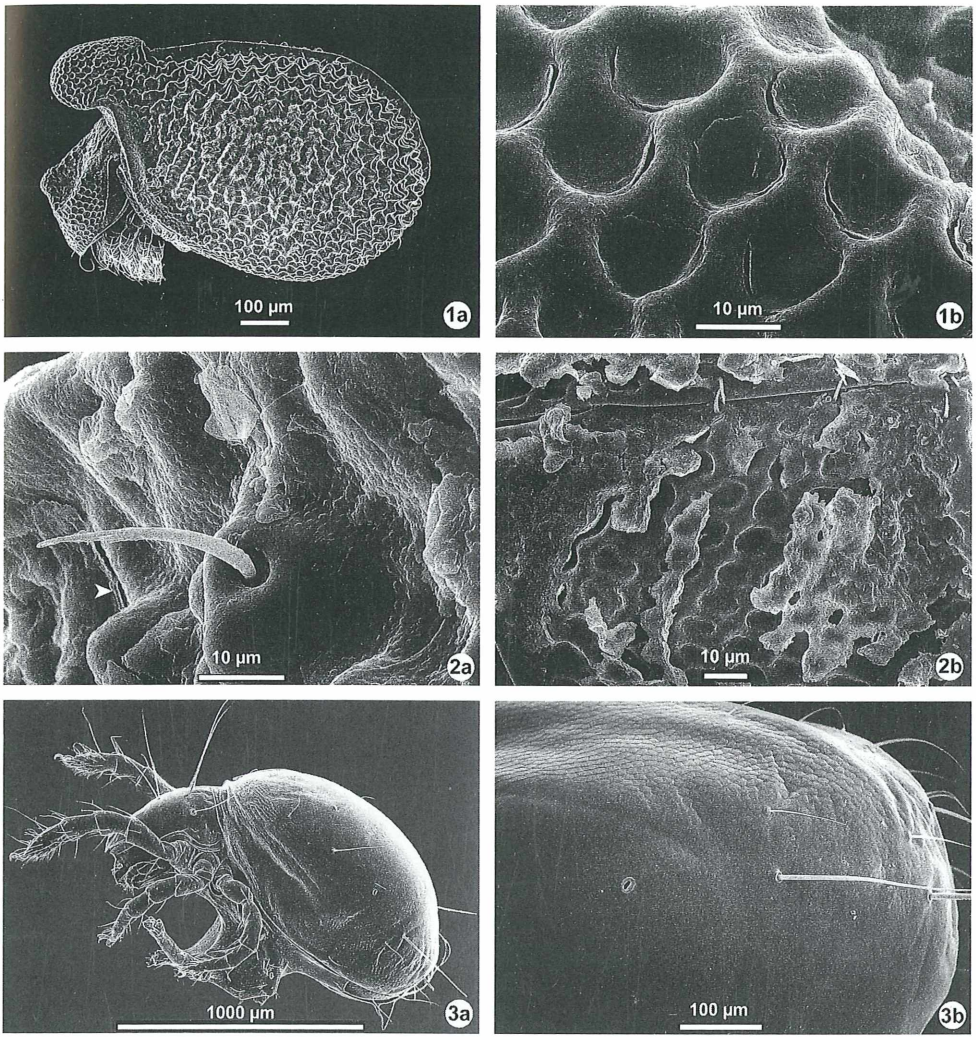

Fig. 1-3: 1, SEM micrographs of *Steganacarus carinatus* after treatment with ethyl acetate; a) Lateral view; b) Detail of the prodorsum with cracked cerotegument. 2, SEM micrographs of *Steganacarus carinatus*; a) Dorsal view; detail of posterior notogaster near the dorsal ridge with pusticulate cuticle surface. Cerotegument partly detached after treatment with ethanol (see arrowhead); b) Ventral view, left genital valve. Note cerotegumental detachment after treatment with hexane. 3, SEM micrographs of *Collohmannia gigantea* after treatment with ethanol, a) Lateral view, b) Lateral view of the posterior part of the notogaster. The cuticle shows a reticulate to squamous surface structure, respectively.

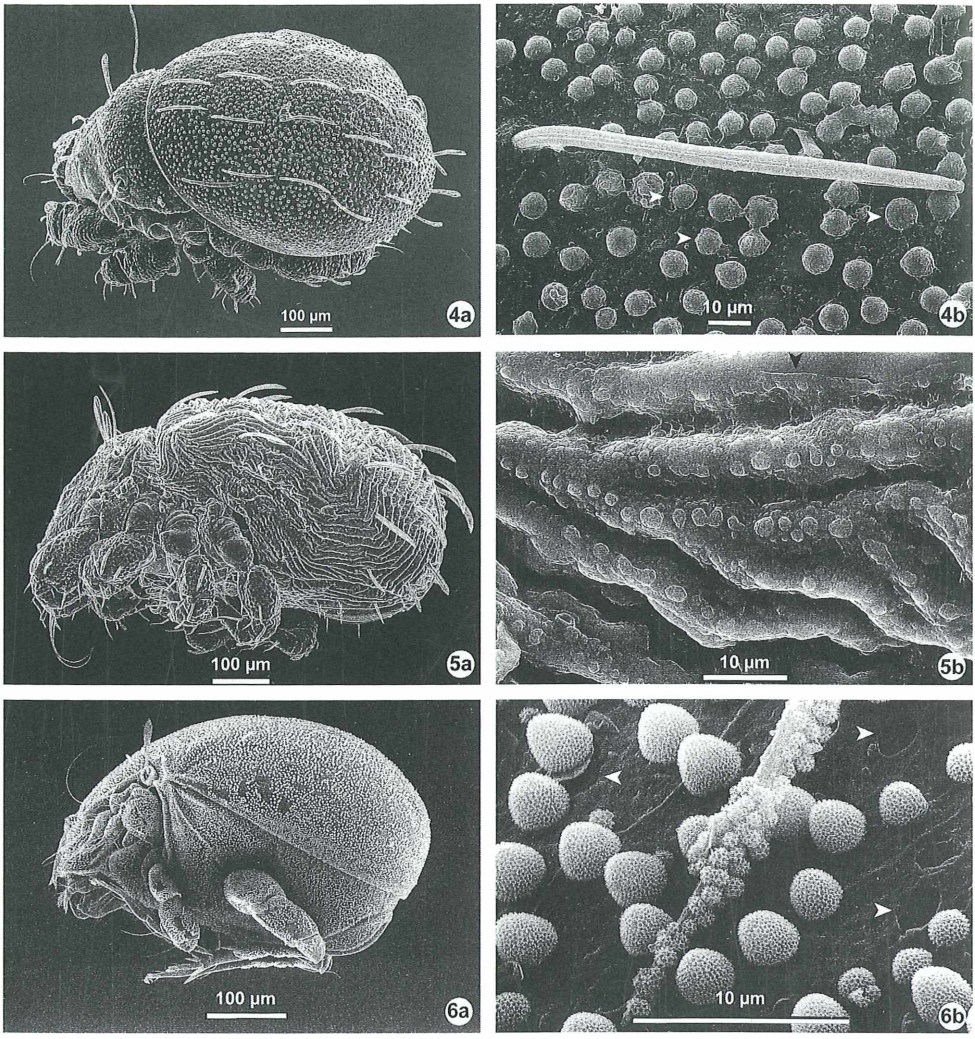

Fig. 4-6: 4, SEM micrographs of *Hermannia gibba* adults after treatment with hexane; a) Dorsolateral view. Note the verrucated cuticular surface; b) Detail of surface structure of the anterior notogaster. Arrowheads point to cerotegumental damages at the tops of the verrucae. 5, SEM micrographs of *Hermannia gibba* tritonymphs after treatment with hexane; a) Lateral view. Surface of the cuticle heterogenously structured; b) Lateral view, detail of the cuticular surface of the anterior notogaster. Wrinkles of the cuticle are covered by a thin cerotegument. Arrowhead points to sites of cerotegumental crack. 6, SEM micrographs of adult *Zetorchestes falzonii*. a) Lateral view after treatment with ethyl acetate. Cerotegument covers body and legs; detachment areas visible; b) Dorsolateral view, detail of the notogaster after treatment with chloroform. Cerotegumental blooms partly broken off the basic cerotegumental layer (see arrowheads). Small blooms upon notogastral setae.

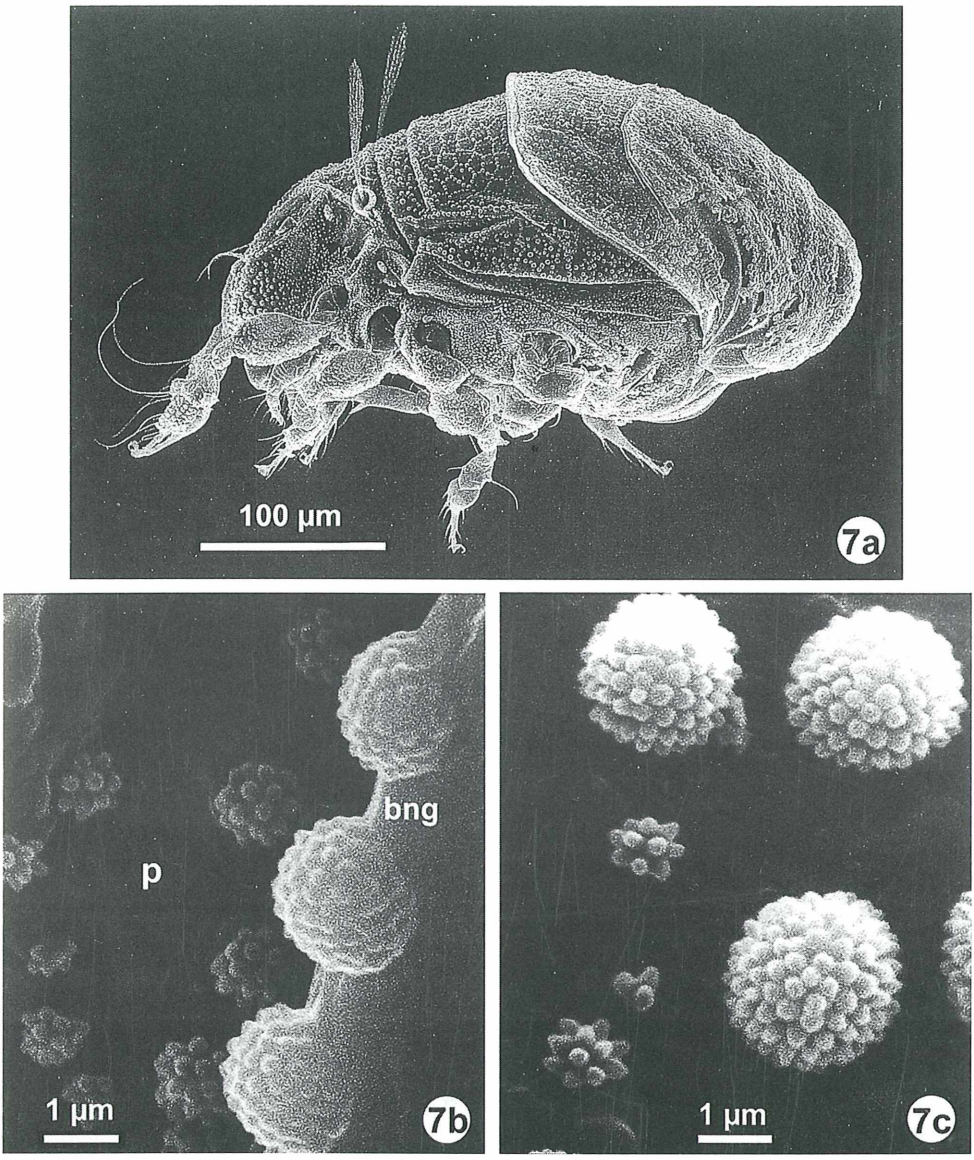

Fig. 7: SEM micrographs of tritonymphs of *Zetorchestes falzonii* after treatment with chloroform; a) Lateral view. Cerotegument detaches in the region behind leg IV and on the larval scalp. Cerotegumental blooms form a reticulate pattern on the notogastral surface; b) Dorsal view of the prodorsum (p) and the anterior border of the notogaster (bng). Cerotegumental blooms of the notogaster are not as clearly structured as blooms of the prodorsum; c) Lateral view. Spiny cerotegumental blooms of the prodorsum near the lamellar setae.

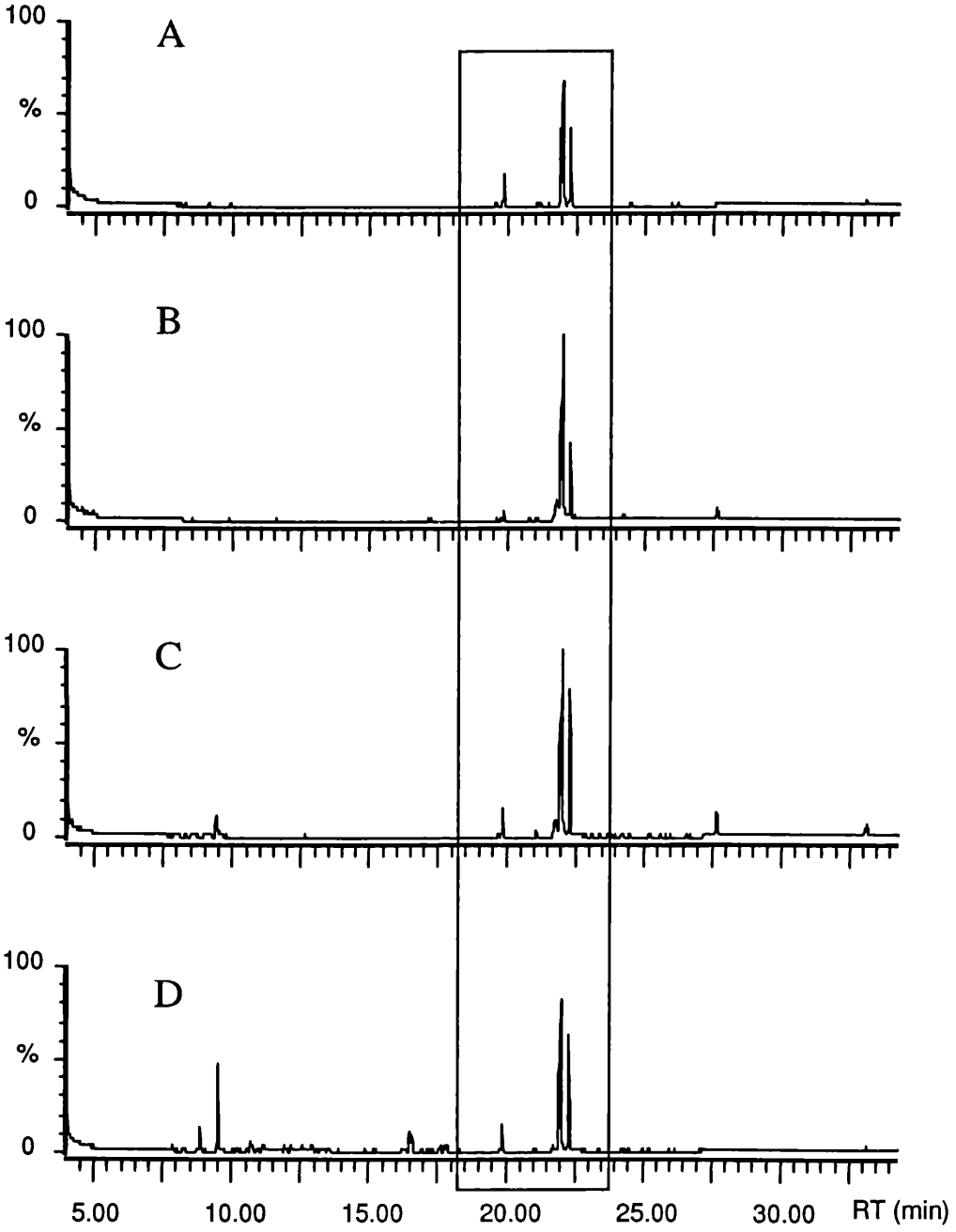

Fig. 8: Typical chromatograms of ethanolic body extracts of different oribatid species obtained with temperature program No.1 (see Materials & Methods). Main cuticular patterns framed (for details see Fig. 9). Peaks in the first half of certain chromatograms (C, D) refer to oil gland components. A) *Zetorchestes falzonii*; B) *Steganacarus carinatus*; C) *Collohmannia gigantea*; D) *Hermannia gibba*.

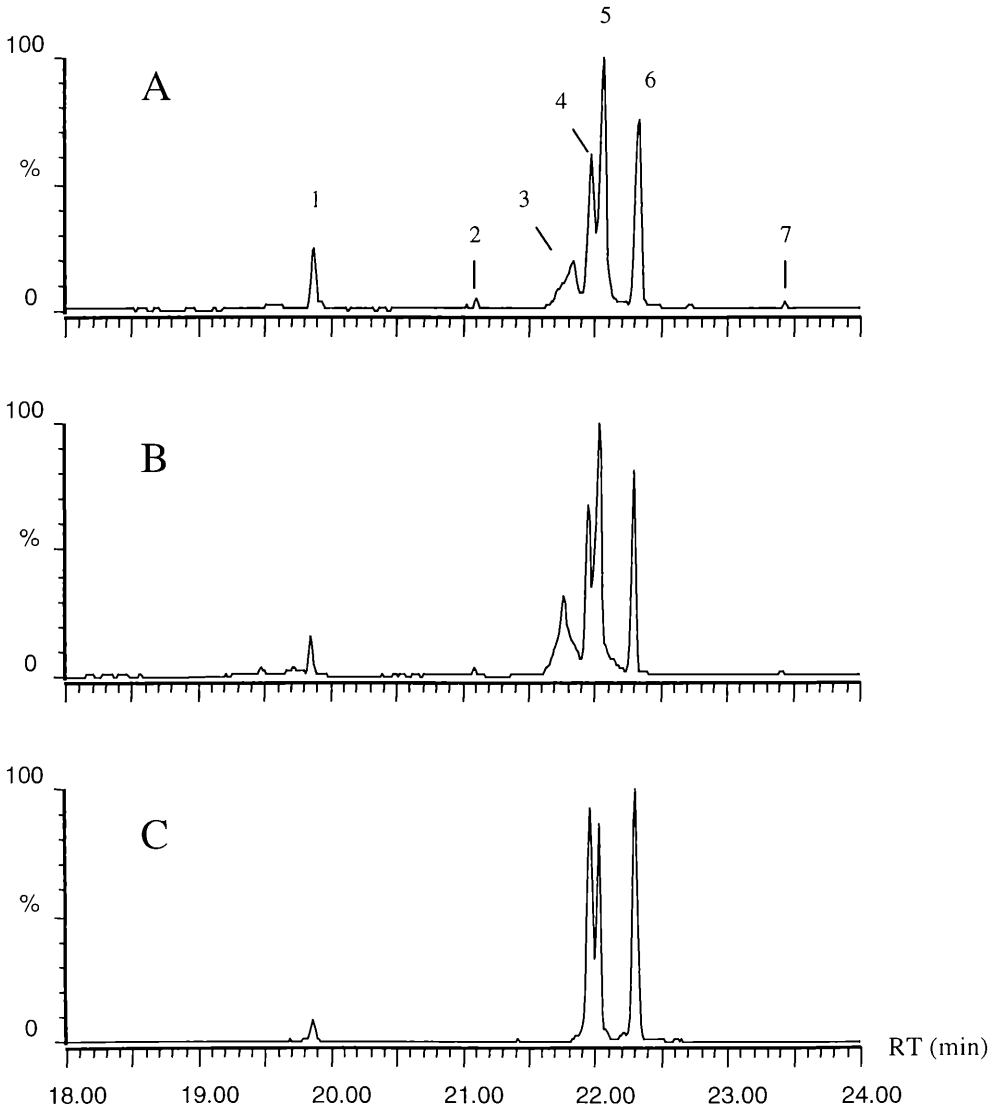

Fig. 9: Details of chromatograms showing the patterns of main components in ethanolic extracts of oribatid mites using temperature program No. 1 (see Materials & Methods); exemplarily demonstrated for *Hermannia gibba*: (1) Palmitic acid ethyl ester, (2) Heptadecanoic acid ethyl ester, (3) Free fatty acid C18-cluster, (4) Linoleic acid ethyl ester, (5) Oleic acid ethyl ester, (6) Stearic acid ethyl ester, (7) Nonadecanoic acid ethyl ester. A) *H. gibba* adults; B) *H. gibba* juveniles; C) Comparison of retention times with an authentic mixture of palmitic-, linoleic-, oleic- and stearic acid ethyl esters.

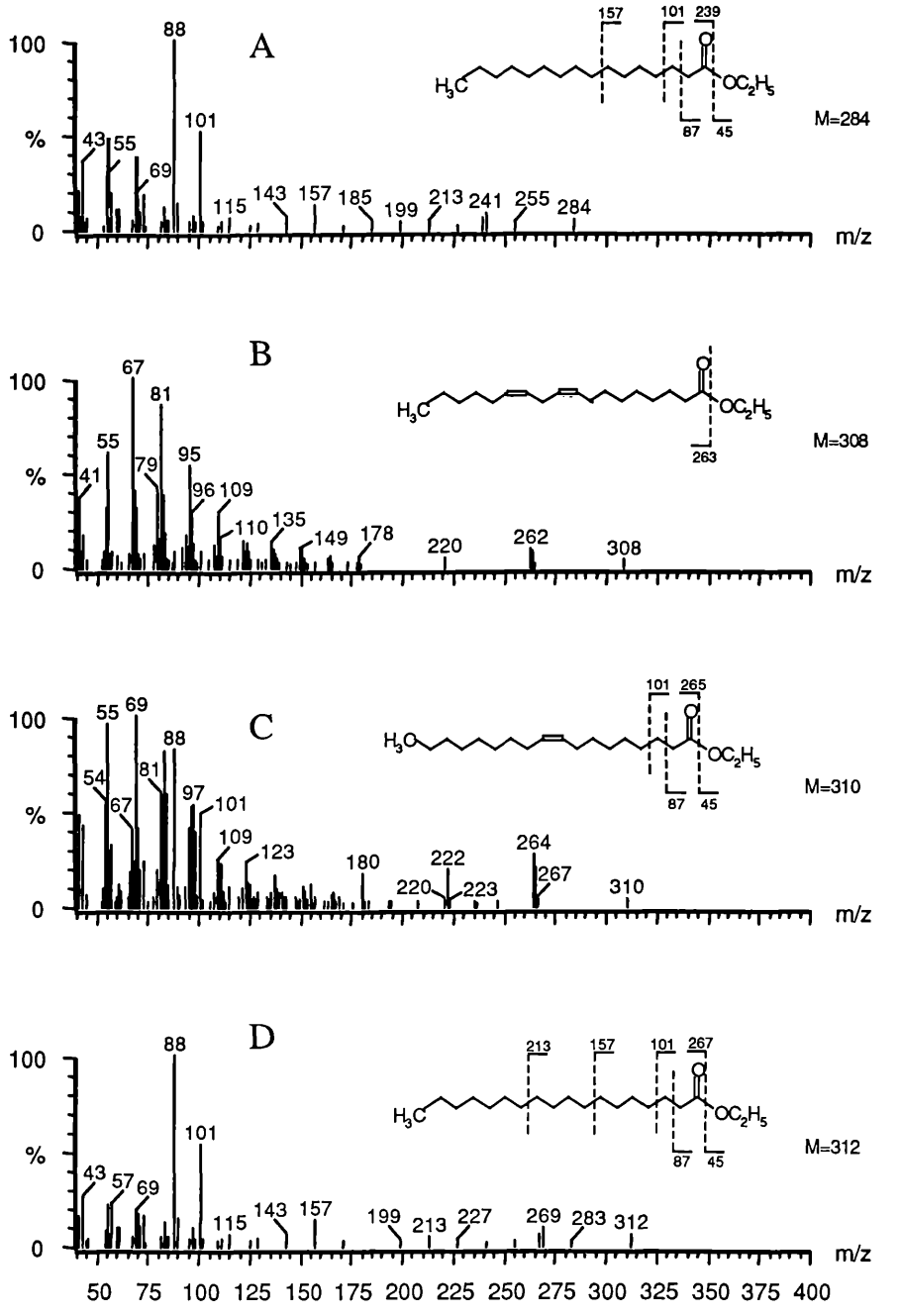

Fig. 10: EI-mass spectra of main components of ethanolic extracts of oribatid mites along with classification of important fragmentation patterns. A) Palmitic acid ethyl ester; B) Linoleic acid ethyl ester; C) Oleic acid ethyl ester; D) Stearic acid ethyl ester.

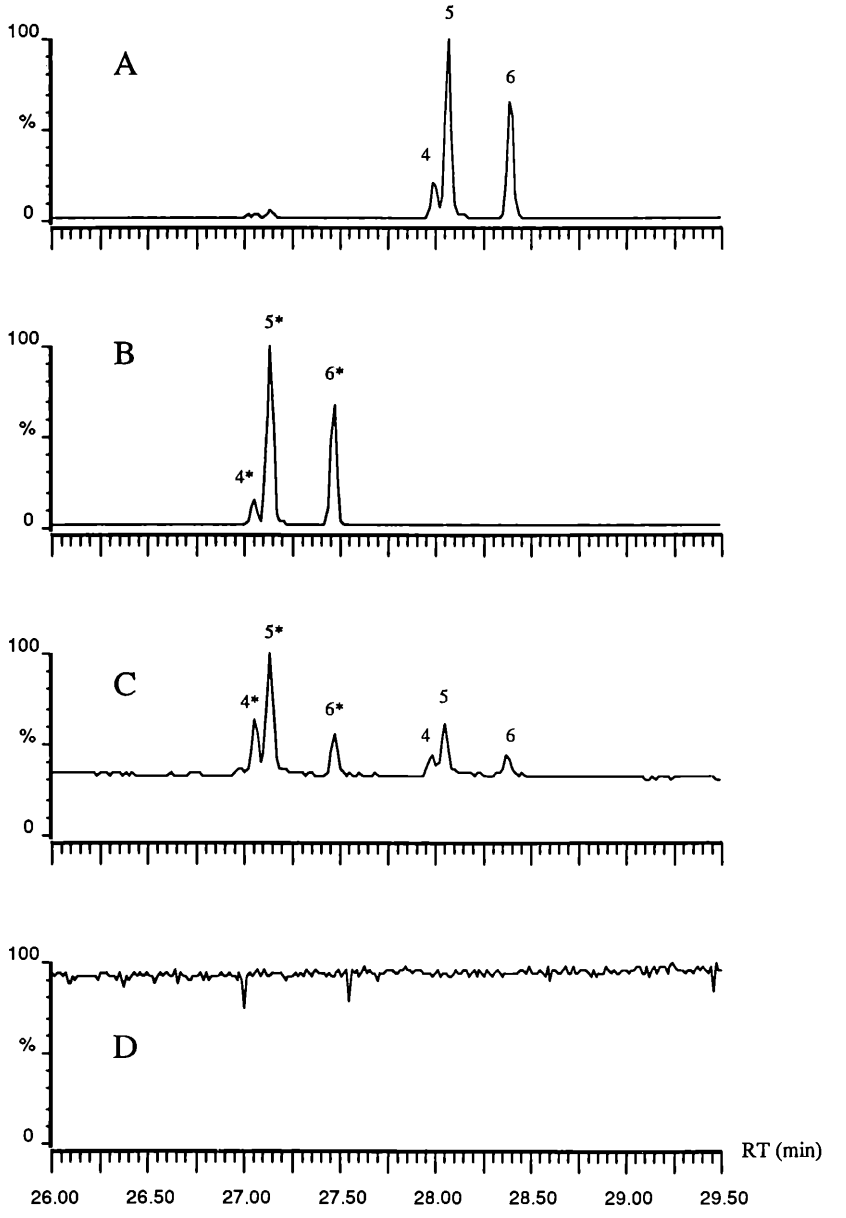

Fig. 11: Identification of originally present free fatty acids. A) Chromatographic detail [recorded with temperature program No. 2 (see Materials & Methods)] of an ethanolic body surface extract of *Collohmannia gigantea* exhibiting three peaks representing (4) linoleic-, (5) oleic- and (6) stearic acid ethyl esters, respectively; B) Methanolic extracts of *C. gigantea* showing the corresponding methyl esters (4*, 5*, 6*) but no traces of ethyl esters; C) Ethanolic extracts of *C. gigantea* after methylation with diazomethane showing both, fatty acid ethyl- and methyl esters; D) Hexane extracts of *C. gigantea* with no peaks visible.

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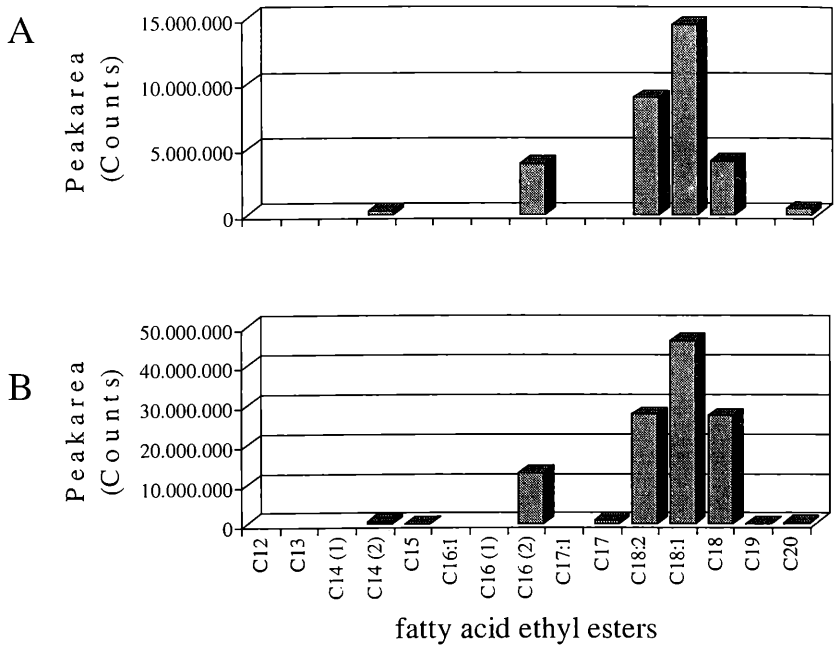

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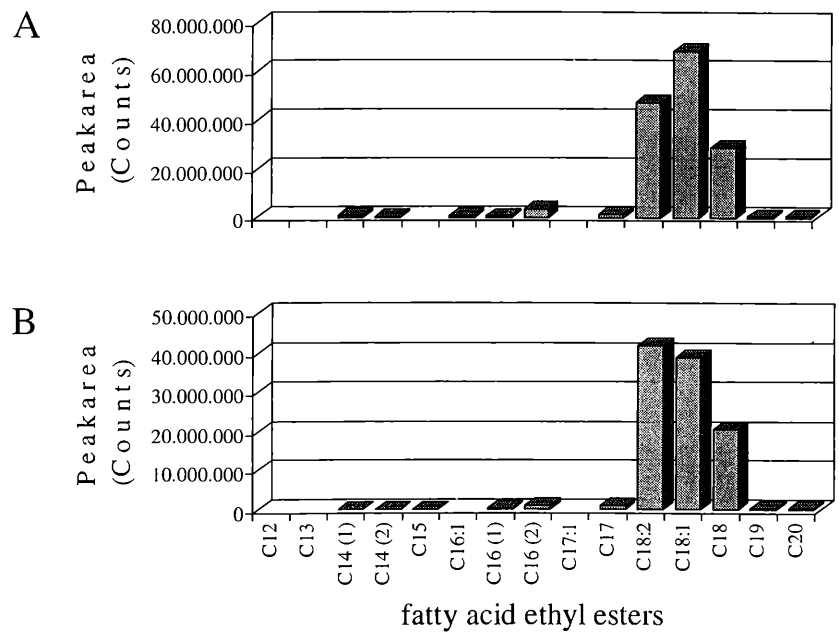

Fig. 12: Cuticle fatty acid patterns (quantified as ethyl esters) of *Zetorchestes falzonii*. A) Adults; B) Juveniles.

Fig. 13: Cuticle fatty acid patterns (quantified as ethyl esters) of *Steganacarus carinatus*. A) Specimens from Styria; B) Specimens from Carinthia.

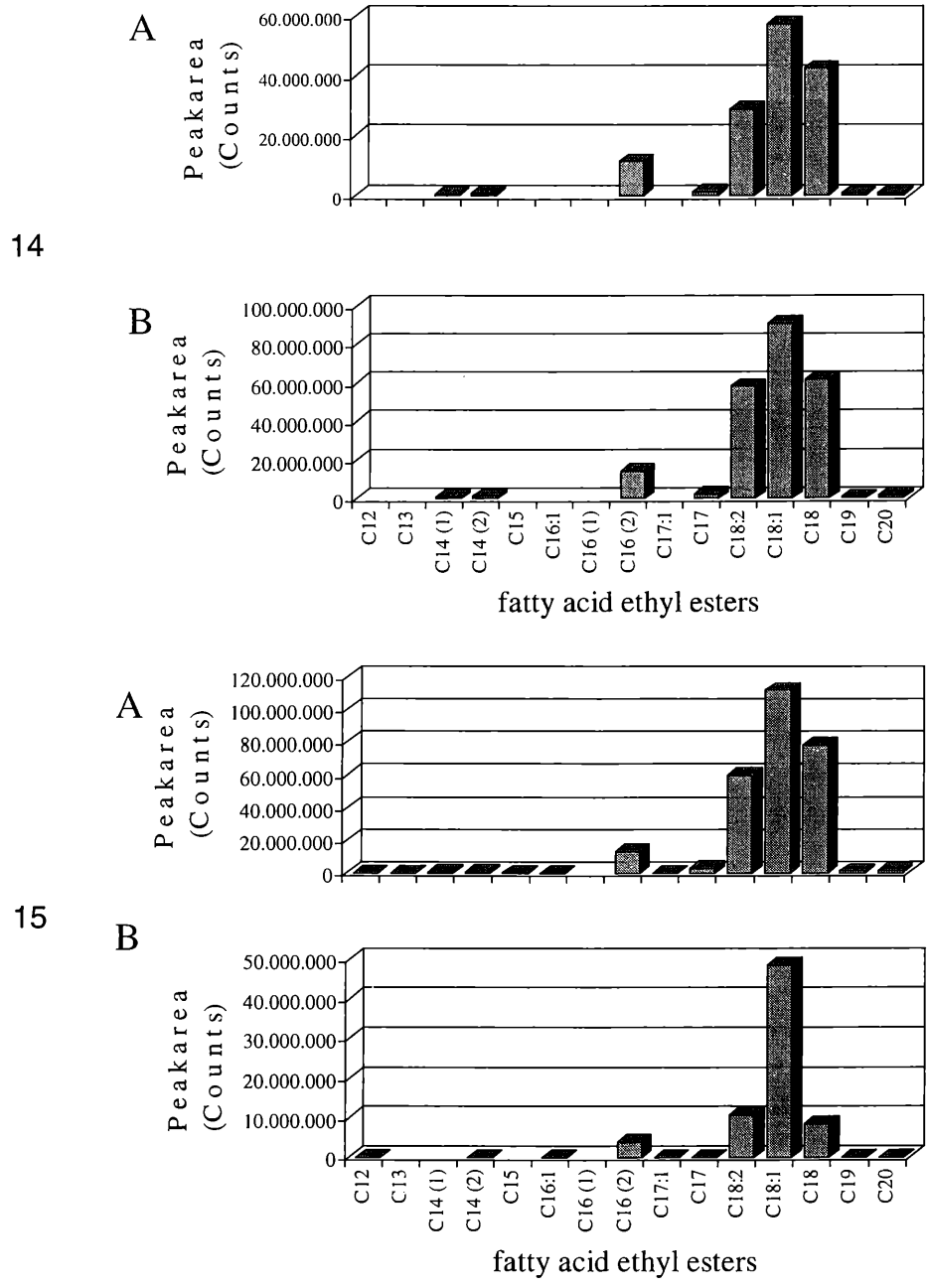

Fig. 14: Cuticle fatty acid patterns (quantified as ethyl esters) of *Hermannia gibba*. A) Adults; B) Juveniles.

Fig. 15: Cuticle fatty acid patterns (quantified as ethyl esters) of *Collohmannia gigantea*. A) Adults; B) Juveniles.