Supplementary information

for

Life as a fortress – Structure, function, and adaptive values of morphological and chemical defense in the oribatid mite *Euphthiracarus reticulatus* (Actinotrichida)

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Morphology

This part is certainly not for everybody, but hardcore morphologists and acarologists might appreciate and even enjoy it.

Results and Discussion

This part describes and discusses mainly the differences between *Euphthiracarus reticulatus* and *Euphthiracarus cooki*, since a detailed morphological description of latter already exists [R1]. Differences between *E. cooki* and other ptyctimous mites have also been described in detail [R2-R6].

Exoskeletal elements

Notogaster - Specimens of *E. reticulatus* used for morphological analyses have a notogaster length between 870 and 940 µm (Fig. 1A - D) and thus it is more than twice as large as that of *E. cooki* (322 - 419 µm;) [R7]. The notogastral fissure (Figs. 1D, J, 5) is long and distinct in both species. A string of soft tissue enters the cuticle in the midline at the posterior end of the notogaster where no seta is present. Thus, we believe it to be the nerve of a mechanoreceptor perceiving the tensile or compressive stress generated by the deformation of the notogaster during ptychosis (*cf.* slit sensilla, lyrifissures in [R8-R11]). In contrast to *E. cooki*, the scale receptacle of *E. reticulatus* is unincisive within the tectonotal notch and is most probably lacking. The lateral anterior tectum ventrally extends into prominent teeth (Fig. 1F, K).

Prodorsum - The prodorsum (Fig. 1, Fig. S7A, B) of *E. reticulatus* is narrower than that of *E. cooki* and seems more oval. It features two carinae of which the dorsal one is more

pronounced than the ventral one (Fig. 1F, S7A). In contrast, the prodorsum of *E. cooki* exhibits 3 carinae.

The bothridial scale is rather long and round, and distally bent upwards (Fig. 1G, Fig. S7A). In the encapsulated state it rests on top of the tectonotal notch (Fig. 1C). It is about as long as that of *E. cooki*, but it is rounder (Fig. S7A, B), which might be connected directly to its function. In *E. cooki* the bothridial scale first acts as a lazy hinge and then enters the tectonotal notch during enptychosis concluding with its anchorage in the scale receptacle. In *E. reticulatus* the bothridial scale also acts as lazy hinge but eventually mounts flush on top of the tectonotal notch instead of inside a receptacle (supplemental video V4, *cf*. Fig. 13 in [R1]).

Since there are well-developed teeth at the lateral anterior tectum and the rostral notch is distinct, with a clear and deep indentation as notogastral counterpart, the scale receptacle might not be needed for keeping the animals encapsulated without muscular activity. *E. reticulatus* has a relatively short and inconspicuous inferior retractor process and is smaller than that of *E. cooki* and the smallest so far found in euphthiracaroid mites [R2, R4, R5].

Holoventral plates - The holoventral and plicature plates of *E. reticulatus* appear more slender than those of *E. cooki*. The holoventral plates have a weakly pronounced anterior interlocking triangle (Fig. 1H), whereas the anterior interlocking triangle of *E. cooki* is more pronounced, seems to be more undulating, and thus is externally better visible. The posterior interlocking triangle is present in *E. reticulatus*, but so weakly pronounced that it is hardly visible in the SRµCT data (data not shown). In contrast, the posterior interlocking triangle is well pronounced in *E. cooki*. Pre- and post-anal apodemes are connected by firm cuticle, most likely due to sclerotization of the formerly soft walls of the anal atrium (Fig. 5, Fig. S3) as found in *E. cooki*. The gladius of the preanal apodeme is anteriorly not connected to any

cuticular structures (*gl*$_{pra}$; Fig. 5, Fig. S3), and probably corresponds to the 'sloped anterior portion' of the preanal apodeme already described. The apodematal complex of the holoventral plates (preanal and postanal apodemes, the sclerotized walls of the anal atrium, and the anteriorly-extending gladius of the preanal apodeme) is not developed in *E. cooki*.

Muscular elements

The coxisternal retractor (*csr*) consists of about 40 muscle fibers (Fig. S7C), and the inferior prodorsal retractor (*ipr*) consists of about 70 muscle fibers inserting via tendons on the intercalary wall induration of the prodorsum (Fig. S7D). These number are much higher than in *E. cooki* (12 and 13, respectively). This might simply relate to body size (length about 900 and 380 µm, respectively), but there is also a functional consideration. The *ipr* inserts on the intercalary wall induration of the prodorsum and the inferior retractor process in *E. cooki*, but only on the intercalary wall induration of the prodorsum in *E. reticulatus*. Accordingly, the inferior retractor process is smaller in *E. reticulatus* compared to *E. cooki*. The absence of the scale receptacle might explain this: a higher number of muscle fibers should yield an overall higher force whereas the insertion on the intercalary wall induration of the prodorsum provides a more direct force transmission onto the notogaster, counterbalancing the absent, energy-saving anchorage of the bothridial scale.

The superior podosomal membrane adjustor (s*ma*; Fig. 2) consists of 3 muscle bands and 1- 2 muscle fibers each. Additionally, we found a muscle (with 3 muscle fibers) originating close to the *sma*, which inserts more anteriorly on the podosomal membrane, close to the coxisternum. The origin of this muscles suggests that, although the direction given by the insertion is slightly different, it might be a split, second portion of the *sma* as we found for example in *Acrotritia ardua* [R3]. Despite not inserting on the sejugal apodeme of the coxisternum but rather on the surrounding podosomal membrane, it might be a

phylogenetic precursor of the coxisternal protractor (*csp*) found in Phthiracaroidea [R4, R5, R6]. Its direction, however, differs and thus an additional role as protractor for the walking legs, as it is the case in Phthiracaroidea, seems unlikely.

There difference in the morphology of the notogaster lateral compressor (*nlc*) between *E. reticulatus* and *E. cooki* is negligible (18 vs. 21 muscle bands, respectively).

The number of muscle fibers of ventral plate adductor (*vpa*) and ventral plate compressor (*vpc*) of both species is similar as well (termed holoventral adductors *hva* and holoventral compressors *hvc* in [R1]; *cf.* [R3]). The *vpa* inserts, however, on the gladius-like appendix of the anal apodeme in *E. reticulatus* in contrast to the preanal apodeme itself.

The lack of the *poam* was confirmed by investigating four other scanned specimens of *E. reticulatus* (data not shown).

The lateral rectal muscle (*lrm*) originates dorsally on the notogaster and inserts dorsolaterally on the rectum. It consists of 3 muscle fibers. Nothing is known of the *lrm* in *E. cooki*.

List of abbreviations

Abbreviation	Meaning
csp	coxisternal protractor
csr	coxisternal retractor
gl_{pra}	gladius of the preanal apodeme
hva	holoventral plate adductor
hvc	holoventral plate compressor
ipr	inferior prodorsal retractor
lrm	lateral rectal muscle
nlc	notogaster lateral compressor muscle
poam	postanal muscle
sma	superior podosomal membrane adjustor
vpa	ventral plate adductor
vpc	ventral plate compressor

Table T1. Landmarks placed on the Synchrotron high-speed radiographies (*cf*. Fig. S2, supplemental video V4).

Radiograph	Landmark description	Landmark number
(A) Lateral view	Prodorsum tip	1
	End point of prodorsum tip movement (1)	2
	Bothridial scale, tip	3
	Tectonotal notch	4
	Notogaster, anterior edge	5
	Holoventral plates, most ventral point	6
	Notogaster, posterior edge	7
	Notogaster, dorsal edge	8
	Ventrosejugal furrow of coxisternum	9
	End point of coxisternum movement (10)	10
(B) Frontal view	Notogaster, dorsal edge	11
	Notogaster, left edge	12
	Notogaster - plicature plate junction, right	13
	Plicature plate - holoventral plate junction, right	14
	Holoventral plates, ventral midpoint	15
	Plicature plate - holoventral plate junction, left	16
	Notogaster - plicature plate junction, left	17
	Notogaster, right edge	18
	Notogaster lateral compressor (*nlc*), top right origin	19
	Notogaster lateral compressor (*nlc*), bottom right origin	20
(C) Dorsal view	Notogaster, left edge	21
	Notogaster, right edge	22
	Notogaster, left anterior edge	23
	Notogaster, right anterior edge	24
	Notogaster, posterior edge	25
	Capitular apodeme of gnathosoma, posterior edge	26
	End point of gnathosoma movement (26)	27
	Notogaster - plicature plate junction, left	28
	Plicature plate - holoventral plate junction, left	29
	Holoventral plates, midpoint	30
	Plicature plate - holoventral plate junction, right	31
	Notogaster - plicature plate junction, right	32

Table T2. Calculated distances and angles based on landmarks placed on the Synchrotron high-speed radiographies (*cf.* Fig. S2, Table T1).

Radiographs	#	Distance	Landmarks used (Table T1, Fig. S2)
(D) Lateral view	A	Distance of prodorsum tip and end point of prodorsum tip movement	1, 2
	A_b	Distance of tectonotal notch and end point of prodorsum tip movement	2, 4
	A_c	Distance of prodorsum tip and tectonotal notch	1, 4
	B	Distance of bothridial scale and tectonotal notch	3, 4
	C	Notogaster length	5, 7
	D	Notogaster height	6, 8
	E	Leg retraction (distance of ventrosejugal furrow and end point of coxisternum movement)	9, 10
	α	Angle enclosed by prodorsum and notogaster (with tectonotal notch as fulcrum point)	1, 2, 4
(E) Frontal view	F	Notogaster width	12, 18
	G	Notogaster height	11, 13, 17
	H	Notogaster gap width (junctions between notogaster and plicature plates)	13, 17
	I	Holoventral plate gap width (junctions between plicature and holoventral plates)	14, 16
	J1 / J2	Distances of notogaster (junctions between notogaster and plicature plates) and midpoint of ventral plates	13, 15 / 15, 17 (X-axis only)
	K1 / K2	Distances of ventral plates (junctions of plicature and holoventral plates) and midpoint of ventral plates	14, 15 / 15, 16 (X-axis only)
	L1 / L2	Distance of nlc origin and junction between plicature and holoventral plates	16, 16 / 16, 20
(F) Ventral view	M	Notogaster width	21, 22 (Y-axis only)
	N	Notogaster length	23, 24, 25 (X-axis only)
	O	Notogaster gap width (junctions between notogaster and plicature plates)	28, 32
	P	Holoventral plate gap width (junctions between plicature and holoventral plates)	29, 31
	Q	Gnathosoma retraction (distance of capitular apodeme of gnathosoma and end point of gnathosoma movement)	26, 27
	R1 / R2	Distances of notogaster (junctions between notogaster and plicature plates) and midpoint of ventral plates	28, 29 / 31, 32
	S1 / S2	Distances of ventral plates (junctions of plicature and holoventral plates, and midpoint of ventral plates)	29, 30 / 30, 31

Chemistry

Materials and Methods

MSTFA derivatization

Derivatization of potential hydroxyl groups to corresponding trimetyl-silyl (=TMCS)-ethers was conducted by adding 150 µl N-methyl-N-(trimethylsilyl)-trifluoracetamid (MSTFA in pyridine 2:1; with 1% trimethylchlorosilane) to 100 µl of the crude extract. After 30 min of reaction at 55°C the mixture was injected into GC-MS. The derivatization of carbonyl groups to corresponding O-methyl oxime derivates (molecular ion + 29 *m/z* for one and + 58 *m/z* for two carbonyl functions) were carried out by using MOX (2% methoxyamine–hydrogen chloride in pyridine). 200 µl of MOX-reagent were added to 100 µl of the pooled extract and subsequently incubated at 70°C for 150 min. After the reaction, the sample was allowed to cool to room temperature and was washed by adding 100 µl hexane and 200 µl deionized water. The organic phase was washed again by adding two 100 µl portions of deionized water. Finally, the organic phase was removed and directly injected into the GC-MS. Derivatization products were measured with a Trace GC, which was directly coupled to a DSQ I MS both from Thermo Fisher Scientific (Waltham, USA) equipped with a ZB-5MS fused silica capillary column (same specifications as noted above). Samples (1.5 µl aliquots) were injected by hand into a split/splitless-injector, which operated in splitless-mode at an injection-temperature of 240 °C. Temperature and ionization settings were the same as described before, helium was used as carrier-gas with a constant flow rate of 1.2 ml/min.

Table T3. Gas chromatography retention index (RI) and mass spectrometric data of compounds extracted from *Euphthiracarus reticulatus*. Peak ID corresponds to Fig. 7; †RI on ZB-5 column

Peak ID	RI†	Mass spectrometric fragmentation (*m/z*)	Identified as
I	1903	**276 (M^+; 3)**, 258 (2), 247 (12), 233 (9), 229 (7), 218 (8), 207 (38), 201(11), 189 (35), 179 (99), 178 (67), 151 (49), 149 (62), 135 (41), 119 (55), 109 (53), 107 (53), 105 (30), 98 (100), 95 (70), 93 (33), 91 (36), 81 (36), 79 (43), 77 (25), 69 (46), 67 (49), 57 (30), 55 (54), 41 (48)	2-(But-1-en-1-yl)-4-butylidene-3-(pent-2-en-1-yl)pentanedial = *δ-acaridial*
II	1921	**272 (M^+; 5),** 257 (4), 229 (4), 215 (2), 201 (3), 187 (9), 179 (7), 161 (16), 147 (13), 135 (10), 134 (10), 133 (30), 121 (15), 120 (22), 119 (22), 109 (10), 107 (19), 105 (10), 95 (17), 93 (33), 91 (11), 81 (30), 79 (16), 69 (100), 67 (17), 55 (12), 41 (18)	7,11,15-Trimethyl-3 methylene-1,6,10,14-hexadecatetraene = *β-springene*

Table T4. Chemical shifts of δ-acaridial measured in CD_2Cl_2 at 274 K, referenced to TMS.

position[a]	δ ^{1}H [ppm]	integral, multiplicity[b]	coupling constant [Hz]	δ ^{13}C [c] [ppm]	^{13}C multiplicity	HMBC correlations [ppm]
1	9.32	1H, d	3.0	200.5	CH	(58.6)
2	3.62*	1H, br	–	(58.6)	n.d.	–
3	2.88*	1H, br	–	n.d.	n.d.	–
4	–	–	–	(141.9)	n.d.	–
5	9.26	1H, d	1.7	195.6	CH	(141.9)
1′	5.21	ovlp	–	123.0	CH	–
2′	5.77	1H, dt	15.3, 6.3	139.6	CH	–
3′	2.10	2H, m	–	26.2	CH_2	139.6, 123.0, 13.4
4′	1.01	3H, t	7.4	13.4	CH_3	139.6, 26.2
1″	2.26*	ovlp	–	32.9	CH_2	–
2″	5.14	1H, dt	15.2, 7.0	126.5	CH	26.0
3″	5.36	ovlp	–	134.4	CH	–
4″	1.92	2H, m	–	25.9	CH_2	134.4, 126.5, 13.6
5″	0.89	ovlp	–	13.6	CH_3	134.4
1‴	6.57	1H, t	7.4	159.8	CH	195.6
2‴	2.34	ovlp.	–	31.6	CH_2	–
3‴	1.53	ovlp.	–	22.1	CH_2	–
4‴	0.98	ovlp.	–	14.0	CH_3	31.6, 22.1

[a] proton resonances of with broad signals are indicated by a *

[b] abbreviations: ovlp = overlapped signals; n.d. = not detectable; br = broad

[c] values in brackets are based on very weak signals

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