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The discovery of *Scutovertex ianus* sp. nov. (Acari, Oribatida) – a combined approach of comparative morphology, morphometry and molecular data

Tobias Pfingstl[1, 2], Sylvia Schäffer[1], Ernst Ebermann[1], Günther Krisper[1]
[1] *Institute of Zoology, Karl-Franzens University, Universitätsplatz 2, A-8010 Graz, Austria*
[2] *E-mail: tobias.pfingstl@uni-graz.at*

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Abstract

Based on morphological, morphometric and genetic data *Scutovertex ianus* sp. nov. is described as a new oribatid mite species. The traditional comparison with the morphologically most similar congeneric *S. minutus* and *S. sculptus* demonstrated that the new species shares certain characters with both species, but can be clearly identified by indistinct cuticular notogastral foveae in combination with short spiniform notogastral setae. Furthermore the eggs of *S. ianus* exhibit a different fine structure of the exochorion. The morphometric analysis of 16 continuous morphological variables separated the three species, *S. minutus*, *S. sculptus* and *S. ianus* with a certain overlap indicating minor size and shape differences in overall morphology. The molecular phylogenetic analysis of mitochondrial COI gene sequences supported the monophyly of all three investigated species and confirmed *S. ianus* as separate species with high bootstrap values. Each performed analysis approves the discreteness of *S. ianus* and the results contradict the formerly supposed large intraspecific variability of the representatives of the genus *Scutovertex*. The records of *S. ianus* are as yet restricted to the Eastern part of Austria and to one location in Germany, but findings of intermediary *Scutovertex* specimens from other European countries may refer to this new species.

Contents

Introduction

At present the oribatid mite genus *Scutovertex* Michael, 1879 consists of 23 species known worldwide (Subías, 2004), whereas more than the half is occurring on the European continent. Many of these live in extreme environments and can be found from the alpine zone to the marine littoral, on sun-exposed rocks and roofs, sparsely covered by lichens and mosses, as well as in saline soils and salt marshes and in inundation meadows (Krisper and Schuster, 2001; Krisper *et al.*, 2002; Smrž, 1992, 1994; Weigmann, 1973). The species of this genus are supposed to exhibit variability in certain morphological features resulting in a difficult classification of some specimens (Weigmann, 2006). But recent publications have shown that intraspecific variation occurs only to a minor extent (Pfingstl *et al.*, 2008) and revealed a few new species in Europe (Schäffer *et al.*, 2008; Pfingstl *et al.*, 2009; Weigmann, 2009) which may have formerly caused a disorder and the idea of certain unstable morphological characters in some members of this genus. In the course of the investigation of Austrian representatives of Scutoverticidae we encountered several *Scutovertex* specimens showing a mixture of *S. minutus* (Koch, 1836) and *S. sculptus* Michael, 1879 specific characters making a clear determination unfeasible. To clarify the status of these 'intermediate forms' and to test again the possibility of large variation within one species a detailed morphological examination and a morphometric as well as a genetic analysis were performed.

Material and methods

Rearing experiments were performed to obtain eggs and juvenile stages, and for this purpose individuals of *Scutovertex ianus* sp. nov. (species description in Appendix) were put into boxes of polystyrol supplied with plaster of Paris and the animals were fed with collected substrate and coccal green algae. For permanent slides the specimens were embedded in BERLESE mountant and for temporary slides lactic acid was used. Measurements and drawings were performed with a differential interference contrast microscope (Olympus BH-2). The SEM-micrographs were taken at the Research Institute for Electron Microscopy and Fine Structure Research, Graz, University of Technology, with a Zeiss Leo Gemini DSM 982.

The morphometric investigation was performed to confirm the discreteness of *S. ianus* and does not represent an identification tool. Multivariate analyses of data were carried out with the software PAST version 1.82b (Hammer *et al*., 2001). To test the intraspecific variability of *S. minutus* and *S. sculptus*, two populations of each species (*S. minutus*: Pogier n=21, Bachsdorf n=40; *S. sculptus*: Fließ n=21, Illmitz n=40) were analysed with a set of 16 continuous morphological characters, the variable body length was excluded (Pfingstl *et al*., 2009). The same set of variables of 40 individuals of each species (*S. ianus*: 18 females, 22 males; *S. minutus*: 20 females, 20 males; *S. sculptus*: 25 females, 15 males) was measured, logarithmized and then used for Principle Component Analysis (PCA) as well as Canonical Variates Analysis (CVA).

For the molecular phylogenetic analysis, the total genomic DNA was extracted from single ethanol-preserved specimens using the modified CTAB (hexadecyltriethylammonium bromide) method after Boyce *et al*. (1989). A 1259 bp fragment of the mitochondrial COI gene was amplified using the primers COI_Fsy (5´-GNTCAACAAWTCATWAAG-3´) and COI_Rsy (5´-TAAACTTCNGGYTGNCCAAAAAATCA-3´) for COI-region 1 (modified after Heethoff *et al*., 2007), Mite COI-2F and Mite COI-2R (Otto and Wilson, 2001) for COI-region 2. PCR amplification and sequencing followed the protocol described in Schäffer *et al*. (2008). Sequences are available from GenBank under the accession numbers specified in Table 1.

Sequences were aligned by eye in MEGA 3.1 (Kumar *et al*., 2004). Phylogenetic reconstruction by neighbour joining (NJ) were conducted in PAUP* 4.02a (Swofford, 2002) and Bayesian inference (BI) in MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003).

Table 1. List of the studied scutoverticid specimens with identification, sampling locality and GenBank Accession Numbers. All sampling sites are located in Austria. Sequences not generated in the framework of this study were obtained from: [a] = Schäffer et al. (in press a); [b] = Schäffer et al. (in press b)

	species identification	sampling locality	accession no.
Scutovertex ianus	SianSt1	Stiwoll	GU937466
	SianSt2	Stiwoll	GU937467
	SianSt6	Stiwoll	GU937468
	SianSt7	Stiwoll	GU937469
	SianSch6	Schladming	GU937470
	SianSch7	Schladming	GU937471
	SianSch8	Schladming	GU208581 [b]
	SianSch9	Schladming	GU937472
	SianAu4	Floodplain of Traun	GU208583 [b]
	SianAu5	Floodplain of Traun	GU208584 [b]
	SianAdm1	Admont	GU208582 [b]
	SianAdm2	Admont	GU937473
	SianAdm3	Admont	GU937474
Scutovertex sculptus	SsI_B5	Lake 'Zicklacke'/Illmitz	GQ890426 [a]
	SsI_C15	Lake 'Oberer Stinker'/Illmitz	GQ890435 [a]
	SsI_C16	Lake 'Oberer Stinker'/Illmitz	GQ890436 [a]
	SsE5	Ernstbrunn	GQ890412 [a]
Scutovertex minutus	SmA6	Asparn	GQ890368 [a]
	SmUsb3	Unterstinkenbrunn	GQ890394 [a]
	SmBach2	Bachsdorf	GQ890380 [a]
	SmKal1	Graz	GQ890371 [a]
Scutovertex pileatus	SpilL2	Laas	GU937475
	SpilSc5	Dobratsch	GU937477
	SpilBH1	Castle Hochosterwitz	GU937476
	SpilBH5	Castle Hochosterwitz	GU208588 [b]
Lamellovertex caelatus	LcE3	Ernstbrunn	GU208605 [b]
	LcE6	Ernstbrunn	GU208606 [b]

We used *Lamellovertex caelatus* (Scutoverticidae) as outgroup. For Bayesian analysis COI gene was partitioned by codon position. Rate heterogeneity was set according to a gamma distribution with six rate categories (GTR model) for each data partition. Bayesian posterior probabilities (BPP) were obtained from a Metropolis-coupled Markov chain Monte Carlo simulation (2 independent runs; 4 chains with 2 million generations each; chain temperature: 0.2; trees sampled every 100 generations), with parameters estimated from the data set. The burn-in fraction was set to 20%. For NJ, the best-fit substitution model selected by the hierarchical likelihood ratio test (hLRT) implemented in Modeltest 3.06 (Posada and Crandall, 1998) was TVM+I+G (base frequencies: A = 0.2551, C = 0.1631, G = 0.1740, T = 0.4077; R-matrix: A↔C =

Table 2a. Loadings of the 16 variables set on the first three principle components. *Scutovertex minutes*, population 'Bachsdorf' versus population 'Pogier'.

variable	PC1	PC2	PC3
bw	0,3433	-0,1142	0,0327
db	0,1983	-0,0120	-0,1622
dc	0,1007	-0,0867	0,0163
cl	0,2571	0,9119	0,1468
pl	0,0520	0,2835	-0,4192
pw	0,1393	-0,0622	-0,0622
nl	0,2701	-0,1370	0,0880
dmw	0,2823	-0,1131	-0,0688
h_3w	0,3149	-0,0901	0,1389
gl	0,2962	-0,0887	0,1487
gw	0,2820	-0,0366	0,2053
dga	0,3797	-0,0503	-0,6826
al	0,2451	-0,0364	0,3355
aw	0,2795	-0,0280	0,2026
dcg	0,0370	-0,0038	-0,2318
ap3	0,2040	-0,0999	-0,0445

Table 2b. Loadings of the 16 variables set on the first three principle components. *Scutovertex sculptus,* population 'Illmitz' versus population 'Fließ'.

variable	PC1	PC2	PC3
bw	-0,2961	0,0895	-0,0515
db	-0,2016	-0,0171	-0,0175
dc	-0,1390	0,1391	0,3394
cl	-0,2756	-0,9186	-0,0916
pl	-0,1601	-0,1933	0,3896
pw	-0,1484	0,0310	-0,0189
nl	-0,2551	0,1388	-0,1838
dmw	-0,2650	0,0689	-0,0015
h_3w	-0,2986	0,1102	-0,1698
gl	-0,3531	0,1009	0,0092
gw	-0,3515	0,1313	0,0324
dga	-0,3278	0,1235	-0,4441
al	-0,1946	0,0647	0,3859
aw	-0,2690	0,0338	0,4989
dcg	-0,0796	-0,0509	-0,1980
ap3	-0,1792	0,0594	-0,1524

Table 3. Measurements (μm) of 16 morphological characters of three *Scutovertex* species used for Principle Component Analysis (PCA) and Canonical Variates Analysis (CVA) (N=40 each species). bw, body width; bd, distance between bothridia; dc, distance between cusps; cl, cuspis length; pl, prodorsum length; pw, prodorsum width; nl, notogaster length; dmw, notogastral width on level of seta *dm*; h_3w, notogastral width on level of seta h_3; gl, genital opening length; gw, genital opening width; dga, distance between genital and anal opening; al, anal opening length; aw, anal opening width; dcg, distance between camerostome and genital opening and ap3, distance between outer borders of apodeme 3.

variable	*S. ianus*	*S. minutus*	*S. sculptus*
bw	304-391 (353.7±22.43)	310-391 (355±22.77)	291-360 (328.6±20.18)
db	157-188 (176±8.07)	148-182 (163.4±8.61)	151-188 (167.2±8.96)
dc	67-87 (78.3±4.36)	67-85 (75.5±4.44)	58-77 (69.8±4.22)
cl	20-33 (27.7±3.13)	15-25 (20.3±2.63)	17-30 (23.1±3.34)
pl	133-167 (151.4±8.73)	117-157 (143.8±9.62)	120-178 (142±11.23)
pw	228-271 (250±8.86)	222-252 (238.1±7.67)	215-243 (226.8±7.86)
nl	403-471 (444.3±18)	415-508 (454.4±25.44)	353-459 (412.4±25.57)
dmw	291-378 (339.9±22.73)	298-366 (336.8±18.72)	285-347 (314±17.07)
h_3w	273-360 (322.7±19.89)	291-360 (324±20.61)	254-335 (297±20.89)
gl	85-107 (96.2±5.46)	83-103 (94.5±6.01)	72-100 (86.6±7.22)
gw	75-98 (87.2±6.02)	78-100 (89±5.68)	68-88 (79.3±6.04)
dga	73-107 (92.4±7.09)	78-107 (91.6±7.77)	72-103 (88.5±6.3)
al	110-147 (120.9±7.36)	108-148 (126.6±7.58)	97-125 (111.7±6.56)
aw	100-122 (113.1±6.76)	100-130 (117.1±6.97)	92-127 (106.7±7.72)
dcg	107-135 (126.6±6.52)	120-137 (127.8±4.36)	110-133 (123.7±5.16)
ap3	255-305 (286.5±12.16)	255-305 (285.8±12.77)	243-286 (262.4±12.12)

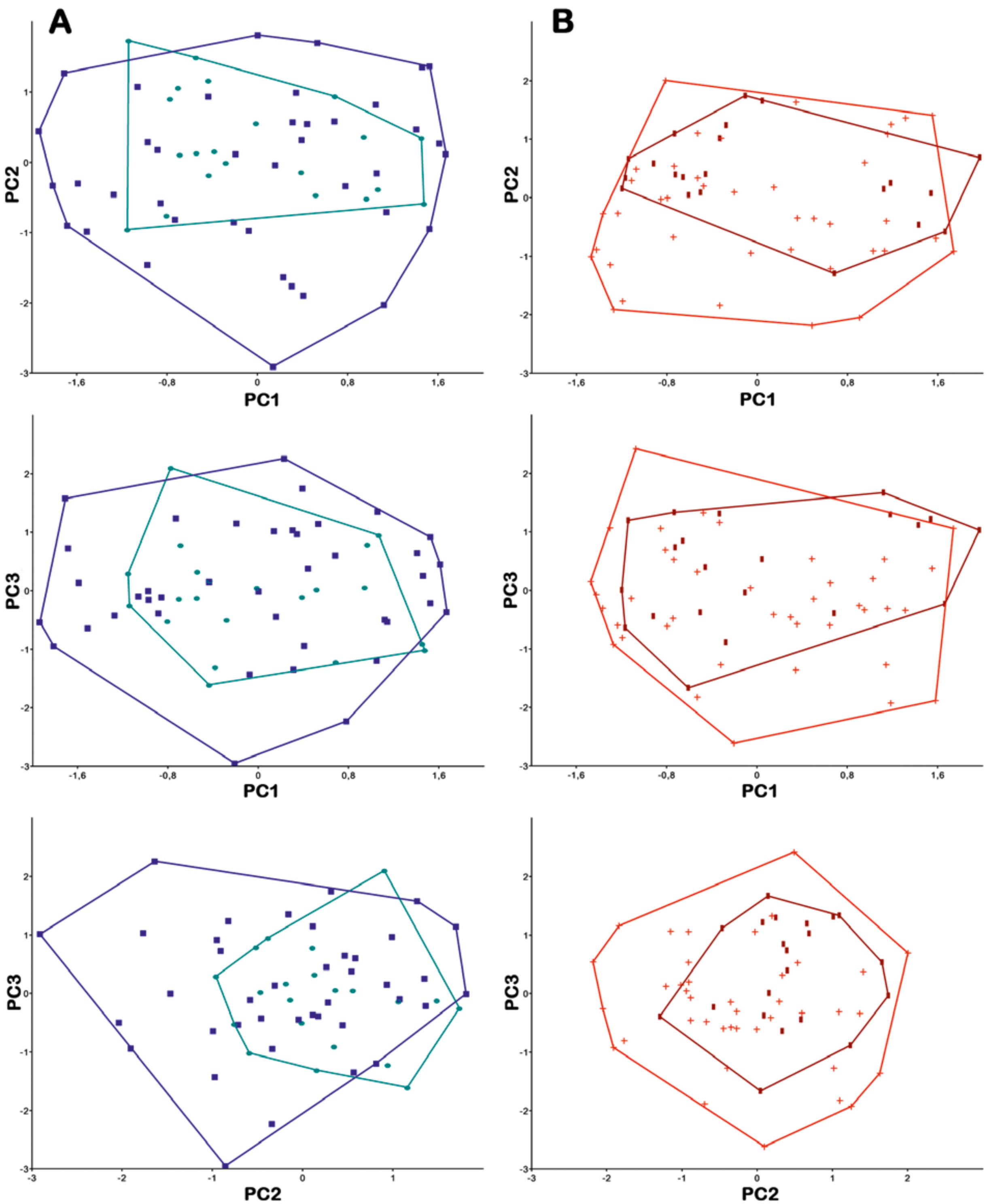

Fig. 1a-b. Projections of the principle component scores: A) *Scutovertex minutus;* ■ = population 'Bachsdorf', ● = population 'Pogier'; B) *S. sculptus;* + = population 'Illmitz', ▮ = population 'Fließ'.

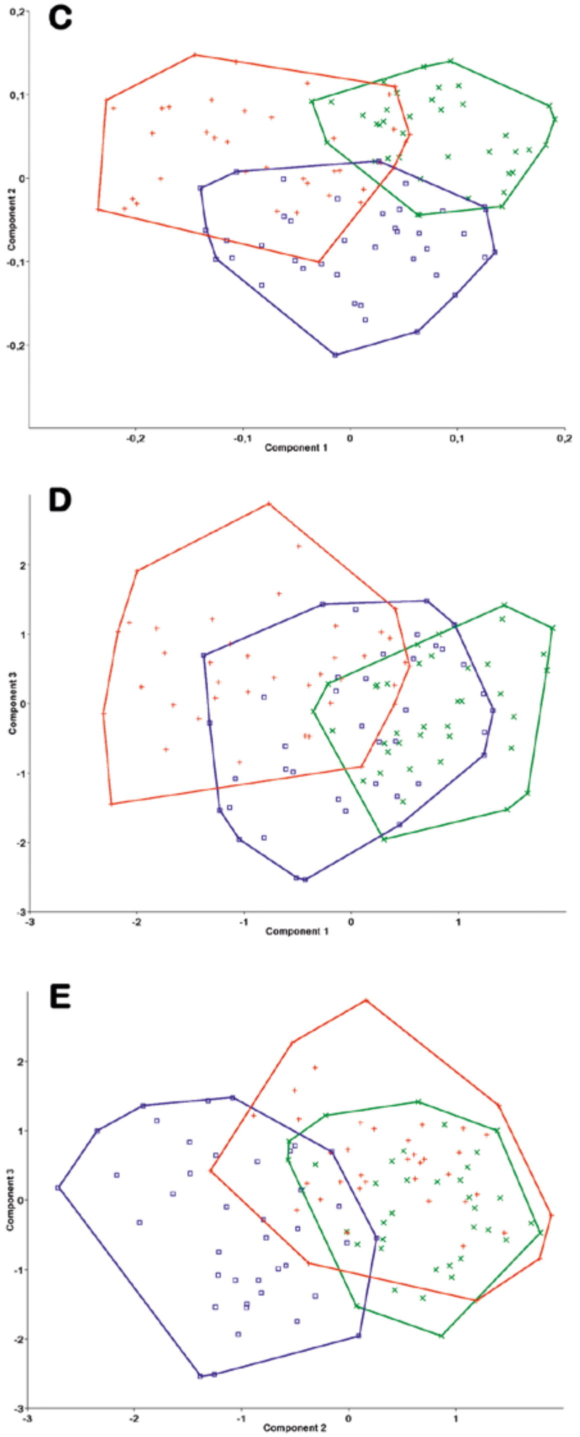

Fig. 1c-e. Scatterplots of the principle component scores: × = *Scutovertex ianus*, + = *S. sculptus*, □ = *S. minutus*.

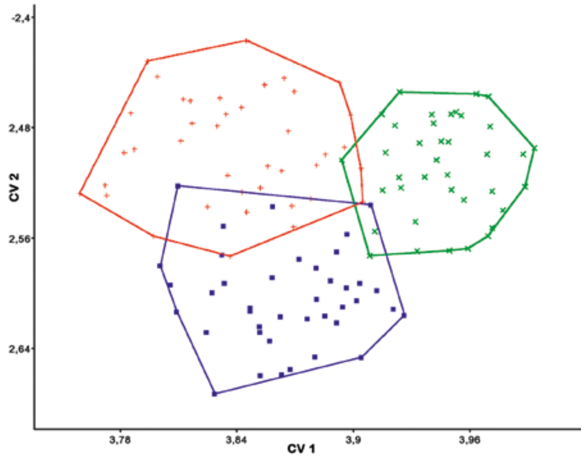

Fig. 2. Plot of canonical variate 1 versus 2: × = *Scutovertex ianus*, + = *S. sculptus*, ■ = *S. minutus*.

0.1754; A↔G = 9.9528; A↔T = 1.3001; C↔G = 0.2368; C↔T = 9.9528; G↔T = 1.0000; proportion of invariable sites: I = 0.5888; gamma shape parameter: α = 2.0069) according to sequencing analyses. Statistical support for the topology was assessed by bootstrapping (1,000 pseudo-replications). Intra- and interspecific pairwise distances (uncorrected *p*-distances) were calculated in MEGA 3.1.

Results

Morphometric analysis of Scutovertex ianus *sp. nov.,* S. minutus *and* S. sculptus

To test the intraspecific variability of *Scutovertex minutus* and *S. sculptus*, two populations of each species were analysed with the 16 variables set. The PCA performed with the two populations of *S. minutus* resulted in three components accounting for 78.7% of total variation. Loadings are given in Table 2a. The projections of the principle component scores show complete overlap of the two clusters (Fig. 1a). The analysis of the two populations of *S. sculptus* produced three components accounting for 81.8% of total variance; loadings are listed in Table 2b. The projections of the principle component scores exhibit also complete overlap of the two populations (Fig. 1b).

The Principle Component Analysis of the same variables set of *S. ianus*, *S. minutus* and *S. sculptus*

Table 4. Loadings of the 16 variables set on the first three principle components. *Scutovertex minutus* versus *S. ianus* versus *S. sculptus*.

variable	PC1	PC2	PC3
bw	0,2708	-0,1383	0,1438
db	0,1803	0,08618	0,2795
dc	0,2057	-0,07807	-0,3455
cl	0,4257	0,8529	0,0004969
pl	0,1727	0,1428	-0,4098
pw	0,1968	-0,00857	-0,01297
nl	0,2297	-0,197	0,09092
dmw	0,2569	-0,1081	0,1377
h_3w	0,2742	-0,1442	0,1883
gl	0,3103	-0,1236	-0,1113
gw	0,3038	-0,1936	-0,1238
dga	0,2313	-0,0842	0,6177
al	0,2375	-0,2093	-0,3382
aw	0,2373	-0,1694	-0,1643
dcg	0,08157	-0,04232	-0,004994
ap3	0,2162	-0,1181	-0,019

produced three components accounting for 84.6% of total variance in the data (Table 3). PC1 (50.4% of cumulative variance) shows moderate loadings for all variables indicating small differences in overall size, the variable cusp length *cl* exhibits the highest value with 0.4257 (Table 4). PC2 accounts for 30% of the total variance and again the cuspis length shows the highest value (0.8529), all the other values are very low. PC3 (4.2%) resulted in high loadings for distance between genital- and anal opening *dga* (-0.6177) and prodorsum length *pl* (0.4098). The projection of PC1 versus PC2 shows three clusters with a small overlap of all three entities (Fig. 1c). Graph PC1 versus PC3 (Fig. 1d) presents large overlaps of all three clusters and the scatterplot of the scores of PC2 versus PC3 (Fig. 1e) illustrates a nearly complete overlap of *S. ianus* and *S. minutus*.

The Canonical Variates Analysis produced a Wilk's lambda of 0.05234. The first canonical function describes 61.99% of the total variability and the second 38.01%. The F-value is 21.07 and p (same) is

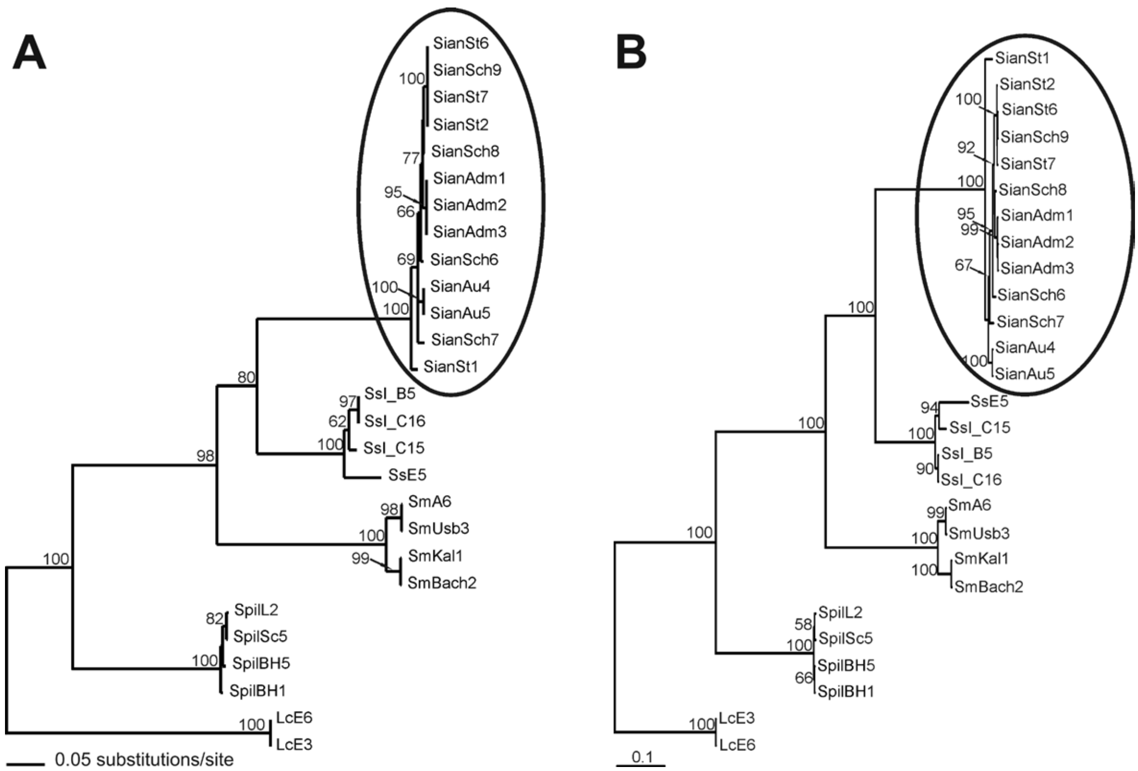

Fig. 3a-b. Phylogenetic trees reflecting genetic relationships among four *Scutovertex* species. *Lamellovertex caelatus* as outgroup: A) NJ-tree using TVM+I+G model; B) Bayesian-50%majority rule consensus-tree. Bootstrap values (NJ) and posterior probabilities (BI) are shown when >50. Encircled clade representing new species *S. ianus*; Ss... = *S. sculptus*, Sm... = *S. minutus*, Spil... = *S. pileatus*, Lc... = *L. caelatus*.

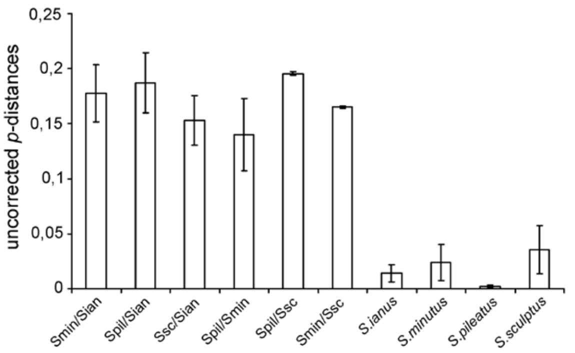

Fig. 4. Inter- and intraspecific uncorrected pairwise distances of four *Scutovertex* species. Smin = *S. minutus*, Sian = *S. ianus*, Spil = *S. pileatus*, Ssc = *S. sculptus*. First six columns displaying interspecific and last three columns representing intraspecific pairwise distances.

4.464×10^{-48}. The projection of the two Canonical Variates displays a clear separation of *S. ianus* with only small areas of overlap with the clusters of *S. minutus* and *S. sculptus* (Fig. 2). An external validation of the data with a discriminant analysis resulted in 97.44% correctly classified specimens (p-same 1.85×10^{-18}) comparing *S. minutus* with *S. ianus* and in 98.72% correctly classified individuals (p-same 1.85×10^{-21}) comparing *S. sculptus* with *S. ianus*.

Table 5. Pairwise distances. n_i = number of analysed individuals, d_{ia} = intraspecific distances (uncorrected), d_{is} = interspecific distances (uncorrected).

	n_i	d_{ia} (averaged)	d_{is} (averaged)		
			S. ianus	*S. sculptus*	*S. minutus*
S. ianus	13	0.014			
S. sculptus	4	0.024	0.157		
S. minutus	4	0.002	0.181	0.165	
S. pileatus	4	0.036	0.191	0.196	0.205

Molecular phylogenetic analysis

The NJ tree supports the monophyly of all investigated species, with bootstrap values of 80-100 for all clades, representing the species *S. ianus* sp. nov., *S. sculptus*, *S. minutus*, *S. pileatus* Schäffer and Krisper, 2007 and *Lamellovertex caelatus* (Berlese, 1895) as the outgroup. The Bayesian tree also shows a clear separation of the species with posterior probabilities of 100 (Fig. 3a-b). Mean pairwise distances within and between the *Scutovertex* species are shown in Table 5. Interspecific distance between *S. ianus* and *L. caelatus* amounted to 23.2%. Intra- and interspecific pairwise distances are plotted as histograms in Fig. 4.

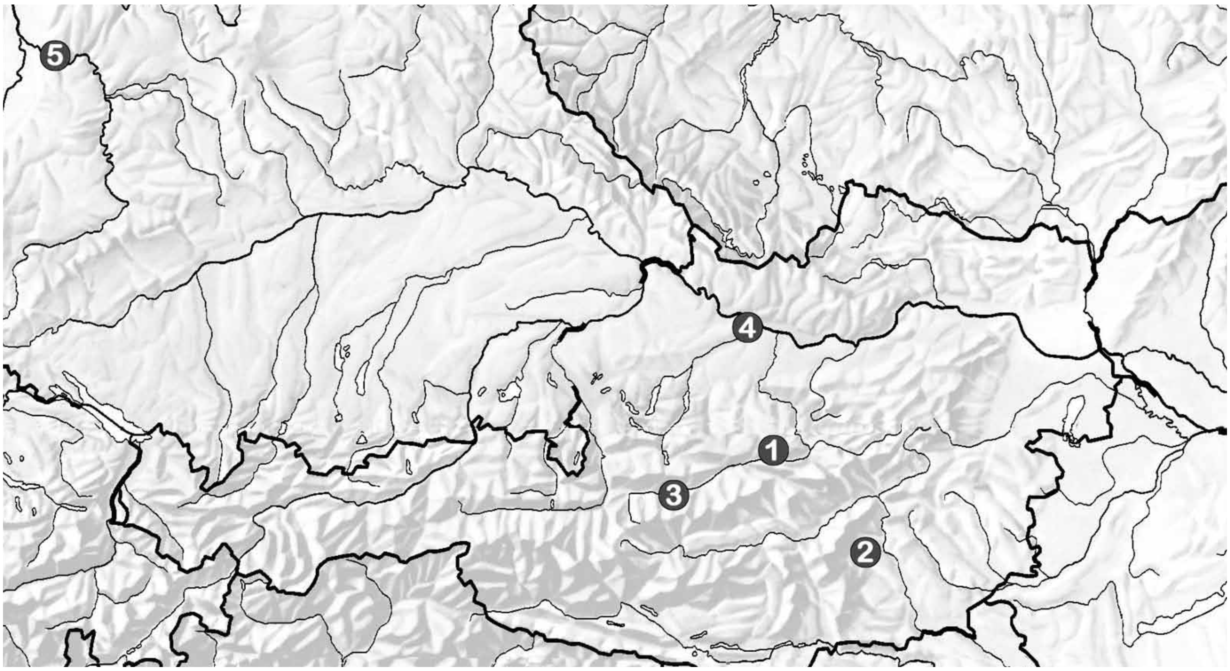

Fig. 5. Geographic distribution of *Scutovertex ianus*, numbers refer to text in results.

Geographic distribution of Scutovertex ianus *sp. nov.*

The occurrence of *S. ianus* is currently limited to areas in Central Europe (numbers in brackets refer to Fig. 5). In Austria there are four records including the 'locus typicus' Admont [1] in Styria and the other three sample locations are Stiwoll [2] and Schladming [3] in Styria and Traun [4] in Upper Austria. The fifth record of this species is Mosbach near Heidelberg [5] in Germany, where only one single specimen was found. *Scutovertex ianus* seems to live in habitats (mosses) with a high permanent humidity as the sample locality in Admont is located at the border of a bog and the area in Traun is a floodplain.

Discussion

The morphological similarity of *S. ianus* sp. nov. with *S. sculptus* and especially with *S. minutus* may lead to the assumption that this new species represents only a deviating population of one of the two above mentioned species. However a recent publication (Pfingstl *et al*., 2008) showed that *S. sculptus* individuals from different European countries are consistent with each other, the found morphological variation is negligible and the differences of *S. ianus* to *S. sculptus* are far beyond this intraspecific range of variability. Schäffer *et al*. (2007) stated that the members of *S. minutus* vary only in the number of notogastral setae, shape of lamellar cusps and of prodorsal ridges and again the members of *S. ianus* exceed these variances. The present results confirm that *S. ianus* is not just a product of the variation within one species. The differences are also not caused by sexual dimorphism, as the males and females of any *Scutovertex* species only vary in body size and the possession of a spermatopositor or ovipositor. The detailed morphological examination showed stable characters of all investigated *S. ianus* individuals and revealed that the combination of indistinct cuticular foveae on the notogaster and spiniform notogastral setae represents the set of characters being specific for this species (Table 6).

Another morphological aspect approving *S. ianus* as valid species is the structure of the exochorion. Krisper *et al*. (2008) already found out that the fine structure of the egg shell of the genus *Scutovertex* is species specific. The comparison of the exochorion of *S. ianus*, *S. minutus* and *S. sculptus* (Fig. 6a-c) reveals differing shapes of fundamental structures. The fungiform structures and granules of *S. minutus* are shaped amorphous whereas *S. ianus* and *S. sculptus* show precise formations but the tops of the fungiform formations of *S. ianus* are conspicuously more flattened than in *S. sculptus*.

The morphometric analysis of different populations of *S. minutus* and *S. sculptus* showed a complete overlap within each species in all graphs. These results demonstrate stable intraspecific morphological

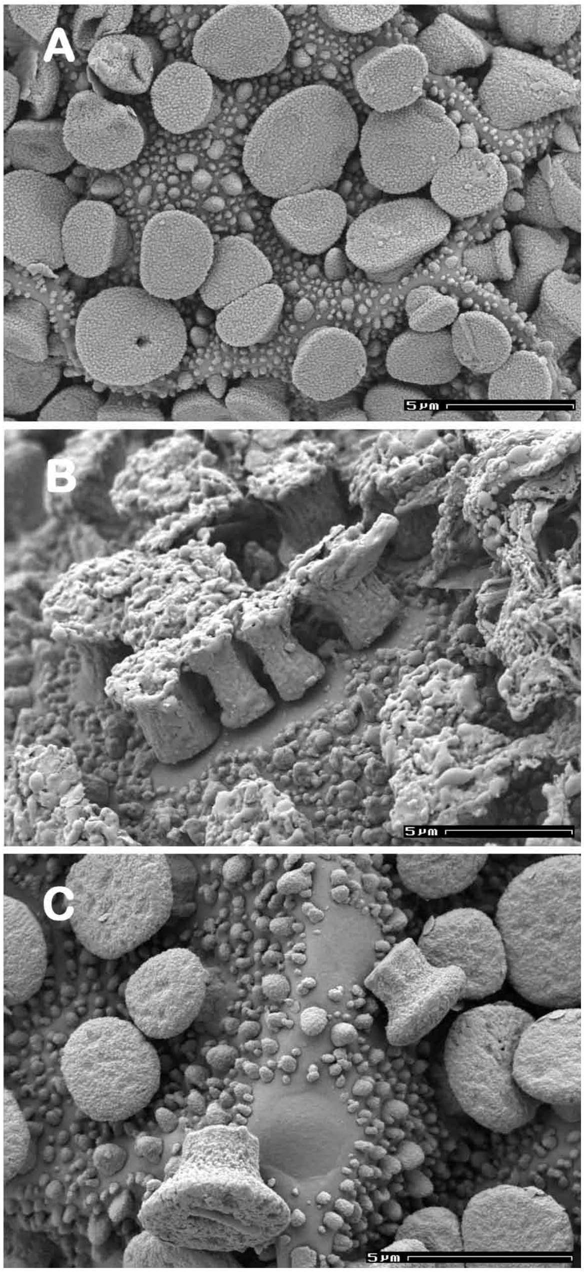

Fig. 6a-c. SEM-micrographs of exochorion structures: A) *Scutovertex ianus*; B) *S. minutus*; C) *S. sculptus*.

Table 6. Comparison of selected morphological characters of 11 *Scutovertex* species. ? = no information available.

	body length µm	prodorsal ridges	pairs of notogastral setae	seta *la*	setae *dm*, *lp*	setae h_1 and ps_1	notogastral cuticular structure	transverse ridge on mentum
S. alpinus	477-527	fused	10	smooth	spiniform, smooth	spiniform, smooth	distinct small foveae	continuous or interrupted
S. arenocolus	527-614	not fused	10	smooth	slightly broadened, spinose	broadened, spinose	indistinct foveae	interrupted
S. ianus	533-640	fused	10	smooth	spiniform, smooth	spiniform, slightly spinose	indistinct foveae	interrupted
S. mikoi	378-445	not fused	9	smooth	spiniform, smooth	slightly broadened, smooth	no foveae	mostly interrupted
S. minutus	550-659	not fused	10-12	smooth	spiniform, smooth	lanceolate, slightly spinose	no foveae	interrupted
S. pannonicus	773-800	not fused	10-11	smooth	spiniform, smooth	slightly broadened, spinose	distinct large foveae	?
S. perforatus	630-680	?	10	smooth	spiniform, slightly spinose	spiniform, slightly spinose	distinct cuticular foveae	?
S. pileatus	481-575	2 pairs not fused	10-11	smooth	spiniform,	spiniform, smooth	no foveae smooth	continuous
S. pilosetosus	655-718	fused	10	smooth	spiniform, slightly spinose	slightly broadened, spinose	indistinct foveae	lacking
S. rugosus	581-630	?	10	spinose	broadened, spinose	broadened, spinose	indistinct foveae	interrupted
S. sculptus	521-671	not fused	10	smooth	broadened, spinose	broadened, spinose	indistinct foveae	interrupted

variables and contradict the formerly supposed high intraspecific variability of these two species (Weigmann, 2006). On the basis of the stable morphology of the latter species, the multivariate analysis of *S. minutus*, *S. ianus* and *S. sculptus* confirmed the existence of a new entity with a good separation. Although there is overlap in all graphs, the projections PC1 versus PC2 and especially the CVA scatter plot exhibit a clear grouping. The projection PC2 versus PC3 fails to discriminate *S. ianus* and *S. sculptus* because the differences in body shape between these two taxa are of minor extent. *Scutovertex minutus* is well separated along the PC2-axis representing significant shape differences. In summary *S. ianus* and *S. sculptus* differ from each other mainly in body size, whereas *S. minutus* diverges from the latter two primarily in body shape. Earlier morphometric investigations of *S. minutus* and two epilittoral *Scutovertex* species (Pfingstl *et al*., 2009) using the same set and even a smaller set of variables resulted in an unambiguous separation and demonstrated clearer size and shape differences in overall morphology of these species. *Scutovertex ianus* represents nevertheless a distinct cluster bearing in mind that the well distinguishable and morphologically stable *S. minutus* and *S. sculptus* show comparable overlapping areas in this analysis.

The molecular phylogenetic analysis of the COI gene highlights the monophyly and the discreteness of all three investigated *Scutovertex* species with high bootstrap values. *Scutovertex ianus* is represented in the NJ tree as the sister group of *S. sculptus* and is therefore more closely related to the latter species than to *S. minutus*. This relationship is not clearly reflected in the morphological similarities of the species; only the exochorion structure of the eggs seems to refer to this correlation.

The combined approach of comparative morphology, morphometry and genetic data justifies *S. ianus* as an indisputable species. Different works (Strenzke, 1943; Schäffer and Krisper, 2007; Pfingstl *et al*., 2008) affirmed the clear distinction between *S. minutus* and

S. sculptus but could not explain the existence of intermediate forms. The detection of *S. ianus* leads us to a possible explanation as former findings of deviant individuals of *S. minutus* or *S. sculptus* may refer to this new species. The existence of as yet undetected species may generally induce confusion about the intraspecific variability of a species. Polderman (1977) discovered the new species *S. pilosetosus* Poldermann, 1977 and revealed that the whole Dutch collection of *S. minutus* consisted of this new species. Pfingstl *et al.* (2009) believe their recently described *S. arenocolus* Pfingstl and Schäffer, 2009 could be responsible for mistaken records of *S. minutus* in certain coastal areas and Weigmann (2009) supposes that findings of a small sized *S. minutus* in Spain may refer to his newly described *S. mikoi* Weigmann, 2009. All these facts point to a more limited intraspecific variability and to a higher species diversity of the genus *Scutovertex* than formerly supposed (Haarlov, 1957; Pérez-Iñigo, 1993; Weigmann, 2006).

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References

Berlese A. 1895. *Scutovertex caelatus* Berl. n. sp. Acari, Myriapoda et Scorpiones hucusque in Italia reperta 74.

Boyce TM, Zwick ME, Aquadro CF. 1989. Mitochondrial DNA in the bark weevils: size, structure and heteroplasmy. *Genetics* 123: 825-836.

Hammer O, Harper DAT, Ryan PD. 2001. PAST: Palaeontological Statistics software package for education and data analysis. *Palaeontologia Electronica* 4: 1-9.

Haarlov N. 1957. Microarthropods from Danish Soils. Systematics. *Spolia Zoologica Musei Hauniensis* 17: 1-60.

Heethoff M, Domes K, Laumann M, Maraun M, Norton RA, Scheu S. 2007. High genetic divergences indicate ancient separation of parthenogenetic lineages of the oribatid mite *Platynothrus peltifer* (Acari, Oribatida). *Journal of Evolutionary Biology* 20: 349-401.

Krisper G, Schuster R. 2001. Umweltansprüche und Verbreitung der Hornmilbe *Provertex kühnelti* Mihelcic, 1959 (Acari, Oribatida) in Österreich. *Mitteilungen des naturwissenschaftlichen Vereines Steiermark* 131: 141-146.

Krisper G, Schmikl M, Ebermann E. 2002. Erstnachweis der felsbewohnenden Hornmilben *Scutovertex pictus* Kunst, 1959 und *Lamellovertex caelatus* (Berlese, 1895) (Acari, Oribatida) für Österreich. *Mitteilungen des naturwissenschaftlichen Vereines Steiermark* 132: 193-196.

Krisper G, Pfingstl T, Ebermann E. 2008. SEM-Investigations on the exochorion of scutoverticid eggs. *Soil Organisms* 80: 217-221.

Koch LC. 1835. *Cepheus minutus*. Deutschlands Crustaceen, Myriapoden und Arachniden (Regensburg), Heft 3, Tab. 12.

Kumar S, Tamura K, Nei M. 2004. MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Briefings Bioinformatics* 5: 150-163.

Michael AD. 1879. A contribution to the knowledge of the British Oribatidae. *Journal of the Royal Microscopical Society London* 2: 225-251.

Mihelčič F. 1957. Oribatiden Südeuropas VII. *Zoologischer Anzeiger* 159: 44-68.

Otto JC, Wilson KJ. 2001. Assessment of the usefulness of ribosomal 18S and mitochondrial COI sequences in Prostigmata phylogeny. Pp. 100-109 in: Halliday RB, Walter DE, Proctor HC, Norton RA, Colloff J, eds., *Acarology. Proceedings of the 10th International Congress*. Melbourne: CSIRO Publishing.

Pérez-Iñigo C. 1993. *Acari, Oribatei, Poronota I. Fauna Iberica*. vol. 3. Madrid: Museo Nacional de Ciencias Naturales.

Pfingstl T, Schäffer S, Ebermann E, Krisper G. 2008. Intraspecific morphological variation of *Scutovertex sculptus* Michael (Acari: Oribatida: Scutoverticidae) and description of its juvenile stages. *Zootaxa* 1829: 31-51.

Pfingstl T, Schäffer S, Ebermann E, Krisper G. 2009. Differentiation between two epilittoral species, *Scutovertex arenocolus* spec. nov. and *Scutovertex pilosetosus* Polderman (Acari: Oribatida) from different European coasts. *Zootaxa* 2153: 35–54.

Polderman PJG. 1977. *Scutovertex pilosetosus* nov. spec. from the Netherlands (Acari, Oribatida). *Entomologische Berichten, Amsterdam* 37: 129-132.

Posada D, Crandall K. 1998. MODELTEST: testing the model of DNA substitution. *Bioinformatics* 14: 817-818.

Ronquist F, Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572-1574.

Schäffer S, Krisper G. 2007. Morphological analysis of the adult and juvenile instars of *Scutovertex minutus* (Acari, Oribatida, Scutoverticidae). *Revue Suisse de Zoologie* 114: 663-683.

Schäffer S, Krisper G, Pfingstl T, Sturmbauer C. 2008. Description of *Scutovertex pileatus* sp. nov. (Acari, Oribatida, Scutoverticidae) and molecular phylogenetic investigation of congeneric species in Austria. *Zoologischer Anzeiger* 247: 249-258.

Schäffer S, Koblmüller S, Pfingstl T, Sturmbauer C, Krisper G. (in press a). Contrasting mitochondrial DNA diversity estimates in Austrian *Scutovertex minutus* and *S. sculptus* (Acari, Oribatida, Brachypylina, Scutoverticidae). *Pedobiologia*, doi:10.1016/j.pedobi.2009.09.004.

Schäffer S., Pfingstl T, Koblmüller S, Winkler KA, Sturmbauer C, Krisper G. (in press b). Phylogenetic analysis of European *Scutovertex* mites (Acari, Oribatida, Scutoverticidae) reveals paraphyly and cryptic diversity: A molecular genetic and morphological approach. *Molecular Phylogenetics and Evolution*, doi:10.1016/j.ympev.2009.11.025.

Schuster R. 1958. Beitrag zur Kenntnis der Milbenfauna (Oribatei) in pannonischen Trockenböden. *Sitzungsberichte der Österreichischen Akademie der Wissenschaften / Mathematisch-Naturwissenschaftliche Klasse*, Abt. 1, 167: 231-235.

Sitnikova LG. 1975. Family Scutoverticidae Grandjean, 1954. Pp. 246-254 in: Ghilarov MS, Krivoluckij DA, eds., *Opredelitel obitajuščih v počve kleščej*. Moskva: Nauka.

Smrž J. 1992. The ecology of the microarthropod community inhabiting the moss cover of roofs. *Pedobiologia* 36: 331-340.

Smrž J. 1994. Survival of *Scutovertex minutus* (Koch) (Acari: Oribatida) under differing humidity conditions. *Pedobiologia* 38: 448-454.

Strenzke K. 1943. Beiträge zur Systematik landlebender Milben, I/II. *Archiv für Hydrobiologie* 40: 57-70.

Subías LS. 2004. Listado sistemático, sinonímico y biogeográphico de los Ácaros Oribátidos (Acariformes: Oribatida) del mundo. *Graellsia* 60: 3-305 (Update 2009: http://www.ucm.es/info/zoo/Artropodos/Catalogo.pdf).

Swofford DL. 2002. *PAUP* Phylogenetic analysis using parsimony (* and other methods),* version 4. Sunderland (MA): Sinauer Associates.

Weigmann G. 1973. Zur Ökologie der Collembolen und Oribatiden im Grenzbereich Land-Meer (Collembola, Insecta - Oribatei, Acari). *Zeitschrift für wissenschaftliche Zoologie* 186: 295-391.

Weigmann G. 2006. Hornmilben (Oribatida). *Die Tierwelt Deutschlands, begründet 1925 von Friedrich Dahl*. 76. Teil. Keltern: Goecke and Evers.

Weigmann G. 2009. Oribatid mites (Acari: Oribatida) from the coastal region of Portugal. III. New species of Scutoverticidae and Scheloribatidae. *Soil Organisms* 81: 107-127.

Willmann C. 1953. Neue Milben aus den östlichen Alpen. *Sitzungsberichte der Österreichischen Akademie der Wissenschaften/Mathematisch-Naturwissenschaftliche Klasse,* Abt. 1, 162: 449-519.

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Appendix

Description of Scutovertex ianus *sp. nov.*
Genus *Scutovertex* Michael, 1879

Holotype. ZMB 48049 (Museum of Natural History, department of Arachnids, Myriapods and Stemgroup Arthropoda, Humboldt-University Berlin), 1 female, Admont (Austria, Styria), moss on a rock at the edge of a bog, August 8th 2007, leg E. McCullough.

Paratypes. ZMB 48050, 1 male, 2 females; 2007/ 37861 (Senckenberg Museum für Naturkunde Görlitz, Sektion Arachnida) 1 male, 3 females; RMNH. ACARI.163 (Netherlands Centre for Biodiversity Naturalis, Leiden) 1 male, 3 females. All paratypes from the same locality as holotype.

Other material. Schladming (Styria), moss from a stone wall in front of town hall, 16th March 2005, leg S. Schäffer (Coll. Pfingstl, slides no. 608, 611); Traun (Upper Austria), moss from rocks in floodplain, June 6th 2006, leg. N. Grafeneder (Coll. Pfingstl, slide no. 1224); Stiwoll (Styria), mosses from a graveyard wall, September 6th 2006, leg. S. Schäffer (Coll. Pfingstl, slides no. 609, 610, 612); Admont (Styria), mosses on rocks, August 8th 2007, leg. E. McCullough (Coll. Pfingstl, slides no. 599-602, 762-772; material in ethanol no. 147-151).

Diagnosis. Habitus corresponding to typical *Scutovertex*. Average length 591 μm, average body width 351 μm. Colour dark brown. Cuticle pusticulate, cerotegument granulate. On anterior part of notogaster irregularly distributed foveae and irregularly shaped ribs. Cusps long, lamellae distinct. Two strongly converging and anteriorly fused ridges on interlamellar field reaching translamella. Sensillus clavate and spinose. 10 pairs of short and spiniform notogastral setae.

Differential diagnosis. *Scutovertex ianus* can be distinguished from *S. alpinus* by the large indistinct notogastral foveae and the different body size. *Scutovertex arenocolus* differs from *S. ianus* in possessing not fused prodorsal ridges and slightly broadened and spinose notogastral setae. *Scutovertex mikoi* can be

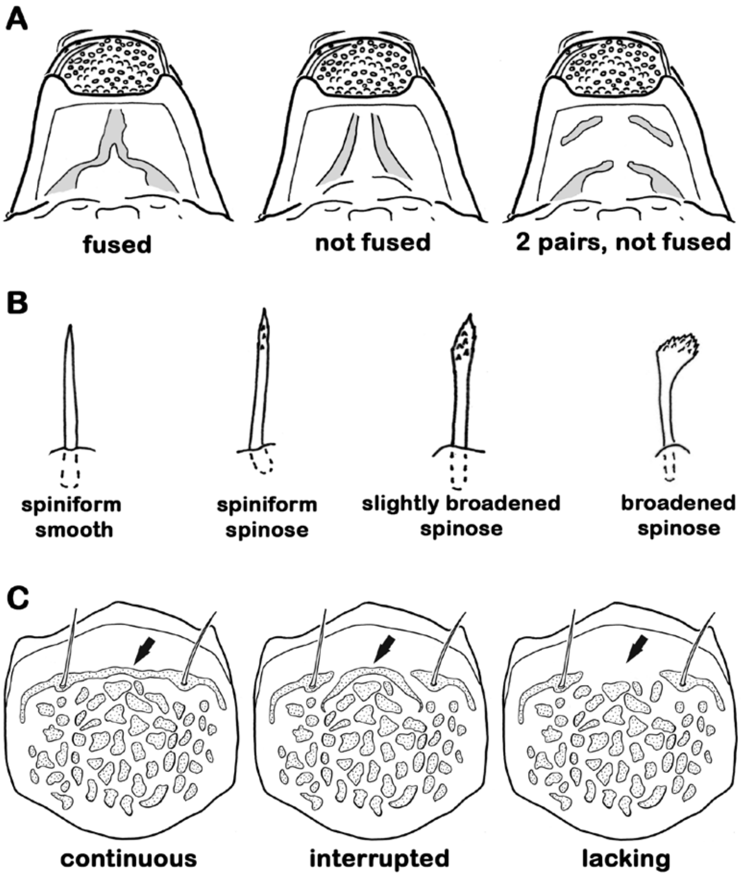

Fig. 7a-c. Examples of different character states in *Scutovertex* species (see Table 6): A) prodorsal ridges; B) shape of notogastral setae; C) transverse ridge on mentum.

distinguished from *S. ianus* by its conspicuous smaller body size, not fused prodorsal ridges, 9 pairs of notogastral setae and the lack of gastronotic foveae. The differences between *S. ianus* and *S. minutus* are the not fused prodorsal ridges and the non-existence of notogastral cuticular foveae in the latter species. *Scutovertex pannonicus* can be distinguished from *S. ianus* by its obvious larger body size and distinct large cuticular foveae on the notogaster. *Scutovertex perforatus* also possesses distinct cuticular foveae and differs in this aspect from *S. ianus*. *Scutovertex pileatus* can be distinguished from *S. ianus* by the not fused two pairs of prodorsal ridges, the absence of cuticular foveae and the continuous transverse ridge on mentum. *Scutovertex pilosetosus* is distinctly larger than *S. ianus* and lacks the median part of the transverse ridge on the mentum. *Scutovertex rugosus* shows different notogastral setae and *S. sculptus* can be distinguished from *S. ianus* by the not fused prodorsal ridges and broadened and spinose posterior notogastral setae (Fig. 7a-c, Table 6).

Description of holotype. Body length (N=49) 533-640 μm. Body width (N=46) 304-391 μm. Prodorsum: cuticle strongly pusticulate. Sensillus (Se) long, clavate, serrate and flattened. Cup-like bothridia with a posterior ridge running caudally (Fig. 8a). Interlamellar and exobothridial setae absent. Lamellae slightly converging, connected by translamella. Long cusps, lamellar setae (*le*) spiniform and serrate. Rostral setae (*ro*) robust and smooth strongly bent inwards. Two strongly converging ridges between lamellae fused in anterior part of interlamellar field reaching translamella. V-shaped tutorium strongly projecting (Fig. 8b). Gastronotic region (Figs 9a, 10a): cuticle pusticulate with indistinct foveae on anterior half of notogastral region, cerotegument granulate. Lenticulus with lateral concave margins, posterior part broadened. Posterior to lenticulus irregularly shaped ridges. Ten pairs of short spiniform notogastral setae: c_2, *la*, *dm*, *lp*, h_1-h_3, ps_1-ps_3 (Fig. 9c). Setae $h_{1\text{-}3}$ and $ps_{1\text{-}3}$ with minimal serration (Fig. 10b), all setae except c_2 and *la* inserted on small humps. Humeral angle well developed. Five pairs of lyrifissures on gastronotic region: *ia*, *im*, *ih*, *ips, ip*; *ia* located laterally on a small cuticular nodule ventrally to humeral angle (Figs 8a, 11a). Lyrifissure *im* slit-shaped, laterally and posterior to seta *la*; *ih* and *ips* next to each other on posterior lateral border of the notogaster and *ip* between setae h_1 and ps_2. Orifice of opisthonotal gland next to seta *lp*, well discernable. Three pairs of small sacculi $S_{1\text{-}3}$ representing octotaxic system, S_A not developed. Subcapitulum and camerostome: cuticle strongly pusticulate. Inner margin of camerostome consisting of rostrophragma. Median rostral lobe forming anterior border of camerostome and longish triangular lamellae flanking rostrophragma laterally (Fig. 9b). Mentum with long spiniform setae *h* and discontinued variable transverse ridge (Fig. 11b). Setae *a* and *m* long acuminate and slightly serrate. Rutellum broad with three teeth, first one largest. Pedipalp pentamerous, chaetome: 0-2-1-3-9 (solenidion excluded), solenidion inclined reaching eupathidium *acm* (Fig. 11c). Longish porous axillary sacculus *as* located at basis of pedipalp. Ventral region of idiosoma (Fig. 9b): cuticle pusticulate forming distinct ribs on posterior part of ventral idiosoma. Epimeral setation (I-IV): 3-1-2-2. Pedotectum I (PtI) large, covering acetabulum I completely. Pedotectum II (PtII) also large, Y-shaped in dorsal view and drop

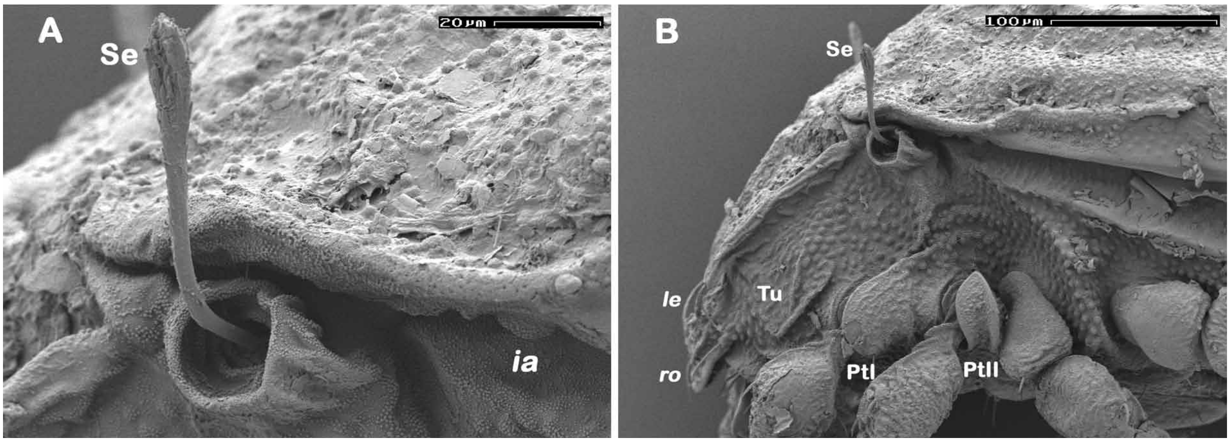

Fig. 8a-b. Scutovertex ianus SEM-micrographs: A) bothridium and sensillus; B) prodorsum in lateral view.

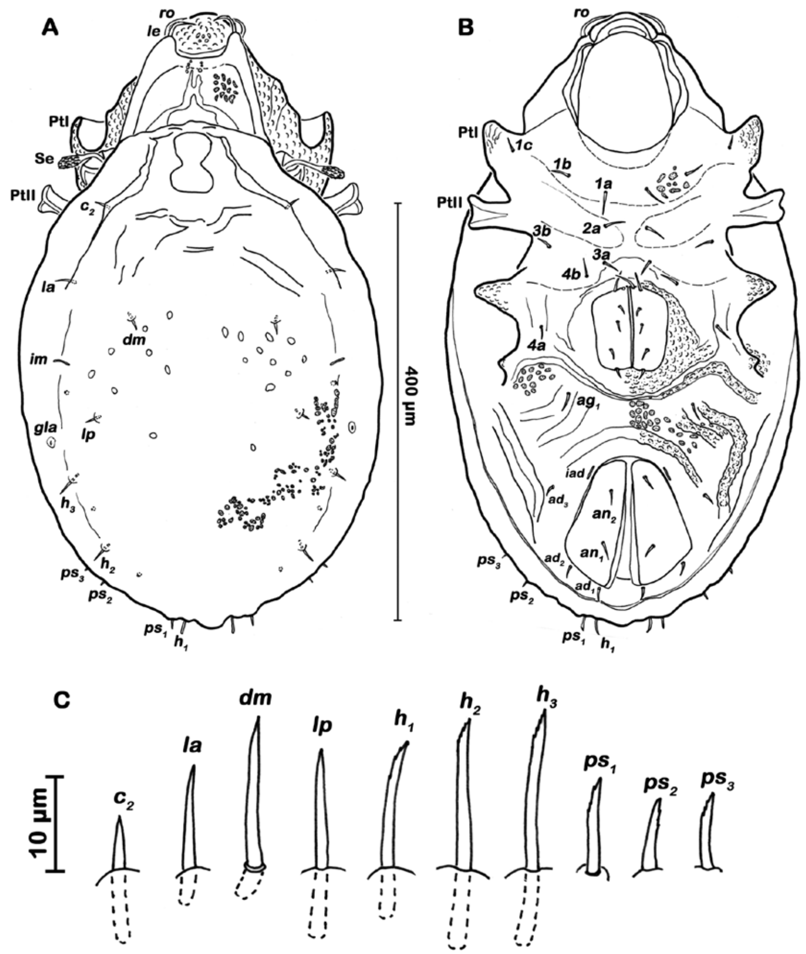

Fig. 9a-c. Scutovertex ianus (female): A) dorsal view; B) ventral view; C) notogastral setae in detail.

shaped in lateral view (Figs 8b, 11a). Genital setation 6+6 (no variations observed). The first two genital setae the longest, inserting side by side, the others arranged in a longitudinal row. Genital valves anteriorly broadened and surrounded by a circular cuticular elevation. A rostrad arcuated cuticular rib anterior to genital opening. One pair of spiniform aggenital setae *ag*. Posterior to genital opening whole ventral region crossed transversely by a narrow and slightly caudad curved rib. Two pairs of anal setae, an_{1-2}. Anal valves posteriorly broadened. Three pairs of acuminate adanal setae ad_{1-3}. Lyrifissure *iad* in paraanal position. Preanal organ cup-shaped. Legs (Fig. 12a-d): tridactyl, heterodactyl, claws dorsally slightly dentate; median claw largest. Cuticle from trochanter to femur pusticulate and from genu to tarsus rugose, forming a few strong ribs. Tibial setae robust and serrate. Ventral and lateral tarsal setae also serrate. Solenidia φ_{1-2} inserting distally on large apophysis of tibia I. Legs showing *Scutovertex*-typical tracheae. Leg chaetome without solenidia: I (1-4-3-4-18), II (1-4-3-4-15), III (2-2-1-3-15), IV (1-2-2-3-12). Solenidia: I (1-2-2), II (1-1-2), III (1-1-0), IV (0-1-0). Eggs (Fig. 13a-b): the exochorion consists of two different structures which are dispersed over the whole endochorion. There are large fungiform formations and smaller cones each of them with a granular surface.

Remarks. Unfortunately, the holotype and paratypes of *S. minutus* of the collection of C.L. Koch are missing (see Schäffer and Krisper, 2007: 676). Furthermore, no information on the whereabouts of the type specimens of *S. sculptus* is available. Therefore the identification of these two species is based on the detailed descriptions given by Schäffer and Krisper (2007) and Pfingstl *et al*. (2008). Sample locations for *S. minutus*: Asparn, Unterstinkenbrunn (Lower Austria), mosses and lichens on a tiled roof, 16th November 1996, leg. E. Ebermann; Graz, Bachsdorf (Styria), mosses on a roof, 8th May 2005, leg. J. Jagersbacher-Baumann; Pogier (Styria),

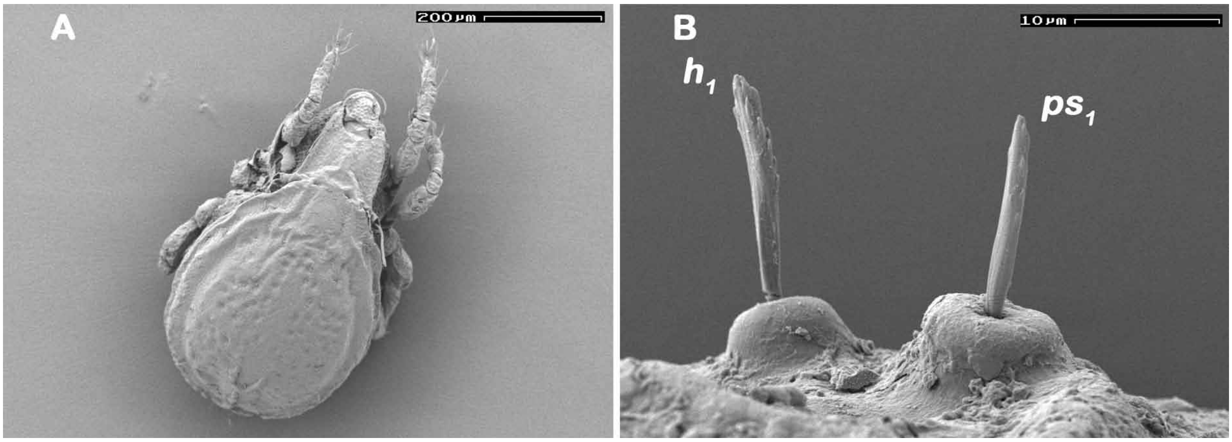

Fig. 10a-b. Scutovertex ianus SEM-micrographs: A) dorsal view; B) Setae h_1 and ps_1 in detail.

mosses on rocks, 12th October 2006, leg. S. Schäffer; *S. sculptus*: Illmitz (Burgenland), shore of lake Zicklacke, 16th September 2005, leg. G. Krisper; Ernstbrunn (Lower Austria), mosses on a rock, 29th March 2005, leg. E. Ebermann; Fließ (Tyrol), sun exposed mosses, 11th June 2007, leg. H. Schatz; *S. pileatus*: all from mosses on rocks, Laas, 28th March 2005, leg. S. Schäffer, Schütt, 8th August 2005, leg. S. Schäffer, Ruin Hochosterwitz, 26th June 2005 (Carinthia), leg. S. Schäffer.

Etymology. The new species shares certain morphological characteristics with *Scutovertex minutus* and

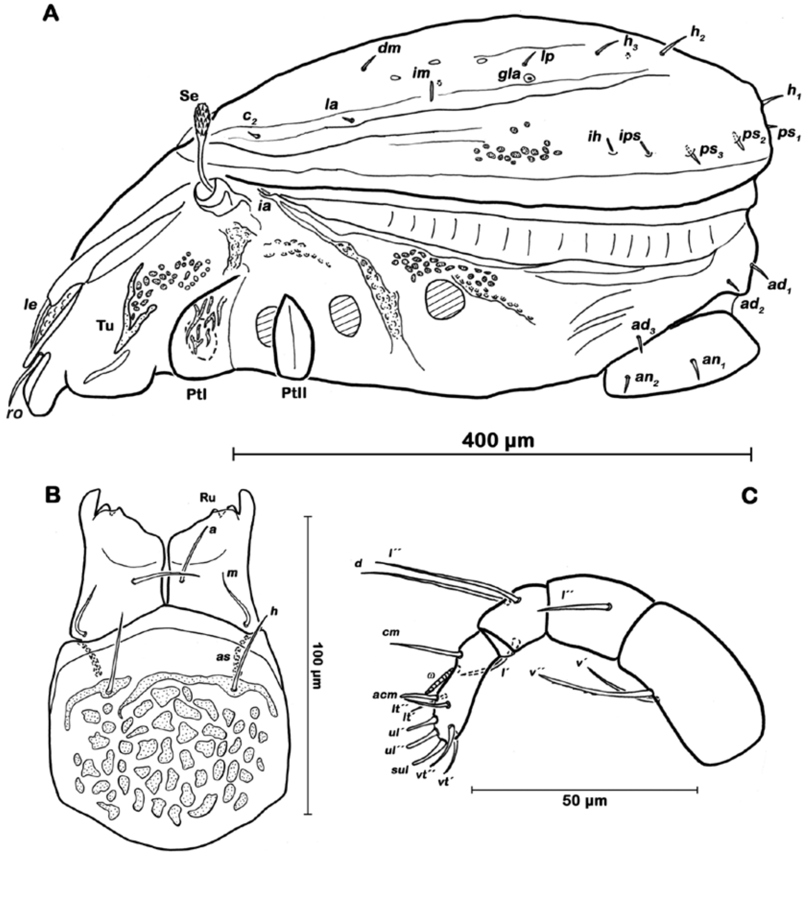

Fig. 11a-c. Scutovertex ianus (male): A) lateral view; B) subcapitulum; C) left pedipalp antiaxial view.

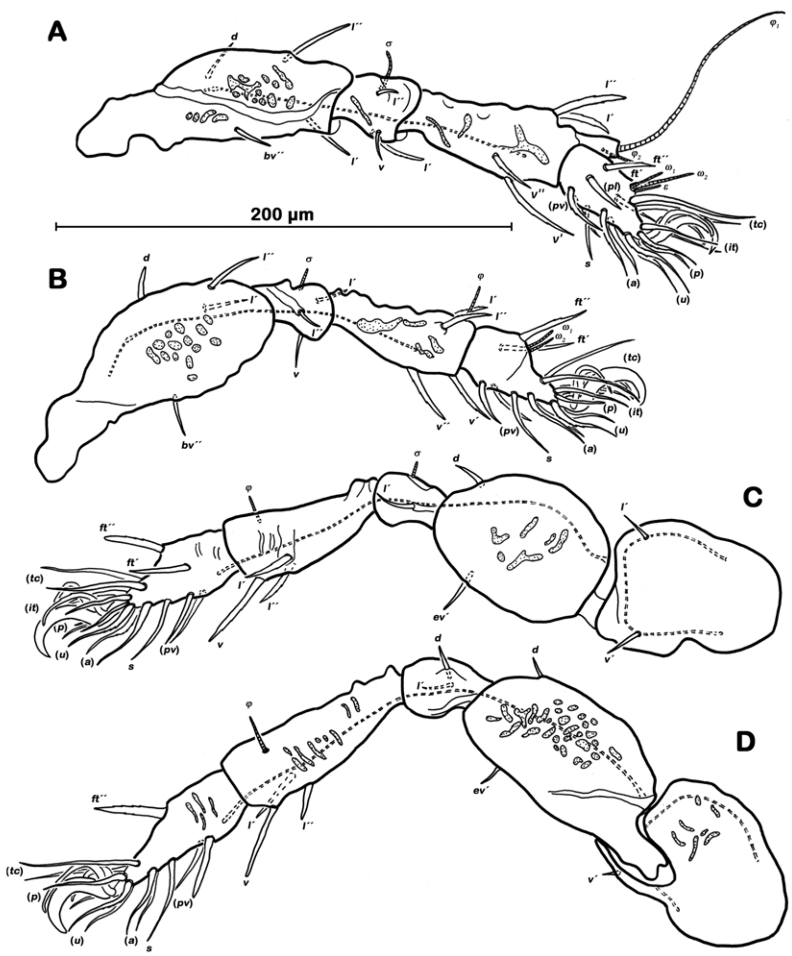

Fig. 12a-d. Scutovertex ianus (female): A) right leg I antiaxial view; B) right leg II antiaxial view; C) right leg III antiaxial view; D) left leg IV paraxial view.

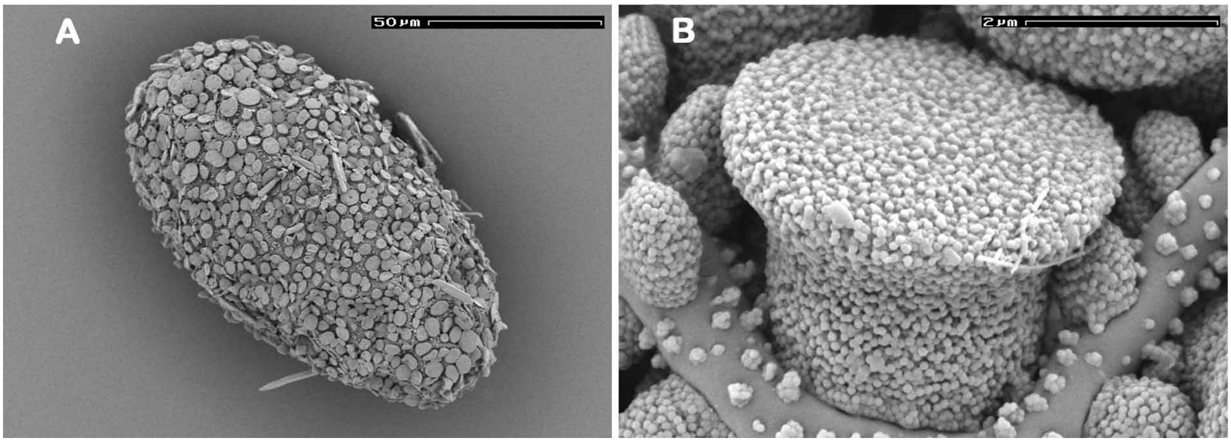

Fig. 13a-b. Scutovertex ianus SEM-micrographs: A) egg; B) exochorion structure in detail.

S. sculptus resulting in an intermediate appearance. Therefore the species is named according to 'Ianus' the two headed Roman god who is also a symbol for ambivalence.

Identification key for European *Scutovertex* species

Diagnosis for the genus *Scutovertex* (according to Weigmann 2009, with minor modification).

Notogaster ovoid and medially fused with prodorsum; lamellae distinct but slender, with cuspis; translamella narrow; interlamellar setae absent; camerostome with separated rostrophragma inside border of rostrum forming a second anterior border, without genal incision; notogaster with longish lenticulus. 9-12 pairs of notogastral setae. Octotaxic system usually present as 3 pairs of saccules; anogenital setation: 6 *g*, 1 *ag*, 2 *an*, 3 *ad*; epimeral setation 3-1-2-2; tutorium weakly developed; pedotecta I and II well developed; legs tri- and heterodactylous; trochanter III and IV with tracheae curving along inner wall; all femora with tracheae reaching into tibia.

1	cuticular foveae on gastronotic region	2
-	cuticular foveae on gastronotic region absent	8
2	(1) foveae indistinct	3
-	foveae distinct	10
3	(2) prodorsal ridges convergent	4
-	prodorsal ridges anteriorly fused, body length 655-718 μm	*S. pilosetosus* Poldermann, 1977
4	(3) notogastral setae broadened	5
-	notogastral setae spiniform, body length 533-640 μm	*S. ianus* sp. nov.
5	(4) cuspis length > 15μm	6
-	cuspis length < 15μm, body length 527-614μm	*S. arenocolus* Pfingstl and Schäffer, 2009
6	(5) setae *la* barbed	7
-	seta *la* smooth, body length 521-671μm	*S. sculptus* Michael, 1879
7	(6) cuticle on gastronotic region granular, body length 630-680μm	*S. perforatus* Sitnikova, 1975
-	cuticle on gastronotic region with oblong lines, body length 581-630μm	*S. rugosus* Mihelčič, 1957
8	(1) cerotegument granular	9
-	cerotegument forming thick nodes, body length 481-575μm	*S. pileatus* Schäffer and Krisper 2007
9	(8) 9 pairs of notogastral setae, body length 378-445μm	*S. mikoi* Weigmann, 2009
-	10-12 pairs of notogastral setae, body length 550-659μm	*S. minutus* (C.L. Koch, 1835)
10	(2) large cuticular foveae on gastronotic region, body length 773-800μm	*S. pannonicus* Schuster, 1958
-	small cuticular foveae on gastronotic region, body length 477–527μm	*S. alpinus* Willmann, 1953