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Morphometrics and SEM analysis of the species pair *Lithobius mutabilis* L. Koch, 1862 and *L. glacialis* Verhoeff, 1937 (Chilopoda: Lithobiomorpha)

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Abstract

The sets of morphological characters of two nominal species in the genus *Lithobius* Leach, 1814, *Lithobius mutabilis* L. Koch, 1862 and *Lithobius glacialis* Verhoeff, 1937, were studied in order to test specific dissimilarity. Morphometrics were examined and analysed statistically. In addition, a comparative SEM analysis of external characters was made. Morphometrically significant differences between the species were found concerning body length, the width ratio of the head to the 5th tergite, the number of antennal articles, and the length/width proportions of certain articles (e.g. the femur) of the 15th legs of males. Because of these and several additional differences, such as in tergite surface structure, modifications to the 15th legs of males, the structure of the female gonopod claws, and between the ecological profiles, *L. glacialis*, which is found in the high Alps at altitudes above timberline (1300 m), can be distinguished clearly from *L. mutabilis*. This study demonstrates another example for a cryptic species pair, a common phenomenon between lowland and alpine populations of small invertebrates. The combination of morphometrics and SEM offers a powerful methodology for resolving previously uncertain questions in the species-level taxonomy of centipedes.

Keywords: Morphometrics; Species pair; *Lithobius glacialis*; *L. mutabilis*; Species distinction; Scanning electron microscopy

Introduction

A very interesting area for taxonomic analyses on European lithobiids is the alpine region, because the species occurring there are barely studied and several taxa, already described from other places or still unknown to science, might await discovery. In an alpine pasture in Bavaria, specimens were collected some years ago (Spelda 1999) that could be assigned to *Lithobius glacialis* Verhoeff, 1937, a species neglected by most subsequent authors.

Using the common keys to species (Brölemann 1930; Matic 1966; Eason 1982; Koren 1992) these alpine lithobiid specimens could not be determined unequivocally. They were very similar to *Lithobius mutabilis* L. Koch, 1862 according to the key by Koren (1992). Males, however, do not show the characteristic modifications to the 13th–15th legs that are typical for *L. mutabilis.* The key by Eason (1982) also fails at a certain point, because further determination can be made on characters of male specimens only. In this case the key

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leads to *Lithobius valesiacus* Verhoeff, 1935, but the spinulation of the 15th legs does not fit, as it is the same as in *L. mutabilis*.

The specimens in question are different from *L. mutabilis* in several aspects; for instance, they exhibit wrinkled rather than smooth tergite surfaces as in *L. mutabilis*. Moreover, they were collected in higher regions of the Alps above 1300 m, where *L. mutabilis* has never been found.

It was noticed by Spelda (1999) that Verhoeff (1937) had already described a species, *L. glacialis*, from two female specimens with wrinkled tergites and the same ventral spinulation of the 15th legs as is characteristic of *L. mutabilis*. Verhoeff (1937) found them under similar geographical and ecological conditions, and thus the female specimens could be determined as *L. glacialis*.

In the recent collection, however, male specimens were found as well. Because of the same ventral spinulation of the 15th legs, the wrinkled tergites, the remarkable ecological profile, and the lack of strong tibial modifications on the 13th legs, they were assigned to *L. glacialis*.

Table 1. Means and standard deviations (separated by slashes) of morphometrics for both sexes pooled

Character	*L. mutabilis*	*L. glacialis*
BL (mm)	14.56/1.097 ($n = 18$)	11.47/1.375 ($n = 17$)
Head/Tg 5	0.931/0.026 ($n = 18$)	0.992/0.017 ($n = 15$)
Ant. art.	41.15/2.154 ($n = 13$)	33.67/2.497 ($n = 18$)

All species differences are statistically significant (see Table 4). BL = body length, Tg 5 = 5th tergite, Ant. art. = antennal articles.

Investigation of the literature resulted in no further records of *L. glacialis* by any author except Spelda (1999). Only in a few faunistic and/or ecological studies (Verhoeff 1940; Attems 1949; Würmli 1972; Spelda 2004) the species is cited in lists among many others.

The purpose of the present study was to look for more characters distinguishing *L. mutabilis* and *L. glacialis*, two nominal species that seem closely related and hence can be seen as a species pair. The males especially deserved a closer look, as they had not been fully described. Some differences already found by Spelda (1999) are examined in greater detail below.

This is done on the one hand by morphometrical analysis as suggested by Folkmanova (1927). Tobias (1969), too, was successful in analysing several morphometrical characters concerning the genus *Lithobius*. In addition, Tobias examined distinctive characters using SEM, a method rarely used by European authors concerning this genus.

Preliminary accounts of the results presented here concerning morphometric analysis were given in Pilz (2005a, b).

Material and methods

Material

The investigated specimens were collected by Barbara Kenter (BK), Joachim Voith and Dorothee Leipold (JVDL), Anja Fenzl (AF), Christian Pilz (CP), and Jörg Spelda (JS). Registration numbers have been assigned to identify single specimens. The specimens analysed in this study are stored at the Bavarian State Collection of Zoology (ZSM).

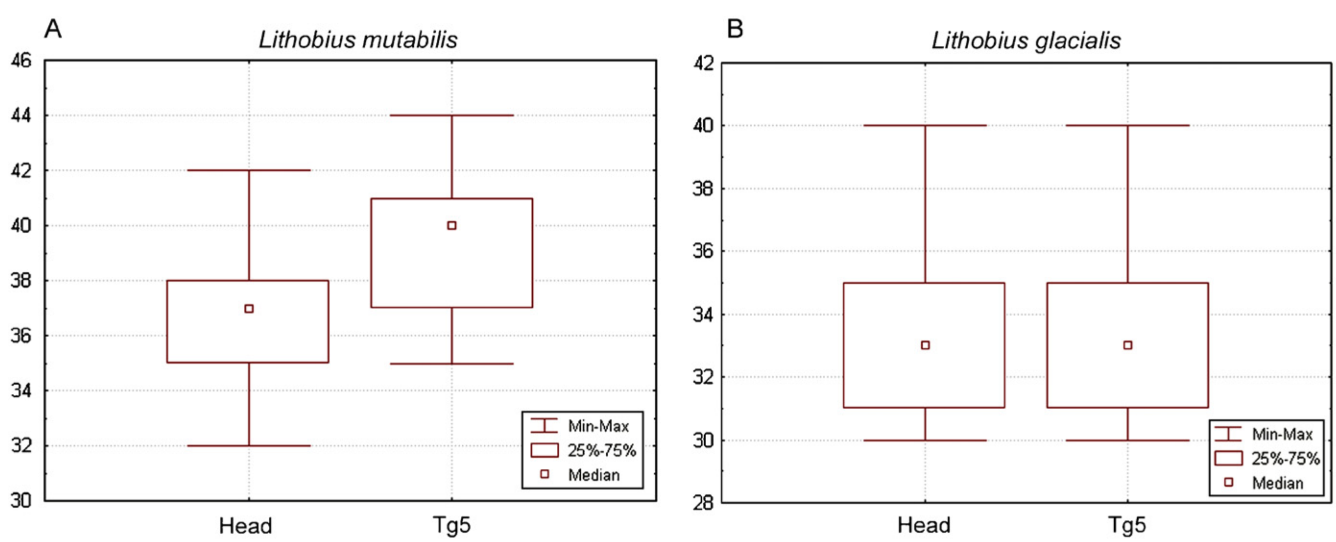

Fig. 1. Boxplot diagrams of widths (in mm) of head and 5th tergite (Tg5), sexes pooled. (A) *Lithobius mutabilis*. (B) *L. glacialis*.

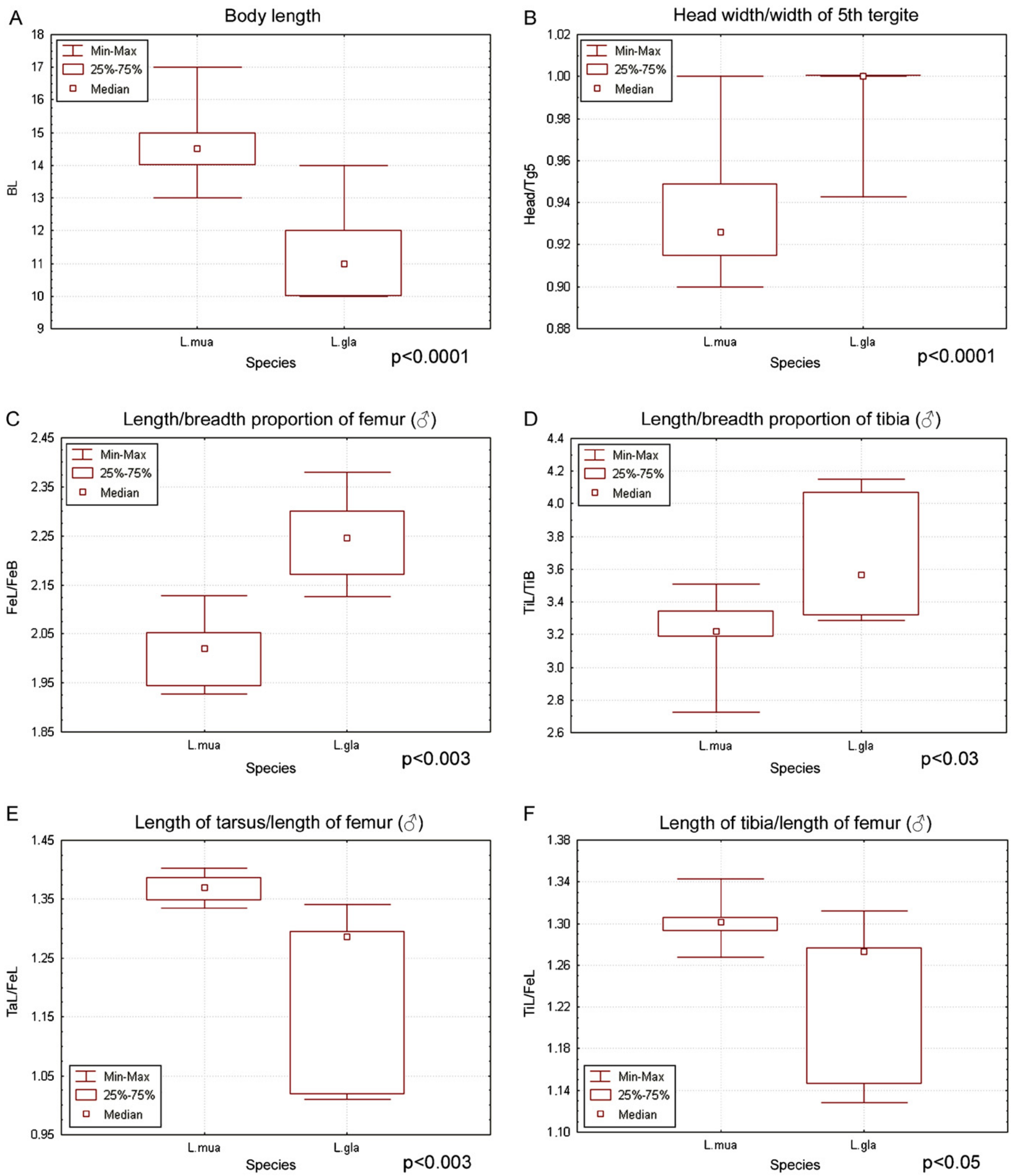

Fig. 2. Boxplot diagrams of morphometrical characters showing significant differences between *Lithobius mutabilis* and *L. glacialis*. (A) Body length (in mm), sexes pooled. (B) Head/5th tergite width ratio, sexes pooled. (C) Length/breadth of male femur. (D) Length/breadth of male tibia. (E) Male tarsus/male femur length ratio. (F) Male tibia/male femur length ratio. Abbreviations: BL = body length, FeB = femur breadth, FeL = femur length, L. gla = *Lithobius glacialis*, L. mua = *Lithobius mutabilis*, TaB = tarsus breadth, TaL = tarsus length, TiB = tibia breadth, TiL = tibia length, Tg5 = tergite 5.

L. glacialis Verhoeff, 1937

AUSTRIA: Salzburg, Fuscher Lacke 8 km N Heiligenblut, ca. 2400 m [47.100°N 12.816°E]: 7 males (CP050127001–CP050127006, CP050607003), 4 females (CP050215001–CP050215002, CP050607002, CP050607004); 07.10.1995; leg. JS.

AUSTRIA: Steiermark, trail above Handlerweg SW Pfaffensattel, ca. 1300 m [47.550°N 15.800°E]: 1 male (CP050131001), 1 female (CP041210002); 24.09.1997; leg. JS.

AUSTRIA: Oberösterreich, Krippenstein to Heilbronner Weg, 4 km S Obertraun, 1850–2100 m [47.516°N 13.683°E]: 4 females (CP050113005, CP050304005, CP050607001, CP041209005); 21.09.1997; leg. JS.

AUSTRIA: Steiermark, Sölkerpaß, Römerwegkehre, 5 km SSE St. Nikolai, 1700–1750 m [47.266°N 14.066°E]: 2 females (CP041209009, CP050704002); 23.09.1997; leg. JS.

AUSTRIA: Oberösterreich, Gablonzer Hütte to Krautgarten-Alm, 6 km SW Gosau, 1250–1485 m [47.533°N 13.483°E]: 1 male (CP050131002); 17.09.1997; leg. JS.

AUSTRIA: Niederösterreich, Raxalpe-Bergstation; 1550–1650 M [47.716°N 15.766°E]: 1 female (CP050113006); 25.09.1997; leg. JS.

AUSTRIA: Steiermark, Hochschwabgipfel (2270 m) [47.616°N 15.266°E]: 1 female (CP050518001); 28.07.1997.

GERMANY: Bayern, Ramsau, Reiteralm/Hirscheck (1600 m) [47.652°N 12.820°E]: 1 male (A20040199), 1 female (A20040200); 26.09.1996; leg. JVDL.

L. mutabilis L. Koch, 1862

GERMANY: Baden-Württemberg, Englenghäu 3 km NO Bernstadt, 5 km NW Langenau, 550–570 m [48.516°N 10.050°E]: 1 male (CP041209002); 20.04.1993: 2 males (CP041209003, CP050113001); 24.05.1993: 2 females (CP050215007, CP050215005); 25.05.1993: 1 male (CP050704004); 27.05.1993: 1 male (CP050308001), 4 females (CP050215004, CP041209008,

Table 2. Means and standard deviations (separated by slashes) of 15th leg proportions in males of *L. mutabilis* and *L. glacialis*

Character	*L. mutabilis* ♂ ($n = 7$)	*L. glacialis* ♂ ($n = 7$)
FeL/FeB	2.010*/0.070*	2.249*/0.086*
TiL/TiB	3.202*/0.238*	3.626*/0.348*
TaL/TaB	4.728/0.567	4.844/0.461
TaL/FeL	1.369*/0.024*	1.215*/0.139*
TiL/FeL	1.302*/0.022*	1.239*/0.072*

*Indicates statistically significant species differences (see Table 4); B = breadth, Fe = femur, L = length, Ta = tarsus, Ti = tibia.

Table 3. Means and standard deviations (separated by slashes) of 15th leg proportions in females of *L. mutabilis* and *L. glacialis*

Character	*L. mutabilis* ♀ ($n = 8$)	*L. glacialis* ♀ ($n = 8$)
FeL/FeB	2.185*/0.105*	2.387*/0.127*
TiL/TiB	3.597/0.191	3.633/0.333
TaL/TaB	5.108/0.671	4.939/0.659
TaL/FeL	1.272/0.099	1.183/0.117
TiL/FeL	1.290/0.036	1.222/0.079

*Indicates statistically significant species differences (see Table 4); B = breadth, Fe = femur, L = length, Ta = tarsus, Ti = tibia.

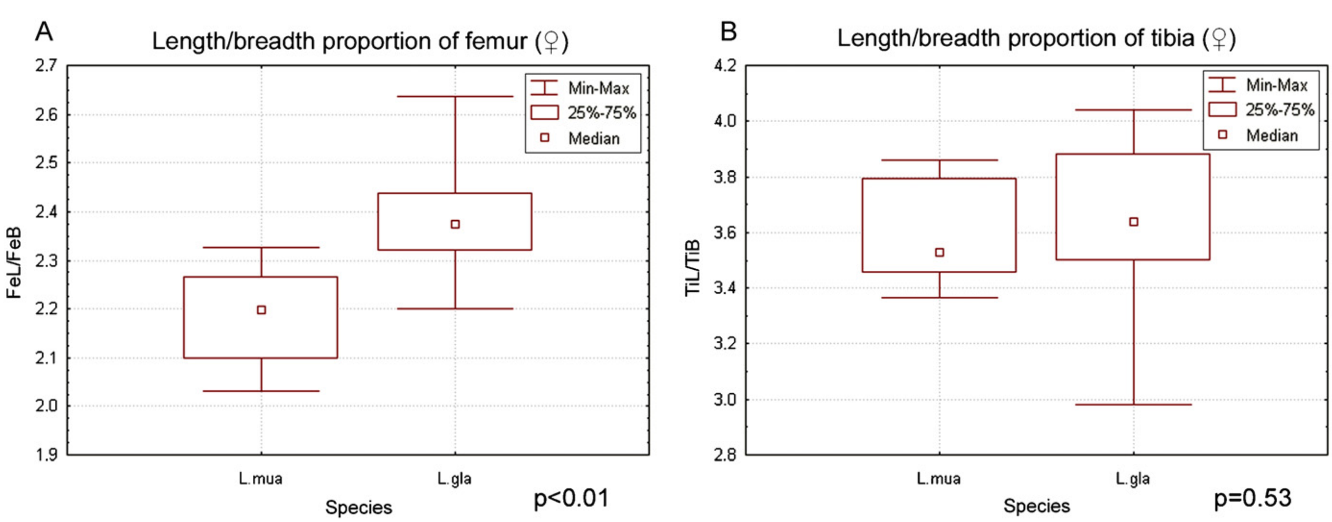

Fig. 3. Boxplot diagrams of differences in 15th leg proportions between females of *Lithobius mutabilis* and *L. glacialis*. (A) Length/breadth of femur. (B) Length/breadth of tibia. Abbreviations: FeB = femur breadth, FeL = femur length, L. gla = *Lithobius glacialis*, L. mua = *Lithobius mutabilis*, TiB = tibia breadth, TiL = tibia length.

Table 4. Summary of *p*-values for all tested morphometrical comparisons between *Lithobius mutabilis* and *L. glacialis*

Character	BL	Head/Tg 5	Ant. art.	FeL/FeB	TiL/TiB	TaL/TaB	TaL/FeL	TiL/FeL
Sex								
♂	0.000005*	0.000009*	0.000006*	0.003*	0.013*	0.850	0.003*	0.048*
♀				0.005*	0.530	1.000	0.090	0.070

*Indicates significant differences; BL = body length, Tg 5 = 5th tergite, Ant. art. = antennal articles; B = breadth, Fe = femur, L = length, Ta = tarsus, Ti = tibia.

CP050215008, CP050215009); 19.08.1993: 3 males (CP050113002–CP050113003, CP041209001), 1 female (CP050304004); 24.03.1993, leg. BK.

GERMANY: Bayern, 1 km SW Papiermühle, 2 km SW Pappenheim, 440–470 m [48.921°N 10.949°E]: 2 females (CP050215003, CP050215006); 01.11.2004; leg. JS.

GERMANY: Baden-Württemberg, Eislingen, 300–500 m [48.698°N 9.725°E]: 1 male (CP050517010); 15.05.2005; leg. CP.

GERMANY: Bayern, München, Obermenzing, Kapuzinerhölzl, 510–520 m [48.167°N 11.494°E]: 4 females (CP050517006–CP050517009); 13.07.2005; leg. AF.

Collecting methods, fixation

The animals were collected either using pitfall traps or by hand sampling. They were fixed in 75% ethanol to which 1–3% glycerol was added in some cases. In order to get straight preparations the animals were put into a wide jar where they would not become twisted before becoming fixed, which would have made it difficult to study them in plain view (Eason 1964; Koren 1986).

Species determination and terminology

The animals were determined using the keys in Eason (1964, 1982), Koren (1992), Brölemann (1930), Verhoeff (1937) and Matic (1966). The terminology of Eason (1964, 1982) was used for taxonomic characters.

Morphometrical studies

In the present work, body length, the head to 5th tergite width ratio, length/breadth proportions of the 15th legs, and the number of antennal articles were used as morphometrical characters. Concerning the last legs some length/length proportions were calculated as well in order to obtain more data.

To avoid circular reasoning the "a priori" groups "*L. mutabilis*" and "*L. glacialis*" were defined by non-morphometric characters (wrinkled tergites, biotope). The hypothesis of dissimilarity was then checked using the morphometrical characters. More generally a set of characters, or even a single character, is used to define groups, while other characters are used to check this grouping. Here this is done by morphometrics.

Table 5. Factor loadings of the discriminant function for males based on 15th leg proportions

Character	Root 1
FeL/FeB	−0.660
TiL/TiB	−0.753
TaL/TaB	0.462
TaL/FeL	1.381
TiL/FeL	−1.255
Eigenvalue	2.720

B = breadth, Fe = femur, L = length, Ta = tarsus, Ti = tibia.

Table 6. Factor loadings of the discriminant function for females based on 15th leg proportions

Character	Root 1
FeL/FeB	1.141
TiL/TiB	0.053
TaL/TaB	−0.559
TaL/FeL	−0.463
TiL/FeL	0.372
Eigenvalue	1.261

B = breadth, Fe = femur, L = length, Ta = tarsus, Ti = tibia.

Table 7. Factor loadings of the discriminant functions based on 15th leg proportions, sexes separate

Character	Root 1	Root 2	Root 3
FeL/FeB	−0.827	−0.863	0.818
TiL/TiB	−0.564	0.781	−1.133
TaL/TaB	0.375	0.533	0.270
TaL/FeL	1.210	−1.542	−0.727
TiL/FeL	−0.912	0.812	1.848
Eigenvalue	3.386	0.369	0.147

B = breadth, Fe = Femur, L = length, Ta = tarsus, Ti = Tibia.

Microslide preparation of last legs

The 15th legs were removed from the animals using two preparation needles. After mechanical cleaning with the same tools the legs were embedded in Berlese mixture on microscope slides. Every slide was labelled with the species name, sex, number and side of the prepared leg. If available and in good condition, the right 15th leg was always used. For measurement of leg proportions the last legs of at least seven specimens of each species and sex were processed in this way.

About 1 day later the preparations on the slides became cleared and were then analysed with an Olympus SZX 12 stereomicroscope. Photographs of the last legs were taken with a ProgRes C12 plus camera.

Measurements

For measurement, pictures of the 15th legs were made using the software "Spot Advanced". Scale bars corresponding to the respective magnification were added, and measurements of length and breadth of femur, tibia and tarsus were taken using this program.

Heads and 5th tergites were studied under a stereomicroscope using a magnification of 25 ×; the widths of both parts were measured with a scaled eyepiece.

Body length was measured with a common ruler accurate to one millimetre, from the margin of the head shield to the end of the telson.

The number of antennal articles was counted as well. Only one complete antenna per animal was evaluated, because on many animals the second antenna was lost, broken or degenerated.

Statistical methods for analysis

Differences or correlations between the two species were tested using the Mann–Whitney *U*-test, Spearman rank correlation or discriminant analysis according to Lamprecht (1999). Where other tests (Wilcoxon for paired samples, Wilcoxon-Lambda) were used, this is mentioned in the text.

Table 8. Comparison of morphological character states in *Lithobius mutabilis* and *L. glacialis*

Body part	*Lithobius mutabilis*	*Lithobius glacialis*
Forcipular coxosternite	Prominent anterior border with 2 + 2 teeth (Fig. 7C, D)	Same (Fig. 10C, D)
	Irregular shoulders lateral to porodont (Fig. 7C, D)	Same (Fig. 10C, D)
Legs	Tibiae of 13th and 14th legs of adult male with group of densely arranged setae situated on a small dorsal swelling at proximal posterior ridge of a dorsal sulcus (Fig. 8C–F)	13th and 14th tibiae of adult male dorsally either with group of very few setae (visible only with SEM) or without modifications; no dorsal swelling, usually no sulcus, sometimes 14th tibia with indistinct sulcus (Fig. 12A–F)
	15th tibiae of adult male with a clearly visible and profound dorsal sulcus (Fig. 8A, B); a mostly indistinct group of setae situated at proximal posterior ridge of sulcus (Fig. 8B)	15th tibiae of adult male mostly without dorsal sulcus (Fig. 11A, B), but sulcus present in some specimens (Fig. 11C, D); sometimes with groups of few setae (Fig. 11D)
	No modifications on 13th, 14th and 15th tibiae of females (Fig. 9E, F)	Same (Fig. 11E)
	15th leg with accessory claw; on opposite side of main claw a very small third accessory claw (sensory spur) (Fig. 7E)	Same (Fig. 10E)
	15th coxae without ventral anterior spine (VaC); 15th legs ventral spinulation of trochanter to tibia: -, m, amp, amp, (a)	Same (Fig. 11E)
Male genitalia	Second genital sternite with two setae (Fig. 7F, F1)	Same (Fig. 10F, F1)
Female genitalia	Gonopod claws with 3 denticles separated from each other (Fig. 9A, C, D)	Gonopod claws with 3 rounded denticles not clearly separated from each other (Fig. 13A, C, D)
	Second article of gonopod with row of dorsolateral setae (Fig. 9C, D)	Same (Fig. 13C, D)
	Gonopod with two conical spurs separated by distance of at least 1/3 of their diameter (Fig. 9A, B)	Gonopod with two blunt conical spurs very close to each other, separated by less than 1/3 their diameter (Fig. 13A, B)

Abbreviations: a = anterior, m = medial, p = posterior (Eason 1964).

SEM analysis of morphology

External features observable in both species were documented using SEM in order to show differences and similarities and to support the results of the morphometrical and statistical examinations. Body parts containing the characters to be studied were severed from the fixed animals. First they were cleaned mechanically with preparation needles; a Pasteur pipette was also used to wash away coarse dirt particles. To remove lighter debris or films some of the preparations were transferred into a mixture of equal parts 75% ethanol and 30% hydrogen peroxide for 10–20 min (after Bolte 1996).

Preparations were then dehydrated using a graded acetone series (70%, 80%, 90%, and 100%). Specimens were soaked for 10 min at each of the first three levels, then twice for 20 min in 100% acetone. Next, the objects were placed into a BAL-TEC CPD 030 apparatus for critical-point drying. Shortly afterwards they were mounted onto SEM stubs covered with a black tape adhesive on both sides, then sputtered with a gold layer for 120 s in a Polaron SEM Coating System. Finally the prepared specimens were examined and photographed using a Leo 1430 VP scanning electron microscope at 11.8 kV.

Results

Morphometrical studies

Body length

Body length of the 18 *L. mutabilis* specimens measured (males and females pooled) ranges from 13 to 17 mm, with a mean of 14.56 mm (Table 1). In *L. glacialis* body length of 17 measured specimens of both sexes is 10–14 mm, with a mean of 11.47 mm (Table 1).

The difference between the two species in pooled body length is highly significant statistically ($p < 0.0001$; Fig. 2A). The mean values and standard deviations are given in Table 1.

Table 9. Shared and specific traits of *Lithobius mutabilis* and *L. glacialis*

Character complex	Character	*L. mutabilis*	*L. glacialis*
Ecology		Lowland species*	Alpine species*
Body length		13–17 mm*	10–14 mm*
Head/Tg 5 width ratio		<1*	$=1$*
Antenna	Number of articles	36–44*	29–37*
	Extending to	Posterior part of Tg 7*	Posterior part of Tg 5*
Tergites	Projections on Tg 9, 11; 13	–/Small, rounded	–/Small, rounded
	Surface	Smooth*	Wrinkled/uneven*
Forcipular coxosternite	Teeth	2+2	2+2
	Shoulders	+	+
Male 13th tibia	Dorsal sulcus	+*	–*
	Dorsal swelling	+*	–*
	Group of setae	+ +	±
Male 14th tibia	Dorsal sulcus	+*	–*
	Dorsal swelling	+*	–*
	Group of setae	+	±
Male 15th tibia	Dorsal sulcus	+	±
	Dorsal swelling	–	–
	Group of setae	+	±
15th legs	Ventral spinulation of trochanter to tibia	–, m, amp, amp, (a)	–, m, amp, amp, (a)
	Accessory claw	+	+
Male genitalia	Setae on second genital sternite	2	2
Female genitalia	Tips of Gp claws	Pointed*	Rounded*
	Distance between spurs	At least 1/3 of diameter*	$<1/3$ of diameter*
	Dorsolateral setae (second Gp article)	+	+
	Dorsomedial setae (first Gp article)	–	–
	Spurs	2+2	2+2

Specifically distinct character states indicated by *; Gp = gonopod, Tg = tergite; a = anterior, m = medial, p = posterior (Eason 1964).

Fig. 4. Stereomicroscope photos of 15th legs of males; left: five *Lithobius mutabilis*, right: six *L. glacialis*; arrows indicate leg articles showing significant differences in length/breadth proportions. Abbreviations: Fe = femur, l = left, Mta = metatarsus, Prf = prefemur, r = right, Ta = tarsus, Ti = tibia, Tr = trochanter.

Fig. 5. Stereomicroscope photos of 15th legs of females; left: six *Lithobius mutabilis*, right: six *L. glacialis*; arrows indicate leg articles showing significant differences in length/breadth proportions. Abbreviations: Fe = femur, l = left, Mta = metatarsus, Prf = prefemur, r = right, Ta = tarsus, Ti = tibia, Tr = trochanter.

Width of head/width of 5th tergite

This character was also examined with males and females pooled.

The head/5th tergite width ratio is lower than 1 in 17 of the 18 *L. mutabilis* measured; the result is statistically significant ($p < 0.001$; Wilcoxon test for paired samples; Fig. 1A). In most of the 14 measured *L. glacialis* specimens the ratio is equal to 1; the statistical test shows no significant difference within *L. glacialis* ($p = 0.35$; Wilcoxon test for paired samples; Fig. 1B).

The difference between the two species in head/5th tergite width ratio is significant ($p < 0.0001$; Fig. 2B). The means and standard deviation are given in Table 1.

Antennal articles

The number of antennal articles of 13 male or female *L. mutabilis* specimens ranged from 36 to 44, with a mean value of 41.15. The antennae of 18 *L. glacialis* animals of both sexes consisted of 29–37 articles, with a mean of 33.67.

The statistical test results show the number of antennal articles to be significantly higher in *L. mutabilis* ($p < 0.00001$; Table 1). Means and standard deviations are given in Table 1.

Proportions of 15th leg articles — males

Length/breadth proportions of the 15th leg articles were measured independently for both sexes, as males show generally more or less modified legs (see also Fig. 4).

Seven males of each species were tested. They show a significant difference in length/breadth proportion of the 15th femur, which is more slender and longer in *L. glacialis* ($p < 0.003$; Fig. 2C). A significant difference was also found concerning the 15th tibia, which is more slender in *L. glacialis* ($p < 0.03$; Fig. 2D). No significant difference in length/breadth proportions was found for the 15th tarsus ($p = 0.85$).

The tarsus/femur length ratio for the 15th leg is significantly larger in *L. mutabilis* ($p < 0.003$; Fig. 2E). A similar result was obtained for tibia/tarsus length of the 15th leg ($p < 0.05$; Fig. 2F). Means and standard deviations are given in Table 2.

Proportions of 15th leg articles — females

Eight female specimens each of *L. mutabilis* and *L. glacialis* were tested. As in the males, the 15th femur is significantly more slender in *L. glacialis* ($p < 0.01$; Fig. 3A). No significant differences were found concerning the remaining 15th leg proportions (Table 3): length/breadth of tibia ($p = 0.53$; Fig. 3B) and tarsus ($p = 1$), tarsus/femur length ($p = 0.09$), and tibia/femur length ($p = 0.07$). Means and standard deviations are given in Table 3 (see also Fig. 5).

In Table 4, a summary of the p-values calculated for all tested morphometrical characters is given.

Discriminant analysis

Because of p-values with partly low significance levels concerning the 15th leg proportions, the morphometrical characters (see above) were combined in a discriminant analysis.

A combination of all 15th leg proportions of males shows significant differences between the two species ($p < 0.03$; Wilcoxon-Lambda test). Concerning females there is no significant difference ($p = 0.1$; Wilcoxon-Lambda test). The factor loadings for the discriminant function are listed in Tables 5 and 6.

For graphical demonstration male and female specimens of both species were pooled in one discriminant analysis. The separation of all four groups by three discriminant functions (roots 1–3; factors in Table 7) is shown in a biplot (Fig. 6). *L. mutabilis* males are clearly separated from all other specimens by root 1. Females of the two species are not clearly separated from each other. Roots 2 and 3 are characterized by very low eigenvalues and do not contribute to any new separation aspects in this case.

Morphological studies with SEM

The morphological characters used — as described by Brölemann (1930), Verhoeff (1937), Eason (1964, 1982), Matic (1966) and Koren (1992) — are summarised in Table 8 to allow direct diagnostic comparison, and are illustrated by high-resolution SEM pictures (Figs. 7–13) referred to in the table.

Discussion

L. mutabilis and *L. glacialis*: characters distinguishing the species

Differences between *L. mutabilis* and *L. glacialis* were found by both, morphometrical and SEM analysis. The

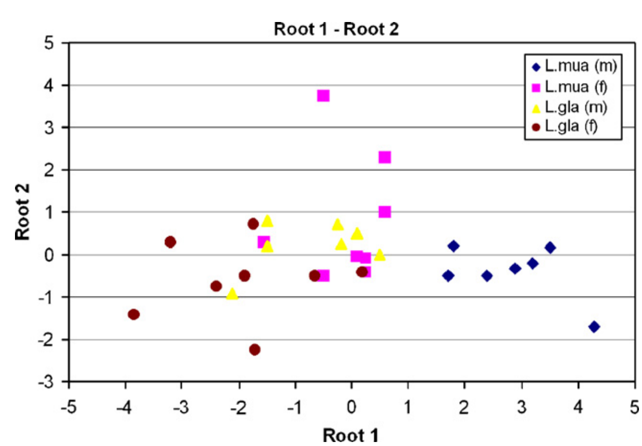

Fig. 6. Biplot of values from discriminant analysis of 15th leg proportions within the species pair *Lithobius mutabilis* (L. mua)/ *L. glacialis* (L. gla). Abbreviations: f = female, m = male.

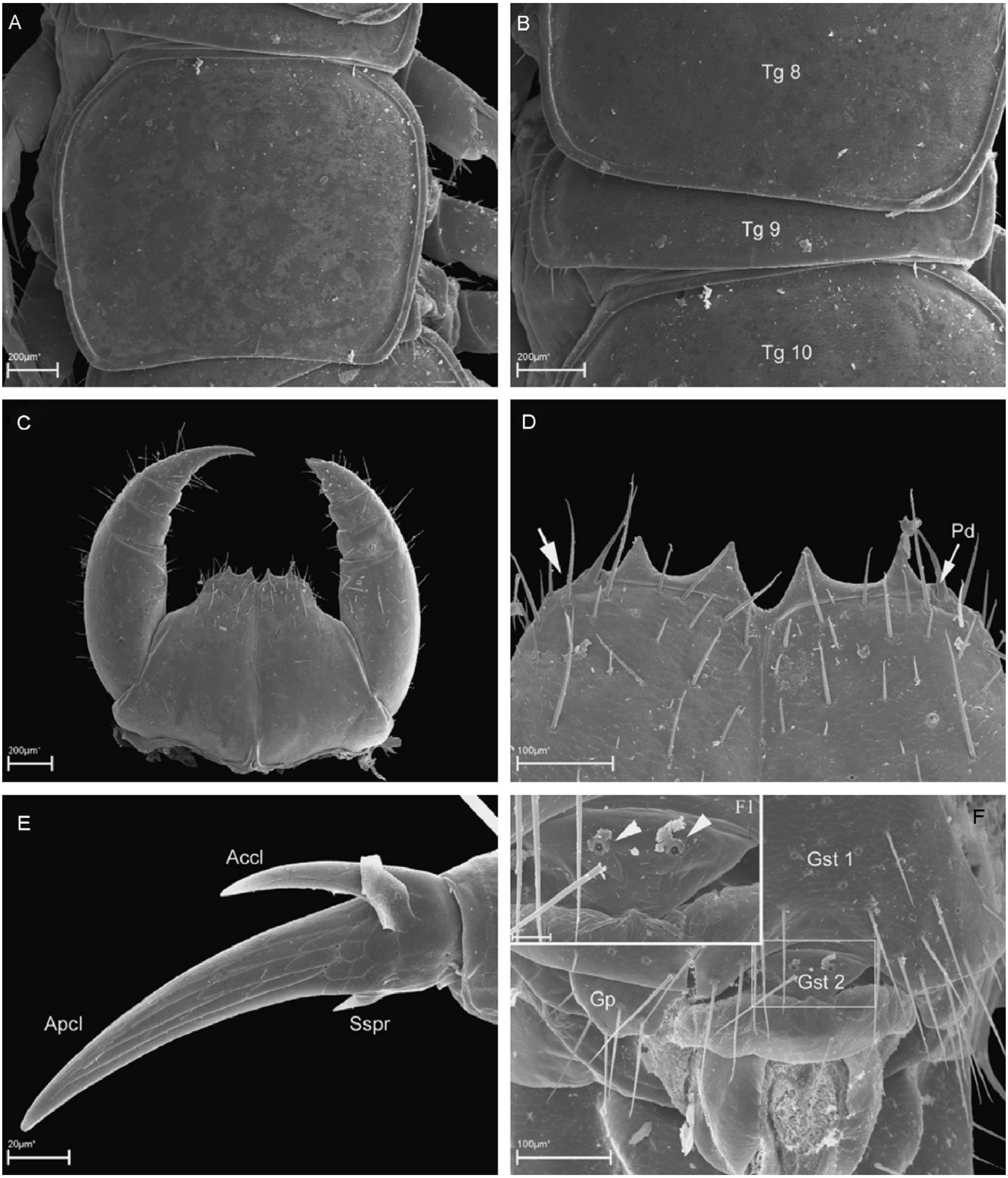

Fig. 7. *Lithobius mutabilis* L. Koch, 1862, scanning electron micrographs. (A) 10th tergite, dorsal. (B) 9th tergite, dorsal. (C) Forcipules, ventral. (D) Forcipular teeth, ventral; left arrow indicates shoulder lateral to porodont. (E) Apical claw of 15th leg. (F) Male genitalia, ventral. (F1) Male genital sternite 2, ventral; arrowheads indicate setae. Abbreviations: Accl = accessory claw, Apcl = apical claw, Gp = gonopod, Gst = genital sternite, Pd = porodont, Sspr = sensory spur, Tg = tergite.

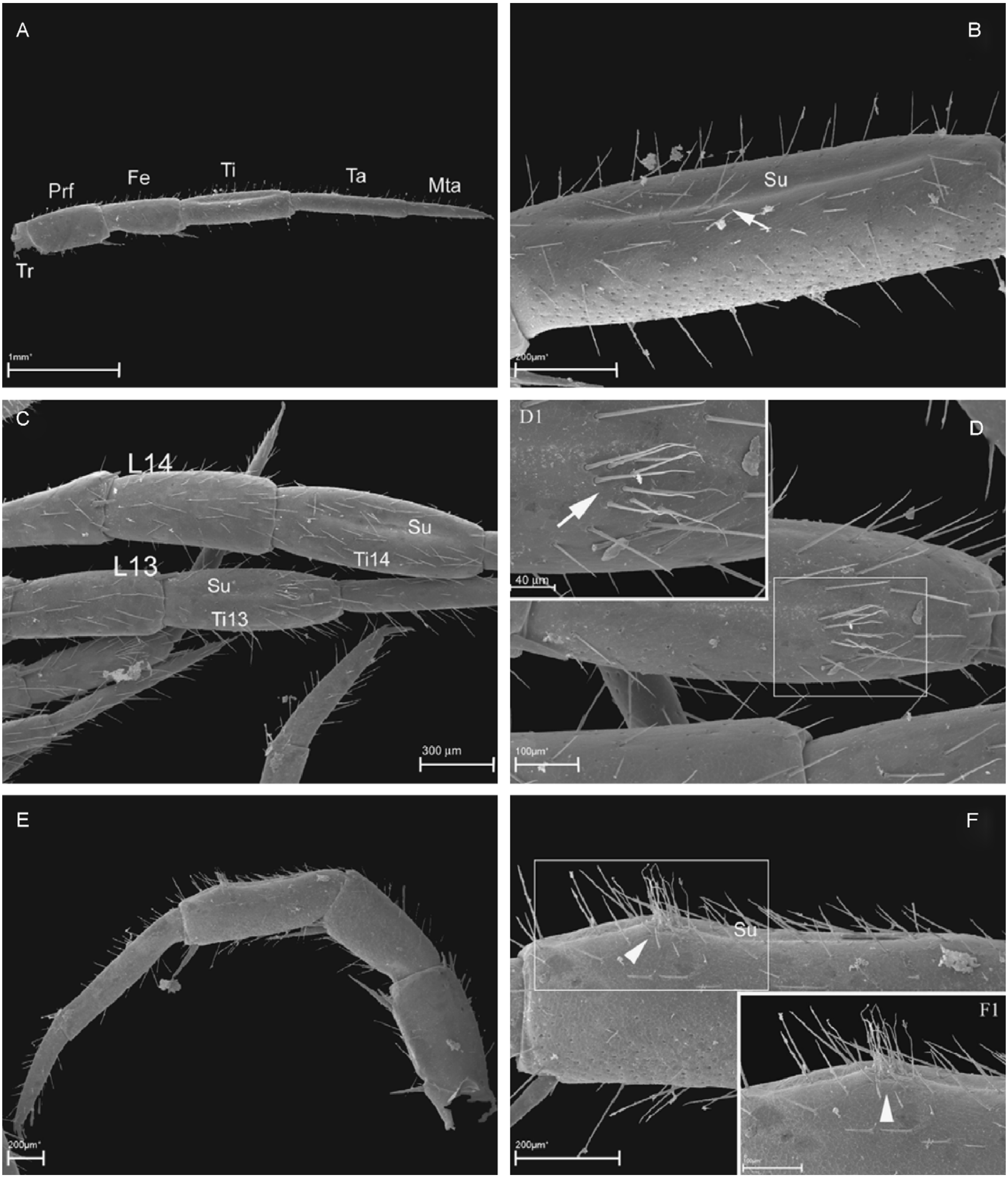

Fig. 8. *Lithobius mutabilis* L. Koch, 1862, scanning electron micrographs. (A) Male 15th leg, lateral. (B) Male 15th tibia, lateral; small arrow indicates group of setae. (C) Male 13th and 14th legs, dorsal. (D) Male 14th tibia, dorsal. (D1) Dorsal swelling on male 14th tibia; large arrow indicates group of setae. (E) Male 13th leg, lateral. (F) Male 13th tibia; arrowhead indicates dense group of setae. (F1) Dorsal swelling on male 13th tibia. Abbreviations: Fe = femur, L = leg, Mta = metatarsus, Prf = prefemur, Su = sulcus, Ta = tarsus, Ti = tibia, Tr = trochanter.

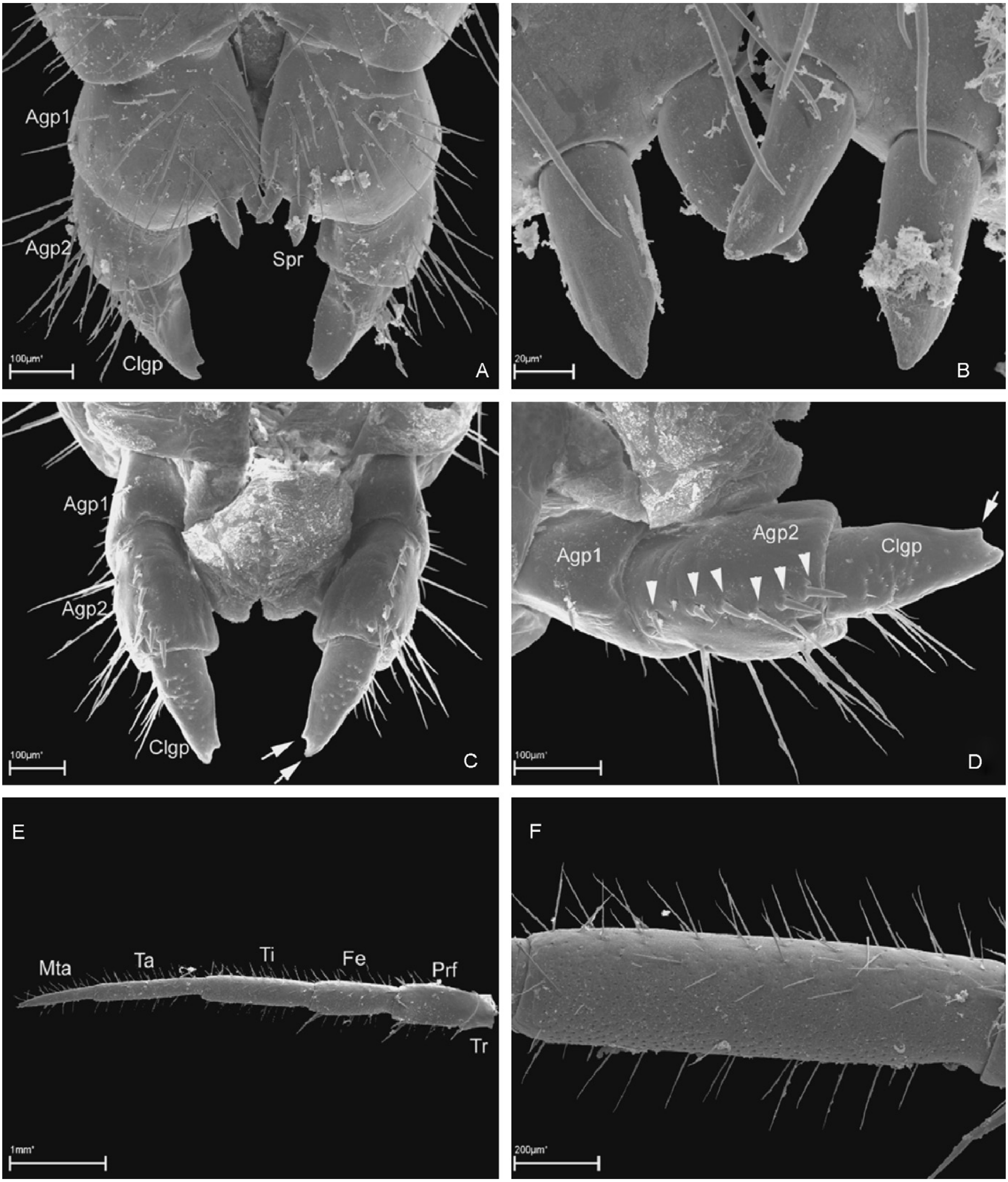

Fig. 9. *Lithobius mutabilis* L. Koch, 1862, scanning electron micrographs. (A) Female gonopod, ventral. (B) Spurs on female gonopods, ventral. (C) Female gonopod, dorsal; arrows indicate denticles of claw. (D) Left female gonopod with claw, dorsal; arrowheads indicate dorsolateral row of setae. (E) Female 15th leg, lateral. (F) Female 15th tibia. Abbreviations: Agp = article of gonopod, Clgp = claw of gonopod, Fe = femur, Mta = metatarsus, Prf = prefemur, Spr = spur, Ta = tarsus, Ti = tibia, Tr = trochanter.

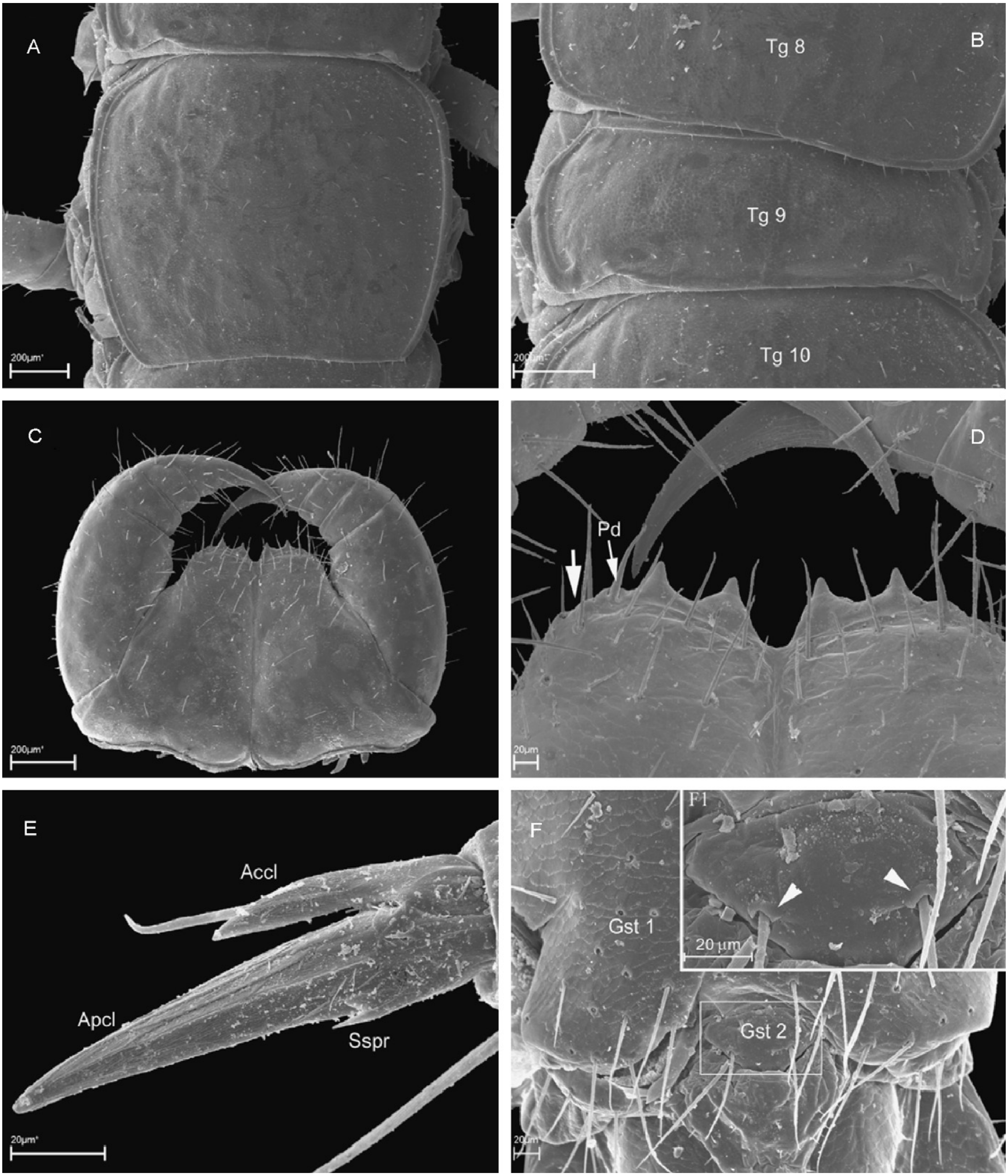

Fig. 10. *Lithobius glacialis* Verhoeff, 1937, scanning electron micrographs. (A) 10th tergite, dorsal. (B) 9th tergite, dorsal. (C) Forcipules, ventral. (D) Forcipular teeth, ventral; left arrow indicates shoulder lateral to porodont. (E) Apical claw of 15th leg. (F) Male genitalia, ventral. (F1) Male genital sternite 2, ventral; arrowheads indicate setae. Abbreviations: Accl = accessory claw, Apcl = apical claw, Gst = genital sternite, Pd = porodont, Sspr = sensory spur, Tg = tergite.

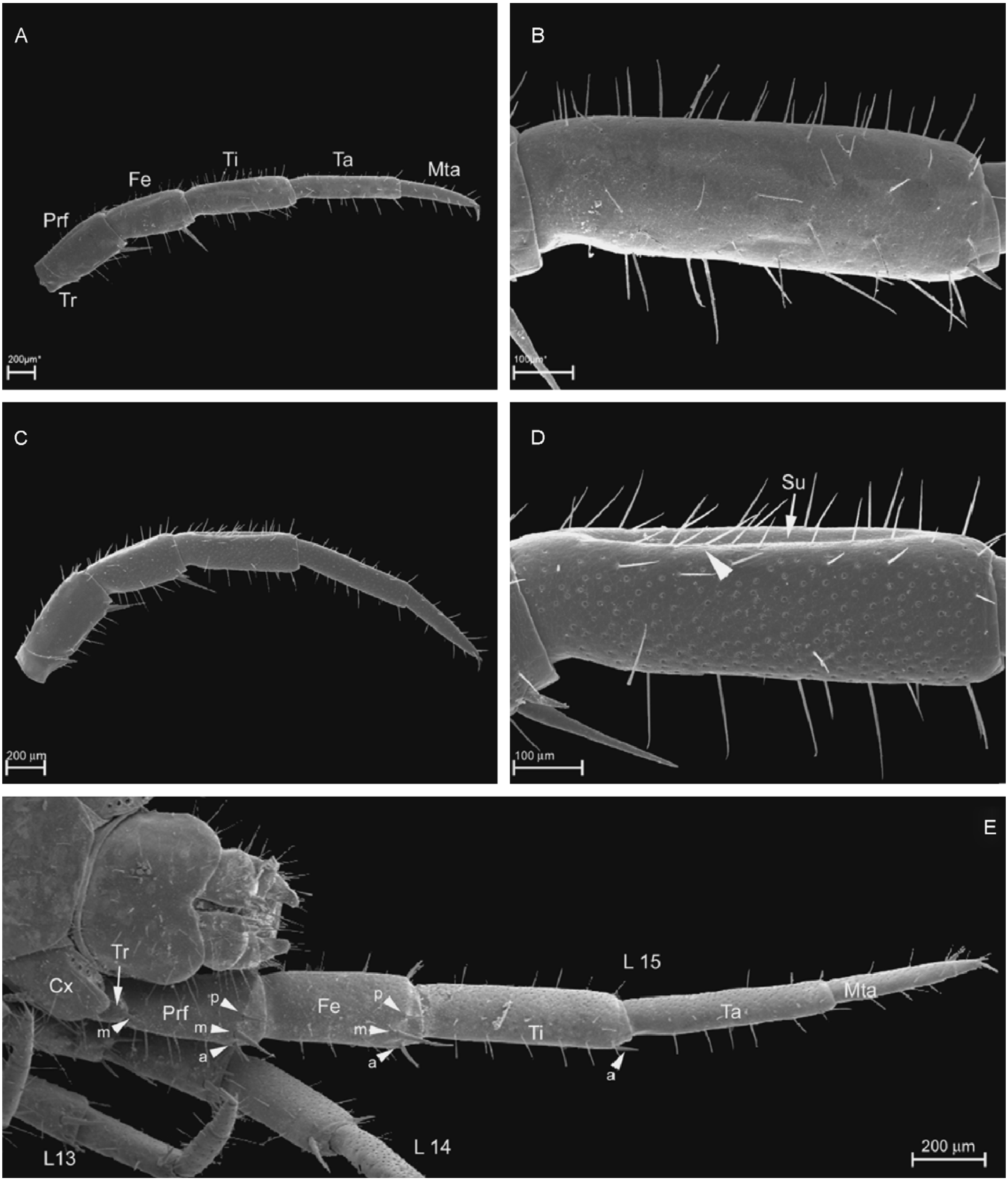

Fig. 11. *Lithobius glacialis* Verhoeff, 1937, scanning electron micrographs. (A) Male 15th leg, lateral. (B) Male 15th tibia, lateral, without tibial sulcus. (C) Male 15th leg, lateral. (D) Male 15th tibia with tibial sulcus; arrowhead indicates group of few setae. (E) Female 15th leg, ventral; small arrowheads indicate ventral anterior (a), medial (m), and posterior (p) spines. Abbreviations: Cx = coxa, Fe = femur, L = leg, Mta = metatarsus, Prf = prefemur, Su = sulcus, Ta = tarsus, Ti = tibia, Tr = trochanter.

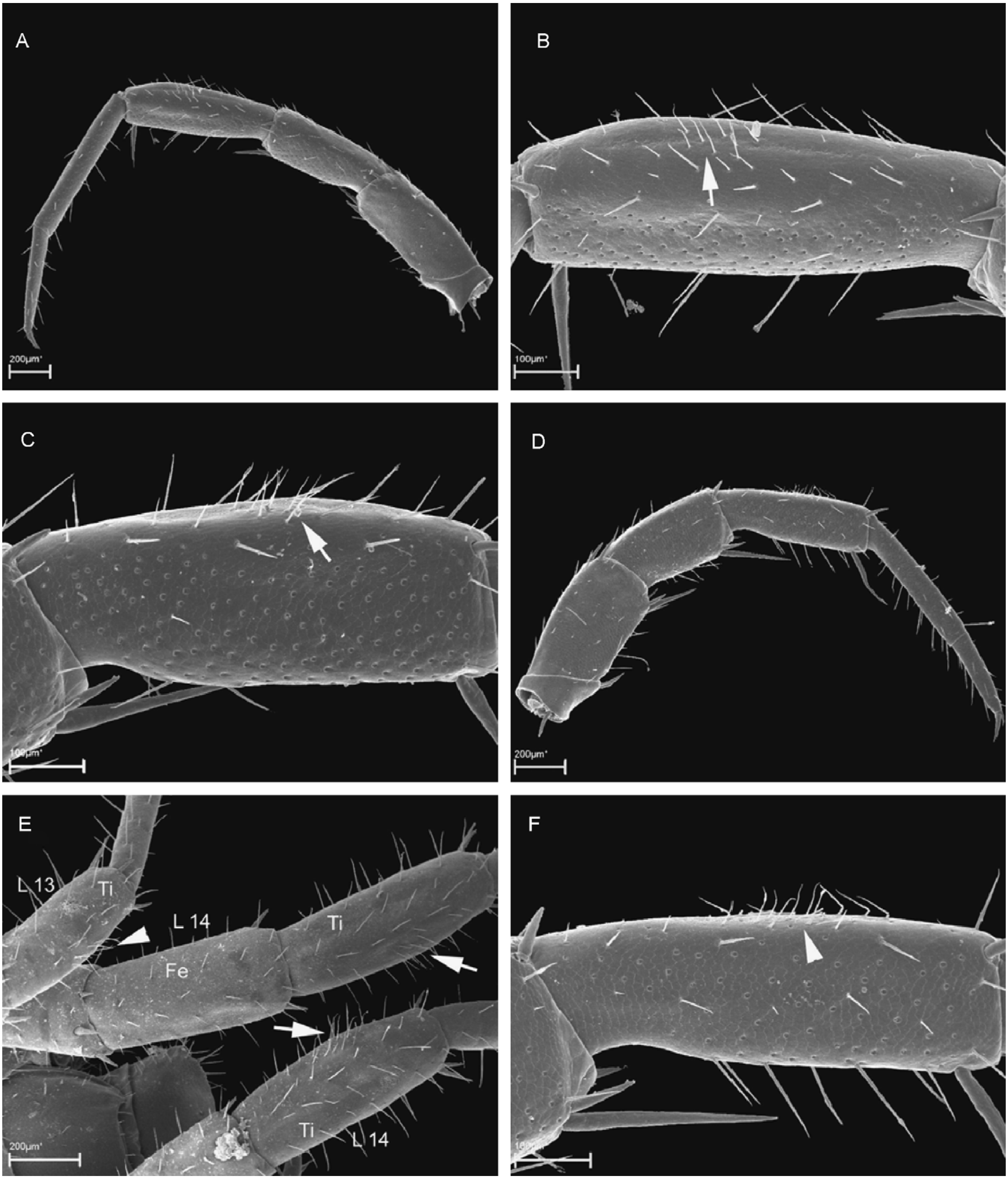

Fig. 12. *Lithobius glacialis* Verhoeff, 1937, scanning electron micrographs. (A) Male 14th leg, lateral. (B) Male 14th tibia; arrow indicates indistinct group of setae. (C) Male 14th tibia; arrow indicates indistinct group of setae. (D) Male 13th leg, lateral. (E) Male 13th and 14th legs, dorsal; arrows and arrowhead indicate indistinct groups of setae. (F) Male 13th tibia; arrowhead indicates indistinct group of setae. Abbreviations: Fe = femur, L = leg, Ti = tibia.

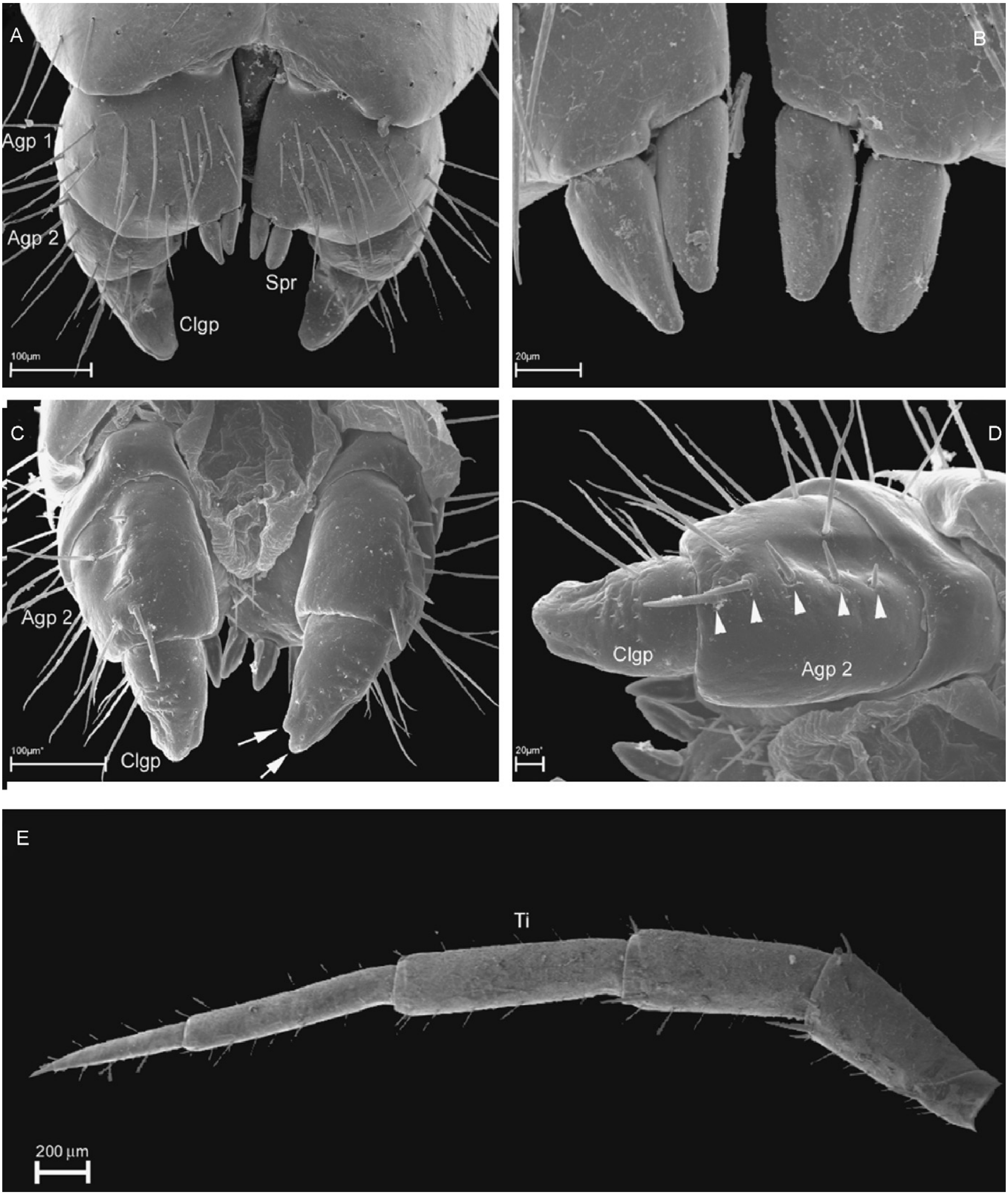

Fig. 13. *Lithobius glacialis* Verhoeff, 1937, scanning electron micrographs. (A) Female gonopods, ventral. (B) Spurs on female gonopods, ventral. (C) Female gonopods, dorsal view; arrows indicate rounded tips of gonopod claw. (D) Left female gonopod with claw, dorsal; arrowheads indicate dorsolateral row of setae. (E) Female 15th leg, lateral. Abbreviations: Agp = article of gonopod, Clgp = claw of gonopod, Spr = spur, Ti = tibia.

characters specific to *L. glacialis* are pointed out in order to justify its status as a distinct species.

The differences in body length, head/5th tergite width ratio, and number of antennal articles pertain to new, distinctive morphometrical characters, and support the hypothesis that *L. mutabilis* and *L. glacialis* are two different species. These evaluations were done with both sexes pooled.

Verhoeff (1937) described *L. glacialis* females as 12 mm long. This fits with the body length (10–14 mm) found in the present study. Hence, the difference in comparison to *L. mutabilis* according to Verhoeff (1937) is confirmed. The number of antennal articles corresponds to the original description as well. Verhoeff reported about 35 articles, within the range found here (29–37).

In spite of the clear statistical distinction by significant *p*-levels, the values measured for the two species overlap to a certain extent concerning body length, head/5th tergite width ratio, and number of antennal articles. Therefore, additional character combinations have to be considered (Table 9).

Regarding the antenna it is better to evaluate its relative length rather than the number of articles. In *L. glacialis*, antennae extend to the anterior or sometimes the posterior margin of the 5th tergite, whereas in *L. mutabilis* they reach the 7th tergite.

The biplot in Fig. 14 shows the distinction made by combining the characters 'number of antennal articles' and 'width of head/width of 5th tergite', which by themselves are uninformative due to visible overlap.

Concerning males, specific dissimilarity can be assumed also because of significant differences in the 15th leg article proportions. Except for tarsus length/breadth, all analysed morphometrics differ between the two species and can be regarded as new, distinctive characters. These differences are also evident in Fig. 4.

The discriminant analysis of all 15th leg proportions further supports clear separation between males within this species pair. Both methods, sexes analysed separately and simultaneously, result in significant differences in 15th leg article proportions. With both methods root 1 separates *L. mutabilis* males from all other specimens, especially from *L. glacialis* males.

Concerning 15th leg article proportions the biplot in Fig. 15 also shows dissimilarity between males of both species by combining length/breath ratio of femur with tarsus/femur length ratio.

Except for femur length/breadth (Fig. 5), the 15th leg article proportions of females are not significantly different and therefore cannot serve as characters distinguishing *L. mutabilis* from *L. glacialis*.

The discriminant analysis of all the 15th leg characters shows no clear separation in females either, regardless of whether or not the sexes are pooled. The scatterplot (Fig. 6) illustrates that neither discriminant function, root 1 or root 2, clearly separates the female specimens in this species pair.

Sexual differences in 15th leg article proportions in *L. mutabilis* were found as well (Fig. 6). Root 1 of the discriminant analysis separates *L. mutabilis* males and females as well as the males of both species. The modifications found on the male 15th tibiae, but not in the females, could be an explanation for this. Hence, concerning the 15th leg article proportions *L. mutabilis* males are different from *L. glacialis* males to the same degree as from females of their own species.

In addition to the newly described morphometrical characters, especially in males, further morphological characters were found and illustrated by SEM studies in order to distinguish between the species. By this method, sets of characters are described that provide a more effective means of examination than separating two

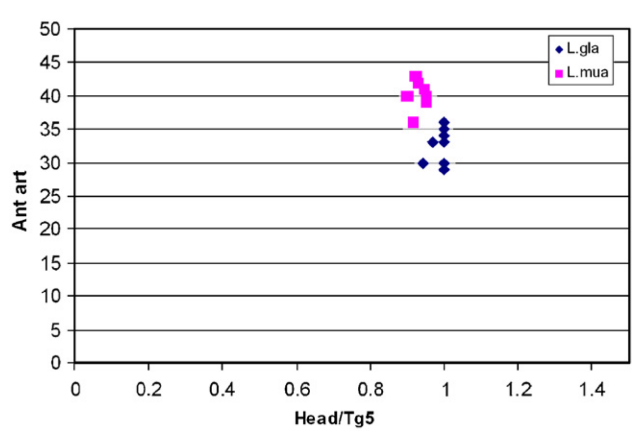

Fig. 14. Species pair *Lithobius mutabilis* (L. mua)/*L. glacialis* (L. gla); biplot of head/5th tergite width ratio against number of antennal articles. Abbreviations: Ant art = antennal articles, Tg5 = tergite 5.

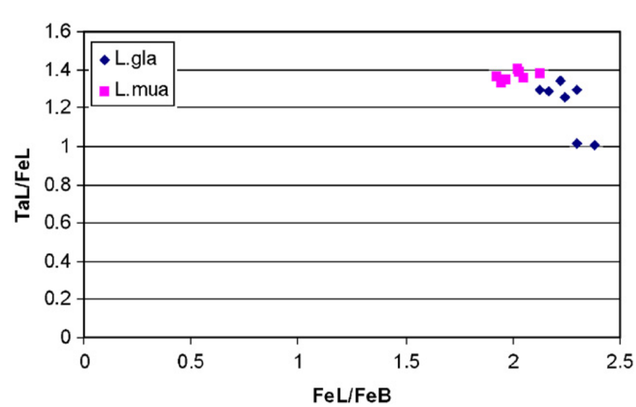

Fig. 15. Species pair *Lithobius mutabilis* (L. mua)/*L. glacialis* (L. gla), males; biplot of length/breadth ratio of femur against tarsus/femur length ratio. Abbreviations: FeB = femur breadth, FeL = femur length, TaL = tarsus length.

chilopod species from each other using one or two differential characters only.

The characters analysed concerning *L. mutabilis* had been described by previous authors already (Brölemann 1930; Verhoeff 1937; Eason 1964, 1982; Matic 1966; Koren 1992), whereas part of the characters for *L. glacialis* had been described by Verhoeff (1937). As listed in the results section, there are several traits shared between *L. mutabilis* and *L. glacialis*, such as the structure of the forcipular coxosternite, the lack of posterior tergite projections, the accessory claw on the 15th leg, the spinulation on the 15th legs, and the two setae on the male second genital sternite. Most of these features are potentially synapomorphic, except for the accessory claw. The latter seems to be a plesiomorphy on this level of comparison (see Edgecombe 2004). Hence, it can be assumed that *L. mutabilis* and *L. glacialis* are closely related species, indeed, and possibly a true species pair, i.e. sister groups.

Nevertheless there are remarkable differences. Some of them were known from earlier studies. Even at first glance, *L. glacialis* can be recognized and distinguished from *L. mutabilis* by the wrinkled surface structure and the colour of the tergites. The latter was the character described by Verhoeff (1937) used to define *L. glacialis* at the onset of the present study.

Verhoeff (1937) further described his female specimens of *L. glacialis* mainly by referring to the gonopods. According to that author the claws of the gonopds are rounded with no dentation in this species. This character state is partly confirmed in the present work, and is different from the one in *L. mutabilis* females. The gonopod claws of *L. mutabilis* are clearly separated from each other, and not rounded. According to the results of the present study the female gonopods of *L. glacialis* are rounded, indeed, but can have small roundish denticles, too.

According to Verhoeff (1937) the spurs of the gonopods of *L. glacialis* stand close to each other and are rather blunt. This can also be confirmed as distinctive in comparison to *L. mutabilis*.

A major deficit of Verhoeff's (1937) description is the lack of male specimens. The present work, however, provides a new list of morphological characters for *L. glacialis*, some of them already proposed by Spelda (1999).

Most characters separating *L. mutabilis* and *L. glacialis* are situated on the male 13th to 15th tibiae. Probably the best distinctive characters can be found on the male 13th and 14th tibiae, as described in the results section and in Table 9.

The male 15th tibiae do not provide good distinction because of the variability in *L. glacialis*. Some specimens show a dorsal sulcus and a group of setae, others do not. On the male 15th tibiae of *L. mutabilis* the dorsal sulcus is always found, but the group of setae is very indistinct in several specimens, especially in subadults.

Sensory spur

During the SEM analysis for this work a small supplementary accessory claw was found anterior to the main apical claw. Eason (1964) referred to this structure as a "sensory spur" and described it for many *Lithobius* species. In more detailed studies of the pretarsal claws Edgecombe (2004) found this structure as well and called it "anteroventral spur". Our own SEM analysis confirms that this spur is also present in *L. mutabilis* and *L. glacialis* (Figs. 7E, 10E). Hence, the assumption by Edgecombe (2004) that this character is synapomorphic for Lithobiidae, probably being a modification of the anterior accessory claw of Henicopidae, is supported by our study.

Summary

Concerning males, the described morphometrical and morphological characters are clearly distinctive between *L. mutabilis* and *L. glacialis*, especially as regards the 15th leg article proportions and the modifications on the 13th–15th legs.

Females of the two species can be separated by several characters such as body length, the head/5th tergite width ratio, and the number of antennal articles. The differences in the female gonopods as well as in the wrinkled tergite structure, both already stated by Verhoeff (1937), are also useful.

The ecological conditions at a given collecting site can also be helpful for determination, because *L. glacialis* is always found in alpine areas at altitudes above timberline (1300 m), whereas *L. mutabilis* is a lowland species.

Because of all these characters, *L. glacialis* should be considered a separate species. There are some additional alpine species that have been neglected (e.g. *L. macrocentrus* Attems, 1949), and still more species may not have been found or described so far, as some of the specimens studied for the present paper (Pilz 2005b) do not completely match available species descriptions.

The close relationship between the two species is probably a hint at what happened during their evolution. One could imagine that *L. mutabilis* was always a continental species found at lower altitudes. After the ice age, however, some specimens may have entered more alpine environments by climbing to higher elevations, and evolved into the species now known as *L. glacialis*.

As another possible pathway of evolution, *L. glacialis* may have evolved from a population of a common ancestor that became isolated on a nunatak during the ice age. This would be an explanation for the present distribution of *L. glacialis* limited to alpine environments.

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