

***Megaphyllum silvaticum* (Verhoeff, 1898) (Diplopoda: Julida), a new species to the Hungarian millipede fauna, with notes on the status of *M. s. discolor* (Verhoeff, 1907) and on their relationship to *M. projectum* Verhoeff, 1894**

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Abstract. The species *Megaphyllum silvaticum* (Verhoeff, 1898) was found in the Aggtelek National Park, northeastern Hungary, as new to the millipede fauna of the country. A short review on the male and female genital morphology and distribution of *M. silvaticum*, *M. s. discolor* (Verhoeff, 1907) and *M. projectum* Verhoeff, 1894 is given. According to our observation the subspecies *discolor* should be regarded only a variation with no taxonomical value. Although the male gonopods of *M. silvaticum* and *M. projectum* are really close to each other, the different female vulvae, observed and illustrated here for the first time, justify their separate specific status.

INTRODUCTION

Megaphyllum silvaticum (Verhoeff, 1898) was described from Mt. Postăvarul (= Schulergebirge), near Braşov, Romania (Verhoeff, 1898: p. 157, Fig. 26). A decade later, Verhoeff described its subspecies *discolor* from Kočevje, Slovenia (= Gottschee) (Verhoeff, 1907: p. 307, Fig. 18). Males of the two subspecies can be distinguished from each other according to the length of the posterior process – „Mesomeritfortsatz” by Verhoeff, or „Paracoxitfortsatz” by Attems (1926) – of the opisthomere. If it exceeds in length the anterior process („Schutzblattfortsatz” by Verhoeff, or „Solänomeritquerlappen” by Ložek & Gulička (1962)), than it is *M. s. discolor*.

M. silvaticum is regarded as a Carpathian species (Golovatch, 1992), mostly found at higher elevations (Verhoeff, 1907). It has already been reported from Austria (Voigtländer *et al.*, 1997), Slovakia (Mock, 2001), Ukraine (Chorny & Golovatch, 1993), Romania (Tăbăcaru, 1976), Croatia (Strasser, 1965), Slovenia (Strasser, 1966), Italy (Foddai *et al.*, 1995), Poland (Stojałowska & Starega, 1961), and Russia (Lokšina & Golovatch, 1979). The new Hungarian record fits very well into this distribution pattern. The diplopod fauna of the Aggtelek National Park has already been surveyed (Lazányi & Korsós, 2009) but due to the

difficult morphological situation of the species group, *M. silvaticum* was omitted from the species list (see Discussion).

One aim of our present study was to make a provisional distribution map of the two subspecies and to reconsider the subspecific status of *M. s. discolor*. Sometimes *M. s. silvaticum* and *M. s. discolor* are found at the same region (Schmölzer-Falkenberg, 1975; Strasser, 1971). Without a precise observation of the gonopods *M. s. silvaticum* can be misidentified as the common species *M. projectum* Verhoeff, 1894. In several parts of their distribution the two species have overlapping areas (e.g. Attems, 1929). Verhoeff (1898) in his original description of *M. silvaticum*, already mentioned the morphological similarity of the two species, and later discussed it in details, too (Verhoeff, 1899, 1907). He found no reliable morphological differences for females, so the other aim of our study was to recheck it, and to find a key for distinguishing females on the basis of vulval morphology.

MATERIAL AND METHODS

The Aggtelek National Park (ANP) is situated at the northeastern border of Hungary, and was founded in 1985. In 1987 the Hungarian Natural History Museum (HNHM) started a collecting

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project which officially lasted for four years, but additional collectings were performed until 2002. Millipedes derived from sifting and hand-sorting are preserved in 70 % ethanol in the Myriapoda Collection of the Department of Zoology, HNHM. For study, we used a Leica M125 stereo microscope, and, if needed, animals were temporarily fixed with lubricant gel in the alcohol.

The *M. silvaticum* male specimen from ANP was compared to the *M. s. silvaticum* type specimen from Museum für Naturkunde, Berlin (ZMB 12976, 1♂: gonopods, holotype, slide preparation. Nr.1254, Coll. Verhoeff, Schuler, Deubel leg.), and to the *M. s. discolor* type specimens from Zoologische Staatssammlung München (1♂: gonopods, regarded as type specimen, slide preparation, A20033660, Bruck). Gonopods of the type specimens were newly drawn at the collections (ZMB and ZSM). We had three additional male specimens in the Myriapoda Collection, HNHM, from Maramureş County, Romania (Korsós & Lazányi, 2008) (2♂: Rodna Mts., Borşa, Staţiunea Borşa, limestone rocks on pasture over the ski course, 1521 m, N47°35'–E24°48', 26.09.2006, leg. Dányi, L., Kontschán, J. & Murányi, D.; 1♂: Maramureş Mts., Borşa-Băile Borşa, Vinişor Valley, middle section, spring in beech forest, 988 m, N47°40'–E24°47', 22.05.-2007, leg. Csuzdi, Cs., Dányi, L., Kontschán, J. & Murányi, D.).

Comparison of females was based upon *Megaphyllum* female specimens from the Maramureş project (Korsós & Lazányi, 2008), from the Aggtelek National Park, and from other parts of Hungary. Vulvae were prepared in Faure-Berlése medium then removed by distilled water and relocated in 70 % ethanol into the collection. After some hours of incubation the internal structure of vulvae (e.g. apodemic tube, ampulla, appendix) were investigated under a light microscope (Leica DM-1000). One of the vulvae was left intact, the other was dissected into the parts bursa and operculum, and these were used to count the setae on their surface.

RESULTS

We compiled a distribution map of *M. s. cf. silvaticum* and *M. s. cf. discolor* (Fig. 1) on the basis of individuals identified in the present study and of literature data. Those data in the literature where exact localities (i.e. at least township names) are given are supplied with data where only wider areas were published.

One male specimen resembling *Megaphyllum s. discolor* was found in the collection from the Aggtelek National Park, northeastern part of Hungary. The sample originated from the valley of the Kecső Stream, collected on the 28th of April, 1989, by Imre Fürjes, assistant curator at that time in the Myriapoda Collection, HNHM. Unfortunately, the label did not contain further data (e.g. habitat type). The specimen is in the developmental stadium XI, with 49+1+T segments, with 44 ocelli on the right, and 45 on the left side. Length is ca. 47.8 mm, height at the 26th segment 2.9 mm. The gonopods (Fig. 2) agree well with the gonopods of the type specimen of *M. s. discolor* (Fig. 3).

The male specimens from Staţiunea Borşa, Maramureş (Fig. 4) are similar to the type specimen of *M. s. silvaticum* (Fig. 5). Details of the two males are as follows: (1) stadium X, 46+1+T segments, 41 ocelli on the left, and 40 on the right side, length ca. 30 mm, height at the 25th segment 2 mm; (2) stadium X, 45+2+T segments, 38 ocelli on the left, and 41 on the right side, length appr. 33.2 mm, height at the 25th segment 2.4 mm. The male specimen from Vinişor Valley, Maramureş, however, has different gonopods (Fig. 6). It is in stadium XI, with 49+1+T segments, 44 ocelli on each side, its length is appr. 43.7 mm, height at the 26th segment: 2.7 mm.

As regards the female vulvae (Figs. 7–8), two types were found in the females of the ANP material, from Szentmargitfalva (southwestern border of Hungary), and from Maramureş.

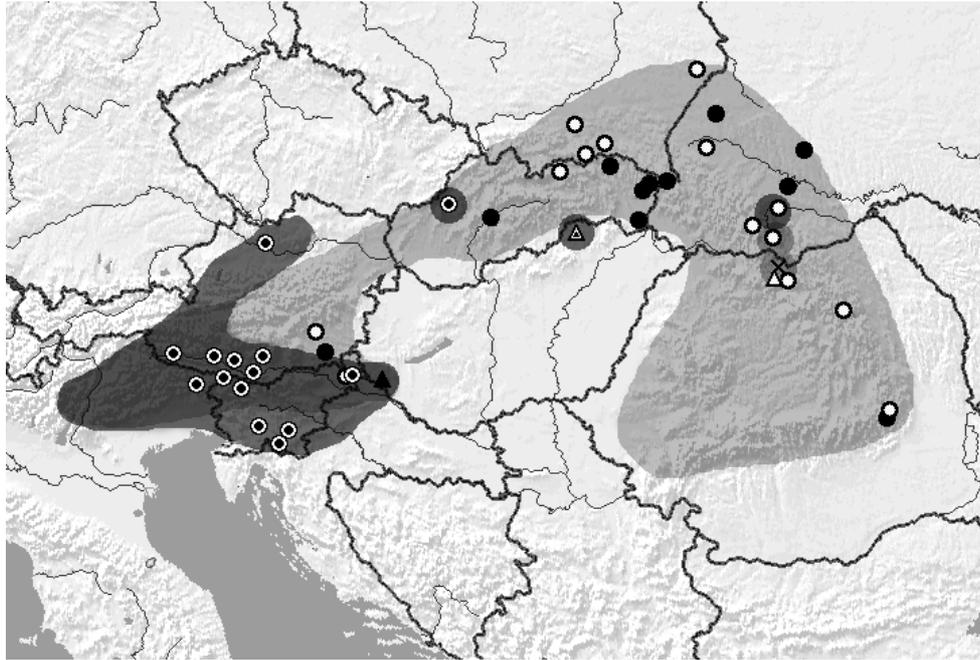


Figure 1. Distribution of *Megaphyllum silvaticum*. Black dots: *M. silvaticum* (without subspecific allocation); white-centered dots: *M. s. cf. silvaticum*; black-centered dots: *M. s. cf. discolor*; black triangle: *M. s.* from Szentmargitfalva; black-centered triangle: *M. s. cf. discolor* from Jósvalfő, Aggteleki NP; white-centered triangle: *M. s. cf. silvaticum* from Stațiunea Borșa, Maramureș; cross-sign: third type of *M. silvaticum* from Vinișor Valley, Maramureș. The light grey distributional area refers to *M. s. cf. silvaticum*, darker grey area to *M. s.* specimens having gonopods with the shortest posterior process; the darkest distributional areas to *M. s. cf. discolor*

Detailed data of one female with the vulva type shown in Fig. 7 (ANP, Szögliget, Patkós valley, 2. May 1988, leg. Z. Korsós): it is in stadium XII, with 49+1+T segments, 47 ocelli on the right and 48 on the left side, its length is ca. 46.7 mm, height at the 26th segment 3.6 mm. Age of females with this vulva type ranged between the stadia XI–XIII. Females of stadium X (and below) had vulvae still in underdeveloped stage. Chaetotaxy of this vulva type is variable, usually about 50, not symmetrically distributed setae can be found on each bursae, and 10–18 setae are both on the anterior and on the caudal half of each operculum.

Detailed data of one female with the other vulva type, shown in Fig. 8 (ANP, Szögliget, Ménes valley, leaf litter, 31. October 1989, leg. Z.

Korsós): it is in stadium X, with 49+2+T segments, 39 ocelli on the right and 38 on the left side, its length is ca. 23.6 mm, height at the 27th segment 2.9 mm. Age of females with this vulva type ranged between stadia X–XI. Chaetotaxy of this vulva type is also variable, usually 4–11 setae are both on the anterior and on the caudal half of each bursae, and 5–3 setae are both on the anterior and on the caudal half of each operculum.

Both species showed the same sexual dimorphism in colour pattern: males dark brown, or dark grey, with one longitudinal black line dorsally; females had three longitudinal lines dorsally, a black one surrounded by two bright yellowish ones, the basic body colour was brighter than males', and varied from light to dark greyish brown.

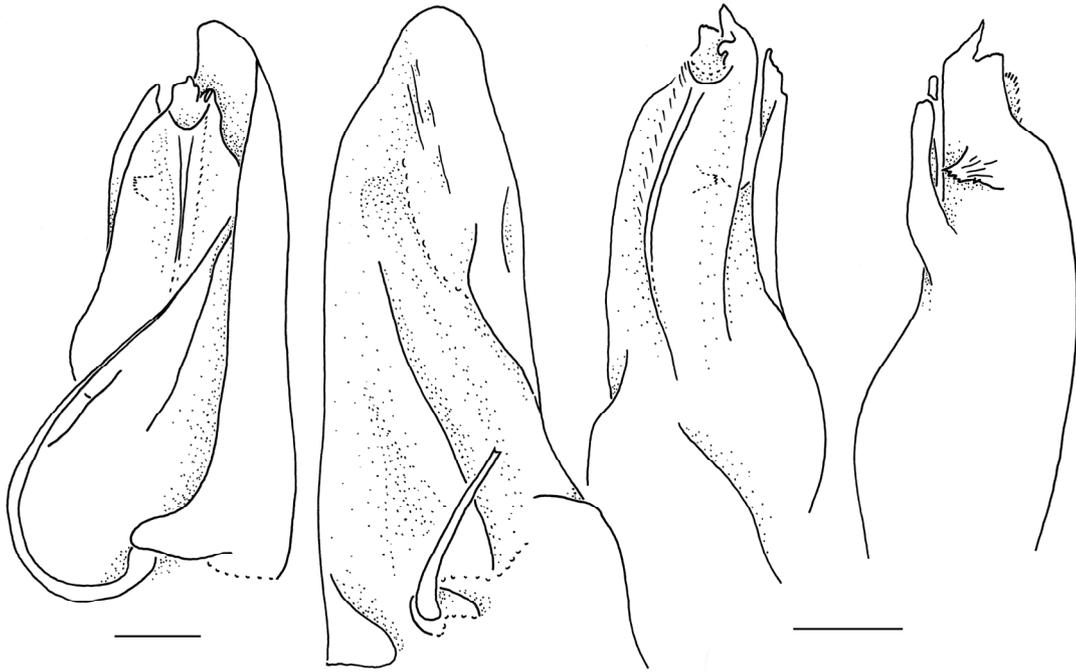


Figure 2. *Megaphyllum silvaticum* male from Jósvalfő, Hungary. Right gonopods as they are joined *in situ*, mesal view (scale bar: 0.2mm); left promere, meso-caudal view; left opisthomere, mesal and lateral view, respectively (posterior process broken) (scale bar: 0.2 mm)

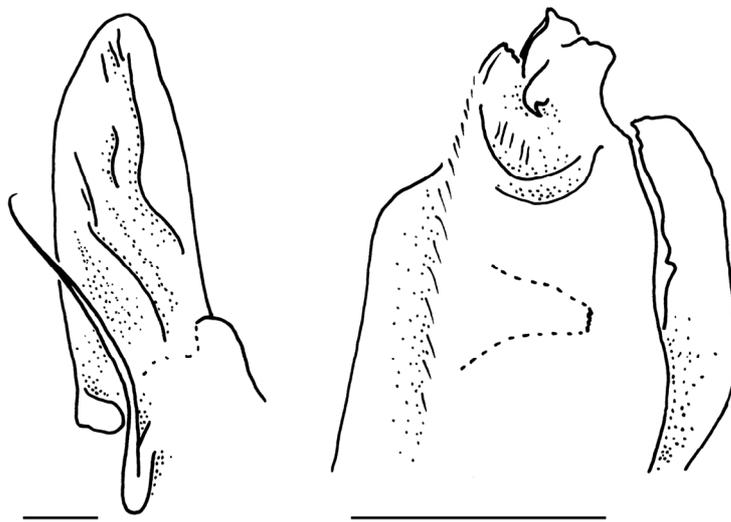


Figure 3. *Megaphyllum silvaticum discolor* male type specimen from Bruck, Austria. Left promere, meso-caudal view, left opisthomere, mesal view (scale bars: 0.2 mm)

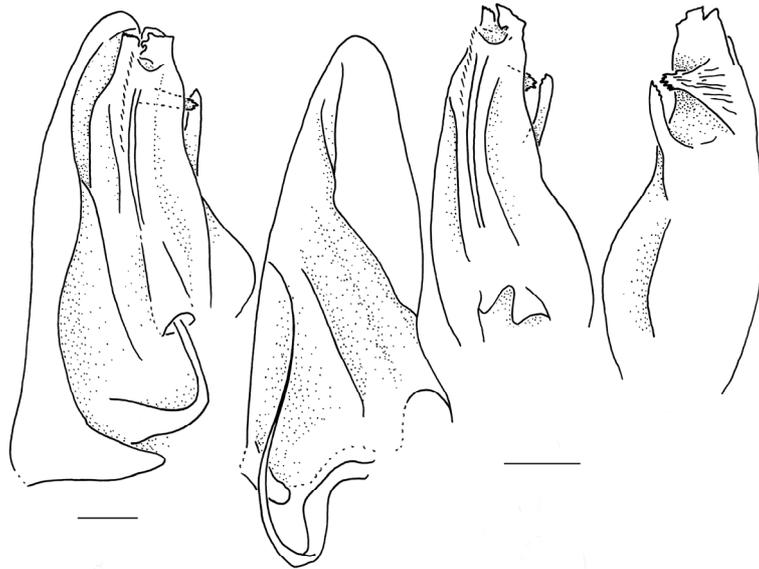


Figure 4. *Megaphyllum silvaticum* male specimen from Statiunea Borşa, Maramureş. Left gonopods as they are joined *in situ*, mesal view (scale bar: 0.2 mm); left promere, meso-caudal view; left opisthomere, mesal and lateral view, respectively (scale bar: 0.2 mm)

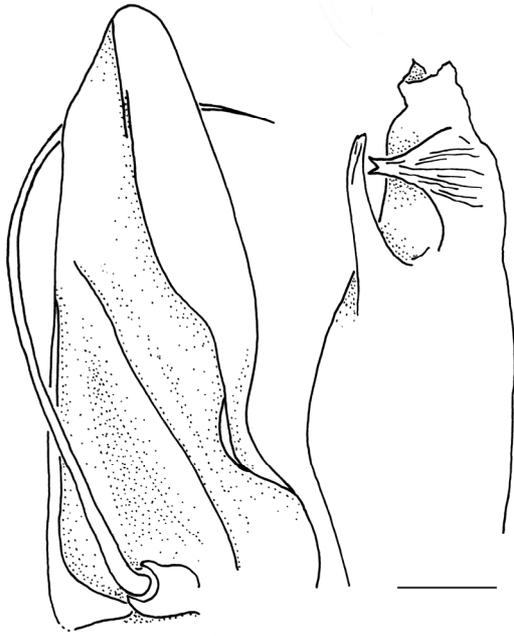
DISCUSSION

The distribution map (Fig. 1) seems to show an ambiguous distribution pattern for *M. s. cf. silvaticum* and *M. s. cf. discolor*. *M. s. cf. discolor* is likely to be restricted to the southwestern part of the distribution area. The problem arises, however, as the Hungarian *M. s. cf. discolor* specimen and the specimen from Skalka (Verhoeff, 1941) are in the centre of the area of *M. s. cf. silvaticum*. If we take into consideration those distributional records from the literature which refer to wider areas, we clearly get two overlapping ranges. The male from the extreme northeast with different gonopods further complicates the concept. Jawłowski already reported remarkable variability in the length of the opisthomere's posterior process from Ukraine (Jawłowski 1936, with figures on three types of gonopods, figs. 10–13). In Zaroślak he also found a gonopod like the one we had from Vinişor Valley. He supposed that the length of the posterior process is in relationship with the altitude above sea level, i.e. the higher is the elevation the smaller the is the process. Ložek & Gulička (1962) in their work on the millipede fauna of NE Slovakia mention that

they found considerable gonopod variation of *M. silvaticum* in the size of the anterior process of the opisthomere. They found no variability in the length of the posterior process with regard to geographical altitude. Neither did our data support Jawłowski's assumption.

Although Verhoeff (1907) gave a detailed morphological description of the two forms, he admittedly could not attach coherent distributional patterns to them. He declared his opinion on the concepts of subspecies and of variation, that he did not consider the geographical distribution as relevant, only morphology. According to the modern concept of subspecies, a disjunct distributional pattern is important, so the form *discolor* can not presently be considered as a subspecies. Our investigation of females supports this statement (see below).

Verhoeff (1898) in his original description stated that regarding to form, sculpture, and size *M. silvaticum* looks like *M. projectum*, so he specified the differences in the gonopods, and gave an illustration of the opisthomere (Verhoeff, 1898, fig. 26).



According to our observations, gonopods of adult males really look differently, even *in situ*, and this can rarely change due to its actual position during fixation in ethanol. Dissected gonopods at high magnification under stereo or biological microscope are clearly different. One year later Verhoeff (1899) gave a description of the females which, nevertheless, fits well to *M. projectum* females, too. He observed one difference between the two species in the striation of the prozonites, but later he rejected it as not reliable (Verhoeff, 1907). He found some differences in the coloration, but even if it was useful, there are many known colour variations of *Megaphyllum* females, and after preservation in ethanol, such colour differences can easily vanish.

Figure 5. *Megaphyllum silvaticum* male holotype, from Schuler (i.e. Mt. Postăvarul). Left promere, meso-caudal view; left opisthomere, lateral view (scale bar: 0.2 mm)



Figure 6. *Megaphyllum silvaticum* male specimen from Vinişor Valley, Maramureş. Right gonopods as they are joined *in situ*, mesal view (scale bar: 0.2 mm); left promere, meso-caudal view; left opisthomere, mesal and lateral view, respectively (scale bar: 0.2 mm)

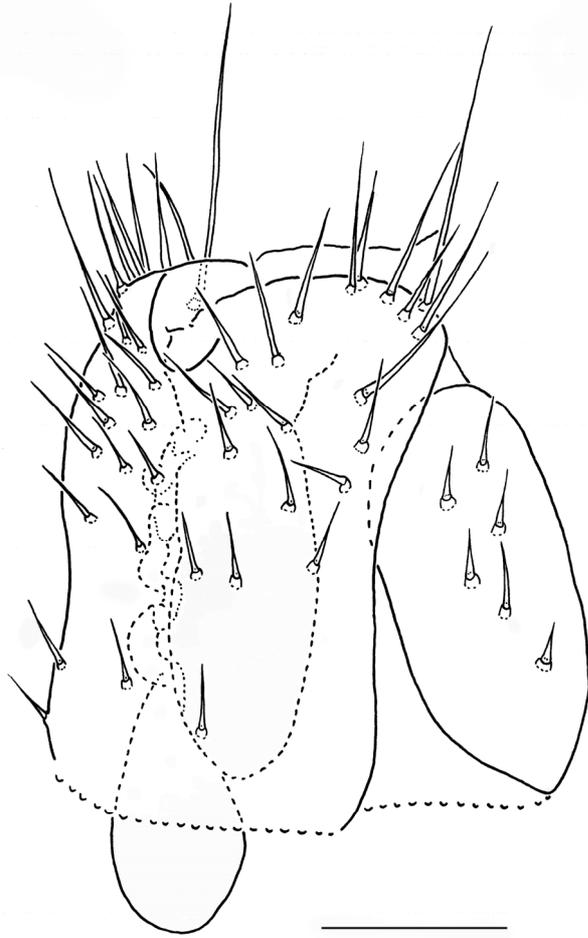


Figure 7. *Megaphyllum projectum* female, left vulva, meso-caudal view (scale bar: 0.2 mm)

Our efforts to find female differences proved to be successful when looking at the female vulvae. There is one early pioneer work on the female characterisation of Hungarian diplopods, but Sziráki (1966) dealt only with the external morphology of vulvae. Based on his work and our present study the *Megaphyllum* females from different parts of Hungary with spiral apodemic tube (Fig. 7) and big ampullas belong to the species *projectum*. The entire vulva is relatively bigger than the other type (Fig. 8, see scale bars), and in most cases, the big ampulla can be seen after dissection, with high magnification, in stereo microscope, too. In Maramureş (Korsós & Lazányi, 2008) only two species of *Megaphyllum* were found, *M. projectum* and *M. silvaticum*, whereas in

the ANP, in our earlier survey (Lazányi & Korsós, 2009), presence of *M. unilineatum* was proved as well. The latter species can easily be identified, so we can state, that the more simple type of vulvae (Fig. 8) belongs to *M. silvaticum*. The inner structure of this vulva type needs preparation in Faure-Berlése medium. An other point which can help at the identification is the age of the individuals. It seems that by *M. silvaticum* the adult female specimens are in the developmental stadia X-XI, while by *M. projectum* they are in the stadia XI-XIII. Thus, a female in the stadia XII-XIII probably belongs to the species *M. projectum*.

In conclusion, we found three different types of *Megaphyllum silvaticum* male gonopods, with no relation to any distribution pattern, and only one type of female vulvae. Thus we can say that all types of males belong to one species, to *M. silvaticum*, which can have a special variability of the length in the posterior process. The form *discolor* henceforward cannot be considered as a subspecies, only a variation with no taxonomical value.

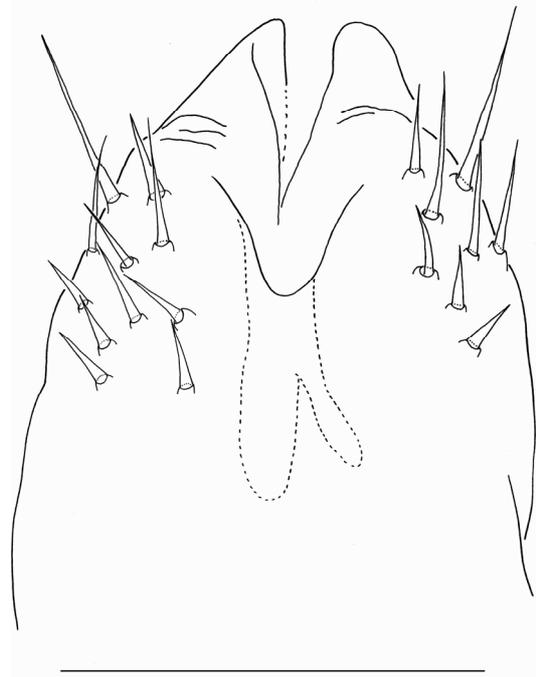


Figure 8. *Megaphyllum silvaticum* female, right vulva, mesal view (scale bar: 0.2 mm)

M. projectum is a very common Central European forest species sometimes in very high abundance, so in general ecological or faunistic studies usually it is difficult to expect to dissect all the individuals for male gonopod inspection. However, as at higher elevations *M. silvaticum* can co-occur with *M. projectum*, and their external morphology is highly similar, the closer investigation of selected males and/or females for their sexual organs is inevitable to prove the presence or absence of one or the other species.

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