

Taxonomic position of *Ceratophysella granulata* STACH, 1949 and *Ceratophysella silvatica* (RUSEK, 1964) in the light of morphological and laboratory hybridization studies

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Abstract. Morphological and laboratory hybridization studies were performed on the problematic springtail species *Ceratophysella granulata* and *C. silvatica*. The studies revealed that the species under consideration are well morphologically defined and well reproductively isolated. Consequently their status of separate species was maintained.

Key words: Collembola, Hypogastruridae, *Ceratophysella granulata*, *C. silvatica*, taxonomy, morphology, laboratory hybridization.

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I. INTRODUCTION

Polymorphism linked with the reproductive cycle (epitoky) is a common phenomenon within the genus *Ceratophysella* BÖRNER, 1932. Transition from the non-reproductive stage to the reproductive one involves strong morphological changes manifested by regression of mouth parts, chaetotaxy, claws, furca and progressive changes of some sensillary structures mostly in males (BOURGEOIS 1974, SKARŻYŃSKI 2000). In the *Ceratophysella armata*-group the reproductive stage may be preceded in suitable ecoclimatic conditions by the pre-reproductive stage characterized by chaetotaxy composed of some spine/spike-like setae (BOURGEOIS 1973, SKARŻYŃSKI 2003). Both polymorphisms are very important from the taxonomic point of view, since different „morphs” of the same species can be recognized as separate species. In this way for example, the pre-reproductive stage (with spine-like setae) of *C. armata* (NICOLET, 1841) from the Alps was described as *C. armatissima* GISIN, 1958 (see SKARŻYŃSKI 2003). In this context, the similar and sympatric species of the *C. armata*-group which differ in the character of setae are suspected to be conspecific. A pair of such species represent *Ceratophysella granulata* STACH, 1949 and *C. silvatica* (RUSEK, 1964). Based on original descriptions (STACH 1949, RUSEK 1964) one can get the impression that the single clear diagnostic feature which separates these European hemiedaphic montane species is the character of setae d_2 on the head (*C. granulata* – normal, *C. silvatica* – spine-like). However, the diagnostic value of this character seems to be low in the light of BABENKO's notes on the variability of *C. silvatica* (BABENKO et al. 1994). According to this author,

C. silvatica can possess either normal or spine-like setae d_2 on the head. For this reason he expressed a presumption that *C. silvatica* may be synonymous with *C. granulata*.

The aim of this work was to verify the taxonomic status of *C. granulata* and *C. silvatica* based on morphological and laboratory hybridization studies.

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II. MATERIAL AND METHODS

Morphological studies

In 2002 the *C. granulata* population from the Tatra Mts (W Carpathians, S Poland) and the *C. silvatica* population from the Beskid Niski Mts (W Carpathians, SE Poland) were studied.

C. granulata was collected from litter of dwarf mountain pine shrubs on the slopes of the Gładkie Uplaziańskie, at an altitude of 1600 m a.s.l., on 14. V, 13. VI, 12. VII, 14. IX, 7. X.

C. silvatica was collected from litter of the Carpathian beech forest on the slopes of the Ostra Góra near village Tylawa, at an altitude of 500 m a.s.l., on 11. V, 16. VI, 14. VII, 12. IX, 28. X.

More than 300 specimens of *C. granulata* (including 22 reproductive females, 16 reproductive males) and 200 specimens of *C. silvatica* (including 16 reproductive females, 11 reproductive males) were collected.

The populations under study were identified on the basis of the original descriptions (STACH 1949, RUSEK 1964). The taxonomic status of the studied population of *C. granulata* was confirmed on the basis of examination of STACH's type materials from the Tatra Mts (for localization data see STACH 1949).

Morphology of collected specimens was examined using a microscope with phase contrast lighting.

Laboratory hybridization studies

Laboratory crosses were set up with *C. granulata* and *C. silvatica* collected in the Tatra Mts and the Beskid Niski Mts respectively (same localization data as above). Pure-species mass cultures of *C. granulata* and *C. silvatica* were set up in July and May 2001 respectively. The ability of studied populations to reproduce was tested for 6-8 months. Since they reproduced satisfactorily, at the beginning of February 2002 adult females originated from the mass cultures were isolated separately. After three weeks of isolation they were mated with heterospecific males originated from the mass cultures. Only females after ecdysis were used. The following interspecific micropopulations were prepared: 10 (3 females *C. granulata* x 2 males *C. silvatica*) and 10 (3 females *C. silvatica* x 2 males *C. granulata*).

In order to check the ability of both species to reproduce by means of parthenogenesis, three micropopulations consisting of three females were set up. The interspecific micropopulations and the isolated females were cultured for five to eight months. The pure-species mass cultures were maintained and observed until the end of the studies.

The interspecific micropopulations, the isolated females and the pure-species mass cultures were kept in tightly closed Petri dishes (35×10 mm) and plastic containers (60×65 mm) respectively, both half filled with a mixture of plaster of Paris and activated charcoal (9:1). The culture vessels were kept in a glass-fronted cooler, in an average temperature of 12°C. A few drops of distilled water were added twice a week to maintain high air humidity.

Yeast was used as food. Old exuvia, yeast and fungi contaminating cultures and inhibiting reproduction were periodically removed.

Eggs produced by interspecific crosses were counted. Dead individuals from all crosses were identified to species.

Crosses were repeated in 2003. At the beginning of May and June females of *C. silvatica* and *C. granulata* respectively collected in the field were isolated separately. The interspecific micro-populations were prepared using the experimental procedure described above. Cultures were maintained until the end of October 2003.

III. RESULTS

Morphological studies

The investigations showed that *C. granulata* and *C. silvatica* passed through only one polymorphism (epitoky) in their life cycles and the morphology of their non-reproductive and reproductive stages was species-specific.

Non-reproductive stages of *C. granulata* (Figs 1-8) and *C. silvatica* (Figs 9-17) proved to be easy to separate on the basis of numerous morphological features (Tab.) e.g. the character of setae d_2 on head, number and shape of sensilla in ventral file on antennal segment IV, presence/absence of setae p_3 on abdominal tergum IV, length of setae (especially microchaetae), distribution and character of strong granulation fields (especially on abdominal terga), length of empodial appendage and others.

Reproductive stages of *C. granulata* and *C. silvatica* differed to a smaller degree than non-reproductive stages. This phenomenon, which seems to be a rule in the genus *Ceratophysella* (see SKARŻYŃSKI 2002), results from the simplified morphology of the reproductive stage. During reproductive stage of both species the ventral sensilla on antennal segment IV became short and blunt at tips, the eversible sac between antennal segments III-IV disappeared, the head of maxilla diminished (lamellae became shortened and their marginal filaments became shorter), the body granulation became more delicate (especially in *C. granulata*), the claws, anal spines, setae lessened, mucro became short and narrow and the cuticular skeleton of furca became delicate.

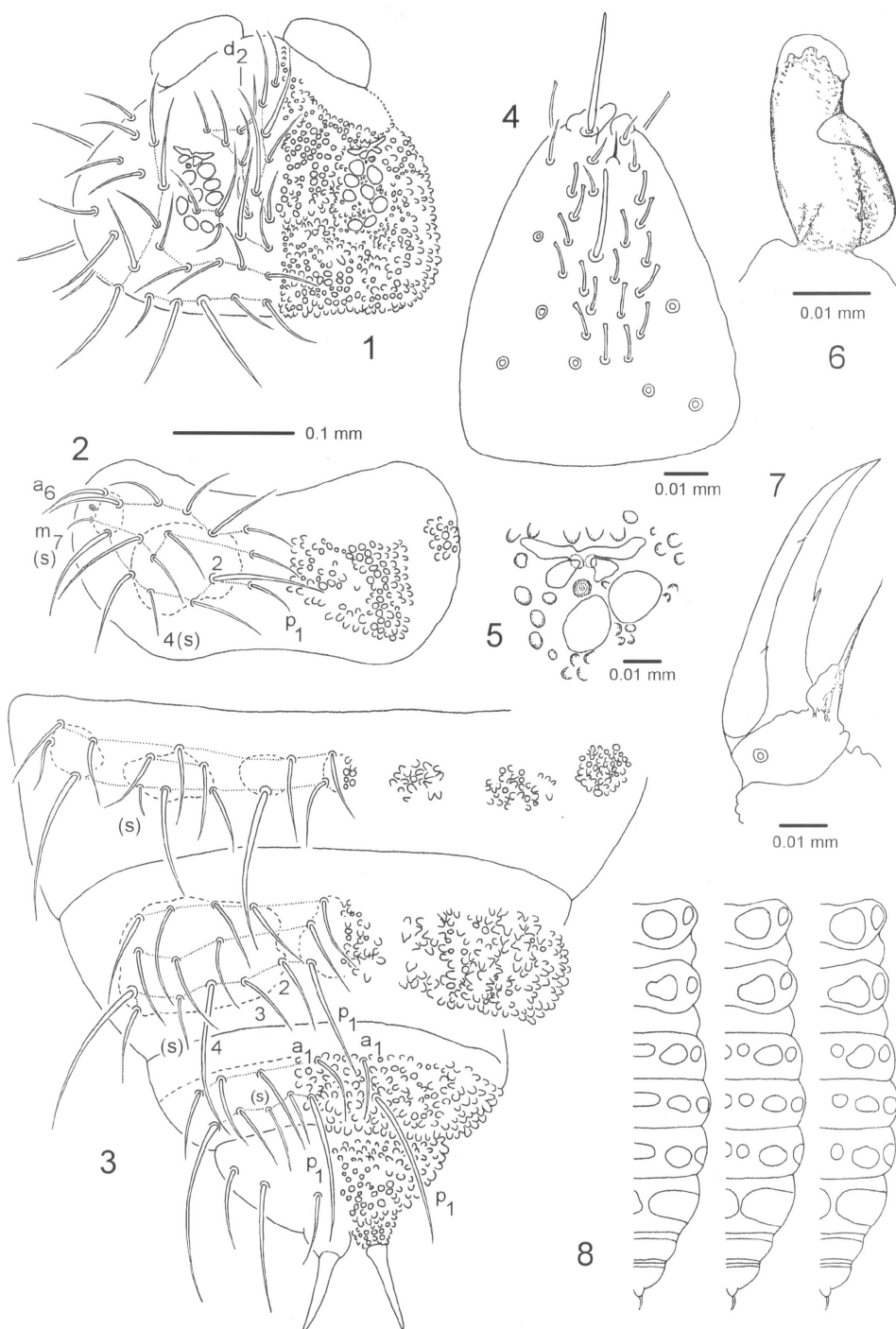
Reproductive individuals of both species showed also some progressive modifications: the fully developed genital plates (with visible internal pouch-like structures in females and swollen ejaculatory duct in males), inflated abdomen in females, enlarged sensilla in antennal III-organ in males, enlarged postantennal organ in males (Fig. 14) and enlarged body sensilla in both sexes (especially in males of *C. silvatica*). Reproductive males of both species had also pouch-like structures inside ventral tubus.

Particular note may be taken of a fact that in *C. silvatica* during reproductive stage spine-like setae d_2 on head became normal. Consequently this feature can not be used in the diagnostics of the mentioned stage. Other characters, i.e. a number of sensilla in ventral file on antennal segment IV, presence/absence of setae p_3 on abdominal tergum IV and distribution of strong granulation fields allow to separate reproductive stages of *C. granulata* and *C. silvatica*.

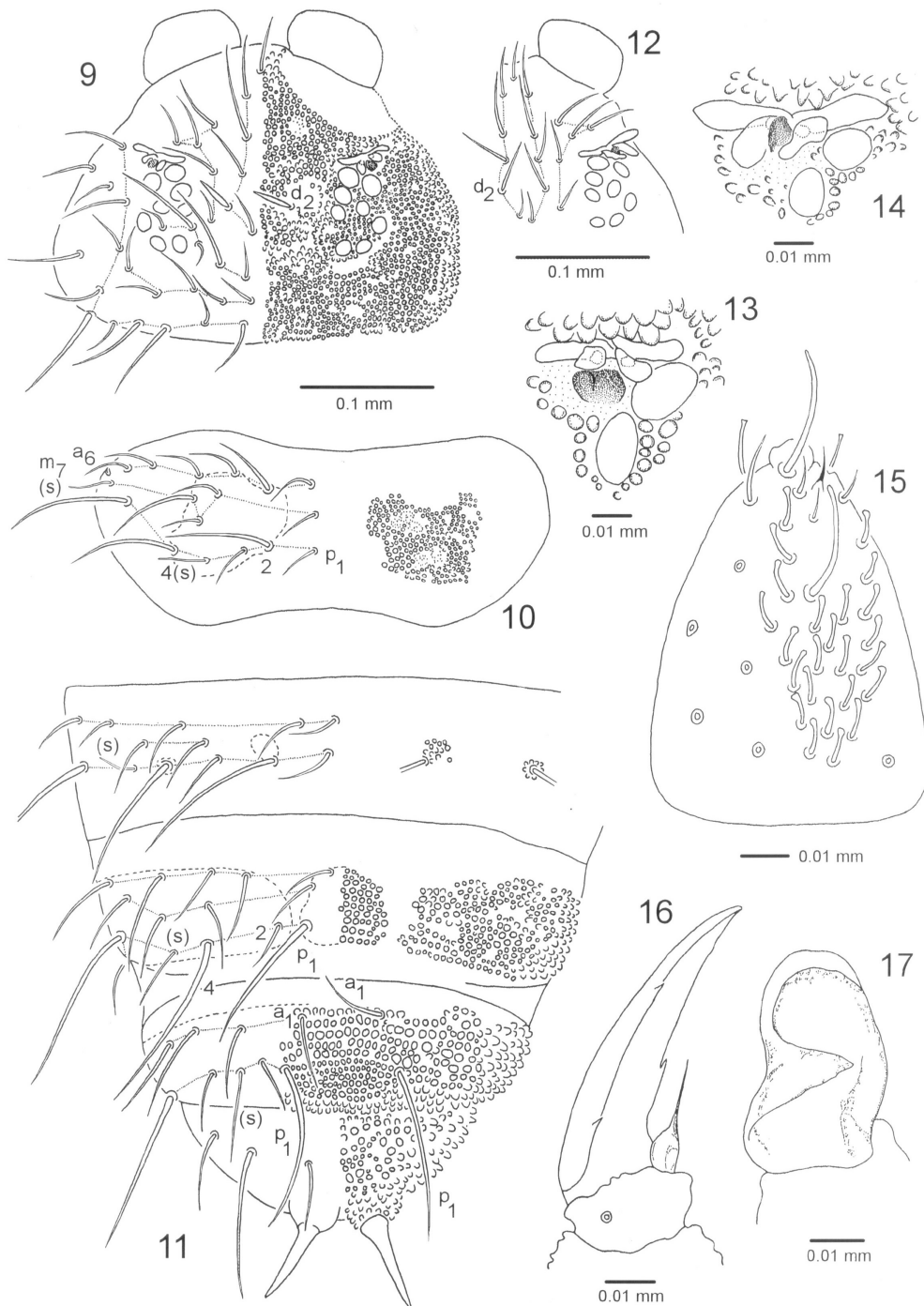
Laboratory hybridization studies

Laboratory investigations revealed that *C. granulata* and *C. silvatica* were not able to hybridize. Interspecific crosses produced some eggs but no offspring. In contrast, eggs and offspring were observed in the pure-species mass cultures throughout the breeding period.

Crosses between *C. silvatica* females and *C. granulata* males produced fewer eggs and less frequently than the reciprocal ones. Only one cross with *C. silvatica* as maternal parent produced 7 eggs in 2002 and two such crosses produced 5 and 9 eggs in 2003. Five reciprocal crosses produced 6-21 (mean 11) eggs in 2002 and six such crosses produced 4-18 (mean 10) eggs in 2003.



Figs 1-8. *Ceratophysella granulata* STACH, 1949. (1-3) – chaetotaxy and distribution of strong granulation fields on: 1 – head, 2 – thoracic tergum II, 3 – abdominal terga III-VI, 4 – ventral side of antennal segment IV, 5 – postantennal organ, accessory boss and neighbor ocelli, 6 – mucro, 7 – empodial appendage and claw III, 8 – patterns of distribution of strong granulation fields on thoracic tergum II – abdominal tergum VI.



Figs 9-17. *Ceratophysella silvatica* (RUSEK, 1964). (9-11) – chaetotaxy and distribution of strong granulation fields on: 9 – head, 10 – thoracic tergum II, 11 – abdominal terga III-VI, 12 – subaxial chaetotaxy of head of reproductive male, 13 – postantennal organ, accessory boss and neighbor ocelli, 14 – postantennal organ, accessory boss and neighbor ocelli of reproductive male, 15 – ventral side of antennal segment IV, 16 – empodial appendage and claw III, 17 – mucro.

Table I

Morphological characteristics of non-reproductive adults of *C. granulata* and *C. silvatica*. Abbreviations: ant. IV – antennal segment IV, th. II-III – thoracic terga II-III, abd. I-VI – abdominal terga I-VI. All measurements in μm

Character	<i>C. granulata</i>	<i>C. silvatica</i>
Number and shape of ventral sensilla on ant. IV	16-23 (m. 18), thin, weakly bent and flattened at tips (Fig. 4)	20-26 (m. 24), thick, distinctly bent and flattened at tips (Fig. 15)
Size and shape of accessory boss in postantennal organ	medium size, globular or oval (Fig. 5)	large, oval, irregularly folded, sometimes subdivided (Fig. 13)
Character of setae d_2 on head	normal (100%) (Fig. 1)	spine-like (100%) (Fig. 9)
Presence of setae p_3 on abd. IV	both setae present (84%), one seta present (16%) (Fig. 3)	both setae absent (90%), one seta present (5%), both setae present (5%) (Fig. 11)
Length of microchaetae p_1 and a_6 on th. II	40-74 (m. 50), 50-90 (m. 66) (Fig. 2)	26-40 (m. 35), 34-56 (m. 43) (Fig. 10)
Length of macrochaetae p_2 on th. II	80-140 (m. 96) (Fig. 2)	60-100 (m. 83) (Fig. 10)
Length of sensilla p_4 and m_7 on th. II	38-50 (m. 44), 20-28 (m. 24) (Fig. 2)	30-40 (m. 36), 22-34 (m. 30) (Fig. 10)
Ratio $a_6:m_7$ on th. II	2.33-3.75 (m. 2.78) (Fig. 2)	1.2-1.75 (m. 1.44) (Fig. 10)
Distances between setae a_1-a_1 and p_1-p_1 on abd. V (μm / number of granules)	30-76 (m. 50)/3-9 (m. 5), 46-80 (m. 62)/6-9 (m. 7) (Fig. 3)	54-84 (m. 67)/6-13 (m. 10), 70-104 (m. 89)/9-17 (m. 13) (Fig. 11)
Distribution of strong granulation fields	Head: large uniform field covering almost whole dorsal side, th. I: 2 medium size lateral fields, th. II-III: 2 large subaxial fields and 2 lateral ones of medium size, abd. I-III: 5-7 fields of medium size, abd. IV: 1 large axial field and 2 lateral large ones, abd. V-VI: large uniform fields covering almost whole dorsal side of terga (Figs 1-3, 8).	Head: large uniform field covering almost whole dorsal side, th. I: fields absent, th. II-III: 2 large subaxial fields, lateral ones absent, abd. I-III: 4 small fields, abd. IV: 1 large axial field and 2 lateral large ones, abd. V-VI: large uniform fields covering almost whole dorsal side of terga (Figs 9-11).
Character of strong granulation fields	Fields are made of irregularly distributed granules, which are broad and high and often bent in different directions (Figs 1-3).	Fields are made of densely and regularly distributed granules, which are comparatively broad and low (Figs 9-11).
Character of strong granulation field on abd. V	Large granules are distributed from the line extended between setae a_1 to the hind margin of tergum (Fig. 3).	Large granules are distributed from the line extended between setae a_1 to the line extended between setae p_1 . Behind this line granules are small and put together in a characteristic pattern (Fig. 11).
Length of empodial appendage and inner edge of claw 3	28-40 (m. 31), 48-68 (m. 55) (Fig. 7)	16-24 (m. 20), 46-60 (m. 51) (Fig. 16)
Ratio empodial appendage: claw 3	0.52-0.68 (m. 0.57) (Fig. 7)	0.33-0.44 (m. 0.4) (Fig. 16)
Length of mucro	30-44 (m. 33) (Fig. 6)	30-42 (m. 37) (Fig. 17)
Width of apical/basal part of mucro	8-19 (m. 13)/12-22 (m. 16) (Fig. 6)	16-22 (m. 19)/18-26 (m. 21) (Fig. 17)
Ratio length: width of terminal part of mucro	0.22-0.52 (m. 39) (Fig. 6)	0.47-0.6 (m. 0.51) (Fig. 17)

All these eggs quickly stopped developing, most likely in a result of abortive heterospecific fertilization after successful matings (isolated females did not lay eggs altogether). However, unfertilized eggs could be spontaneously produced by *C. granulata* and *C. silvatica* females which is less probable. Such a phenomenon was detected in *Ceratophysella denisana* YOSII, 1956 (KOJIMA 1985).

Considering all these facts one can ascertain that *C. granulata* and *C. silvatica* are well reproductively isolated. Prezygotic barriers incompletely prevented matings, but completely prevented fertilization.

IV. DISCUSSION

Morphological and laboratory hybridization studies showed that *C. granulata* and *C. silvatica* are separate species under the morphological and biological species concepts, since they are well morphologically defined and well reproductively isolated. Moreover, epitoky in a typical form was the one polymorphism detected in the life cycles of both species.

The presumption expressed by BABENKO et al. (1994) that *C. silvatica* may be synonymous with *C. granulata* proves to be false. This author probably compared reproductive specimens of *C. silvatica* with normal setae d_2 on head or juveniles which share this character with the specimens of *C. granulata*.

Remarkable morphological similarity and incomplete premating isolation between the studied species suggest their comparatively close relationship. Although fertilization and developmental systems of *C. granulata* and *C. silvatica* are strongly incompatible, their mating systems are compatible to a certain degree (if one accepts the assumption that females were activated to egg production by the heterospecific males' action). Even such a low degree of gene pools coadaptation suggests the common origin of *C. granulata* and *C. silvatica*.

Previous laboratory studies revealed that sibling (closely related) species of the genus *Ceratophysella*, *C. denticulata* (BAGNALL, 1941) and *C. engadinensis* (GISIN, 1949) can hybridize (SKARŻYŃSKI 2004b). Crosses between comparatively similar (comparatively closely related) species *C. cavicola* (BÖRNER, 1901) and *C. impedita* SKARŻYŃSKI, 2002 and *C. denticulata* and *C. stercoraria* STACH, 1963 produced embryo and unfertilized eggs respectively (SKARŻYŃSKI 2004c, b). In contrast, crosses between morphologically distinct (distantly related) species *C. impedita* and *C. engadinensis* did not produce even eggs (SKARŻYŃSKI 2004b).

With respect to all these data *C. granulata* and *C. silvatica* can be regarded as comparatively closely related, to the same degree as *C. denticulata* and *C. stercoraria*.

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