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Clinopodes vesubiensis: New species of centipede (Chilopoda: Geophilomorpha: Geophilidae) from Slovenia

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Abstract. Centipedes are a well studied and diverse goup of invertebrates with 98 confirmed species from Slovenia. During field surveys many specimens of unknown species ahave beenwere found, which awere recorded as *Clinopodes* n. sp. and deposited in the Department of Biology, Biotechnical Faculty of the University of Ljubljana. We examined 195 of these specimens and compared their morphology with the original description ofthe *Clinopodes vesubiensis* (Bonato, Iorio et Minelli, 2011). Furthermore, we analyzed the variation and its dependence on size of some of the morphological characters. Our results confirm that *Clinopodes vesubiensis* is a new species for the Slovenian fauna. By inspecting many specimens collected at seven locations throughout the year, we increased the knowledge of this species. We noticed substantial variation in some morphological characters. Distance from the previously known locations for this species in southeastern France is remarkable, which raises questions about the history of this species and its taxonomic status.

Key words. Taxonomic characters, size-dependent morphological characters, Chilopoda, Geophilomorpha, Geophilidae, *Clinopodes vesubiensis*, Slovenia.

INTRODUCTION

Studies on centipedes in Slovenia reach far back in history, with first records over 300 years ago, when J. V. Valvasor (1641–1693) depicted a lithobiid centipede (Valvasor 2004). In the 18th century Giovanni Antonio Scopoli collected similar specimens from Idrija and sent them to C. L. Koch, who on this basis described *Lithobius grossipes* (Koch, 1847), now *Eupolybothrus grossipes* (Eason, 1970). First extensive studies in the area of northwest Balkans date back to Latzel (1880, 1882), Attems (1895, 1929, 1949, 1959) and Verhoeff (1929, 1934, 1937) and resulted in the gathering of essential faunistical data and descriptions of some species with type localities in this region. This area is part of the Mediterranean basin and one of the hotspots of biodiversity (Myers et al. 2000). Centipede diversity in some areas in Slovenia has been intensively studied (Bagola 1997, Grgič 2005, Grgič & Kos 2003, 2005, Kohek 2012, Kos 1988, 1992, 1995a, 1995b, 1996, Kos & Praprotnik 2000, Kos et al. 2015, Pagon 2006, Ravnjak 2006, Ravnjak & Kos 2015, Vode & Kos 2014) during which unidentified species were occassionally found. Overall we can claim that the Slovenian centipede fauna is thoroughly studied and diverse, with 98 confirmed species (Ravnjak & Kos 2015). Geophilid centipedes are well represented in samples from different habitats, including three species in the genus *Clinopodes* C. L. Koch, 1847; *C. carinthiacus* (synonym *C. trebevicensis*), *C. flavidus* and an unidentified species. Determination of taxa of Geophilomorpha is an arduous task, due to the fact that the descriptions of most species are based on just a few specimens, often from only one population (Bonato et al. 2014). Thus variation within and between populations, as well as age-dependant variation are poorly studied (Misioch

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1978). Consequently, their taxonomy is often confusing with their taxonomic status unclear and many synonyms (Bonato & Minnelli 2014). For this reason, many of the master's theses presented in the Biotechnical faculty, University of Ljubljana focus on morphological characters of centipedes, including some geophilid species (Huzminec 2009, Mikoš 1989, Mulej 2016, Lesar 2002, Schoss 1991).

In an effort to clarify the taxonomy, Bonato et al. (2011) reviewed the genus *Clinopodes* (Chilopoda, Geophilomorpha, Geophilidae) and described a new species to science *Clinopodes vesubiensis* (Bonato, Iorio & Minelli, 2011) based on six specimens (four females and two males), found in the Maritime Alps in southeastern France. Its eye-catching elongated forcipular denticles led us to further investigate the similarities between *C. vesubiensis* and specimens in our collection, which were until now stored and reported as *Clinopodes* n. sp. We examined the specimens and compared their morphology with the original description of *Clinopodes vesubiensis*. Moreover, we analyzed the variation within and between populations and age/size-dependent variation of some morphological characters. We included many specimens sampled at different localities and at different times in a year, in order to obtain a good overview of the variability of characters in specimens from Slovenia.

MATERIAL AND METHODS

We examined 195 specimens (50 males, 47 females, 94 larvae, 4 ex) stored in the Department of Biology of the Biotechnical Faculty of the University of Ljubljana, labelled as *Clinopes* n. sp. These specimens were collected at seven localities in Slovenia since 2000 (Fig. 1). Soil samples were collected using a soil corer (fi 21 cm) and later extracted using a modified Berlese-Tullgren funnel.

Specimens, stored as microscopic preparates in Swann media, were examined under an Olympus CX41 (100–400×) microscope. A few specimens that were stored in 70% ethanol were examined under an Olympus SZX7 (11.5×) stereomicroscope. Photographs and measurements were taken using a microscope camera Olympus XC30 and programme Cell'A.

We measured the following characters: number of leg-bearing segments, head length, distance between coxosternal condyles, number of pores on 1st, 7th and antepenultimate sternite, number of short and long setae on the 1st and 7th

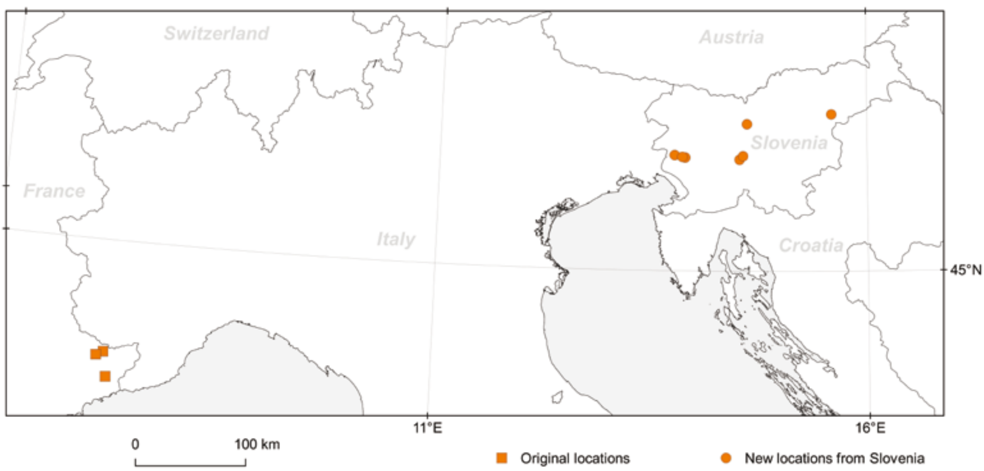

Fig. 1. Map showing the locations of *Clinopodes vesubiensis* (Bonato, Iorio et Minelli, 2011) in southeastern France (squares) and the new locations in Slovenia (circles).

Table 1. Range of values for some of the morphological characters of specimens of *Clinopodes vesubiensis* from Slovenia. Those marked with * are size-dependent

character	range
number of leg-bearing segments	♂♂ 55–61 / ♀♀ 59–63
head length (µm)*	365–1230
distance between coxosternal condyles (µm)*	220–715
number of pores on 1st sternite*	2–11
number of pores on 7th sternite*	8–49
number of pores on antepenultimate leg-bearing sternite*	12–115
number of long setae on 1st sternite	5–7
number of long setae on 7st sternite	4–8
number of short setae on 1st sternite*	2–18
number of short setae on 7th sternite*	6–66
number of coxal pores (on both coxae)*	2–29
number of setae on first genital sternite*	2–36

sternite, number of coxal pores (numbers given are sums of the pores on both legs) and number of setae on first genital sternite. We also examined the shape of denticles on the forcipular coxosternite in order to determine if the chitin lines reach the condyle, size of basal denticle on the tarsungulum and number and size of labral denticles.

Material examined

19 ♂♂, 19 ♀♀, 43 larvae, 2 ex, Municipality of Ig, Iška village, 45°54'44"N, 14°30'39"E, 2000–2003, 409–455 m a. s. l., forest dominated by *Fagus sylvatica*, leg. F. Kljun, I. Kos & T. Grgič;

16 ♂♂, 14 ♀♀, 34 larvae, 1 ex, Municipality of Poljčane, Boč hill, 46°17'00"N, 15°35'02"E, 2005, 610–675 m a. s. l., *Ostryo-Fagetum*, *Carici albae-Fagetum*, *Fraxino orni-Ostryetum*, leg. B. Ravnjak;

2 ♂♂, 7 ♀♀, 2 larvae, 1 ex., landfill, Duplica village by Kamnik, 46°12'12"N, 14°35'23"E, 2007, 360 m a. s. l., parks (planted trees), leg. F. Kljun & T. Grgič;

4 ♂♂, 2 ♀♀, Municipality of Nova Gorica, Šempas village, 45°56'46"N, 13°44'41"E, 2 April 2009, 294 m a. s. l., thermophilic forest, leg. F. Kljun & B. Ravnjak;

3 ♂♂, 2 ♀♀, 1 larva, Municipality of Ajdovščina, Križec hill, 45°55'36"N, 13°52'10"E, 24 April 2009, 630 m a. s. l., thermophilic forest, leg. F. Kljun & B. Ravnjak;

1 ♂, Municipality of Ajdovščina, Čaven hill, 45°55'54"N, 13°50'07"E, 24 April 2009, 1175 m a. s. l., forest dominated by *Fagus sylvatica*, leg. F. Kljun & B. Ravnjak;

5 ♂♂, 3 ♀♀, 14 larvae, Municipality of Ig, Draga village by Ig, 45°56'04"N, 14°32'57"E, 17 May 2017, 340 m a. s. l., mixed forest, leg. F. Kljun.

We determined the sex of 76 of the 94 specimens labelled as larvae (38 males, 38 females).

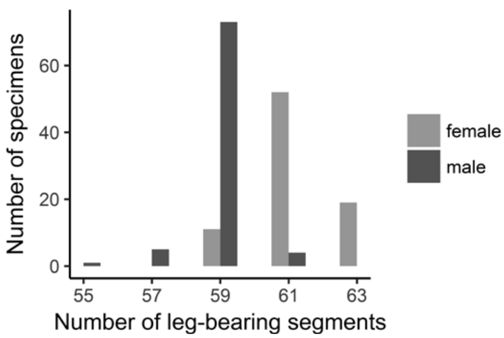

Fig. 2. Number of leg-bearing segments in males and females of *Clinopodes vesubiensis* from Slovenia.

RESULTS

General description of the specimens examined: Denticles on the forcipular coxosternite are distinctly elongated, ca. as long as wide, although the variation in size and shape is quite large (Figs. 3B–D, Fig. 4). The number of leg-bearing segments is 55–63, average 59 in males and 59–63, average 61 in females (Fig. 2). The sternal pore-fields on the posterior leg-bearing segments reach half way along the metasternites. Pore fields are divided in two on middle segments (from 22–23 to last 7–8). Coxal pores open independently, sometimes in 2 or 3 weakly recognizable clusters with isolated smaller pores lateral to the others (Fig. 3A). Chitin-line mostly does not reach the condyle. Labrum has tubercules and is without or with one denticle. Range of values for some morphological characters is given in Table 1.

There were a few specimens that were generally similar, but differed in one of the characters, presumingly because they were deformed. One specimen had two isolated pores instead of the diagnostic one. Three specimens had two denticles on labrum along with tubercules.

Of the size-dependent characters, positive correlations with distance between condyles were found for head length, number of pores on first, seventh and antepenultimate sternite, number

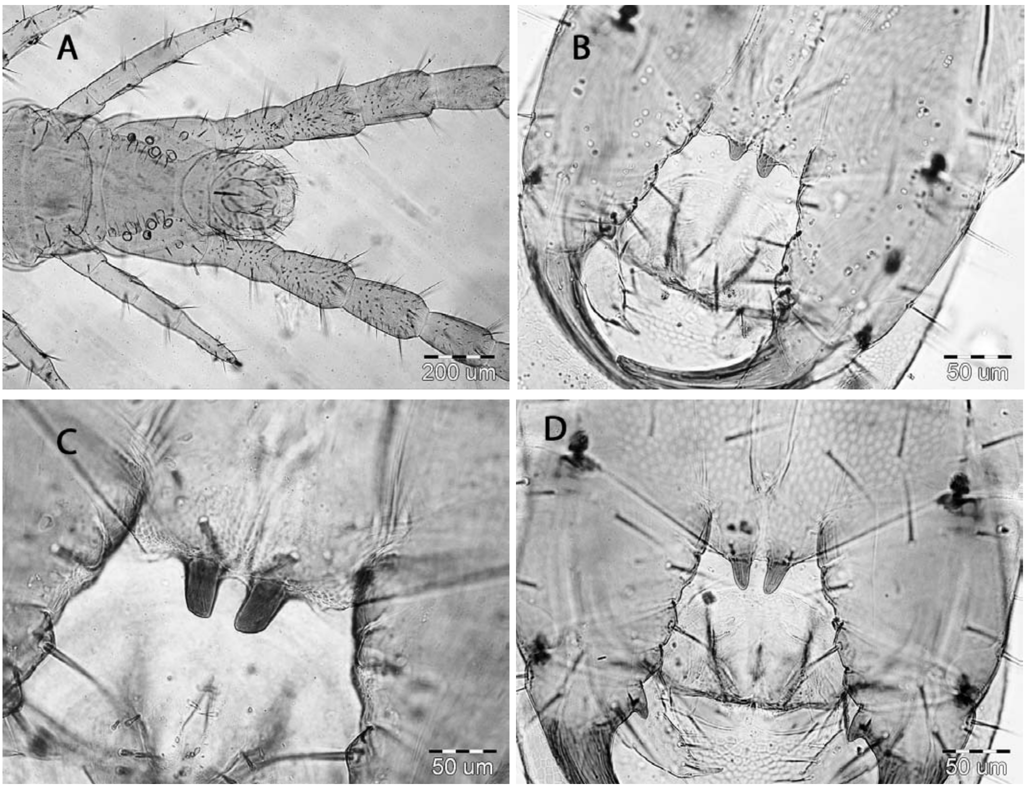

Fig. 3. *Clinopodes vesubiensis* from Slovenia. A – posterior segments with coxal pores, ventral view; B–D – anterior denticles on the forcipular coxosternite,ventral view.

of short setae on first and seventh sternite, number of coxal pores and number of setae on first genital sternite (Figs 5, 6).

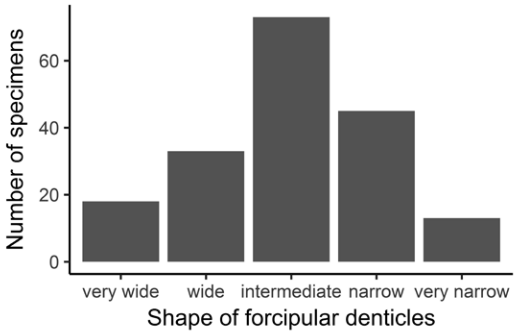

Fig. 4. Variation in the shape of the forcipular denticles on *Clinopodes vesubiensis* from Slovenia.

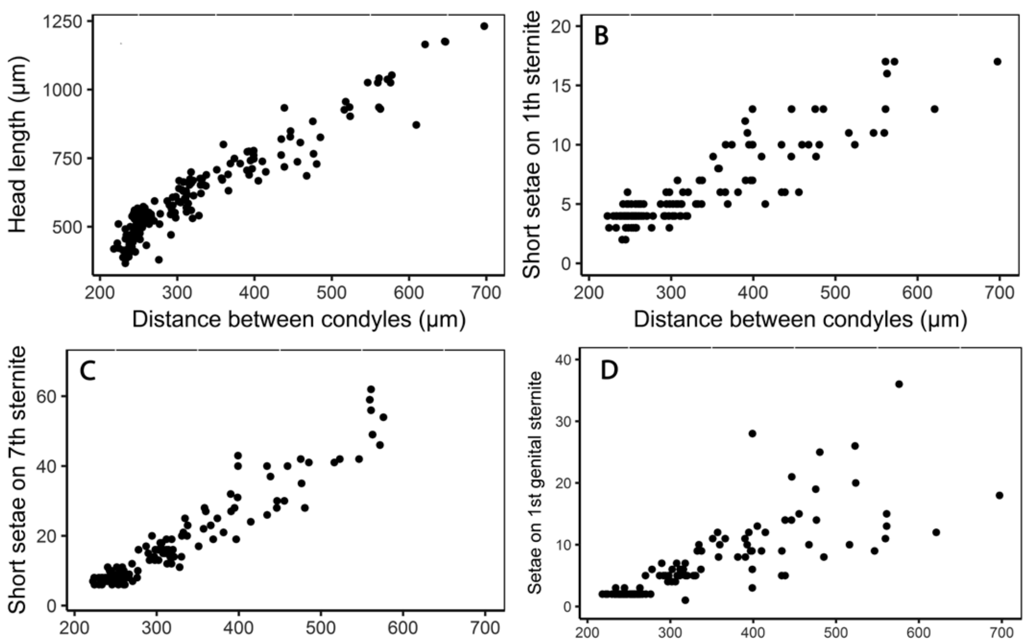

Fig. 5. A – Head length in relation to distance between condyles (n=185, r_p=,0.937 r_s=0.890). B – Number of short setae on first sternite in relation to distance between condyles (n=153, r_p=0.852, r_s=0.762). C – Number of short setae on seventh sternite in relation to distance between condyles (n=146, r_p=0.952, r_s=0.893). D – Number of setae on first genital sternite in relation to distance between condyles (n=153, r_p=0.854, r_s=0.888).

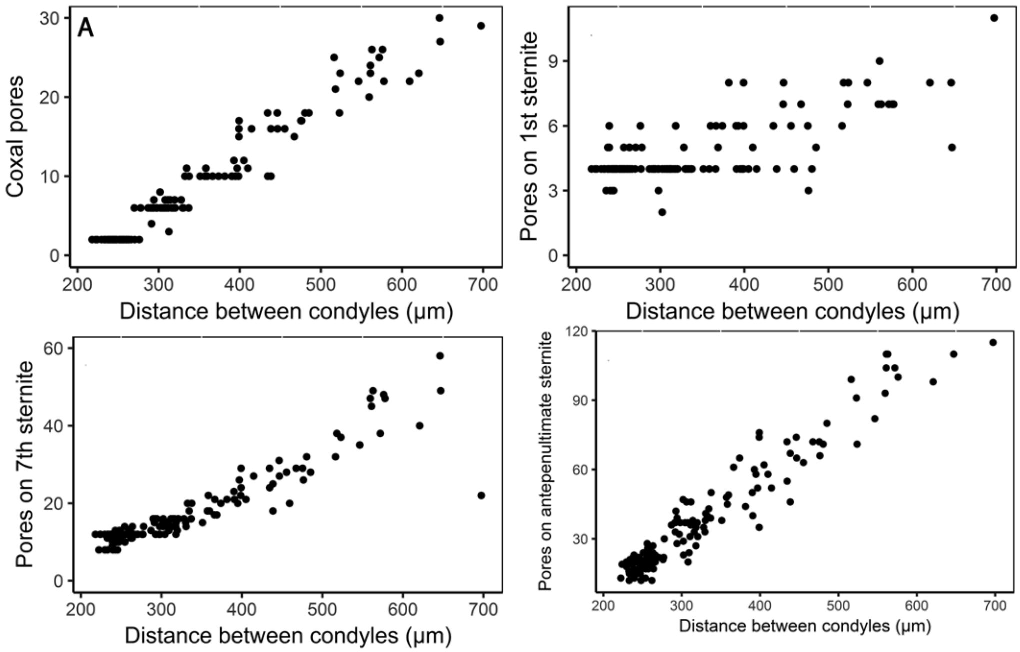

Fig. 6. A – Relation between the number of coxal pores (sum of both sides) and the distance between condyles (n=173, r_p=0.978, r_s=0.905). B – Number of pores on first sternite in relation to distance between condyles (n=176, r_p=0.678, r_s=0.469). C – Number of pores on seventh sternite in relation to distance between condyles (n=168, r_p=0.956, r_s=0.891). D – Number of pores on antepenultimate sternite in relation to distance between condyles (n=156, r_p=0.961, r_s=0.878).

DISCUSSION

On the basis of matching the main identification characters, we confirm a new species for Slovenia – *Clinopodes vesubiensis*. Morphological similarities indicate it is the same species as reported from France (Bonato et al. 2011), but the size and shape of its forcipular denticles are very variable. Nevertheless they still protrude more than in other species of *Clinopodes.* Some variation was also detected in in the basal denticle on the tarsungulum, which in most cases is even larger than cited in the original description. Furthermore, our specimens have no, one or two denticles on the labrum, but in the original description it is reported to have no denticles.

We noticed a discrepancy from the description in the shape of pore fields, which in our specimens are divided in two on the middle segments. This differences might be taxonomically important.

Further research is required to confirm that our speciemens are truly *Clinopodes vesubiensis* and thus can be used to define the diagnostic characters for this genus as suggested by Bonato et al. (2011). It might be that the Slovenian and French specimens belong to two populations of the same species or to two separate, yet morphologically very similar species. Their phylogenetic relationship needs to be resolved using molecular methods and a detailed morphological analysis of individuals from both populations (Bonato et al. 2014, Koch & Edgecombe 2008, Pilz et al. 2008). Perhaps due to recent divergence specimens from Slovenia lack noticeable morphological differences, but still belong to a different, yet cryptic species, as similarly reported by Fišer et al. (2018).

We noticed substantial variation in some characters that researchers find taxonomically important, for example the shape and size of the forcipular teeth. However, variation is present even at the same location, thus these differences may not be taxonomically significant. Similarly, the chitin line is an unstable character, often allowing different interpretations regarding (dis)continuity. Such characters can be useful when combined with others, but one should keep in mind that in some cases individuals deviate from the description of the identification traits (Misioch 1978). Furthermore, it would be more convenient if some additional more stable characters could be fiound. Another alternative is to combine more size-dependent attributes, though this would require more research on the development of characters in different species. Population-wide morphological studies reveal the quality of morphological traits and enable a more detailed comparison of different populations and perhaps even reveal cryptic species (Huzimec 2009, Kos 1997, Lesar 2002, Misioch 1978, Mulej 2016).

Some characters are size- as well as age-dependent and thus provide some insight into post--embryonic or post-larval development (Andersson 1978, Kos 1997, Mikoš 1989, Mulej 2016, Schoss 1991). A size-dependent attribute as such has little taxonomic value, but when combined with several other characteristics (number of coxal pores and distance between condyles) it can indicate a different species (Misioch 1978, Mulej 2016). For example misidentified *C. flavidus* have (among other diagnostic differences) a lower number of coxal pores in specimens with the same distance between condyles, compared to *C. vesubiensis* (Kos, unpubl.).

Up till now *C. vesubiensis* was known only form three localities in the Maritime Alps, France. New records from Slovenia indicate a wider and probably disjunct distribution of this species. This raises the question, what biogeographical history could account for this distribution. Perhaps, these populations were divided for long period existing in different refugia during the last glaciation. As suggested by Bonato, Iorio and Minelli (2011), the French population might have survived the Alpine glaciation as a relic or in refugial areas in the nearby Maritime Alps, whereas populations in Slovenia had a refugium in the Balkan peninsula, which is well known as refugium for other taxa (Kos 2000, Hewitt 2000). Another possible explanation is that the source of both populations was the same refugial area in the Balkans with some individuals spreading towards the French Alps. To account for the current distribution, this would require a colonisation path via areas in the south of the Alps. Microlocations with southern exposure in the Alps might also play an important role as refugia, as is the case for many plants and animals (Brus 2010, Dobrowski 2011).

Because of this it is possible it occurs in the area in between, perhaps even throughout the Alpine range, especially bearing in mind that this species was described less than ten years ago. Further molecular studies on individuals from both populations might provide insights into the biogeography of these taxa and that of other similar cases.

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