Towards elucidating species diversity of European inland *Strigamia* (Chilopoda: Geophilomorpha): a first reassessment integrating multiple evidence

Lucio Bonato[1], Francesca Bortolin[1], Giada De Zen[1], Peter Decker[2], E. Norman Lindner[3], Marco Orlando[4], Jörg Spelda[5], Karin Voigtländer[2], Thomas Wesener[6]

[1]*Department of Biology, University of Padova, Padova, Italy; National Biodiversity Future Center, Italy*
[2] *Senckenberg Museum of Natural History, Görlitz, Germany*
[3] *Leipzig, Germany*
[4] *Department of Biotechnology and Life Sciences, University of Insubria, Varese, Italy*
[5] *Staatliche Naturwissenschaftliche Sammlungen Bayerns, Zoologische Staatssammlung München, München, Germany*
[6] *Zoologisches Forschungsmuseum A. Koenig, Leibniz Institute for Analysis of Biodiversity Change, Bonn, Germany*

running title: integrative taxonomy of European *Strigamia*

ABSTRACT

Strigamia centipedes are widespread in European forest soils, however a complex inconsistent taxonomy developed over time. Based on a modern species concept, we evaluated multiple lines of evidence for speciation among inland populations from the Italian to the Baltic region. Hypotheses of species delimitation were drawn independently from: (a) morphological differentiation, by means of model-based cluster analyses of 52 specimens, after controlling for allometry and sexual dimorphism; (b) syntopy of morphologically distinct individuals, assumed as representatives of coexistent species, from >700 sites; (c) molecular differentiation, by various methods applied to COI sequences of the same 52 specimens. Diagnoses and geographical distributions were revised by re-examining >2000 specimens and the entire literature. We found preliminary evidence for: a *S. acuminata* species-complex, widespread, including a candidate species from the Eastern Alps differing in the forcipules; a *S. carniolensis* species-complex, often called *S. crassipes*, widespread, but absent from Sicily; a *S. crassipes* species-complex, often called *S. transsilvanica*, more widespread than previously known, including 3 candidate species co-occurring in the Western Alps and differing in body size and leg number despite little genetic divergence; and *S. engadina*, exclusive of a narrow part of the Central Alps.

Keywords: COI – integrative taxonomy – morphometrics – speciation – species delimitation – soil animals

INTRODUCTION

Recent decades have witnessed great progress in developing conceptual and methodological tools for taxonomy, i.e., for the description of organismal diversity. Different definitions of "species" have been reconciled as complementary criteria of recognition, within a broader concept of evolutionary species, often referred to as a "unified" or "general lineage species concept" (e.g.: De Queiroz, 2007; Zachos, 2016). Moreover, consensus has emerged that the practice of detecting, circumscribing, and diagnosing species should be based on multiple lines of evidence for speciation, under the methodological framework of "integrative taxonomy" (e.g.: Dayrat, 2005; Schlick-Steiner et al., 2010; Padial et al., 2010). This is motivated by the fact that different sources of information may reveal different expected outcomes of speciation: genetic divergence, morphological differentiation, reproductive isolation when in syntopy, ecological divergence, and others. Especially for molecular data, different methods for species delimitation have been

developed and assessed (e.g.: Sites & Marshall, 2004; Flot, 2015; Fontaneto et al., 2015). Built on statistical theory and explicit models of evolution, they ensure scientific standards of repeatability and falsifiability (Popper, 2002; Padial et al., 2010; Gill, 2014).

This theoretical and operational taxonomic toolkit is being increasingly applied to a variety of organisms and ecological contexts. However, a bias persists against small soil-dwelling animals, which however comprise a large portion of the extant biodiversity (e.g., Orgiazzi et al., 2016). This bias is evident even within the European biota, which is acknowledged to be the most investigated in the world (Fontaine et al., 2012). Largely neglected and yet diverse groups of soil animals include the myriapods, for most of which we are still far from understanding the true pattern of species-level differentiation (e.g., Bonato & Minelli, 2009; Kime & Enghoff, 2017).

Centipedes of the genus *Strigamia* Gray, 1843 (Geophilidae: Linotaeniinae; Fig. 1) are among the most widespread and ecologically significant centipedes in the forest soils of the northern hemisphere (Voigtländer, 2009; Bonato et al., 2017). They are among the most frequently recorded centipedes even in the forests throughout Central Europe, where they are currently referred to a total of five species: two apparently widespread species that are almost invariantly called *S. acuminata* (Leach, 1816) and *S. crassipes* (C.L Koch, 1835), and three apparently more localized species called *S. transsilvanica* (Verhoeff, 1928), *S. cottiana* (Verhoeff, 1935) and *S. engadina* (Verhoeff, 1935) (e.g.: Koren, 1986; Geoffroy & Iorio, 2009; Bonato et al., 2012, 2014a; Reip et al., 2012; Bonato & Minelli, 2014; Iorio, 2014). However, many inconsistencies and uncertainties in the recent taxonomic and faunistic literature reveal that our understanding of the true species diversity of these animals is still unsatisfactory.

We applied a modern theoretical and operational approach for a preliminary taxonomic reassessment of forest-dwelling *Strigamia* across a broad sector of Europe. Our primary aim was (i) detecting, (ii) circumscribing and (iii) diagnosing all evolutionary lineages at the level of species or species-complexes. To produce evidence-based hypotheses, we gathered indirect evidence of speciation from:

(a) morphological differentiation: differences between organisms may be taken as evidence of phenotypic differentiation of lineages after speciation, after distinguishing the component of variation associated with body size (ontogenetic and static allometry) and sex (sexual dimorphism);

(b) syntopy of morphologically distinct organisms: the local co-occurrence of organisms with alternative discrete phenotypes may be taken as evidence of coexistence of lineages with substantial reproductive isolation, after ruling out alternative explanations;

(c) molecular differentiation: molecular differences between organisms may be taken as evidence for genetic differentiation of lineages after speciation, after considering within-population and intra-specific genetic variation.

MATERIAL AND METHODS

Study area

We considered a broad sector across Europe, including the entire Italian region, the whole of the Alps and the northern European lowlands within the conventional boundaries indicated in Fig. 2. Only inland areas were considered, so that we ignored littoral habitats, which are inhabited by the congeneric but distantly related *S. maritima* (Leach, 1817) (Bonato et al., 2017).

Species delimitation from morphological differentiation

Preliminary evaluation of intraspecific variation and character selection

We considered all morphological characters proposed by previous authors as distinguishing species or infraspecific taxa in European inland *Strigamia* (Table S1). However, we ignored characters that were later found to be ineffective, either because they are much more variable between individuals than between populations or because they are hard to evaluate objectively. All

retained characters (13) were defined operationally as binary, meristic, or continuous morphometric (Table 1; Fig. 3). For the morphological terminology we followed Bonato et al. (2010).

As a preliminary step, we evaluated the inter-individual variation of each character within 11 samples of individuals (Table S2; Fig. 2). Each sample included 13–23 confidently conspecific specimens collected from a single site or a few nearby sites without obvious intervening barriers, and is hereinafter referred to as a "population". For the sake of comparability, we selected specimens >15 mm long, with gonopods at least partially developed, and without anomalies or evident damage preventing character evaluation. The 11 populations were representative of most of the currently distinguished species of inland *Strigamia* within the study area, and of their overall morphological diversity. Referring to the taxonomic names currently in use, we considered 3–4 populations for each of the species *S. acuminata*, *S. crassipes* and *S. transsilvanica*, and one population for *S. cottiana*.

All 13 characters were evaluated in all 172 specimens by a single person (GDZ). Some characters were evaluated by transmitted light with a biological microscope (Leica DMLB), others by incident light with a stereomicroscope (Leica MZ12.5) (Table 1). Measurements were taken by means of a micrometer applied to the ocular lens, to the nearest 5 µm (for the diameter of the coxal pores) or 10 µm (for all other measures). The maximum width of the head was taken as an index of body size, following Horneland & Meidell (2009). Sex assignment was made based on the shape of gonopods only (e.g., Brolemann, 1930), without considering any other putative secondary sexual character.

The variation of each character with body size and sex was evaluated by building a generalized linear mixed model (GLMM). Body size and sex were treated as fixed effects, including their interaction, whereas the population was treated as a random effect. For binary characters, a binomial distribution was assumed, and the link function was set as logistic. For meristic characters, a Poisson distribution was assumed, and the link function was set as logarithmic. For continuous characters, a normal distribution was assumed, and the link function was set as identity. Models were fitted with the R package 'lme4' (Bates et al., 2015). The statistical significance of the effects of body size, sex and their interaction was tested with the Wald χ^2 test, as implemented in the R package 'car' (Fox et al., 2012), after controlling for overdispersion with the test implemented in the R package 'glmmTMB' (Magnusson et al., 2017).

Whenever a character was found to be significantly associated with body size and/or sex, it was tentatively corrected to minimize these effects. In detail, meristic and continuous characters associated with body size were divided for the body size or its square (Table 1). Moreover, characters differing between sexes were corrected estimating the expected condition in a single sex. In particular, as the number of legs was found on average higher in females than in males with a modal difference of 2 pairs, despite heritable variation between individuals and some temperature-induced plasticity (Vedel et al., 2010), values in males were corrected by adding 2 pairs. In the same way, as the number of coxal pores was found on average higher in females than in males with a modal difference of 1 for each coxopleuron, values in males were corrected by adding 1 (Table 1).

Model-based cluster analysis based on selected characters

For the species delimitation analysis we considered only the characters that were not found either significantly associated with body size, or with sex, in the preliminary analysis (see above; Table 1). All these characters (9) were evaluated on a sample of 52 specimens (Table S3), including 49 specimens collected by the authors throughout the study area (Fig. 2) and representative of most of the known morphological and ecological diversity of *Strigamia* in the area. The three other specimens are from other sites in Western Europe (Table S3). Upon collection, specimens were fixed and maintained exclusively in either 70% or absolute ethanol, and the geographical position of the site was registered in WGS 84, in decimal degrees, with precision of 0.001° (about 0.1 km), by means of GPS devices or web-GIS applications. For the analysis of species delimitation, all characters were evaluated on the 52 specimens by two persons only (MO, JS) and strictly following a common protocol.

To obtain an optimal partition of the 52 specimens in morphologically different candidate species, we carried out a model-based cluster analysis of the specimens considering the 9 selected characters. In detail, assuming a hypothetical number of species N and different probability distributions of the characters among the N species, we estimated for each specimen the probability of belonging to each species. Normal distributions were assumed for the continuous characters, which were also standardized, and Poisson distributions for the meristic characters. The parameters of the distributions and the assignment probabilities of the specimens were estimated with the Expectation Maximization algorithm (Witten & Frank, 2005). To determine the most likely number of candidate species N, a measure of likelihood was estimated for each hypothetical N ranging between 1 (all specimens belonging to a single species) and 52 (each specimen in a separate species). This was done by applying a 10-fold cross-validation procedure: for each value of N, we repeated the Expectation Maximization algorithm 10 times, each time excluding a random sample of 1/10 of the specimens and using the latter for validation, and then averaging likelihoods among repetitions. The analysis was performed with the software STATISTICA 8.0 (Statsoft, 2008) and was repeated 10 times with different 'seed numbers' to test for the stability of the results, as suboptimal results are expected from some runs. The analyses were repeated considering the absolute values of the continuous characters instead of their standardized values. To explore evidence for further partition within the candidate species, additional analyses were run separately for each of the major clusters of specimens obtained from the primary analyses, however using a 5-fold cross-validation procedure because of the lower number of specimens.

Species delimitation from syntopy of morphologically distinct individuals

In addition to the specimens employed for the analyses of morphological differentiation (see above), we considered many other specimens of *Strigamia* collected within the study area and preserved in different collections, for a total of 2111 specimens from 786 sites (Fig. 2).

Whenever multiple specimens were collected from a single site in a single day (as indicated by their preservation as a single sample in a single tube with a common label), we compared all specimens of the sample for the 9 characters employed for the species delimitation analysis from morphological differentiation (see above; Table 1). We also compared the specimens for the developmental condition of the gonopods in relation to the body length. Whenever we detected discrete differences between subgroups of specimens within a single sample and these differences were larger than expected for within-population variation (including sexual dimorphism and variation with body size, based on the preliminary analysis of intraspecific variation; see above), we hypothesised that those different subsamples belong to morphologically differentiated species coexisting in a site. An alternative explanation would claim the existence of discrete phenotypic polymorphism within a population of a single species, but this appears much less likely because there is no evidence of polymorphism in any population of *Strigamia*.

Based on the morphological characters, we eventually correlate the putative species found in syntopy in a site with those found in syntopy in other sites and with the candidate species indicated by the other lines of evidence (see below).

Species delimitation from molecular differentiation

<u>COI sequencing</u>

Sequences of COI were obtained for all the same 52 specimens employed for the species delimitation analysis by morphological differentiation (Table S3; Fig. 2).

To the exclusion of 5 already available sequences (Bonato et al., 2014b, 2017), all other sequences were obtained de novo. DNA extraction and amplification were performed in different laboratories for different specimens: Department of Biology, University of Padova (PD), with the primer pair LCOI490/HCO2198 or COI StrigFor/COI StrigRev (Bonato et al., 2017); Zoologische Staatssammlung München (ZSM) via Canadian Centre for DNA Barcoding, Guelph (CCDB), with

the primer pair Folmer LepF/LepR; Zoologisches Forschungsmuseum Alexander Koenig, Bonn (ZFMK), with the degenerated primer LCO1490-JJ and HCO2198-JJ (Astrin & Stüben, 2008).

At PD, total DNA was extracted from a few body trunk segments using the DNeasy Blood and Tissue kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. PCRs were performed in 20 µl reactions containing 4.0 µl of 5X Flexi Buffer, 0.4 µl of 10 mM dNTPs, 1.2 µl of 25mM MgCl2, 0.5 µl of 100% DMSO, 0.5–1.0 µl of each 10 µM primer, 0.1 µl of 5U/µl GoTaq Flexi DNA Polymerase (Promega, Madison, USA), 1 µl of template DNA and purified water. The reaction was carried out as follows: 1 step at 95 °C for 5 min; 27–35 cycles consisting of 1 min at 94 °C, 1 min at 40–42 °C (for LCOI490/HCO2198 primer) or at 49–51 °C (for COI StrigFor/COI StrigRev primer), and 1 min 30 s at 72 °C; a final step at 72° C for 7 min. The double-stranded PCR products were verified by 1% agarose gel electrophoresis and purified using the MinElute PCR Purification Kit (Qiagen). The purified PCR products were sequenced directly on both strands with the same primer pairs as used for amplification through BMR Genomics (Padova, Italy). Sequencing was performed by BMR Genomics, Padova.

At CCDB and ZFMK, DNA extraction, amplification and sequencing were carried out as described by Astrin et al. (2016) and Wesener et al. (2015), respectively.

Sequence reliability was verified by translating the nucleotide sequence into amino acid sequence with MEGA 7 (Kumar et al., 2016).

Estimation of gene tree

As some methods of species delimitation require a gene tree or even an ultrametric gene tree (i.e., with branch lengths proportional to divergence times), a phylogenetic analysis was performed on the COI sequences.

To the 52 sequences we added two other already available sequences, which were obtained from two specimens from the study area but unavailable for morphological analysis: a specimen from Hainich, Thuringian Forest, previously identified as *S. acuminata* (GenBank accession code JQ801586) and a specimen from Vilm, on the Baltic Sea, previously identified as *S. crassipes* (OQ38444). Moreover, identical sequences were removed to avoid biases in the inference of model parameters and to avoid zero-length tree branches, which are not tractable by some species delimitation methods.

Already available sequences of *Strigamia maritima* (KP173664 and NC_026557) and *Pleurogeophilus mediterraneus* (Meinert, 1870) (KF569299) were added as outgroups. *S. maritima* and *P. mediterraneus* were chosen because they are among the most closely related species to the European inland species of *Strigamia* according to previous molecular phylogenetic analyses (Bonato et al., 2014b, 2017) and they outperformed other candidate outgroup species (*Clinopodes flavidus* C.L. Koch, 1847, *Geophilus electricus* (Linnaeus, 1758), *Henia vesuviana* (Newport, 1845), *Stenotaenia sorrentina* (Attems, 1903)) according to the Evolutionary Placement Algorithm (Berger et al., 2011) implemented in RAxML 8.2.1 (Stamatakis, 2014).

Sequences were aligned with MAFFT 7 using L-INS-i algorithm (Katoh & Standley, 2013), and the alignments were corroborated with Clustal W and MUSCLE as implemented in MEGA.

ML analyses were performed with PhyML v.3.1 (Guindon et al., 2010; Criscuolo, 2011) with averaged models selected by jModelTest 2 (Darriba et al., 2012) according to different criteria (AIC, AICc, BIC). Model parameters were estimated from both empirical and optimized base frequencies. Alternative analyses were performed considering one or the other outgroup species or both together. Node statistical supports were assessed with 1000 non-parametric bootstrap replicates and the stabilization of bootstrap frequencies was checked with RAxML through the –I option. "Rogue taxa", i.e., sequences resolved at variable positions on the trees generated through bootstrapping, were assessed with RogueNaRok (Aberer et al., 2013). A compositional test was also performed with the p4 program (Foster, 2004).

An ultrametric tree was produced by a relative time calibration with the RelTime algorithm implemented in MEGA, using the ML tree previously obtained with PhyML and the best-fit substitution model selected with jModelTest.

Molecular methods of species delimitation

To estimate the optimal partition of the COI haplotypes in candidate species, we applied the Automatic Barcoding Gap Discovery algorithm (ABGD; Puillandre et al., 2012), the Assemble Species by Automatic Partition algorithm (ASAP; Puillandre et al., 2021), the K/θ method (Birky et al., 2010), the Generalized Mixed Yule Coalescent method (GMYC; Pons et al., 2006; Fujisawa & Barraclough, 2013) and the Poisson Tree Processes method (PTP; Zhang et al., 2013).

Both the ABGD and ASAP methods are based on the expectation that interspecific pairwise distances are higher than intraspecific pairwise distances, and on the coalescent model (Puillandre et al., 2012, 2021). Alternative hypotheses of species delimitation are produced depending on the prior value of the maximum intraspecific distance. In addition, ASAP ranks the alternative hypotheses according to the estimated probability that some candidate species are instead a single panmictic species, as well as the relative width of the estimated gap between intra- and interspecific distances (Puillandre et al., 2021). Both ABGD and ASAP were applied on distances corrected both with the Jukes-Cantor substitution model (JC69-distances) and the Kimura-2-parameter substitution model (K2P-distances). For ABGD, we considered 1000 prior values of the maximum intraspecific distance ranging between 0.1% and 15% (Steps = 1000, P_{min} = 0.001, P_{max} = 0.15), which encompass all four apparent minima in the frequency distribution of the pairwise distances (Fig. S1) and also lower values that are often assumed or retrieved for other animal groups (Puillandre et al., 2012). Instead, we did not constrain the minimum relative breadth of the barcoding gap (parameter X = no value). For ASAP, we kept the default threshold value of 0.01 for the probability of species panmixia for splitting species.

The K/θ method, also known as the "4x rule", is also based on the coalescent model. In particular, it is based on the expectation that, for each pair of sister species, the sequence divergence between species (K) is >4 times (with confidence level >95%) as large as the sequence divergence within the species (θ) (Birky et al., 2010). We tested all supported clades in the gene tree, estimating K as the average pairwise distance from its sister clade and θ as the average pairwise distance within the clade, considering both JC69- and K2P-distances.

The GMYC method is based on the expectation that, considering the timing of branching in a gene tree, the rate of branching within a species is higher than the apparent rate of branching between species (Pons et al., 2006). Hypotheses of species delimitation are produced by estimating either a single (ST-GMYC) or multiple (MT-GMYC) transitions in the rate of branching (Monaghan et al., 2009).

The PTP method is based on the expectation that the number of nucleotide substitutions accumulated within any species is distinctly lower that the number of substitutions between species (Zhang et al., 2013). We employed this method under both its Maximum Likelihood implementation (PTP) and its Bayesian implementation (bPTP). We performed a rjMCMC of 100,000 steps, discarding the first 10,000 steps as burn-in, and retaining one step every 1000 post burn-in steps. We checked the estimated parameters visually for convergence and good mixing of the chains.

Differences between species and geographical distribution

Following the results of the species delimitation analyses on the 52 specimens (see above), we tentatively (re-)identified all other 2059 specimens sampled from the study area (Fig. 2), based on the major morphological differences found between the candidate species in the 9 characters examined (see above). In addition, we browsed the entire taxonomic and faunistic literature to retrieve all published records of *Strigamia* from the study area (Fig. 2), and we revised all published identifications scrutinizing all accompanying information.

Finally, we revised the morphological diagnoses and the geographical distribution of the candidate species based on the confidently identified specimens and the validated published records.

Whenever coordinates were not given, they were estimated from the textual indications given either on the specimen labels or in the publications, with variable precision. Records were mapped with QGIS, excluding those with lower precision (> 0.1°, which correspond to about 10 km). For describing the geographical occurrence of the candidate species, we referred to the names of major physical areas (mountains ranges, lowlands) currently in use.

RESULTS

Species delimitation from morphological differentiation

Of the 13 morphological characters evaluated for their inter-individual variation within 11 populations of *Strigamia* (Table 1), six characters were found to be significantly associated with body size (GLMMs: Wald χ^2 test: $p<0.05$): the head elongation, the relative separation of the forcipules and the relative size of the forcipular tarsungulum were found to decrease with body size, whereas the relative size of the forcipular tergite and both the number and the size of the coxal pores were found to increase with body size (Tables 1 and S4). Moreover, three characters were found to differ on average between sexes (GLMMs: Wald χ^2 test: $p<0.05$): the head elongation was slightly but significantly higher in males than in females, whereas the number of legs and the number of coxal pores were generally higher in females with marginal statistical significance (Table S4). Instead, no significant interactions were found between body size and sex for any character (Table S4). After applying corrections, for the purpose of species delimitation we retained 9 characters that were not significantly affected by either body size or sex (Tables 1 and S4).

Considering the 9 selected characters in 52 specimens of *Strigamia* sampled from the study area (Fig. 2), the model-based cluster analysis with cross-validation (see Material and Methods) retrieved an optimal partition of the specimens into two clusters in most of the iterations, with high assignment probabilities (>0.999) for all specimens ("primary cluster analysis" in Fig. 4). Both clusters included specimens sparsely distributed through most of the study area: a cluster (A in Fig. 4) including all specimens previously identified as *S. crassipes*, *S. transsilvanica* and *S. cottiana*, two specimens of uncertain identity and a specimen from the South-Western Alps previously identified as *S. acuminata*; a cluster (B) including all other specimens previously identified as *S. acuminata*. The same partition was retrieved in all iterations run after standardizing the continuous characters.

When repeating the analysis within the cluster A, the specimens were separated into two candidate species in most of the iterations ("secondary cluster analysis" in Fig. 4), distinguishing some specimens previously identified as *S. crassipes* (A") from all other specimens (A'). The same partition was retrieved also in some iterations of the primary analysis and rarely also in the secondary analysis after standardizing the continuous characters.

When repeating the analysis within the cluster B, a further partition was retrieved only in some iterations ("secondary cluster analysis" in Fig. 4): some specimens from different sites of the Central and Eastern Alps (B') were sometimes separated from all others (B"), with high assignment probabilities for most of them. The same partition was also rarely retrieved in the primary analysis, but not in the secondary analysis after standardizing the continuous characters.

Species delimitation from syntopy of morphologically distinct individuals

Considering the 786 sites where specimens of *Strigamia* were collected and examined by us (Fig. 2), we detected at least 106 sites where morphologically well-distinguishable specimens were found together, on the same day, suggesting the syntopy of 2–4 species in each of these sites (Table S5). Syntopic candidate species were distinguished most often by disjunct ranges of variation of the

number of legs and frequently also by discrete differences in the arrangement of the clypeal setae, the relative size and shape of the forcipular denticles, the presence of the anterior ventral sclerotized stripe, and sometimes also the different shape of the margins of the forcipular tarsungula and the adult body length. Comparing and matching the morphology of the syntopic candidate species between different sites, we found evidence for the existence of at least 7 morphologically distinct species in the study area (Fig. 5). Three of these candidate species correspond to species retrieved by the model-based cluster analysis of morphological differentiation (A", B', B"). Three other candidate species (A'_1, A'_2, A'_3) were not distinguished in the previous analysis. The seventh candidate species (C) was apparently not represented among the 52 specimens analysed for morphological differentiation (see above).

Species delimitation from molecular differentiation

Reliable COI sequences were obtained for all the same 52 specimens that were employed for the analysis of morphological differentiation (Fig. 2). After adding two other already available sequences (see Material and Methods), a multiple alignment without indels was obtained consistently with different methods, with a total length of 497 positions. The maximum pairwise p-distance was 19.3% and the maximum pairwise K2P-distance was 22.7%. A total of 40 haplotypes were found, including 7 haplotypes shared by multiple specimens (Table S3).

The ML phylogenetic analysis with a BIC-averaged substitution model, *S. maritima* as outgroup and relative time calibration produced a tree with all nodes supported by ≥ 89% bootstrap values (Fig. 4). Using alternative criteria of model selection (AIC, AICc) produced the same topology but with slightly lower bootstrap supports. Using *P. mediterraneus* as an alternative outgroup or both outgroups produced only minor differences in the topology and with lower bootstrap supports. No “rogue taxa” were detected.

The tree indicated that most candidate species hypothesized by the previous analyses (based on morphological differentiation and on syntopy of different individuals) correspond to clades, except for A'_1 (paraphyletic with respect to A'_2 and A'_3) and B" (paraphyletic with respect to B').

The ABGD method partitioned the haplotypes into 5–12 candidate species for prior values of the maximum intraspecific distance between 6.7% and 9.6%, for both JC69- and K2P-distances, whereas only 2 species for higher distances and no fewer than 19 species for lower distances. The 2-species hypothesis separated three specimens from all others, approximately mirroring the separation of A" from all other species retrieved by the previous analyses. All other hypotheses additionally separated one or more candidate species corresponding to A’ from two or more candidate species corresponding to B (e.g., “ABGD 5 spp.” in Fig. 4). Under the hypotheses of ≥19 species, most candidate species comprised single or pairs of specimens from the southern part of the study area (e.g., “ABGD 19 spp.” in Fig. 4).

The ASAP method indicated no fewer than 19 species in all the first eight optimal hypotheses of species delimitation, with 24 species in the very first hypothesis (“ASAP” in Fig. 4).

The K/θ method suggested a partition into candidate species only for some sequences (“K/θ” in Fig. 4). Within the clade A, only two small subclades were retrieved as candidate species, whereas most of the sequences remained unresolved. Instead, within the clade B, three of the four major subclades were retrieved as candidate species.

The GMYC method indicated between 18 and 24 species, and most probably 24, when allowing multiple thresholds (column “MT-GMYC” in Fig. 4). Further splits were suggested when assuming a single threshold, for a total of 30 species.

The PTP method indicated between 14 and 32 species, most probably 26 in the Bayesian implementation (“bPTP” in Fig. 4) and 28 in the ML implementation.

Candidate species

Despite the diversity of species delimitation hypotheses produced by different analyses and different lines of evidence (Fig. 4), all candidate species grouped consistently into three species-

complexes, each comprising an uncertain number of morphologically similar and phylogenetically related species. These three species-complexes (labeled A', A" and B in Fig. 4) correspond only partially to the taxonomy and nomenclature in use: A' comprises all specimens previously referred to *S. transsilvanica* and other specimens previously referred to *S. crassipes*, *S. cottiana* and *S. acuminata*, but it should be called the *Strigamia crassipes* species-complex according to the rules of zoological nomenclature; A" comprises part of the specimens previously referred to *S. crassipes*, it should be called the *S. carniolensis* species-complex; B comprises most of the specimens previously referred to *S. acuminata* and should be called the *S. acuminata* species-complex. The additional candidate species indicated by the analysis of syntopy (C in Fig. 5) should be called *S. engadina*, but its relationships with the three species-complexes above mentioned remain unknown. Detailed notes on the valid scientific names are given in the Appendix in the Supporting Information.

Both when comparing only the specimens employed in the species delimitation analyses (see above) and when extending the comparison to all examined and confidently assigned specimens (97% of the 2111 specimens from the study area) and to all validated published records (59% of >2736 specimens from the study area, reported in 211 publications), the three species-complexes and *S. engadina* can be unambiguously diagnosed by a combination of a few characters (Table 2).

Moreover, all three species-complexes are similarly widespread in most of the study area, whereas *S. engadina* is limited to a small area in the Central Alps (Figs 6-8).

Strigamia acuminata (Leach, 1816) species-complex

Diagnosis of adult individuals: usually up to 2–3 cm long; clypeal setae arranged in three groups, i.e. with distinct gaps between intermediate and lateral groups of setae; forcipular tergite usually 30–40% of the head length; forcipules relatively slender and relatively separated from each other (distance between the basal condyles >1.6 times the basal width of the forcipules); forcipular tibia usually without a distinct projection, at most a very small tubercle or a shallow bump; tarsungulum elongate (on average 80% of the distance between the basal condyles, rarely <70%), with the outlines of the intermediate part either distinctly converging or sub-parallel; forcipular denticle relatively short (usually 40–50% of the tarsungulum length) and its outlines usually straight; 39–43 leg pairs, almost invariantly 41 in females and 39 in males; metasternites of the anterior third of trunk without a mid-longitudinal sclerotized stripe, only a shallow groove; each coxopleuron with relatively few coxal pores in proportion to body size, i.e. usually no more than 15 pores in individuals up to 25 mm long, and no more than 25 pores in longer individuals; coxal pores relatively small in proportion to body size, diameter of the largest pore usually less than 4% of the head width, and usually distinctly narrower than their canals.

Geographical distribution

Within the study area, species of the *S. acuminata* complex are widespread from the northern coastal plain, through the Alps, to the Apennines, southwards to the Nebrodi mountains in Sicily (Fig. 6). They are apparently missing from Corsica and Sardinia.

Outside the study area, species of the *S. acuminata* complex inhabit a broad part of Europe: westwards to the Pyrenees (Barace & Herrera, 1980), Brittany (Iorio, 2014) and Great Britain (Barber, 2022); northwards to the southern part of the Jutland Peninsula (Lohmander, 1957; Andersson et al., 2008); eastwards to the Volga basin and Caucasus (summarized in Bonato et al., 2012); southwards to Anatolia (Zapparoli, 1999) and southern Dinarides (Kaczmarek, 1969; Stoev, 2002), but also Crete (Simaiakis et al., 2004). Other published records from other areas have been questioned (e.g., Bonato et al., 2012) or need confirmation.

Candidate species

Different lines of evidence suggest at least two species. A candidate species (B' in Fig. 4) seems to differ from the other/s species in the shape of the forcipules: the tarsungula are much more

elongate than in the other/s species (usually >85% of the distance between the basal condyles, on average 88%, vs. usually <85%, on average 78%; Mann-Whitney test: U = 371, z = -4.26, p < 0.00001, n= 28 vs. 46; Fig. S2), the outlines of the intermediate part of each tarsungulum are often sub-parallel whereas they are uniformly converging in the other/s species, and the denticles are relatively shorter than in the other/s species (usually <45% of the tarsungulum length, on average 43%, vs. usually >45%, on average 49%; Mann-Whitney test: U = 10, z = 6.94, p < 0.00001, n= 27 vs. 45; Fig. S2). Reliable records of this candidate species are distributed along the South-Eastern part of the Alps, from the Bergamasque Prealps to at least the Julian Alps and Prealps (Fig. 6). It is largely parapatric with respect to other candidate species in the same complex, and evidence for their syntopic occurrence is weak. The valid name of this candidate species should be *S. microdon* (see Appendix).

Strigamia carniolensis (Verhoeff, 1895) species-complex

Diagnosis of adult individuals: usually up to 3–6 cm long; clypeal setae usually uniformly spaced in a continuous row, rarely with two recognizable gaps between intermediate and lateral groups of setae; forcipular tergite usually 35–50% of the head length; forcipules relatively broad and little separated from each other (distance between the basal condyles <1.8 times the basal width of the forcipules); forcipular tibia usually with a distinct projection; tarsungulum little elongate (on average 70% of the distance between the basal condyles, rarely >75%), with the outlines of the intermediate part fully converging; forcipular denticle relatively long (usually 55–65% of the length of the tarsungulum, on average 60%) and its outlines usually distinctly curved; 45–57 leg pairs, often 49–51 in females and often 47–49 in males; metasternites of the anterior third of trunk with a distinct mid-longitudinal sclerotized stripe; each coxopleuron with many coxal pores in proportion to body size, i.e. usually no fewer than 25 pores in individuals longer than 25 mm, and up to more than 50 pores in longer individuals; coxal pores relatively small in proportion to body size, diameter of the largest pore usually less than 4% of the head width, and usually distinctly narrower than their canals.

Geographical range:

Within the study area, species of the *S. carniolensis* complex are widespread at least along the southern part of the Alps and the entire Apennines, southwards to the Aspromonte Massif (Fig. 7). North of the Alps, they occur also along the Rhine Valley, northwards to the Rhenish Massif (Fig. 7). They are missing from Corsica, Sardinia, and Sicily.

Outside the study area, the actual distribution of species of the *S. carniolensis* complex needs deep revision, because of the broad variation of taxonomic opinions and nomenclature employed by different authors (see below, under Discussion, and Appendix). As a matter of fact, most published records cannot be interpreted confidently as referring to either the *S. carniolensis* complex or the *S. crassipes* complex. Those that can be confidently assigned to the *S. carniolensis* complex indicate that species of the latter complex inhabit a broad area in Western and Southern Europe: westwards to the North-Western part of the Iberian Peninsula (Gregory & Lewis 2015, Cabanillas, 2019, both sub *S. crassipes*; see below, under Discussion, and Appendix); northwards at least to the northernmost part of France (Iorio, 2014, sub *S. crassipes*); eastwards at least to the western coasts of the Black Sea (Matic 1972, sub *S. crassipes*); southwards at least to the Rhodopes in the Balkan Peninsula (Kaczmarek, 1969; Stoev, 2002; both sub *S. crassipes*), the entire Italian Peninsula, and the central part of the Iberian Peninsula (Cabanillas 2020, sub *S. crassipes*). Other published records from other areas need confirmation.

Candidate species

Evidence of multiple species is weak.

Strigamia crassipes (C.L Koch, 1835) species-complex

Diagnosis of adult individuals: usually up to 4 cm long, but in some lineages up to 2 cm and in other lineages up to 6 cm long; clypeal setae uniformly spaced in a continuous array, without

recognizable gaps between intermediate and lateral groups of setae; forcipular tergite usually 35–50% of the length of the head; forcipules variable in size and separation, usually moderately separated (distance between the basal condyles 1.5–2.0 times the basal width of the forcipules); forcipular tibia usually with a distinct projection; tarsungulum moderately elongate (usually 70–80% of the distance between the basal condyles, on average 75%), with the outlines of the intermediate part fully converging; forcipular denticle of moderate length (usually 50–60% of the tarsungulum length, on average 55%) and its outlines distinctly curved; variable number of leg pairs, any odd number in the range 37–65, more frequently 47–49 in females and 45–47 in males; metasternites of the anterior third of trunk without a mid-longitudinal sclerotized stripe, only a shallow groove; each coxopleuron with relatively few coxal pores in proportion to body size, i.e. usually no more than 15 pores in individuals up to 25 mm long, and no more than 25 pores in longer individuals; coxal pores relatively large in proportion to body size, diameter of the largest pore usually more than 4% of the head width, and usually about as wide as their canals.

Geographical range:

Within the study area, species of the *S. crassipes* complex are widespread from the northern coastal plain, through the entire Alps, to Corsica and the Apennines, southwards to the Nebrodi mountains in Sicily (Fig. 8). They are missing from Sardinia.

Outside the study area, the actual distribution of species of the *S. crassipes* complex needs deep revision, because of the broad variation of taxonomic opinions and nomenclature employed by different authors (see below, under Discussion, and Appendix). As a matter of fact, most published records cannot be confidently interpreted as referring to either the *S. carniolensis* complex or the *S. crassipes* complex. Those that can be confidently assigned to the *S. crassipes* complex indicate that species of the latter complex inhabit a broad area in part of Europe: westwards at least to the Provence Prealps (Iorio & Berg, 2007, sub *S. transsilvanica*; see below, under Discussion, and Appendix), the Meuse river (Iorio, 2014, sub *S. transsilvanica*; Jeekel & Brugge, 2009) and Great Britain (orig.; Fig. 4); northwards to the southern part of the Scandinavian Peninsula (Porat, 1889; Andersson et al., 2008) and the lands East of the Baltic Sea (Bonato et al., 2005, 2012, given as uncertain, sub *S. transsilvanica*, but here confirmed; Sammet et al., 2018, sub *S. transsilvanica*); eastwards at least to the Carpathians (Verhoeff, 1935, sub *Sc. transsilvanicus*; Matic & Dărăbanţu, 1968, sub *S. transsilvanica*); southwards to the Peloponnese (Zapparoli, 1994, sub *S. transsilvanica*). Other published records from other areas have been questioned (e.g., Bonato et al., 2012) or need confirmation, including recent records from the Azores (e.g., Borges & Enghoff, 2005; Borges et al., 2016) and from European Russia (Zuev & Evsyukov, 2016).

Candidate species

Different lines of evidence indicate at least three species.

A candidate species (A'_3 in Fig. 4) differs from other species of the same complex by a remarkably shorter body (rarely more than 20 mm) and fewer body segments (39–43 leg pairs in females and 37–41 leg pairs in males). It seems to be distributed along the Western Alps and the northern Apennines, between the Pennine Alps and the northernmost part of the Tuscan-Emilian Apennines, as well as in Corsica (Fig. 8). No names are available for this species.

Another candidate species (A'_2) differs from other species of the same complex by a remarkably longer body (surpassing 40 mm) and more numerous body segments (55–65 pairs in both sexes). It seems to be distributed in a narrower part of the South-Western Alps, between the Cottian and the Ligurian Alps (Fig. 8). It is already distinguished as *S. cottiana* (see Appendix for detailed notes on the nomenclature).

Strigamia engadina (Verhoeff, 1935)

Diagnosis of adult individuals: up to 5 cm long or longer; clypeal setae uniformly spaced in a continuous row; forcipular tergite 40–50% of the head length; forcipules relatively broad and little

separated from each other (distance between the basal condyles <1.8 times the basal width of the forcipules); forcipular tibia with a distinct projection; tarsungulum elongate, > 80% of the distance between the basal condyles, with the outlines of the intermediate part sub-parallel; forcipular denticle relatively short (usually 40–55% of the tarsungulum length) and its outlines distinctly curved; around 51–55 leg pairs, 53–55 in females and 51–53 in males; metasternites of the anterior third of trunk without a distinct mid-longitudinal sclerotized stripe; each coxopleuron with relatively few coxal pores in proportion to body size, no more than 15 pores in individuals up to 25 mm long, and no more than 25 pores in longer individuals; coxal pores relatively large in proportion to body size, diameter of the largest pore more than 4% of the head width, about as wide as their canals.

Geographical range:

The species is apparently limited to a small area in the Central Alps, between the Bergamasque Prealps and the Western and Southern Rhaetian Alps (Fig. 7).

Published records from other areas (Pyrenees, remaining Alps, central Apennines, Dinarides, Carpathians and other areas in the Balkan Peninsula) are certainly or probably based on misidentifications of the candidate species *S. microdon* (see above) or other species (e.g., Matic & Dărăbantzu, 1971; Matic, 1975). We revised some of these records by re-examining the voucher specimens. The few other published records need confirmation because they are almost invariantly from within the known range of *S. microdon* and, when reporting *S. engadina* from a composite sample of specimens from this area, authors assigned other specimens to *S. crassipes* or to *S. transsilvanica*, but not to *S. acuminata* (Marcuzzi & Minelli, 1971; Matic & Dărăbantzu, 1971; Minelli, 1979). Moreover, the record from the Carpathian Mountains based on the type material of *Scolioplanes engadinus rodnaensis* (Verhoeff, 1935) also needs confirmation, because the little reported morphological information on the single specimen is not enough to confirm its species identity.

Candidate species

There is no evidence of multiple species.

DISCUSSION

Novel views on central European *Strigamia* diversity

Our investigation on the diversity of *Strigamia* centipedes in a broad part of Europe disclosed a much more complex pattern of species differentiation than that envisioned so far, and drafted novel views of the evolution of these animals.

For instance, within the *S. acuminata* species-complex, we found evidence for a candidate species (*S. microdon*) with particularly shaped forcipules, and apparently substituting all other related species throughout the South-Eastern Prealps.

In the same way, within the *S. crassipes* species-complex, we found evidence for a candidate species (A'3) with reduced body size and modularity (i.e., the body is smaller at full growth and is has fewer segments), and coexisting with other related species in the South-Western Alps.

More generally, within the *S. crassipes* complex, we documented a broader diversity in body size and modularity, and even habitat, than previously known, especially within the Alps. The diversity spans from the candidate species A'3, which does not grow longer than 2.5 cm, has around 40 pairs of legs and lives in montane soils covered by beech forests, to the similarly epigean and often syntopic *S. cottiana*, which surpasses 4 cm in total length and has around 60 pairs of legs, and even to some cave-dwelling populations, which grow even longer than 6 cm and have 50–60 pairs of legs.

On the other hand, the two other widespread species complexes, namely the *S. acuminata* complex and the *S. carniolensis* complex, showed a remarkably lower level of morphological and ecological divergence within the study area, and especially within the Alps. However, the COI sequences suggest that all three species complexes may have accumulated similar levels of molecular diversity, which may be decoupled from ecological-phenotypic disparity.

Our results also provided compelling evidence for the South-Western Alps as a major hotspot of diversity for *Strigamia*. Here we found the highest amount of molecular diversity, of morphological disparity and of coexisting species. For instance, up to three candidate species of the *S. crassipes* complex have been recorded as strictly syntopic in multiple sites between the Cottian and Maritime Alps, often together with other species of other complexes. Additionally, this area hosts narrow endemics that are strongly differentiated morphologically, including *S. cottiana* and the species A'3.

Towards a revised taxonomy for central European *Strigamia*

Our investigation revealed that the taxonomic and nomenclatural scheme currently in use for the *Strigamia* centipedes in Europe is far from matching the real pattern of diversity, and in some respect it is even inconsistent and misleading.

The results allowed us to draw a comprehensive taxonomy at the level of species-complexes, and to point to some candidate species within these complexes.

We managed to spot and settle inconsistencies in defining some of the major morphological characters so far given high diagnostic values in *Strigamia* taxonomy. For instance, we found that different species complexes differ in the presence vs. absence of sclerotized narrow stripes on the sternites of the anterior part of the body. However, up to now this character has been intended in different ways and often misunderstood: some authors misinterpreted the stripes with shallow grooves (e.g.: Eason, 1964; Barber, 2008, 2009; Andersson et al., 2005; Matic, 1972; Tuf & Kupca, 2015), others gave diagnostic value to both stripes and grooves (Iorio, 2005; Iorio & Labroche, 2015; Iorio et al., 2022), and most authors did not restrict the evaluation on the anterior part of the body. Additional confusion arose when it was suggested that similar stripes on the posterior part of the body could help to distinguish among otherwise similar putative species within the *S. crassipes* complex (Spelda, 2005; Voigtländer & Spelda, 2019).

Our results also allowed resolution of some uncertainties and incongruences in the circumscription and valid names of the *Strigamia* taxa. For instance, we managed to disclose and settle a long-lasting issue with the application of the name *crassipes*, which had caused the accumulation of much incongruence and ambiguity on the true morphology and geographical occurrence of two of the major species complexes (see Appendix). We found that the name *crassipes* should be applied to a species complex without sclerotized stripes on the anterior sternites and with relatively few coxal pores, not to a different species complex with those stripes and more numerous coxal pores. Up to now, this usage has been endorsed unambiguously only by very few authors (Jeekel, 1964; Spelda, 2005), while most authors applied the name *crassipes* to the other species complex, which is here called *carniolensis*, whereas they often adopted the name *transsilvanica* for species in the *S. crassipes* complex.

Furthermore, the large number of new records here presented and the critical reassessment of all published ones allowed us to revise substantially previous views on the geographical distribution of different *Strigamia* taxa. For instance, we found that species of the *S. crassipes* complex cover a much broader range than previously thought: up to now, there were no reports south of the Northern Apennines (Zapparoli & Minelli, 2007, sub *S. transsilvanica*), the presence was often considered scarce north of the Alps (e.g., Bonato et al., 2005, and Reip et al., 2012, both sub *S. transsilvanica*; but see, e.g., Jeekel, 1964, sub *S. crassipes*, and Voigtländer & Dunger, 1992, sub *S. transsilvanica*) and populations in the British Isles were misinterpreted due to the confusion with the application of the name *crassipes* (see above, and Jeekel, 1964). On the other hand, we found that *S. engadina* is most probably limited to the Central Alps, as all the sparse published records between the Pyrenees and the Balkan Peninsula turned out probably or certainly erroneous.

However, our investigations did not allow drawing a complete and consistent taxonomy of *Strigamia* at the species level.

For instance, within the *S. acuminata* species complex, it remains to be clarified whether – besides the morphological differentiated candidate species inhabiting the South-Eastern Prealps –

the remaining areas are inhabited by a single species, as suggested by phenotypic uniformity, or multiple parapatric species, as suggested by some genetic differentiation.

In the same way, it remains to be clarified how many other species comprise the *S. crassipes* complex, besides the two candidate species here recognized in the South-Western Alps. Diversity in body size, in body modularity and in other characters claims for further investigations. For instance, populations living north of the Alps have often evident, but variable, darker mid-longitudinal stripes on the sternites of the posterior part of the body (Spelda, 2005), while this feature is found less frequently in the populations living in the Eastern Alps. Additionally, a few specimens collected from caves in the South-Eastern Prealps show larger body size, more numerous legs, longer antennae and relatively wider coxal pores, when compared to the epigean populations living in the surrounding areas. This contrast may be explained by the existence of differentiated species, or by the presence of locally adapted cave-dwelling populations, or merely by phenotypic plasticity in hypogean vs. epigean habitats (e.g., Bilandžija et al., 2020).

Despite of an unprecedented sampling effort (overall 2111 specimens from 786 sites, including 52 specimens employed in strictly integrative analyses) and a broad array of methods applied to different lines of evidence (morphological and molecular differentiation, and coexistence of distinct morphologies; Fig. 9), addressing the taxonomy within any species-complex will require an even more intense sampling effort, especially within the Alps, and a more thorough evaluation of different lines of evidence.

With respect to genetic differentiation, our preliminary analysis based on the single mitochondrial marker COI should be expanded to multiple loci from the entire genome, leveraging recently developed methodologies allowing phylogenomic analyses and population genomics (e.g., Noguerales et al., 2018; Stanton et al., 2019). Preliminary genomic data sequenced as Illumina short reads, as well as complete mitochondrial genomes are already available for some specimens representative of different *Strigamia* species complexes (Collins et al., 2023), but more data are needed.

Additionally, with respect to morphological differentiation, despite that we already aligned with best practices of integrative taxonomy (e.g., controlling characters for within-population variation, and drawing morphological-based species delimitation hypotheses independently from molecular-based hypotheses and not only to corroborate the latter; see also Peretti et al., 2022), advances are expected by expanding the suite of the investigated characters. Indeed, other characters have been suggested by previous authors as putative different between *Strigamia* populations, but they have not been included in our study because they are hard to evaluate through a protocol granting precision and repeatability. These characters include: adult body size, body color of living animals (e.g. Fig. 1), elongation of the antennal articles, shape of venom calyx in the forcipules, pattern of sclerification and shallow depressions on the sternites, shape of the pore-fields, shape and setation of the sternite of the ultimate leg-bearing segment (Table S1 in Supporting Information). Additionally, subtle shape variation could be assessed through an explorative approach, e.g., by applying geometric morphometrics to selected body parts (e.g., Siriwut et al., 2015; Chaplin et al., 2020).

Our study integrated evidence from morphological and molecular variation with an additional line of evidence that is much rarely employed in integrative taxonomy in an explicit and rigorous way: the syntopic occurrence of discretely morphologically distinct organisms as an additional hint of speciation (Padial et al., 2010; Solís-Lemus et al., 2015; Cadena et al., 2018; Hausdorf & Hennig, 2020). However, additional insights are expected by exploring other lines of evidence, like the ecological niche differentiation (e.g., Rissler & Apodaca, 2007; Wang et al., 2019).

Finally, advances are expected from expanding the sampling to the entire geographical range of the *Strigamia* lineages under study. Both Western and Eastern Europe are inhabited by some of the species detected by our study in Middle Europe, however additional field surveys are needed to collate an adequate sampling scheme. Especially Eastern Europe is known to host additional

lineages, which have been so far rarely sampled and investigated (e.g., Dobroruka, 1977; Matic, 1985).

Final remarks

Our investigation on the diversity of *Strigamia* centipedes in a broad part of Europe showcases how the taxonomy in use for soil-dwelling animals may turn out substantially unsatisfactory, even in long investigated continents, when we endorse a modern theoretical view of species and speciation and when we scrutinize different lines of evidence for species and speciation within an integrative taxonomic methodology and a rigorous statistical approach.

ACKNOWLEDGMENTS

We are grateful to: Marco Giacomazzo, Bernhard A. Huber, Thorsten Klug, Giulia Moresco, Jan-Philip Oeyen, Christian Owen and Hans Reip, for helping us with field sampling and preliminary examinations of specimens; Leandro Drago, for helping in DNA extraction and amplification; David Cabanillas, for sharing information on published records; Emiliano Peretti and two anonymous reviewer for helping us in improving the manuscript. Some molecular sequences were obtained within the GBOL project "German Barcode of Life", funded by the German Federal Ministry of Education and Research (BMBF FKZ01LI1101A, FKZ01LI1101B), and within the project "Barcoding Fauna Bavarica" funded by the Bavarian State Ministry of Education and Culture, Science and the Arts. The sequencing work at ZSM was supported, in part, by the Government of Canada to Genome Canada through the Ontario Genomics Institute, while the Ontario Ministry of Research and Innovation and NSERC supported development of the BOLD informatics platform.

REFERENCES

Aberer AJ, Krompass D, Stamatakis A. 2013. Pruning rogue taxa improves phylogenetic accuracy: an efficient algorithm and webservice. *Systematic Biology* **62**: 162–166.

Andersson G, Djursvoll P, Scheller U. 2008. Katalog över Nordens mångfotingar över Nordens mångfotingar. *Entomologisk Tidskrift* **129**: 173–190.

Andersson G, Meidell B, Scheller U, Djursvoll P, Gärdenfors U, Budd G, Bergström J, Xianguang H. 2005. Mångfotingar (Myriapods). *Nationalnyckeln till Sveriges flora och fauna*. Artdatabanken, SLU, Uppsala, 351 pp.

Astrin JJ, Höfer H, Spelda J, Holstein J, Bayer S, Hendrich L, *et al.* 2016. Towards a DNA Barcode Reference Database for Spiders and Harvestmen of Germany. *PLoS ONE* **11**: e0162624.

Astrin JJ, Stüben PE. 2008. Phylogeny in cryptic weevils: molecules, morphology and new genera of western Palaearctic Cryptorhynchinae (Coleoptera: Curculionidae). *Invertebrate Systematics* **22**: 503–522.

Barace J, Herrera L. 1980. Estudio faunistico del macizo de Quinto Real. III. Miriápodos quilópodos (Myriapoda Chilopoda). *Publicaciones de Biologa de la Universidad de Navarra* **4**: 1–26.

Barber AD. 2008. Key to the identification of British centipedes. *Field Study Council Occasional Publications* **130**: 96 pp.

Barber AD. 2009. Centipedes. *Synopses of the British Fauna* **58**: 228 pp.

Barber AD. 2022. *Atlas of the centipedes of Britain and Ireland*. Field Studies Council Publications, 389 pp.

Bates D, Mächler M, Bolker B, Walker S. 2015. Fitting Linear Mixed–Effects Models Using lme4. *Journal of Statistical Software* **67**: 1–48.

Berger SA, Krompass D, Stamatakis A. 2011. Performance, accuracy, and web server for evolutionary placement of short sequence reads under maximum likelihood. *Systematic Biology* **60**: 291–302.

Bilandžija H, Hollifield B, Steck M, Meng G, Ng M, Koch AD, Gračan R, Ćetković H, Porter ML, Renner KJ, Jeffery W. 2020. Phenotypic plasticity as a mechanism of cave colonization and adaptation. *eLife* **9**: e51830

Birky Jr CW, Adams J, Gemmel M, Perry J. 2010. Using population genetic theory and DNA sequences for species detection and identification in asexual organisms. *PLoS one* **5**: e10609.

Bonato L, Bortolin F, Drago L, Orlando M, Dányi L. 2017. Evolution of *Strigamia* centipedes (Chilopoda): a first molecular assessment of phylogeny and divergence times. *Zoologica Scripta* **46**: 486–495.

Bonato L, Dányi L, Socci AA, Minelli A. 2012. Species diversity of *Strigamia* Gray, 1843 (Chilopoda: Linotaeniidae): a preliminary synthesis. *Zootaxa* **3593**: 1–39.

Bonato L, Drago L, Murienne J. 2014b. Phylogeny of Geophilomorpha (Chilopoda) inferred from new morphological and molecular evidence. *Cladistics* **30**: 485–507.

Bonato L, Edgecombe GD, Lewis JGE, Minelli A, Pereira LA, Shelley RM, Zapparoli M. 2010. A common terminology for the external anatomy of centipedes (Chilopoda). *ZooKeys* **69**: 17–51.

Bonato L, Lopresti M, Minelli A, Cerretti P. 2014a. ChiloKey, an interactive identification tool for the geophilomorph centipedes of Europe (Chilopoda, Geophilomorpha). *ZooKeys* **443**: 1–9.

Bonato L, Minelli A. 2009. Geophilomorph centipedes in the Mediterranean region: revisiting taxonomy opens new evolutionary vistas. *Soil Organisms* **81**: 489–503.

Bonato L, Minelli A. 2014. Chilopoda Geophilomorpha of Europe: a revised list of species, with taxonomic and nomenclatorial notes. *Zootaxa* **3770**: 1–136.

Bonato L, Minelli A, Spungis V. 2005. Geophilomorph centipedes of Latvia. *Latvijas Entomologs* **42**: 5–15.

Borges PAV, Enghoff H. 2005. Chilopoda. *In:* Borges PAV, Cuhna R, Gabriel R, Frias Martins A, Silva L, Vieira V, eds, Listagem da fauna e flora (Mollusca e Arthropoda) (Bryophyta, Pteridophyta e Spermatophyta) terrestres dos Açores. Universidade dos Açores, Direção Regional do Ambiente, Horta: p. 184.

Borges PAV, Gaspar C, Fonseca Crespo LC, Rigal F, Cardoso P, Pereira F, et al. 2016. New records and detailed distribution and abundance of selected arthropod species collected between 1999 and 2011 in Azorean native forests. *Biodiversity Data Journal* **4**: e10948.

Brolemann HW. 1930. *Éléments d'une faune des myriapodes de France*. *Chilopodes*. Imprimérie Toulousaine, Toulouse, 405 pp.

Cabanillas D. 2019. Centipede biodiversity (Myriapoda, Chilopoda) in two *Eucalyptus* plantations in northern Spain and other records from Vegadeo (Asturias, Spain). *Boletín de la Asociación española de Entomología* **43**: 287–303.

Cabanillas D. 2020. Centipede fauna (Myriapoda, Chilopoda) from saproxylic environments of Salamanca (Castilla y León, Spain). *Boletín de la Asociación española de Entomología* **44**: 517–527.

Cadena CD, Zapata F, Jiménez I. 2018. Issues and perspectives in species delimitation using phenotypic data: Atlantean evolution in Darwin's finches. *Systematic Biology* **67**: 181–194.

Chaplin K, Sumner J, Hipsley CA, Melville J. 2020. An integrative approach using phylogenomics and high-resolution x-ray computed tomography for species delimitation in cryptic taxa. *Systematic Biology* **69**: 294–307.

Collins G, Schneider C, Boštjančić LL et al. 2023. MetaInvert: A new soil invertebrate genome resource provides insights into below-ground biodiversity and evolution. PREPRINT. *Research Square*. https://doi.org/10.21203/rs.3.rs-2706746/v1

Criscuolo A. 2011. MorePhyML: improving the phylogenetic tree space exploration with PhyML 3. *Molecular Phylogenetics and Evolution* **61**: 944–948.

Darriba D, Taboada GL, Doallo R, Posada D. 2012. JModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* **9**: 772.
Dayrat B. 2005. Towards integrative taxonomy. *Biological Journal of the Linnean Society* **85**: 407–415.
De Queiroz K. 2007. Species concepts and species delimitation. *Systematic Biology* 56: 879–886.
Dobroruka LJ. 1977. Bulgarische Chilopoden in der Sammlung von Nationalmuseum Praha. *Vestnik Ceskoslovenské Spolecnosti Zoologické* **41**: 5–7.
Eason EH. 1964. *Centipedes of the British Isles*. F. Warne & Co, London, 294 pp.
Flot JF. 2015. Species delimitation's coming of age. *Systematic Biology* **64**: 897–899.
Fontaine B, van Achterberg K, Alonso-Zarazaga MA, Araujo R, Asche M, Aspöck H, *et al*. 2012. New Species in the Old World: Europe as a Frontier in Biodiversity Exploration, a Test Bed for 21st Century Taxonomy. *PLoS ONE* **7**: e36881.
Fontaneto D, Flot JF, Tang CQ. 2015. Guidelines for DNA taxonomy, with a focus on the meiofauna. *Marine Biodiversity* **45**: 433–451.
Foster PG. 2004. Modeling compositional heterogeneity. *Systematic Biology* **53**: 485–495.
Fox J, Weisberg S, Adler D, Bates D, Baud-Bovy G, Ellison S, ... Heiberger R. 2012. Package 'car'. R Foundation for Statistical Computing, Vienna.
Fujisawa T, Barraclough TG. 2013. Delimiting species using single-locus data and the Generalized Mixed Yule Coalescent approach: a revised method and evaluation on simulated data sets. *Systematic Biology* **62**: 707–724.
Geoffroy JJ, Iorio E. 2009. The French centipede fauna (Chilopoda): updated checklist and distribution in mainland France, Corsica and Monaco. *Soil Organisms* **81**: 671–694.
Gill FB. 2014. Species taxonomy of birds: Which null hypothesis? *The Auk 131*: 150–161.
Gregory SJ, Lewis JGE. 2015. Geophilomorph centipedes (Chilopoda: Geophilomorpha) from north-west Spain and northern Portugal collected by the British Myriapod and Isopod group in 2004. *Bulletin of the British Myriapod & Isopod Group* **28**: 15–30.
Guindon S, Dufayard J, Lefort V, Anisimova M, Hordijk W, Gascuel O. 2010. New algorithms and methods to estimate maximum–likelihood phylogenies: assessing the performance of PhyML 3.0. *Systematic Biology* **59**: 307–321.
Hausdorf B, Hennig C. 2020. Species delimitation and geography. *Molecular Ecology Resources* **20**: 950–960.
Horneland EO, Meidell B. 2009. Postembryonic development of *Strigamia maritima* (Leach, 1817) (Chilopoda: Geophilomorpha: Linotaeniidae) with emphasis on how to separate the different stadia. *Soil Organisms* **81**: 373–386.
Iorio E. 2005. *Strigamia transsilvanica* (Verhoeff, 1928), espece nouvelle pour la faune de France (Chilopoda, Geophilomorpha, Linotaeniidae). *Bulletin de la Société Linnéenne de Bordeaux* **33**: 195–198.
Iorio E. 2014. Catalogue biogéographique et taxonomique des chilopodes (Chilopoda) de France métropolitaine. *Mèmoires de la Société Linnéenne de Bordeaux* **15**: 372 pp.
Iorio E, Berg M. 2007. Première contribution a l'etude des chilopodes (Chilopoda) de Provence et description d'une nouvelle sous-espèce. *Bulletin de la Société Linnéenne de Provence* **58**: 21–36.
Iorio E, Labroche A. 2015. Les chilopodes (Chilopoda) de la moitié nord de la France: toutes les bases pour débuter l'étude de ce groupe et identifier facilement les espèces. *Invertébrés Armoricains* **13**: 108 pp.
Iorio E, Labroche A, Jacquemin G. 2022. Les chilopodes (Chilopoda) de la moitié nord de la France: toutes les bases pour débuter l'étude de ce groupe et identifier facilement les espèces. Version 2. Distributed by the authors: 91 pp.
Jeekel CAW. 1964. Beitrag zur Kenntnis der Systematik und Ökologie der Hundertfüsser (Chilopoda) Nordwestdeutschlands. *Abhandlungen und Verhandlungen des Naturwissenschaftlichen Vereins in Hamburg* **8**: 111–153.

Jeekel CAW, Brugge BJH. 2009. Millipedes and centipedes of the Kleimeer Polder, Province N. Holland, The Netherlands. *Myriapod Memoranda* **3**: 72–93.
Kaczmarek J. 1969. Beiträge zur Kenntnis bulgarischer Chilopoden, Teil 1. *Bulletin de la Societé des Amis des Sciences et des Lettres de Poznan, Serie D, Sciences Biologiques* **9**: 263–277.
Katoh K, Standley DM. 2013. MAFFT Multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* **30**: 772–780.
Kime RD, Enghoff H. 2017. Atlas of European millipedes 2: Order Julida (Class Diplopoda). *European Journal of Taxonomy* **346**.
Koren A. 1986. Die Chilopoden–Fauna von Kärnten und Osttirol. 1. Geophilomorpha Scolopendromorpha. *Carinthia II* **43** (suppl.): 1–85.
Kumar S, Stecher G, Tamura K. 2016. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* 33: 1870–1874.
Lohmander H. 1957. Faunistiskt Fältarbete i Nordoch Västjylland 1954 och 1956. *Göteborgs Musei Årstryck* 1957: 29–86.
Magnusson A, Skaug H, Nielsen A, Berg C, Kristensen K, Maechler M, … Brooks MM. 2017. Package 'glmmTMB'. R Package Version 0.2. 0.
Marcuzzi G, Minelli S. 1971. Osservazioni ecologiche sulla fauna geofila del Cansiglio. *Atti e Memorie. Accademia Patavina di Scienze Lettere ed Arti* **83**: 55–105.
Matic Z. 1972. *Fauna Republicii Socialiste România. Clasa Chilopoda Subclasa Epimorpha*. Academia Republicii Socialiste România, Bucureşti, 220 pp.
Matic Z. 1975. Contributo alla conoscenza dei chilopodi della Francia Meridionale. *Bollettino del Museo Civico di Storia Naturale di Verona* **2**: 155–166.
Matic Z. 1985. Note sur les chilopodes du genre *Strigamia* Gray (Chilopoda: Geophilomorpha) de la réserve scientifique de «Retezat». *Revue Roumaine de Biologie, Série de Biologie Animale* **30**: 11–15.
Matic Z, Dărăbanţu C. 1968. Contributii la cunoaşterea genurilor *Strigamia, Pachymerium* şi *Dicellophilus* (Chilopoda – Epimorfa [sic]) din fauna României. *Studia Universitatis Babeş–Bolyai, Series Biologia* **1**: 91–94.
Matic Z, Dărăbantzu [sic] **C. 1971.** Chilopodi raccolti dal dr. Giuseppe Osella in Italia. *Memorie del Museo Civico di Storia Naturale di Verona* **18**: 389–422.
Minelli A. 1979. Centipedes from the burrows of *Talpa europaea* L. in Italy north of Po River. *Bollettino del Museo Civico di Storia Naturale di Verona* **5**(1978): 573–579.
Monaghan MT, Wild R, Elliot M, Fujisawa T, Balke M, Inward DJG, Lees DC, Ranaivosolo R, Eggleton P, Barraclough TG, Vogler AP. 2009. Accelerated species inventory on Madagascar using coalescent–based models of species delineation. *Systematic Biology* **58**: 298–311.
Noguerales V, Cordero PJ, Ortego J. 2018. Integrating genomic and phenotypic data to evaluate alternative phylogenetic and species delimitation hypotheses in a recent evolutionary radiation of grasshoppers. *Molecular Ecology* **27**: 1229–1244.
Orgiazzi A, Bardgett RD, Barrios E, Behan–Pelletier V, Briones MJI, Chotte J–L, De Deyn GB, Eggleton P, Fierer N, Fraser T, Hedlund K, Jeffery S, Johnson NC, Jones A, Kandeler E, Kaneko N, Lavelle P, Lemanceau P, Miko L, Montanarella L, Moreira F, Ramirez KS, Scheu S, Singh BK, Six J, van der Putten WH, Wall DH (eds.). 2016. *Global Soil Biodiversity Atlas*. Luxembourg: European Commission, Publications Office of the European Union.
Padial JM, Miralles A, De la Riva I, Vences M. 2010. The integrative future of taxonomy. *Frontiers in Zoology* **7**: 1–14.
Peretti E, Cecchin C, Fusco G, Gregnanin L, Kos I, Bonato L. 2022. Shedding light on species boundaries in small endogeic animals through an integrative approach: species delimitation in the centipede *Clinopodes carinthiacus* (Chilopoda: Geophilidae) in the south-eastern Alps. *Zoological Journal of the Linnean Society* **196**: 902–923.

Pons J, Barraclough TG, Gomez–Zurita J, Cardoso A, Duran DP, Hazell S, Kamoun S, Sumlin WD, Vogler AP. 2006. Sequence-based species delimitation for the DNA taxonomy of undescribed insects. *Systematic Biology* **55**: 595–609.

Popper K. 2002. *The Logic of Scientific Discovery*. Routledge, New York.

Porat C.O. 1889. Nya bidrag till Skandinaviska halföns Myriopodologi. *Entomologisk Tidskrift* **10**: 1–68.

Puillandre N, Brouillet S, Achaz G. 2021. ASAP: assemble species by automatic partitioning. *Molecular Ecology Resources* **21**: 609–620.

Puillandre N, Lambert A, Brouillet S, Achaz G. 2012. ABGD, Automatic Barcode Gap Discovery for primary species delimitation. *Molecular Ecology* **21**: 1864–1877.

Reip HS, Decker P, Voigtländer K, Lindner EN, Hannig K, Spelda J. 2012. Seltene Myriapoden Deutschlands (Diplopoda, Chilopoda). *Schubartiana* **5**: 49–112.

Rissler LJ, Apodaca JJ. 2007. Adding more ecology into species delimitation: ecological niche models and phylogeography help define cryptic species in the black salamander (*Aneides flavipunctatus*). *Systematic Biology* **56**: 924–942.

Sammet K, Ivask M, Kurina O. 2018. A synopsis of Estonian myriapod fauna (Myriapoda: Chilopoda, Diplopoda, Symphyla and Pauropoda). *ZooKeys* **793**: 63–96.

Schlick–Steiner BC, Steiner FM, Seifert B, Stauffer C, Christian E, Crozier RH. 2010. Integrative Taxonomy: a multisource approach to exploring biodiversity. *Annual Reviews of Entomology* **55**: 421–438.

Simaiakis S, Minelli A, Mylonas M. 2004. The centipede fauna (Chilopoda) of Crete and its satellite islands (Greece, Eastern Mediterranean). *Israel Journal of Zoology* **50**: 367–418.

Siriwut W, Edgecombe GD, Sutcharit C, Panha S. 2015. The centipede genus *Scolopendra* in mainland Southeast Asia: molecular phylogenetics, geometric morphometrics and external morphology as tools for species delimitation. *PLoS ONE* **10**: e0135355

Sites JW, Marshall JC. 2004. Operational criteria for delimiting species. *Annual Review of Ecology, Evolution and Systematics* **35**, 199–227.

Solís-Lemus C, Knowles LL, Ané C. 2015. Bayesian species delimitation combining multiple genes and traits in a unified framework. *Evolution* **69**: 492–507.

Spelda J. 2005. Improvements in the knowledge of the myriapod fauna of southern Germany between 1988 and 2005 (Myriapoda: Chilopoda, Diplopoda, Pauropoda, Symphyla). *Peckiana* **4**: 101–129.

Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post–analysis of large phylogenies. *Bioinformatics* **30**: 1312–1313.

Stanton DWG, Frandsen P, Waples RK, Heller R, Russo IM, Orozco-terWengel PA, Tingskov Pedersen CE, Siegismund HR, Bruford MW. 2019. More grist for the mill? Species delimitation in the genomic era and its implications for conservation. *Conservation Genetics* 20: 101–113.

Statsoft. 2008. *STATISTICA (data analysis software system)*. Version 8.0. www.statsoft.com.

Stoev P. 2002. A Catalogue and Key to the Centipede (Chilopoda) of Bulgaria. *Pensoft Series Faunistica* **25**: 103 pp.

Tuf IH, Kupka J. 2015. The first record of *Strigamia pusilla* from the Czech Republic (Chilopoda: Geophilomorpha). *Acta Carpathica Occidentalis* **6**: 108–110.

Vedel V, Apostolou Z, Arthur W, Akam M, Brena C. 2010. An early temperature-sensitive period for the plasticity of segment number in the centipede *Strigamia maritima*. *Evolution & Development* **12**: 347–352.

Verhoeff KW. 1935. Über *Scolioplanes* (Chilopoda), *Zoologischer Anzeiger* **111**: 10–23.

Voigtländer K. 2009. Ökologie. *In*: Rosenberg J. (Ed.), Die Hundertfüßer. Chilopoda. *Die Neue Brehm–Bücherei* **285**: 385–409.

Voigtländer K, Dunger W. 1992. Long-term observations of the effects of increasing dry pollution on the myriapod fauna of the Neiße Valley (East Germany). *Bericht des Naturwissenschaftlich–Medizinischen Vereins in Innsbruck* suppl. **10**: 251–256.

Voigtländer K, Spelda J. 2019. Myriapoda – Vielfüßer, Tausendfüßer. *In*: Klausnitzer B, ed. Stresemann – Exkursionsfauna von Deutschland. Band 1: Wirbellose (ohne Insekten). Springer Spektrum, Berlin, Heidelberg: pp. 573–603.

Wang X, Que P, Heckel G, Hu J, Zhang X, Chiang CY, Zhang N, Huang Q, Liu S, Martinez J, Pagani-Núñez E, Dingle C, Leung YY, Székely T, Zhang Z, Liu Y. 2019. Genetic, phenotypic and ecological differentiation suggests incipient speciation in two *Charadrius* plovers along the Chinese coast. *BMC Evolutionary Biology* **19**: 135.

Wesener T, Voigtländer K, Decker P, Oeyen JP, Spelda J, Lindner EN. 2015. First results of the German Barcode of Life (GBOL) – Myriapoda project: Cryptic lineages in German *Stenotaenia linearis* (Koch, 1835) (Chilopoda, Geophilomorpha). *Zookeys* **510**: 15–29.

Witten IH, Frank E. 2005. *Data Mining: Practical machine learning tools and techniques*, 2nd Edition. Morgan Kaufmann, San Francisco.

Zachos FE. 2016. *Species concepts in biology*. Cham: Springer.

Zapparoli M. 1994. Note tassonomiche e faunistiche su Chilopodi della Grecia (Chilopoda). *Fragmenta Entomologica* **26**: 11–66.

Zapparoli M. 1999. The present knowledge of the centipede fauna of Anatolia (Chilopoda). *Biogeographia* **20**: 105–177.

Zapparoli M, Minelli A. 2007. Chilopoda. *In*: Ruffo S, Stoch F., eds, Checklist e distribuzione della fauna italiana. *Memorie del Museo civico di Storia naturale di Verona. 2a serie, Sezione Scienze della Vita* **17** [2006]: 123–125.

Zhang J, Kapli P, Pavlidis P, Stamatakis A. 2013. A general species delimitation method with applications to phylogenetic placements. *Bioinformatics* **29**: 2869–2876.

Zuev RV, Evsyukov AP. 2016. Centipedes (Chilopoda) from the Rostov-on-Don Region, southern Russia. *Russian Entomological Journal* **25**: 417–426.

Data Availability Statement: *Data underlying this article are partly in the Supporting Information and partly available from the corresponding author upon request.*

Tables

Table 1. Morphological characters analyzed for their variation in 11 populations of *Strigamia* (Tables S2 and S4) and employed in the species delimitation analysis in 52 specimens (Table S3). Characters are listed in anatomical order, anterior to posterior, and the evaluation method is indicated for each of them: i = by incident light with stereomicroscope; t = by transmitted light with biological microscope. Variation with body size and differences between sexes are indicated when their effects were statistically significant in the GLMMs (see Material and Methods; also, Table S4). Arrows indicate either increase (↑) or decrease (↓) with body size.

character	operational definition	variable type	evaluation method	variation with body size	differences between sexes	considered for species delimitation	correction for species delimitation
head elongation	head length / width	continuous	t	yes ↓	yes	no	-
arrangement of clypeal setae	setae on anterior clypeus: three groups vs. continuous row	binary	t	no	no	yes	no
relative size of forcipular tergite	forcipular tergite / head length	continuous	t	yes ↑	no	no	-
relative separation of forcipules	distance between condyles of coxosternite / basal width of trochanteroprefemur	continuous	t	yes ↓	no	no	-
presence of projection on forcipular tibia	internal margin of forcipular tibia: only small tubercle or shallow bump vs. large projection	binary	t	no	no	yes	no
relative size of forcipular tarsungulum	distance of tip of tarsungulum from forcipular external hinge / distance between condyles of coxosternite	continuous	t	yes ↓	no	no	-
relative size of forcipular denticle	distance of tip of forcipular denticle from forcipular external hinge / distance of tip of tarsungulum from forcipular external hinge	continuous	t	no	no	yes	no
shape of forcipular denticle	external and internal margins of denticle of forcipular tarsungulum: approximately straight vs. distinctly curved	binary	t	no	no	yes	no
shape of forcipular ungulum	intermediate part of external and internal margins of forcipular tarsungulum: uniformly convergent vs. sub-parallel	binary	t	no	no	yes	no
number of legs	number of leg-bearing segments	meristic	i	no	yes	yes	+ 2 if male
presence of stripe on anterior sternites	mid-longitudinal sclerotized stripe at 20% of the series of trunk sternites: absent vs. present	binary	i	no	no	yes	no
number of coxal pores	maximum number of coxal pores between the two coxopleura	meristic	t	yes ↑	yes	yes	+ 1 if male / $(\text{head width})^2$
size of coxal pores	maximum diameter of coxal pores	continuous	t	yes ↑	no	yes	/ head width

Table 2. Main differential characters between the species-complexes of *Strigamia* in the study area, according to the results of the species delimitation analyses and data from reliably assigned specimens and validated published records (see Material and Methods). The sample size "n" includes both specimens examined directly and specimens reported in publications. Notes: *, character evaluated only in specimens >15 mm long; **, evaluated only in specimens 15–25 mm long.

Name	**operational definition**	***S. acuminata* species-complex**	***S. carniolensis* species-complex**	***S. crassipes* species-complex**	***S. engadina***
body length	length without appendages: mode, max	mode 21, max 35 mm (n=631)	mode 31, max 60 mm (n=390)	mode 20, max 65 mm (n=529)	max 50 mm (n=5)
arrangement of clypeal setae	setae on anterior clypeus: three groups vs. continuous row *	three groups: 100% (n=301)	continuous row: 92% (n=83)	continuous row: 99% (n=235)	continuous row: 100% (n=2)
relative size of forcipular tergite	forcipular tergite/head length: mean [min-max] *	0.34 [0.28-0.41] (n=72)	0.41 [0.34-0.51] (n=47)	0.43 [0.33-0.53] (n=97)	0.45 [0.41-0.48] (n=2)
relative separation of forcipules	distance between condyles of coxosternite/max width of trochanteroprefemur: mean [min-max] *	1.9 [1.6-2.3] (n=70)	1.6 [1.4-1.8] (n=47)	1.8 [1.5-2.1] (n=94)	1.6 [1.6-1.6] (n=2)
presence of projection on forcipular tibia	internal margin of forcipular tibia: only small tubercle or shallow bump vs. large projection *	small: 99% (n=70)	large: 94% (n=52)	large: 97% (n=90)	large: 100% (n=2)
relative size of forcipular tarsungulum	distance of tip of tarsungulum from external hinge / distance between condyles of coxosternite: mean [min-max] *	0.82 [0.71-0.94] (n=97)	0.68 [0.63-0.77] (n=47)	0.75 [0.68-0.85] (n=95)	0.89 [0.84-0.94] (n=2)
relative size of forcipular denticle	distance of tip of denticle from forcipular external hinge / distance of tip of tarsungulum from external hinge: mean [min-max] *	0.46 [0.39-0.52] (n=95)	0.62 [0.56-0.67] (n=47)	0.55 [0.48-0.62] (n=96)	0.50 [0.44-0.53] (n=3)
shape of forcipular denticle	external and internal margins of denticle of forcipular tarsungulum: approximately straight vs. distinctly curved	straight: 99% (n=335)	curved: 94% (n=95)	curved: 100% (n=298):	curved: 100% (n=5)
shape of forcipular tarsungulum	intermediate part of external and internal margins of forcipular tarsungulum: uniformly convergent vs. sub-parallel	convergent: 91% sub-parallel: 19% (n=310)	convergent: 100% (n=402)	convergent: 100% (n=473)	sub-parallel: 100% (n=5)
number of legs	number of leg-bearing segments	37-43 [males 37-41, females 39-43] (n=1169)	45-57 [males 45-55, females 47-57] (n=554)	37-65 [males 37-65, females 39-65] (n=676)	51-55 [males 51-53, females 53-55] (n=5)
presence of stripe on anterior sternites	mid-longitudinal sclerotized stripe at 20% of the series of trunk sternites: absent vs. present	absent: 100% (n=587)	present: 100% (n=402)	absent: 100% (n=475)	absent: 100% (n=5)
average number of coxal pores	number of coxal pores on a coxopleuron at intermediate body size (about 25 mm long)	usually ≤ 15 (n=552)	usually ≥ 25 (n=288)	usually ≤ 15 (n=508)	usually ≤ 15 (n=5)
maximum number of coxal pores	maximum number of coxal pores on a coxopleuron	22 (n=592)	56 (n=314)	31 (n=569)	21 (n=5)
relative size of coxal pores	maximum diameter of coxal pores / head width	0.03 [0.02-0.04] (n=74)	0.03 [0.02-0.05] (n=47)	0.06 [0.04-0.10] (n=100)	0.08 [0.07-0.08] (n=2)
shape of coxal canals	diameter of opening / diameter of canal: distinctly	< 1: 79%	< 1: 98%	~ 1: 85%	~ 1: 100%

	< 1 vs. ~ 1 *	(n=89)	(n=57)	(n=117)	(n=5)

FIGURES

Figure 1. Representative individuals of *Strigamia* from the study area: males (a, c, e) and females (b, d, f) belonging to the *S. acuminata* species-complex (a, b), *S. carniolensis* species-complex (c, d), and *S. crassipes* species-complex (e, f). Photos: a, München, 9.VI.2013, J. Spelda; b, Venetian Prealps, Mt. Pasubio, 27.VI.2017, L. Bonato; c, Venetian Prealps, Montello, 27.IV.2012, L. Bonato; d, Dolomites, Val di Medon, 30.V.2005, L. Bonato; e, Danube Valley, Kelheim, 19.V.2010, J. Spelda; f, Northern European Plain, Landgrafen Jena, 11.XI.2017, H. Reip.

Figure 2. Study area (elevation in gray shades) and sampling sites of the specimens examined for intra-population variation (code letters as in Table S2), of the specimens employed in the species delimitation analyses by morphological and molecular differentiation (code numbers as in Table S3), of all other specimens examined for morphology, and all other published records.

Figure 3. Selected anatomical parts of representative specimens of *Strigamia*: (a) head and forcipular segment; (b, c) anterior part of head; (d) posterior tip of the body; (e, f) forcipules. Photos taken through the microscope, from the ventral side: a, Northern European Plain, Dubringer Moor, 4.IV.2012, K. Voigtländer, ZFMK MYR 3557, *S. acuminata* species-complex; b, e, Abruzzi Apennines, Ovindoli, 7.IX.1999, G. Osella, PD-G 2884, *S. acuminata* species-complex; c, f, Lucan Apennines, Novi Velia, 10.VII.1973, R. Pace, PD-G 2480, *S. crassipes* species-complex; d, Swabian Jura, Leipheim, 27.V.2013, J. Spelda, ZSM-JSP150201-106, *S. crassipes* species-complex.

Figure 4. Hypotheses of species delimitation of *Strigamia* obtained from different lines of evidence and different methods, illustrated upon the ultrametric tree of the COI. Bootstrap values are indicated whenever different from 100%. Tree tips represents haplotypes, coded as in Table S3. Both the previous species identification of the specimens and the revised names of the species-complexes are indicated. Intraspecific genetic distances are given in Table S6.

Figure 5. Candidate species of *Strigamia* indicated by the syntopy of morphologically distinct individuals. Each species is represented by a circle, whose size is proportional to the number of sites. The syntopy between two species is represented by a line, whose width is proportional to the number of sites where the syntopy has been found (indicated along the line). Characters differing between the candidate species are indicated by symbols along the lines (see also Table 1). Each species is coded as in Fig. 4 and indicated with its revised name.

Figure 6. Geographical distribution of the *Strigamia acuminata* species-complex and candidate species within the study area, based on direct examination of specimens and critically revised published records.

Figure 7. Geographical distribution of the *Strigamia carniolensis* species-complex and the species *S. engadina* within the study area, based on direct examination of specimens and critically revised published records.

Figure 8. Geographical distribution of the *Strigamia crassipes* species-complex and candidate species within the study area, based on direct examination of specimens and critically revised published records.

Figure 9. Summary of the different evidence of speciation, sources of evidence, and analyses performed.

SUPPORTING INFORMATION

Figure S1. Frequency distribution of pairwise COI genetic distances.

Figure S2. Relation between relative size of forcipular tarsungulum and relative size of forcipular denticle in *S. acuminata* species-complex, distinguishing the candidate species *S. microdon*.

Figure S3. Specimens of the *S. crassipes* species-complex collected near the type locality of *crassipes* C.L. Koch, 1835: A, ♀ with 49 leg pairs, from NW Tegernheim (5 km ENE Regensburg), 1.XI.2013 J. Spelda lg, ZSM-JSP131101-055; B, with 49 leg pairs, ♀ from W Keilberg (4 km NE Regensburg), 13.XI.2015 J. Spelda lg, ZSM-JSP151117-004.

Figure S4. Representative specimen of the *S. carniolensis* species-complex: ♀ with 51 leg pairs, from Caverna Mainarda (6 km NNE Travesio), showing the mid-longitudinal sclerotized stripe of the anterior metasternites.

Table S1. Characters proposed by previous authors as distinguishing species or infraspecific taxa in European inland *Strigamia*, with the main references suggesting their diagnostic validity (the putatively distinguished nominal taxa are in parentheses).

Table S2. Specimens of *Strigamia* employed in the preliminary analysis of intraspecific morphological variation before the species delimitation analysis. Populations are listed in geographical order approximately North to South, and West to East (Fig. 2). The maximum distance between collection sites was estimated at the nearest 5 km.

Table S3. Specimens of *Strigamia* employed in the analysis of morphological and molecular differentiation for species delimitation. Specimens are listed in geographical order approximately North to South, and West to East. Collection codes: Bonato-Minelli collection, Department of Biology, University of Padova (PD); Forschungsmuseum Alexander Koenig, Bonn (ZFMK); Zoologische Staatssammlung München (ZSM). COI haplotypes shared by multiple specimens are coded with the prefix h-.

Table S4. Variation of morphological characters in relation to body size and sex, analyzed in 172 specimens of *Strigamia* from 11 populations (Table S2) by means of GLMMs, with body size and sex as fixed effects, and population as random effect. Characters are ordered anterior to posterior. Measurements are in mm. Coefficients of statistically significant effects are in bold.

Table S5. Specimens of *Strigamia* collected together from a single site, in a single date, and showing remarkable morphological differences that suggest the syntopy of two or more species (see Material and Methods). Altitudes are approximated at ± 100m.

Table S6. Intraspecific pairwise p-distances of COI sequences in *Strigamia* species-complexes and candidate species, according to the results of the species delimitation analyses. We considered only the specimens from within the study area (Fig. 2; n=51).

Appendix. Taxonomic and nomenclatorial notes on the *Strigamia* species-complexes and candidate species.

Additional references for Supporting Information