The "five divergent amino acids rule" and evolution analysis of cytochrome *c* oxidase as new methods for *Scolopendra* Linnaeus, 1758 species differentiation (Chilopoda. Scolopendromorpha).

Carles Doménech[1, 5], Víctor M. Barberá[2, 3] & Eduardo Larriba[4]

[1]*Departament de Ciències Ambientals i Recursos Naturals, Facultat de ciències, Universitat d'Alacant. Carretera de Sant Vicent del Raspeig s/n C.P. 03690, San Vicent del Raspeig, Alacant (Spain).* https://orcid.org/0000-0003-1890-9434

[2]*Unidad de Genética Molecular, Hospital General Universitario de Elche, Fundación para el Fomento de la Investigación Sanitaria y Biomédica de la Comunidad Valenciana (FISABIO). Camí de l'Almazara, 11, C.P. 03203 Elche, Alicante, (Spain).* https://orcid.org/0000-0002-4012-6973

[3]*Departamento de Fisiología, Genética y Microbiología, Universidad de Alicante. Carretera de Sant Vicent del Raspeig s/n C.P. 03690, San Vicent del Raspeig, Alacant (Spain).*

[4]*Instituto de Bioingeniería, Universidad Miguel Hernández. Avda. de la Universidad, s/n C.P. 03202 Elche, (Spain).* https://orcid.org/0000-0001-8838-4974

[5]*Corresponding author: E-mail: carles.domenech.perez@gmail.com*

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Abstract

Scolopendra Linnaeus, 1758 is a genus of centipede showing reasonably good morpho-molecular correlation. This often allows the acceptable species differentiation and phylogenetic positioning by just using the partial sequence of the cytochrome *c* oxidase (COI) gene. However, for biological, statistical, or technical reasons, several exceptions to that fact have been observed, which together with a lack of a solid criterion to differentiate species at the molecular level, make difficult the interpretation of some taxonomic and systematic outcomes.

With the scope of providing a standardized system improving the molecular *Scolopendra* species delimitation and surpassing some issues related with the mtDNA's use, a total of 45 representative COI sequences belonging to 22 taxa are tested, but for the first time, performing distinct amino acid's chains evolution analyses. To illustrate this, a general evaluation of the genus is firstly provided, while to exemplify some of the deeper analysis, the cases of *S. paradoxa* Doménech, 2018 and *S. spinosissima* Kraepelin, 1903 are then here re-explored using some alternative tools.

As a result, the partial COI protein sequences analysis identified, at least, ten amino acid residues' positions as useful for *Scolopendra* species differentiation, being generally five divergent residues enough to distinguish taxa ["the five divergent amino acid rule"]. When this premise wasn't fulfilled, the amino acid frequency, exclusivity, or electrochemical properties and especially the *r* substitution probability test, helped solve the cases. Among *S. paradoxa* and *S. spinosissima* five diverging residues were found, with one of them being exclusive to the entire genus for the former taxon. Also, the substitution probability (*r*) analysis showed strong positive selection for three of these five divergent amino acids.

As exceptions, *S. dawydoffi* Kronmüller, 2012 has been found indistinguishable from *S. multidens* Newport, 1844 by this method, suggesting its eventual synonymy, while *S. cataracta* Siriwut, Edgecombe & Panha, 2016 showed an intraspecific maximum divergence of 5 amino acids. Finally and at nomenclatural level, the species name *S. hainanum* Kronmüller, 2012 is emended as *S. hainanensis* while the taxon *S. mojiangica* Zhang & Chi, is here declared *nomen nudum*, due its original description remains unavailable.

Introduction

The class Chilopoda is among the oldest groups of terrestrial arthropods (Edgecombe & Giribet 2007). These soil dweller invertebrates' (Undheim & King 2011; Undheim et al. 2015) are venomous predators widely distributed across the warmer and tropical ecosystems. They are classified into five distinct orders, which currently include a total of 3,300 described taxa (Edgecombe & Giribet 2007). Among the 700 valid species of the order Scolopendromorpha, the most recognizable genus is *Scolopendra* Linnaeus, 1758, which currently comprises around 100 accepted members (Bonato et al. 2016; Schileyko et al. 2020).

Like many other metazoans and since their first descriptions, species of this genus have undergone multiple taxonomic and nomenclatural changes (see, for example, Linnaeus 1758; Kraepelin 1903; Attems 1930; Kronmüller 2012). However, these classical classification systems, based mainly on morphological and geographical differences, have been significantly improved by integrating them with more modern molecular techniques. As a paradigm of this progress, cytochrome *c* oxidase (COI) has proven to be a reliable bioindicator capable of establishing consistent morpho-molecular

phylogenies across various groups. These have helped resolve some of the difficult relationships within the genus *Scolopendra* (Edgecombe & Giribet 2008; Vahtera et al. 2012, 2013; Oeyen et al. 2014; Siriwut et al. 2015). Likewise, through the quantitative analysis of genetic distances in partial COI sequences, it has also been possible to distinguish species with a reasonable degree of confidence (Siriwut et al. 2016; Doménech et al. 2018). However, despite generally working well, these methods do not always reflect an expected correlation between the morphology of all taxa and their percentage of divergence or phylogenetic position. For example, some studies have revealed that two congeners, despite being morphologically very different, appear to be more molecularly similar to each other than to their morphologically closer relatives. In some cases, even species placed in different genera turned out to be more similar to each other at the molecular level than to their actual congeners (Vahtera et al. 2012, 2013; Vahtera & Edgecombe 2014; Siriwut et al. 2016; Doménech et al. 2018).

Three main reasons could explain these occasional inconsistencies: first, technical issues that may affect the preprocessing of samples, PCR, and/or DNA sequencing, which could ultimately compromise sequence quality. The second reason relates to the statistical methodology used to carry out the systematic analysis. These tests may yield significantly different results depending on several factors, such as the number and length of sequences used, the number and type of species or genera studied, the alignment method, the statistical approach, or the number of applied replicates and biases caused by certain bioinformatic phenomena such as long branch attraction (Susko et al. 2021). However, the third and most important reason may lie in various biological causes, such as the accumulation of neutral mutations, their saturation, or differing nucleotide substitution rates (heterotachy) in the COI sequences of each species (Kimura 1968, 1983; Zuckerkandl & Pauling 1962; Xia 2009).

Furthermore, molecular studies of the genus have generally been conducted partially; this is by dividing analyses by geographical region (see Oeyen et al. 2014; Siriwut et al. 2015, 2016; Doménech et al. 2018). This adds to the issue, as there is no clearly defined threshold to distinguish intra- from interspecific levels for species of the genus *Scolopendra*. As a result, it is sometimes observed that the same percentage of molecular divergence between two individuals can be considered either intra- or interspecific depending on the source taken as reference (compare Oeyen et al. 2014; Siriwut et al. 2015, 2016; Doménech et al. 2018). Lastly, when comparing different species, this lack of consensus can also occasionally make it difficult to coherently reconcile morphological results with various molecular studies.

On the other hand, molecular studies on COI have generally focused on genetic positioning in trees and complemented by the percentage analysis of genetic divergences. These studies, without exception, have always been based on nucleotide sequences. However, phenotypic determination—and consequently, the external morphology—is given by the proteins translated from these and other genes, with the peptide chain carrying the "true" evolutionary weight as it determines the adaptation or elimination of individuals in a specific environment. The peptide chains of the partial COI sequence have not been studied taxonomically, yet they may offer certain advantages over nucleotide sequences. Quantitatively, amino acid chains, being two-thirds shorter than nucleotide chains, could facilitate faster reading and interpretation of different alignments. Likewise, the analysis of amino acid substitutions, where fewer changes are expected compared to nucleotide analysis (Kimura 1968, 1983), could be easier to analyze. This might translate into the use of whole numbers instead of divergence percentages obtained through more complex statistical methods subject to other statistical biases. Qualitatively, amino acid chains are more diverse than

nucleotides (20 possible residues vs. only four possible bases), which allows easier discrimination of changes; making it possible to observe whether these changes are exclusive to a species or a group of species. These changes, involving electrochemical and structural modifications in the protein, can be measured, providing new differential data between sequences. Similarly, amino acid changes carry greater evolutionary and taxonomic significance than nucleotide changes, as they tend to be more inherently discriminative (Kimura 1968, 1983). Finally, protein studies would present much less bias and "noise" than nucleotide-based studies, as they would be minimally influenced by factors such as neutral mutations or substitution saturation, potentially offering molecular divergences more consistent with morphology.

Not being the main objective of this study to discern the probable causes generating the morphomolecular incongruences observed in previous articles based on mtDNA (Vahtera et al. 2012, 2013; Vahtera & Edgecombe 2014; Siriwut et al. 2016; Doménech et al. 2018), the present work analyzes for the first time the advantages and disadvantages of using translated partial COI sequences when applied to the systematics of the genus *Scolopendra*. To illustrate this, the translated partial COI fragment from a representative sample set of 21 *Scolopendra* species will be compared under this alternative molecular perspective. First, general results obtained from various bioinformatic tests will be presented, along with general conclusions for discriminating between different species of the genus. Next, as a guide, the methodology for resolving cases of difficult interpretation from an evolutionary standpoint will be outlined, exemplified by two species recently redefined by this research group — *S. spinosissima* Kraepelin, 1903 and *S. paradoxa* Doménech, 2018 — (Doménech et al. 2018; Doménech 2024). Finally, a simple summary scheme will be provided for the easy application of the aforementioned methods. The work will conclude with a discussion of

the pros and cons of using these new methods and will present some taxonomic and nomenclatural observations involving some of the species studied here.

Methods

Pre-processing of materials, nomenclatural considerations, and image treatment.

To elongate the previously trimmed terminal ends and reaffirm their suitability, the sequences of *Scolopendra spinosissima* Kraepelin, 1903 (KY888682.1), *S. paradoxa* Doménech, 2018 (MH087425.1, KY888684.1, KY888685.2, KY888686.2), and *S. subscrustalis* Kronmüller, 2009 (KY888683.2) were re-sequenced following the protocol outlined in Doménech et al. (2018).

The specific epithet of the taxon *Scolopendra hainanum* Kronmüller, 2012 ***nom. imperfect.*** is considered an original incorrect spelling (Art. 32.5 ICZN) due to grammatical gender disagreement (Art. 11.9.1.3, 31.1.2, 31.2, 32.2, and 32.5 ICZN). Therefore, the name is justifiably emended here (Art. 19.2 ICZN) and is consistently presented throughout the text as *S. hainanensis* ***nom. correct***.—in agreement with the species' author, C. Kronmüller; May/June 2020, pers. comm. Likewise, the *taxon S. mojiangica* attributed to Zhang & Chi is here considered a ***nomen nudum*** (Art. 12 and 14, ICZN) as its original description is unavailable (NCBI: txid2023220). Since morphologically this species is not clearly distinguishable from other Asian congeners, this taxonomic unit is provisionally kept separate from the rest to avoid attribution bias.

The creation and editing of images (contrast and brightness adjustment, notes and references in illustrations) were carried out using Adobe Photoshop CS6.

Bioinformatic analysis of partial COI sequences.

A total of 383 COI sequences assigned to the genus *Scolopendra* were retrieved from the BOLD V4 database (Ratnasingham & Hebert 2007). The partial COI sequences were batch-translated into proteins using the getorf program from the EMBOSS tools (Rice et al. 2007), applying the invertebrate mitochondrial genetic code and discarding ORFs with fewer than 200 residues. To align the selected partial COI sequences translated into proteins, the ClustalW program implemented in BioEdit (Hall 2011) and MEGA X was used (Figure 1A, Supplementary files 1–3). The protein alignments were manually checked, discarding sequences with gaps, ambiguous residues, or those that did not align properly. Subsequently, some representative sequences from each species were selected proportionally to the number of available sequences and with the greatest possible geographical/geological distance between them. The taxonomic identity of the selected sequences was verified or modified according to current nomenclature. The divergence table of the selected protein products was generated using MEGA X (Supplementary file 4).

The amino acid substitution probability (*r*) (Table 1) was calculated using a trimmed alignment of 41 partial COI protein sequences and a substitution matrix estimated and implemented in MEGA X (Kumar et al. 2018). The sequence logos (Figure 1B) were generated using the WebLogo online tool (https://weblogo.berkeley.edu/logo.cgi). Amino acid frequencies were calculated using MEGA X, and the heat map of amino acid frequencies along with hierarchical clustering (HCL) was generated using MeV software (Multiple Experiment Viewer, www.mev.tm4.org) (Figure 1C). Amino acid sequence changes were evaluated with reference to the haplotype of the translated partial sequence belonging to the holotype of *S. paradoxa* (S. paradoxa.CEUA017-MR0000).

The statistical selection of the models that best fit the amino acid substitution study was performed using the Find Best Substitution Model tool implemented in MEGA X, assuming the lowest AICc scores (corrected Akaike Information Criterion). Evolutionary analysis was carried out in MEGA X. The maximum likelihood (ML) phylogenetic tree (Guindon et al. 2010) was constructed using the mitochondrial general reversible model (mtREV) (Le et al. 2017) with 1000 bootstrap replicates (Figure 2). A consensus tree was subsequently obtained and rooted with selected outgroups. In the final stage, node support was evaluated, and genetic affinities were discussed in relation to current nomenclature and taxonomy.

The summary diagram outlining the intra- and interspecific boundaries of COI partial sequences translated into proteins for the genus *Scolopendra* was created using PowerPoint (Microsoft Office 365) (Figure 3).

Abbreviations

Institutions:

BMB-DENR - Biodiversity Management Bureau - Department of Environment and Natural Resources, Philippines.

CEUA- Colección Entomológica de la Universidad de Alicante San Vicent del Raspeig, Alacant, Spain.

PAE - Philippines Association of Entomologists, Inc., Philippines.

PNM - Philippine National Museum, Philippines.

PNU - Philippine Normal University, Philippines.

WRD-DENR - Wildlife Resources Division - Department of Environment and Natural Resources, Philippines.

Molecular Section:

A, alanine; **COI**, cytochrome *c* oxidase; **E**, glutamic acid; **L**, leucine; **M,** methionine; **N**, asparagine; **S**, serine; **T**, threonine.

Others: ICZN; International Code of Zoological Nomenclature.

Results

Sequence Filtering

The first step in the bioinformatic analysis was to approach *bona fide* sequences and eliminate those with the evident errors. To do so, strict quality control criteria were applied to the sequences extracted from the BOLD database (see Materials section). Briefly, of the 383 sequences initially obtained, 22 sequences were discarded as they did not actually belong to the genus *Scolopendra*. Of the "29" *Scolopendra* species initially available, 14 sequences were removed for having fewer than 200 residues, including two representative species: *S. cf. amazonica* Bücherl, 1946 and *S. leki* (Waldock and Edgecombe, 2012). Additionally, another 17 sequences were eliminated for being non-translatable into a coding protein. A third filter removed 61 sequences and five more species (*S. lufengia* Kang, Liu, Zeng, Deng, Luo, Chen & Chen, 2017; *S. nigrocapitis* Zhang & Wang, 1999; *S. mojiangica*; *S. valida* Lucas, 1840; and the remaining sequences of *S. cretica* Lucas, 1853), as they yielded defective protein alignments.

Finally, from the extracted dataset, only a total of 269 sequences were deemed suitable, from which 45 sequences belonging to 21 different species were selected (Supplementary File 1).

Partial COI protein sequences variation in the genus *Scolopendra*.

Although we have found a high amino acid identity regions in our *Scolopendra* partial protein COI genes' alignment, we have identified ten residues' sites that present a great variability in the relative position number: 21, 24, 52, 104, 105, 112, 115, 129, 131 and 176; being also the relatives positions 249, 255, 256 and 265 other probable amino acid variable hot spots (Fig. 1B, Supplementary files 2 and 3).

Constantly, determinate amino acid combinations in the proteins' variable fraction have being observed at intraspecific and interspecific levels. These let to establish differences between species with a quite elevate degree of confidence As a general rule, when comparing two sequences, five or more amino acid residue changes in the studied COI fragment (equivalent to 2.2% or more) indicate an interspecific range between the two amino acid chains. However, as an exception to this clear intra/interspecific division — equivalent to a mitochondrial DNA divergence gap — only 14 exceptions were observed among the 820 alignments analyzed. Therefore, this simple method of counting divergent amino acids is capable of distinguishing different species of the genus *Scolopendra* with an approximate accuracy of 98.29% (Supplementary file 4)

At the intraspecific level, all alignments uniformly showed between zero and four amino acid changes. The only exception was found in the alignment between the holotype of *S. cataracta* from Laos and a conspecific individual from China, which showed five residue substitutions. Additionally, although not counted here to avoid potential attribution/sequencing biases, two singular cases were detected far beyond these general observations: 1) *S. subspinipes* Leach, 1816, despite aligning correctly, showed either only two or nine changes respectively, depending on whether the unique CUMZ sequence was used or not; and 2) *S. pinguis* Pocock, 1891, a species under suspected cryptic speciation (Siriwut et al. 2015, 2016), which exhibited an extremely unusual range of between two and fourteen amino acid variations in the studied COI fragment (Supplementary file 4).

At the interspecific level, all alignments consistently showed five or more substitutions (hereafter referred to as the "5 divergent amino acid rule") (Supplementary file 4). However, two different types of exceptions were detected: 1) Presumed distinct, but morphologically very similar species, with all their alignments falling within the intraspecific range (four or fewer divergent residues), such as *S. multidens* Newport, 1844 / *S. dawydoffi* Kronmüller, 2012, which consistently aligned with zero to three amino acid substitutions; and 2) Clearly distinct species whose respective alignments fell within both the intra- and interspecific range (all with between four and five substitutions): *S. cataracta* / *S. hainanensis*, *S. lufengia* / *S. dawydoffi* and *S. cingulata* when aligned with *S. lufengia* and *S. cataracta*. In the interspecific range, no divergences involving zero to three residue substitutions were observed between morphologically distinct species, leading to the conclusion that this number of changes occurs exclusively within the intraspecific range. Moreover, without exception, all morphologically distinct species with overlapping substitutions in the intra/inter range consistently had at least one alignment with four or five, or more divergent residues, depending on the sequences analyzed. This suggested that when this rare ranges overlap occurs, the analyzed taxa consistently support the presence of two distinct species (Supplementary file 4).

Moreover, upon examining the amino acid sequence alignments (Supplementary files 2 and 3), it was also found that 12 out of the 21 *Scolopendra* species had one or more unique residues in their sequence. These exclusive variations were observed consistently in species represented by multiple sequences, suggesting that the presence of such unique amino acids is, by itself, diagnostic of the species possessing them. Other amino acids were shared only by specific groups of morphologically related species, such as *S. subcrustalis* Kronmüller, 2009 and *S. laeta* Haase, 1887, or by

morphologically and geographically distant species such as *S. mutilans* L. Koch, 1878, *S. polymorpha* Wood, 1861, and *S. viridis* Say, 1821, indicating a character shared by a group of species. When these group-characteristic but not species-exclusive amino acids were found, they were also observed to be useful for distinguishing species—especially when the taxa were not morphologically and/nor geographically related.

On the other hand, in terms of amino acid composition, the different translated partial COI protein sequences also showed significant similarity among *Scolopendra* species (Figure 1C). However, slight variations in composition were observed, which made it possible to establish hierarchical groupings and, in turn, distinguish species by positioning them in a way that differs from the phylogenies (Figure 2). By this method, only some species showed non-monophyletic groupings.

At the phylogenetic level, the tree inferred based on protein chains (Figure 2) showed similar results in species placement when compared to mtDNA trees (check Siriwut et al. 2015, 2016; Oeyen et al. 2014; Doménech et al. 2018). However, branch positioning was more sensitive to amino acid variations, while node supports were correspondingly weaker in protein-based phylogenetic reconstructions. Ultimately, it was found that trees based on COI protein-coding sequences were somewhat less useful for establishing phylogenies than those based on mtDNA (see Siriwut et al. 2015, 2016; Oeyen et al. 2014; Doménech et al. 2018).

At the taxonomic level, the molecular results showed a significant number of amino acid substitutions (>4) between *S. mutilans* and its closest relative, *S. subspinipes*, reaffirming that these entities are not conspecific taxa, contrary to what has been suggested in recent literature (Vahtera et al. 2013; Kang et al. 2017; Han et al. 2018) (Supplementary File 4). Conversely, the findings by Kang et al. (2017) which reported no molecular differences between *S. subspinipes* and *S. hainanensis*, are not

supported here (residue differentiation >4; Supplementary File 4), as our results were consistent with them being distinct species, aligning with their clear morphological differences (Kronmüller 2012; Siriwut et al. 2016).

In parallel, experimental alignments (unpublished), which include relatively short but partially well-conserved sequences, revealed that the morphological similarity between *S. morsitans* Linnaeus, 1758 and *S. cf. amazonica* is strongly supported at the molecular level, suggesting a likely synonymy (residue substitution = 1). Meanwhile, species that appear morphologically similar, such as *S. japonica* and *S. negrocapitis* Zhang & Wang, 1999, as well as *S. mojiangica*, produced inconclusive results (three to five amino acid substitutions), showing the unsolved taxonomical and nomenclature situation for these cases.

COI protein sequence variations between *S. paradoxa* and *S. spinosissima*, and final considerations

The analysis of COI protein sequence variations was conducted using the DNA sequences of *S. paradoxa* and *S. spinosissima* obtained in Doménech et al. (2018) (Supplementary File 1). We found that the protein sequences derived from the holotype and paratypes of *S. paradoxa* encoded exactly the same protein sequence (Supplementary Files 2 and 3). Therefore, only one consensus sequence of *S. paradoxa* was used for the following analyses. Figure 1A shows the pairwise alignment of the partial COI protein sequences of *S. paradoxa* and *S. spinosissima*. As shown in the alignment, the partial COI proteins displayed a high degree of identity (97.8% of aligned amino acids in Supplementary Files 2 and 3). However, we identified five amino acid residue differences between the two species: 19T>A, 105T>S, 108L>M, 115N>S, and 169E>S (Figures 1A–B, Table 1). Of these five substitutions, two—105T>S and 115N>S—were located in regions of high variability observed across the

sequences (Supplementary File 2). The variations 19T>A, 108L>M, and 169E>S involved relevant changes in the physicochemical properties of the amino acids (Table 1), which could suggest changes in the structure, function, and adaptation of the resulting COI protein.

To ensure that the evolutionary process between these two species—of which few sequences are available—is not random or an exception to the "five amino acid rule," we calculated the substitution probability of these five residues using a total of 41 partial COI protein sequences of *Scolopendra* (Table 1). We found that substitutions 19T>A, 105T>S, and 115N>S had a substitution probability greater than one, suggesting strong positive selection (adaptive or diversifying) at these three amino acid positions (Table 1). In contrast, the substitutions at residues 108L>M and 169E>S showed substitution probabilities below one, suggesting moderate and strong negative (purifying) selection, respectively (Table 1). These data support the conclusion that the observed amino acid divergence cannot be explained by genetic drift alone and highlight the selective pressures involved in the diversification of these two species.

Among all the *Scolopendra* species represented in this study, *S. paradoxa* consistently and exclusively exhibited a glutamic acid (E) at relative position 169, making this amino acid a standalone diagnostic character for the species (Supplementary File 3). As a group, *S. paradoxa*-*S. spinosissima* did not exhibit unique group-specific amino acids that differentiated them from the rest of the species. However, *S. spinosissima* shared characteristic amino acids with *S. calcarata* Porat, 1876, while *S. paradoxa* shared group-specific amino acids with *S. laeta*—none of which were found together in any other species.

In the hierarchical classification of amino acid frequencies (Figure 1C), *S. paradoxa* and *S. spinosissima* were grouped into non-sister sections, suggesting the

separation of these species by the use of this method. Similarly to this, the inferred protein phylogenetic reconstruction showed *S. paradoxa* and *S. spinosissima* as close but not strictly sister species. *Scolopendra paradoxa* displayed strong monophyletic node support including its four sequences, whereas the position of *S. spinosissima* remained weakly supported—precisely as reported in previous literature (Doménech et al. 2018).

In line with earlier findings (Doménech et al. 2012), all these showed molecular analyses concerning *S. spinosissima* and *S. paradoxa* once again conclude that these taxonomic entities are objectively and definitively two distinct species, separated by evolutionary selective pressures rather than by chance.

Finally, to facilitate species discrimination within *Scolopendra* using this alternative molecular method based on partial proteins translated from COI fragments, we provide a summary diagram of the results obtained, including noted exceptions and recommendations for particular situations (Figure 3).

Discussion

In this study, the translated partial COI gene has been confirmed as a valuable biomarker for the identification and differentiation of species within the genus *Scolopendra*. The analysis of amino acids in the partial COI protein fragment showed that sequences belonging to different species differ by five or more residues. However, some exceptions to this “rule” were detected. To clarify most of these ambiguous inter/intraspecific cases, additional analysis of exclusive and characteristic divergent amino acids—their frequency, electrochemical properties, and especially the *r* substitution probability test—proved to be useful tools for resolving these exceptional scenarios.

Compared to traditional divergence studies based on mtDNA percentage differences, the intra/interspecific discrimination of amino acid substitutions expressed as natural numbers proved to be more easily interpretable, computationally less demanding, and less affected by methodological and biological variation. Likewise, the *r* substitution probability test showed a high degree of confidence in resolving complex cases of taxon differentiation, providing measurable insights into the evolutionary history of these species. On the other hand, the phylogenetic tree inferred from amino acid chains, while yielding good results on its own, also proved to be no more accurate than phylogenetic reconstructions based on mtDNA (Figure 2). In this case, the shorter and more similar sequences, along with the statistical weight of any amino acid substitution, made this type of analysis—though robust—more prone to fluctuations than the more "tolerant" trees built from mtDNA data. Meanwhile, heat map and hierarchical clustering, although useful in distinguishing species by amino acid composition in most cases, were not as precise as divergence studies, but still served as valuable supporting tools for the overall analysis. In contrast, the study of exclusive or group-characteristic amino acids proved to be significant for the separation and discrimination of *Scolopendra* species. However, this latter method should be interpreted with caution in species with a low number of sequences analyzed and/or a restricted geographic distribution.

Proportionally, it has been shown that interspecific mtDNA divergence percentages do not correspond to those of amino acids (i.e., compare the divergences of *S. spinosissima* and *S. paradoxa*, with respective differences of 19% for mtDNA and 2.2% for the coding protein; see Supplementary File 4 and Doménech et al. 2018). This result aligns with Kimura's neutral theory (1983), which states that most DNA mutations do not affect codon translation. Therefore, compared to most nucleotide

421 changes, an amino acid substitution is much more influential on the structure and
422 function of the resulting protein, and consequently, on species decline or differentiation.
423 Thus, due to its inherently lower susceptibility to the random effects of evolution, a
424 species differentiation method based on proteins, such as the one proposed here, could
425 represent an alternative to mtDNA-based methods, offering certain interpretative
426 advantages and a high level of reliability.

427 On the other hand, from a general perspective, it is noteworthy that
428 approximately one-third of the available COI sequences of Scolopendra exhibited
429 various issues that hindered proper alignment. The intrinsic difficulty of COI
430 sequencing—which includes artifacts such as nuclear mitochondrial DNA segments
431 (NUMTs) (Rogers & Griffiths-Jones 2012)—combined with human-related factors like
432 species misidentification, statistical errors, and biological elements such as the
433 accumulation of neutral mutations, excessive substitution saturation, or the phenomenon
434 of heterotachy (Kimura 1968, 1983; Zuckerkandl & Pauling 1962; Xia 2009), may
435 ultimately disrupt molecular analysis. All of these elements should be evaluated to
436 improve the quality of future analyses aimed at species differentiation. Furthermore,
437 aside from the sequences reviewed directly in this study, the quality of the remaining
438 protein sequences filtered using different methods can only be approximated here, and
439 cannot be guaranteed with certainty. For this reason, some of the "irregular" cases
440 tentatively treated as exceptions in this study (see Results section) may, in fact, be
441 explained by the aforementioned errors. A higher standard of rigor and standardized
442 filtering applied prior to sequence publication could be key to improving the overall
443 quality of COI databases.

444 In the species-level analysis, our study found that some sequences of *S.*
445 *cataracta* exhibited the highest molecular variability within the genus, consistent with

the findings of Siriwut et al. (2015), making it the only intraspecific exception to the "five amino acid rule." However, despite passing the selection filters, this exception could potentially be linked to an error, given the geographic-molecular discrepancies observed among the available sequences of this species, thus requiring further study. On the other hand, the morphologically very similar S. multidens and S. dawydoffi (Siriwut et al. 2016) also could not be separated using this method, which might suggest that the minor morphological differences actually represent intrinsic variability within a single taxon. The possible synonymy of these two species should be confirmed through a study of the type specimens. Likewise, the sequences of *S. subspinipes* and *S. pinguis* should be re-examined due to the excessively high intraspecific distances observed in this study. Additionally, the validity of the few sequences available for *S. cingulata*, *S. cataracta*, and *S. lufengia* should also be assessed, as their placement falls ambiguously within either the intra- or interspecific range. If no errors are confirmed in these few cases, such deviations should be interpreted solely as a reflection of the progressive evolutionary effect in certain specimens—representing a transitional and certainly exceptional stage relative to the divergence ranges clearly defined by the five amino acid rule.

Regarding *S. spinosissima* and *S. paradoxa*, the comparison of the translated COI ortholog gene revealed five clear and consistent differences in their amino acid composition and in certain electrochemical properties (Figure 1A and B, Table 1, Supplementary Files 2 and 3). Moreover, the *r* substitution probability analysis rejected the null hypothesis (genetic drift) for all amino acid substitutions and confirmed that none of them could be explained by any random process. All these genetic results provide evidence of a speciation process driven by selection between *S. spinosissima*

and *S. paradoxa*, findings that further support the morphological differences and taxonomic separation of both species (Doménech et al. 2018, Doménech 2024).

The genetic results presented here represent a step forward in the distinction of *Scolopendra* species. Although consistent, the conclusions of this analysis were based on representative sequences from approximately 20–25% of the described species within the genus, and are therefore subject to further refinement. Revisiting the questionable and excluded sequences, along with incorporating more sequences from both these and other unexamined taxa, could enhance the accuracy of this new and simple methodology aimed at improving the molecular differentiation of *Scolopendra* species.

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Tables

Table 1. Position and chemical proprieties of divergent amino acid residues between *S. paradoxa* and *S. spinosissima* COI protein sequence. Colum *r* is probability of substitution. The numbering of the amino acids refers to the positions indicated in the supplementary file 1. *=essential amino acids.

Residue position	**Location on highly variable regions**	***S. paradoxa***		***S. spinosissima***		**Probability of substitution *(r* value)**
		Amino acids	**Electrochemical Characteristics**	**Amino acids**	**Electrochemical Characteristics**	
19	No	T*	Polar, non-charged	A	Non-polar	1.83
105	Si	T*	Polar, non-charged	S	Polar, non-charged	1.69
108	No	L*	Non-polar	M*	Polar, non-charged	0.47
115	Si	N	Polar, non-charged	S	Polar, non-charged	1.79
169	No	E	Negatively charged; Polar	S	Polar, non-charged	0.11

Figures

Figure 1. Molecular analysis of the partial COI protein sequence. **A** Pairwise alignment of the partial COI protein sequence of *S. paradoxa* / *S. spinosissima*. Identical amino acid residues are shaded in black, and residues with more than 70% similarity with the rest of the studied sequences are shaded in grey. Asterisks indicate amino acid substitutions between these two species. Blue lines mark sites where residues show high variability within the genus *Scolopendra*. **B** Logos representing 11 polymorphic residues found in the 41 partial COI protein sequences (blue asterisk). Polymorphic residues from the partial COI protein sequences of *S. paradoxa* / *S. spinosissima* are included (red asterisk). Red asterisks underlined in blue indicate polymorphic residues shared between the 22 *Scolopendra* spp. and the *S. paradoxa* / *S. spinosissima* COI protein sequences. **C** Heatmap and hierarchical clustering of amino acid frequency composition from the 45 partial COI protein sequences of *Scolopendra*. Hierarchical clustering was calculated using Pearson's average correlation of amino acid frequency composition for each species. Colored branches highlight the close amino acid frequency relationships of *S. paradoxa* (orange) and *S. spinosissima* (green).

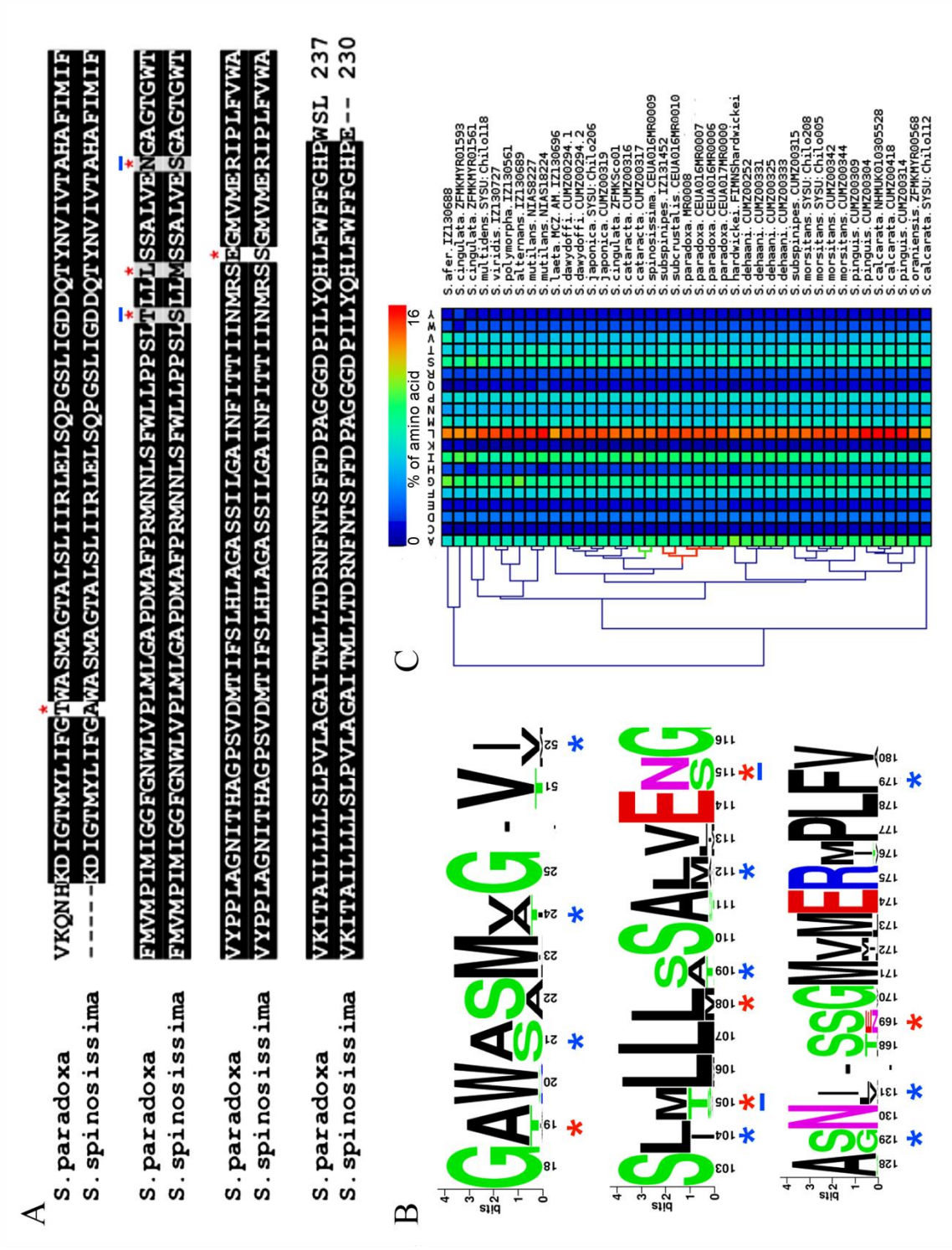

A
S.paradoxa
S.spinosissima
S.paradoxa
S.spinosissima
S.paradoxa
S.spinosissima
S.paradoxa
S.spinosissima
237
230
B
bits
C
% of amino acid
0
16

Figure 2. Maximum Likelihood phylogenetic reconstruction based on the protein-coding region of the partial COI gene translated into proteins, including 45 selected *Scolopendra* taxa along with six additional members of the tribe Scolopendrini and two species from the subfamily Otostigminae. Bootstrap values are shown for each node. Colored branches in the tree represent the genetic affinities of *S. paradoxa* (blue) and *S. spinosissima* (red) in relation to their congeners (thin black branches). Bold branches and thick black branches indicate the positions of other members of the *Scolopendrini* tribe and the Otostigminae species (outgroups), respectively.

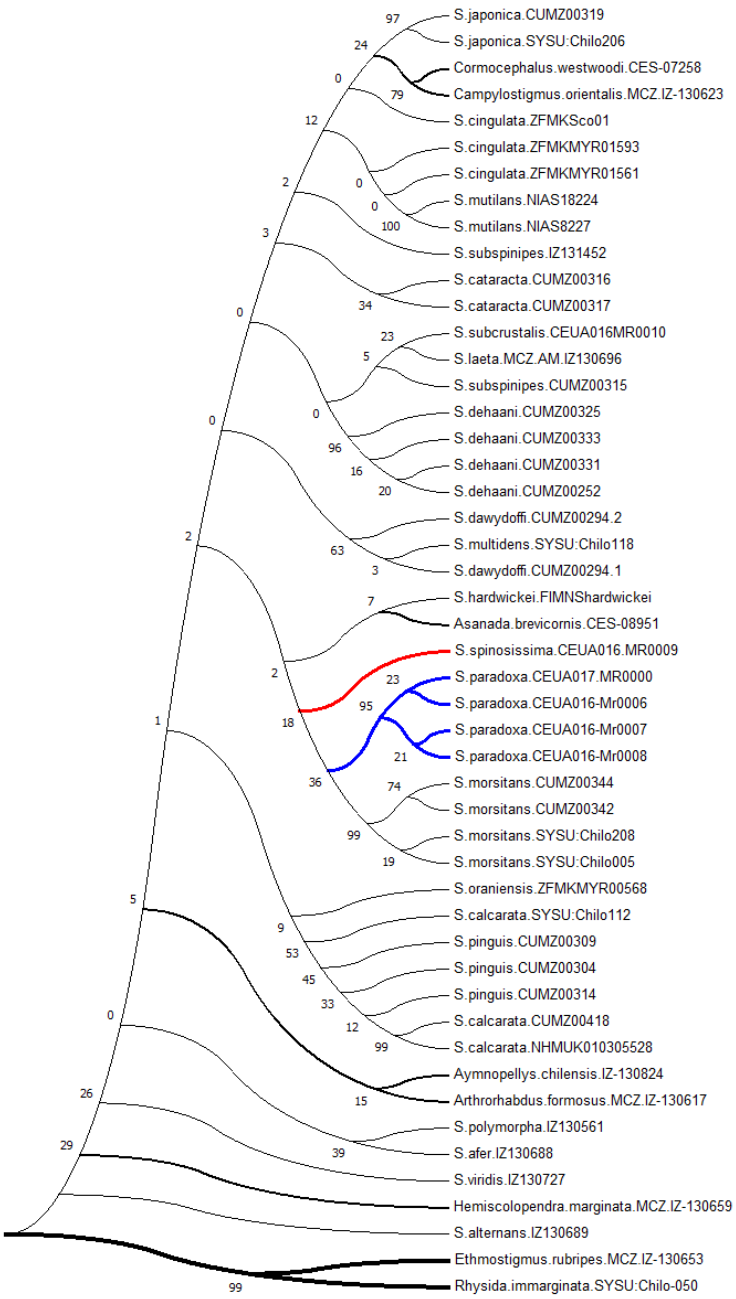
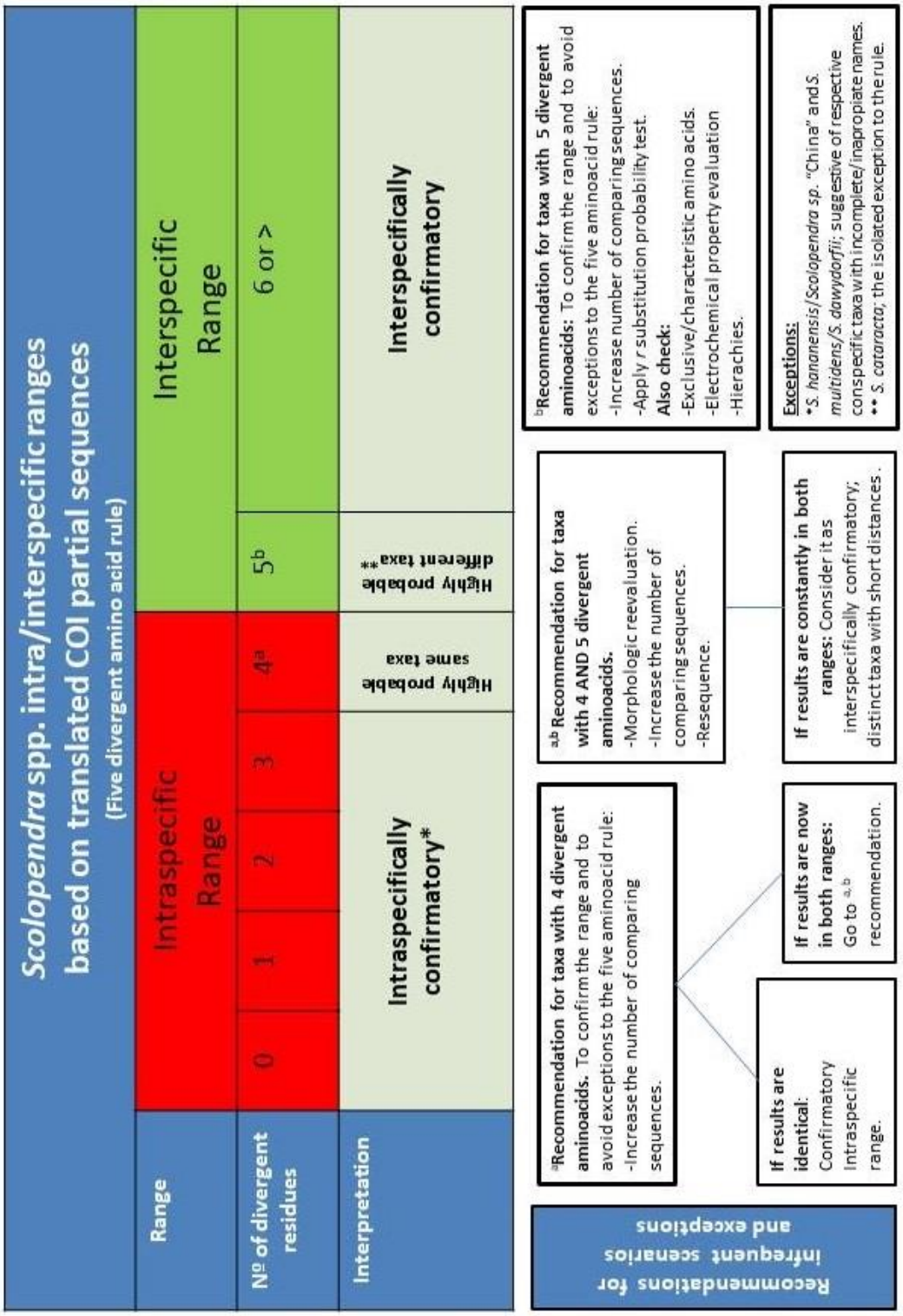


Figura 3. Summary scheme of the results, exceptions, and recommendations for determining intra- or interspecific differences between two or more translated protein sequences from the partial COI gene in the genus *Scolopendra*.



Supplementary files

Supplementary file 1. Summary of the 45 COI sequences from *Scolopendra* species, along with eight from other Scolopendromorpha, obtained from the Barcode of Life Data System (BOLD) and used in the molecular analyses. Each sample includes the institutional abbreviation of the voucher, type status, voucher code, locality, and GenBank accession number for the selected partial COI gene sequence.

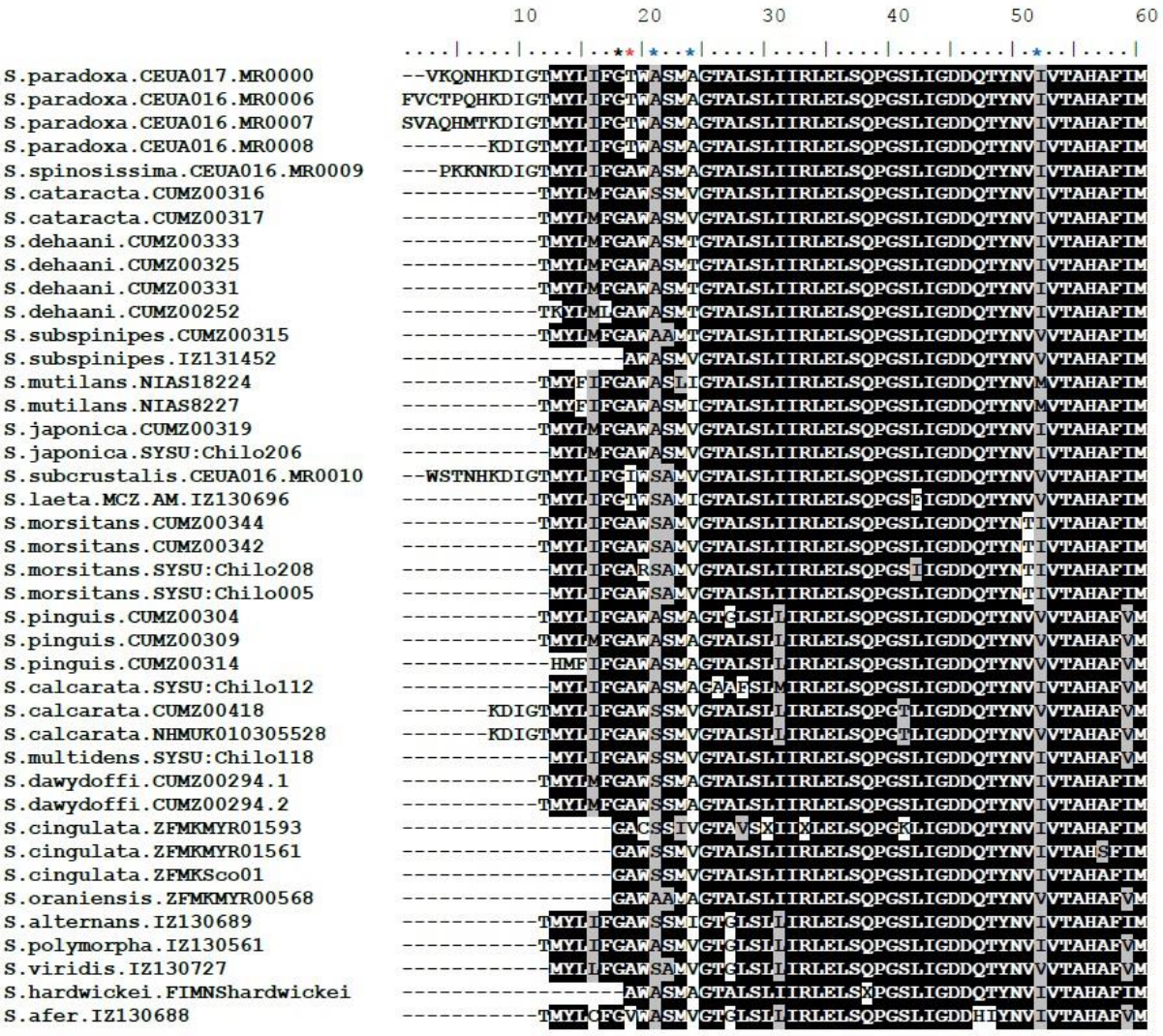
Sample name (species and specimens' voucher)	**Locality**	**COI GenBank accession**
***Scolopendra spinosissima* Kraepelin, 1903**		
S.spinosissima.CEUA016.Mr0009	Philippines	KY888682.1
***Scolopendra paradoxa* Doménech, 2018**		
S.paradoxa.CEUA017.Mr0000 (Holotype)	Philippines	MH087425.1
S.paradoxa.CEUA016.Mr0006 (Paratype 9)	Philippines	KY888684.1
S.paradoxa.CEUA016.Mr0007 (Paratype 10)	Philippines	KY888685.2
S.paradoxa.CEUA016.Mr0008 (Paratype 11)	Philippines	KY888686.2
***Scolopendra afer* (Meinert,1886)**		
S.afer.IZ130688	Tanzania	KF676520.1
Scolopendra alternans Leach, 1816		
S.alternans.IZ130689	United States of America	KF676521.1
***Scolopendra calcarata* Porat, 1876**		
S.calcarata.SYSU:Chilo112	Vietnam	AB614399.1
S.calcarata.CUMZ00418	Thailand	KU512630.1
***Scolopendra cataracta* Siriwut, Edgecombe & Panha , 2016**		
S.cataracta.CUMZ00316 (Holotype)	Laos	KR705672.1
S.cataracta.CUMZ00317 (Paratype)	Laos	KR705633.1
***Scolopendra cingulata* Latreille, 1829**		
S.cingulata.ZFMKMYR01593	France	KJ812087.1

S.cingulata.ZFMKMYR01561	Hungary	KJ812079.1
S.cingulata.ZFMKSco01	Austria	KJ812067.1
***Scolopendra dawydoffi* Kronmüller, 2012**		
S.dawydoffi.CUMZ00294.1	Thailand	KR705635.1
S.dawydoffi.CUMZ00294.2	Thailand	KR705634.1
***Scolopendra dehaani* Brandt, 1840**		
S.dehaani.CUMZ00333	Laos	KR705673.1
S.dehaani.CUMZ00325	Thailand	KR705656.1
S.dehaani.CUMZ00331	Cambodia	KR705686.1
S.dehaani.CUMZ00252	Thailand	KR705683.1
***Scolopendra hardwickei* Newport, 1844**		
S.hardwickei.FIMN-Shardwickei	N/a (¿India?)	KF676523.1
***Scolopendra japonica* L. Koch, 1878**		
S.japonica.CUMZ00319	Japan	KR705679.1
S.japonica.SYSU:Chilo206	Japan	AB672741.1
***Scolopendra laeta* Haase, 1887**		
S.laeta.MCZ.AM.IZ130696	Australia	KF676541.1
***Scolopendra morsitans* Linnaeus, 1758**		
S.morsitans.CUMZ00344	Thailand	KR705664.1
S.morsitans.CUMZ00342	Thailand	KR705666.1
S.morsitans.SYSU:Chilo208	Taiwan	AB672739.1
S.morsitans.SYSU:Chilo005	Taiwan	AB614397.1
***Scolopendra multidens* Newport, 1844**		
S.multidens.SYSU:Chilo118	Vietnam	AB672738.1

***Scolopendra mutilans* L. Koch, 1878**		
S.mutilans.NIAS18224	Korea	MG991739.1
S.mutilans.NIAS8227	Korea	MG991742.1
***Scolopendra oraniensis* Lucas, 1846**		
S.oraniensis.ZFMKMYR00568	Morocco	KJ812088.1
***Scolopendra pinguis* Pocock, 1891**		
S.pinguis.CUMZ00304	Laos	KR705642.1
S.pinguis.CUMZ00309	Laos	KR705646.1
S.pinguis.CUMZ00314	Thailand	KR705648.1
***Scolopendra polymorpha* Wood, 1861**		
S.polymorpha.IZ130561	United States of America	KF676525.1
***Scolopendra subcrustalis* Kronmüller, 2009**		
S.subcrustalis.CEUA016.MR0010	Philippines	KY888683.2
***Scolopendra subspinipes* Leach, 1816**		
S.subspinipes.IZ131452	Martinique	HQ402554.1
S.subspinipes.CUMZ00315	Singapur	KR705636.1
***Scolopendra viridis* Say, 1821**		
S.viridis.IZ130727	United States of America	DQ201431.1
Scolopendromorpha sequences used for the inferred phylogenetic tree		
***Asanada brevicornis* Meinert, 1886**		
Asanada.brevicornis.CES-08951	India	JN004014.1
***Akymnopellis chilensis* (Gervais, 1847)**		
Akymnopellis.chilensis.IZ-130824	Chile	KF676538.1
***Cormocephalus* (*Cormocephalus*) *westwoodi* (Newport, 1844)**		

Cormocephalus.westwoodi.CES-07258	Australia	JN004004.1
***Cormocephalus* (*Campylostigmus*) *orientalis* Ribaut, 1923**		
Campylostigmus.orientalis.MCZ.IZ-130623	New Caledonia	HQ402542.1
***Arthrorhabdus formosus* Pocock, 1891**		
Arthrorhabdus.formosus.MCZ.IZ-130617	South Africa	HQ402539.1
***Hemiscolopendra marginata* (Say, 1821)**		
Hemiscolopendra.marginata.MCZ.IZ-130659	United States of America	HQ402548.1
***Ethmostigmus rubripes* Brandt, 1840**		
Ethmostigmus.rubripes.MCZ.IZ-130653	Australia	KF676542.1
***Rhysida immarginata* Porat, 1876**		
Rhysida.immarginata.SYSU:Chilo-050	Taiwan: Kinmen, Lieyu	AB614648.1

Supplementary file 2. Alignment of the translated amino acid sequence from 45 partial DNA sequences of the COI gene. The alignment was performed using the ClustalW algorithm in the BioEdit package. Black asterisks indicate the first and last amino acid positions of the trimmed alignment used in the molecular analysis. Red asterisks indicate the five differing amino acid residues between the COI protein sequences of *S. paradoxa* and S. spinosissima. Blue asterisks indicate variable amino acid residues in the partial COI sequence within the genus *Scolopendra*. Black boxes mark conserved amino acid sites. White boxes indicate variable sites with <70% similarity, while gray boxes indicate sites with ≥70% similarity.



```
                                   70        80        90       100       110       120
                                   ....|....|....|....|....|....|....|....|...**..**|.*..*....|
S.paradoxa.CEUA017.MR0000          IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLTLLLSSALVENGAGTG
S.paradoxa.CEUA016.MR0006          IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLTLLLSSALVENGAGTG
S.paradoxa.CEUA016.MR0007          IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLTLLLSSALVENGAGTG
S.paradoxa.CEUA016.MR0008          IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLTLLLSSALVENGAGTG
S.spinosissima.CEUA016.MR0009      IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLSLLMSSALVESGAGTG
S.cataracta.CUMZ00316              IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSIMLLLTSALVESGAGTG
S.cataracta.CUMZ00317              IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSIMLLLSSALVESGAGTG
S.dehaani.CUMZ00333                IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSIMLLLASALVENGAGTG
S.dehaani.CUMZ00325                IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSIMLLLASALVENGAGTG
S.dehaani.CUMZ00331                IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSIMLLLASALVENGAGTG
S.dehaani.CUMZ00252                IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSIMLLLASALVENGAGTG
S.subspinipes.CUMZ00315            IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSITLLLASTLMENGAGTG
S.subspinipes.IZ131452             IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSIMLLLTSALVENGAGTG
S.mutilans.NIAS18224               IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSIMLLLTSALIENGAGTG
S.mutilans.NIAS8227                IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSIMLLLTSALIENGAGTG
S.japonica.CUMZ00319               IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLMLLLSSAMVENGAGTG
S.japonica.SYSU:Chilo206           IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLMLLLSSAMVENGAGTG
S.subcrustalis.CEUA016.MR0010      IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLTLLLASALVENGAGTG
S.laeta.MCZ.AM.IZ130696            IFFMVMPIMIGGFGNWLIPLMLGAPDMAFPRMNNLSFWLLPPSLALLLASAMVESGAGTG
S.morsitans.CUMZ00344              IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLTLLLSSAMVENGAGTG
S.morsitans.CUMZ00342              IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLTLLLSSAMVENGAGTG
S.morsitans.SYSU:Chilo208          IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLTLLLSSAMVENGAGTG
S.morsitans.SYSU:Chilo005          IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLTLLLSSAMVENGAGTG
S.pinguis.CUMZ00304                IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLTLLLASAMLESGAGTG
S.pinguis.CUMZ00309                IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLTLLMTSAMMENGAGTG
S.pinguis.CUMZ00314                IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLTLLLASAVLESGAGTG
S.calcarata.SYSU:Chilo112          IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLEPPSLMLLLSSAMLESGASTG
S.calcarata.CUMZ00418              IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLSLLLASAALESGAGTG
S.calcarata.NHMUK010305528         IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLSLLLASAALESGAGTG
S.multidens.SYSU:Chilo118          IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLMLLLSSALVENGAGTG
S.dawydoffi.CUMZ00294.1            IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLMLLLSSALVENGAGTG
S.dawydoffi.CUMZ00294.2            IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLMLLLSSALVENGAGTG
S.cingulata.ZFMKMYR01593           IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSIMLLLSSALVENGAGTG
S.cingulata.ZFMKMYR01561           IF-MVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSIMLLLSSALVENGAGTG
S.cingulata.ZFMKSco01              IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSIMLLLSSALVENGAGTG
S.oraniensis.ZFMKMYR00568          IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLMLLMSSALVESGAGTG
S.alternans.IZ130689               IFFMVMPIMMGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLMLLLSSGLVESGAGTG
S.polymorpha.IZ130561              IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLMLLLSSALVESGAGTG
S.viridis.IZ130727                 IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLILLLSSAMVENGAGTG
S.hardwickei.FIMNShardwickei       IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLTLLMASAMVESGAGTG
S.afer.IZ130688                    IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLMMLMGSSIVESGAGTG
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                                  70        80        90       100       110       120
                                  ....|....|....|....|....|....|....|....|...**..**|.*..*....|
S.paradoxa.CEUA017.MR0000         IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLTLLLSSALVENGAGTG
S.paradoxa.CEUA016.MR0006         IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLTLLLSSALVENGAGTG
S.paradoxa.CEUA016.MR0007         IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLTLLLSSALVENGAGTG
S.paradoxa.CEUA016.MR0008         IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLTLLLSSALVENGAGTG
S.spinosissima.CEUA016.MR0009     IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLSLLMSSALVESGAGTG
S.cataracta.CUMZ00316             IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSIMLLLTSALVESGAGTG
S.cataracta.CUMZ00317             IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSIMLLLSSALVESGAGTG
S.dehaani.CUMZ00333               IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSIMLLLASALVENGAGTG
S.dehaani.CUMZ00325               IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSIMLLLASALVENGAGTG
S.dehaani.CUMZ00331               IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSIMLLLASALVENGAGTG
S.dehaani.CUMZ00252               IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSIMLLLASALVENGAGTG
S.subspinipes.CUMZ00315           IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSITLLLASTLMENGAGTG
S.subspinipes.IZ131452            IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSIMLLLTSALVENGAGTG
S.mutilans.NIAS18224              IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSIMLLLTSALIENGAGTG
S.mutilans.NIAS8227               IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSIMLLLTSALIENGAGTG
S.japonica.CUMZ00319              IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLMLLLSSAMVENGAGTG
S.japonica.SYSU:Chilo206          IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLMLLLSSAMVENGAGTG
S.subcrustalis.CEUA016.MR0010     IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLTLLLASALVENGAGTG
S.laeta.MCZ.AM.IZ130696           IFFMVMPIMIGGFGNWLIPLMLGAPDMAFPRMNNLSFWLLPPSLALLLASAMVESGAGTG
S.morsitans.CUMZ00344             IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLTLLLSSAMVENGAGTG
S.morsitans.CUMZ00342             IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLTLLLSSAMVENGAGTG
S.morsitans.SYSU:Chilo208         IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLTLLLSSAMVENGAGTG
S.morsitans.SYSU:Chilo005         IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLTLLLSSAMVENGAGTG
S.pinguis.CUMZ00304               IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLTLLLASAMLESGAGTG
S.pinguis.CUMZ00309               IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLTLLMTSAMMENGAGTG
S.pinguis.CUMZ00314               IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLTLLLASAVLESGAGTG
S.calcarata.SYSU:Chilo112         IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLEPPSLMLLLSSAMLESGASTG
S.calcarata.CUMZ00418             IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLSLLLASAALESGAGTG
S.calcarata.NHMUK010305528        IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLSLLLASAALESGAGTG
S.multidens.SYSU:Chilo118         IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLMLLLSSALVENGAGTG
S.dawydoffi.CUMZ00294.1           IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLMLLLSSALVENGAGTG
S.dawydoffi.CUMZ00294.2           IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLMLLLSSALVENGAGTG
S.cingulata.ZFMKMYR01593          IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSIMLLLSSALVENGAGTG
S.cingulata.ZFMKMYR01561          IF-MVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSIMLLLSSALVENGAGTG
S.cingulata.ZFMKSco01             IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSIMLLLSSALVENGAGTG
S.oraniensis.ZFMKMYR00568         IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLMLLMSSALVESGAGTG
S.alternans.IZ130689              IFFMVMPIMMGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLMLLLSSGLVESGAGTG
S.polymorpha.IZ130561             IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLMLLLSSALVESGAGTG
S.viridis.IZ130727                IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLILLLSSAMVENGAGTG
S.hardwickei.FIMNShardwickei      IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLTLLMASAMVESGAGTG
S.afer.IZ130688                   IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLMMLMGSSIVESGAGTG
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717

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                                          190       200       210       220       230       240
                                   ...*|....|....|....|....|....|....|....|....|....*....|....|
S.paradoxa.CEUA017.MR0000          WAVKITAILLLLSLPVLAGAITMLLTDRNFNTSFFDPAGGGDPILYQHLFWFFGHPWSL-
S.paradoxa.CEUA016.MR0006          WAVKITAILLLLSLPVLAGAITMLLTDRNFNTSFFDPAGGGDPILYQHLFWFFGH-----
S.paradoxa.CEUA016.MR0007          WAVKITAILLLLSLPVLAGAITMLLTDRNFNTSFFDPAGGGDPILYQHLFWFFGH-----
S.paradoxa.CEUA016.MR0008          WAVKITAILLLLSLPVLAGAITMLLTDRNFNTSFFDPAGGGDPILYQHLFWFFG------
S.spinosissima.CEUA016.MR0009      WAVKITAILLLLSLPVLAGAITMLLTDRNFNTSFFDPAGGGDPILYQHLFWFFGHPE---
S.cataracta.CUMZ00316              WAVKITAILLLLSLPVLAGAITMLLTDRNFNTSFFDPAGGGDPILYQHLFWFFGHPEVYI
S.cataracta.CUMZ00317              WAVKITAILLLLSLPVLAGAITMLLTDRNFNTSFFDPAGGGDPILYQHLFWFFGHPEVYI
S.dehaani.CUMZ00333                WAVMITAILLLLSLPVLAGAITMLLTDRNFNTSFFDPAGGGDPILYQHLFWFFGHPEVYI
S.dehaani.CUMZ00325                WAVMITAILLLLSLPVLAGAITMLLTDRNFNTSFFDPAGGGDPILYQHLFWFFGHPEVYI
S.dehaani.CUMZ00331                WAVMITAILLLLSLPVLAGAITMLLTDRNFNTSFFDPAGGGDPILYQHLFWFFGHPEVYI
S.dehaani.CUMZ00252                WAVMITAILLLLSLPVLAGAITMLLTDRNFNTSFFDPAGGGDPILYQHLFWFFGHPEVYI
S.subspinipes.CUMZ00315            WAVKITAILLLLSLPVLAGAITMLLTDRNFNTSFFDPAGGGDPILYQHLFWFFGHPEVYI
S.subspinipes.IZ131452             WAVKITAILLLLSLPVLAGAITMLLTDRNFNTSFFDPAGGGDPILYQHLFWFFGHPEVYI
S.mutilans.NIAS18224               WSVLITTILLLLSLPVLAGAITMLLTDRNFNTSFFDPAGGGDPILYQHLF----------
S.mutilans.NIAS8227                WSVLITTILLLLSLPVLAGAITMLLTDRNFNTSFFDPAGGGDPILYQHLF----------
S.japonica.CUMZ00319               WSVKITAILLLLSLPVLAGAITMLLTDRNFNTSFFDPAGGGDPILYQHLFWFFGHPEVYI
S.japonica.SYSU:Chilo206           WSVKITAILLLLSLPVLAGAITMLLTDRNFNTSFFDPAGGGDPILYQHLF----------
S.subcrustalis.CEUA016.MR0010      WAVFITAILLLLSLPVLAGAITMLLTDRNFNTSFFDPTGGGDPILYQHLFWFFGHPE---
S.laeta.MCZ.AM.IZ130696            WAVFITAILLLFSLPVLAGAITMLLTDRNFNTSFFDPTGGGDPILYQHLFWFFGHPEVYI
S.morsitans.CUMZ00344              WAVFITAILLLLSLPVLAGAITMLLTDRNFNTSFFDPAGGGDPILYQHLFWFFGHPEVYI
S.morsitans.CUMZ00342              WAVFITAILLLLSLPVLAGAITMLLTDRNFNTSFFDPAGGGDPILYQHLFWFFGHPEVYI
S.morsitans.SYSU:Chilo208          WAVFITAILLLLSLPVLAGAITMLLTDRNFNTSFFDPAGGGDPILYQHLF----------
S.morsitans.SYSU:Chilo005          WAVFITAILLLLSLPVLAGAITMLLTDRNFNTSFFDPAGGGDPILYQHLF----------
S.pinguis.CUMZ00304                WAVLITAILLLLSLPVLAGAITMLLTDRNFNTSFFDPAGGGDPVLYQHLFWFFGHPEVYI
S.pinguis.CUMZ00309                WAVLITAILLLLSLPVLAGAITMLLTDRNFNTSFFDPAGGGDPILYQHLFWFFGHPEVYI
S.pinguis.CUMZ00314                WAVLITAILLLLSLPVLAGAITMLLTDRNFNTSFFDPAGGGDPILYQHLFWFFGHPEVYI
S.calcarata.SYSU:Chilo112          WAVLITTILLLLSLPVEAGAITMLLTDPNFNTSFFDPAGGGDPILYQHMF----------
S.calcarata.CUMZ00418              WAVLITAILLLLSLPVLAGAITMLLTDRNFNTSFFDPAGGGDPILYQHLFWFFGHPEVYI
S.calcarata.NHMUK010305528         WAVLITAILLLLSLPVLAGAITMLLTDRNFNTSFFDPAGGGDPILYQHLFWFFGHPEVYI
S.multidens.SYSU:Chilo118          WAVKITAILLLLSLPVLAGAITMLLTDRNFNTSFFDPAGGGDPILYQHLF----------
S.dawydoffi.CUMZ00294.1            WAVKITAILLLLSLPVLAGAITMLLTDRNFNTSFFDPAGGGDPILYQHLFWFFGHPEVYI
S.dawydoffi.CUMZ00294.2            WAVKITAILLLLSLPVLAGAITMLLTDRNFNTSFFDPAGGGDPILYQHLFWFFGHPEVYI
S.cingulata.ZFMKMYR01593           GSVKITAILLLLSLPVLAGXITMLLKDRNFNTSFFDPAGGGDPILYHHLYWX--------
S.cingulata.ZFMKMYR01561           WSVKITAILLLLSLPVLAGAITMLLTDRNFNTSFFDPAGGGD------------------
S.cingulata.ZFMKSco01              WSVKITAILLLLSLPVLAGAITMLLTDRNFNTSFFDPAGGGDPILYQHLFW---------
S.oraniensis.ZFMKMYR00568          WAVKITTILLLLSLPVLAGAITMLLTDRNFNTSFFDPAGGGDPILYQHLFWF--------
S.alternans.IZ130689               WAVKITAILLLLSLPVLAGAITMLLTDRNFNTSFFDPAGGGDPILYQHLFWFFGHPEVYI
S.polymorpha.IZ130561              WAVKITTILLLLSLPVLAGAITMLLTDRNFNTSFFDPAGGGDPILYQHLFWFFGHPEVYI
S.viridis.IZ130727                 WAVKITTILLLLSLPVLAGAITMLLTDRNFNTSFFDPAGGGDPILYQHLFWFFGHPEVYI
S.hardwickei.FIMNShardwickei       WAVKITAILLLLSLPVLAGAITMLLTDRNFNTSFFDPAGGGDP-----------------
S.afer.IZ130688                    WAVGITTVLLLLSLPVLAGAITMLLTDRNFNTSFFDPAGGGDPILYXHLFWFFGHPEVYI
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718

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                                        250       260       270       280
                                 ....|....|....|....|....|....|....|....|..
S.paradoxa.CEUA017.MR0000        ----------------------------------------
S.paradoxa.CEUA016.MR0006        ----------------------------------------
S.paradoxa.CEUA016.MR0007        ----------------------------------------
S.paradoxa.CEUA016.MR0008        ----------------------------------------
S.spinosissima.CEUA016.MR0009    ----------------------------------------
S.cataracta.CUMZ00316            LILPGFGMISHIISQQSGKKEPFGTLGMIYAMAAIGNLGFVV
S.cataracta.CUMZ00317            LILPGFGMISHIISQQSGKKESFGTLGMMYAMGAIGILGFVV
S.dehaani.CUMZ00333              LILPGFGMISHIISQQSGKKEPFGTLGMIYAMAAIGILGFVV
S.dehaani.CUMZ00325              LILPGFGMISHIISQQSGKKEPFGTLGMIYAMAAIGILGFVV
S.dehaani.CUMZ00331              LILPGFGMISHIISQQSGKKEPFGTLGMIYAMAAIGILGFVV
S.dehaani.CUMZ00252              LILPGFGMISHIISQQSGKKETFGTLGMIYAMAAIAILGFVV
S.subspinipes.CUMZ00315          LILPGFGMISHIISQQSGKKEPFGTLGMIYAMAAIGILGFVV
S.subspinipes.IZ131452           LILPGFGMISHIISQQSGKKEPF-------------------
S.mutilans.NIAS18224             ----------------------------------------
S.mutilans.NIAS8227              ----------------------------------------
S.japonica.CUMZ00319             LILPGFGMISHIISQQSGKKEPFGTLGMIYAMAAIGILGFVV
S.japonica.SYSU:Chilo206         ----------------------------------------
S.subcrustalis.CEUA016.MR0010    ----------------------------------------
S.laeta.MCZ.AM.IZ130696          LILPGFGMISHIISHYSGKKEPFGTLGMIYAMAAIGILGFVV
S.morsitans.CUMZ00344            LILPGFGMISHIISHYSGKKEPFGTLGMIYAMAAIGILGFVV
S.morsitans.CUMZ00342            LILPGFGMISHIISHYSGKKEPFGTLGMIYAMAAIGILGFVV
S.morsitans.SYSU:Chilo208        ----------------------------------------
S.morsitans.SYSU:Chilo005        ----------------------------------------
S.pinguis.CUMZ00304              LILPGFGMISHIISQQSGKKEPFGTLGMIYAMAAIGILGFVV
S.pinguis.CUMZ00309              LILPGFGMISHIISQQSGKKEPFGTLGMIYAMAAIGILGFVV
S.pinguis.CUMZ00314              LILPGFGMISHIISQQSGKKEPFGTLGMIYAMAAIGILGFVV
S.calcarata.SYSU:Chilo112        ----------------------------------------
S.calcarata.CUMZ00418            LILPGFGMISHIISQQSGKKEPFGTLGMIYAMAAIGILGFVV
S.calcarata.NHMUK010305528       LILPGFGMISHIISQQSGKKEPFGTLGMIYAMAAIGILGFVV
S.multidens.SYSU:Chilo118        ----------------------------------------
S.dawydoffi.CUMZ00294.1          LILPGFGMISHIISQQSGKKEPFGTLGMIYAMAAIGILGFVV
S.dawydoffi.CUMZ00294.2          LILPGFGMISHIISQQSGKKEPFGTLGMIYAMAAIGILGFVV
S.cingulata.ZFMKMYR01593         ----------------------------------------
S.cingulata.ZFMKMYR01561         ----------------------------------------
S.cingulata.ZFMKSco01            ----------------------------------------
S.oraniensis.ZFMKMYR00568        ----------------------------------------
S.alternans.IZ130689             LILPGFGMVSHIISQQSGKKEPFGALGMIYAMAAIGILGFVV
S.polymorpha.IZ130561            LILPGFGMVSHIISQQSGKKEPFGSLGMIYAMAAIGILGFVV
S.viridis.IZ130727               LILPGFGMVSHIISHQCGKKEPFGALGMIYAMASIGILGFVV
S.hardwickei.FIMNShardwickei     ----------------------------------------
S.afer.IZ130688                  LILPGFGMVSHIISQESGSSEP--------------------
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Supplementary file 3. Alignment of 45 amino acid sequences from the partial COI gene belonging to 22 different species of the genus *Scolopendra*. Asterisks indicate residues common to all studied species. The color coding helps identify amino acids that are unique to a species and characteristic of a group of species.

Species/Abbrv	
1. S.paradoxa.CEUA017.MR0000	MYLIFGTWASMAGTALSLIIRLELSQPGSLIGDDQTYNVIVTAHAFIMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLT
2. S.paradoxa.CEUA016-Mr0006	MYLIFGTWASMAGTALSLIIRLELSQPGSLIGDDQTYNVIVTAHAFIMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLT
3. S.paradoxa.CEUA016-Mr0007	MYLIFGTWASMAGTALSLIIRLELSQPGSLIGDDQTYNVIVTAHAFIMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLT
4. S.paradoxa.CEUA016-Mr0008	MYLIFGTWASMAGTALSLIIRLELSQPGSLIGDDQTYNVIVTAHAFIMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLT
5. S.spinosissima.CEUA016.MR0009	MYLIFGAWASMAGTALSLIIRLELSQPGSLIGDDQTYNVIVTAHAFIMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLS
6. S.cataracta.CUMZ00316	MYLMFGAWSSMVGTALSLIIRLELSQPGSLIGDDQTYNVIVTAHAFIMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSIM
7. S.cataracta.CUMZ00317	MYLMFGAWASMVGTALSLIIRLELSQPGSLIGDDQTYNVIVTAHAFIMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSIM
8. S.dehaani.CUMZ00333	MYLMFGAWASMTGTALSLIIRLELSQPGSLIGDDQTYNVIVTAHAFIMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSIM
9. S.dehaani.CUMZ00325	MYLMFGAWASMTGTALSLIIRLELSQPGSLIGDDQTYNVIVTAHAFIMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSIM
10. S.dehaani.CUMZ00331	MYLMFGAWASMTGTALSLIIRLELSQPGSLIGDDQTYNVIVTAHAFIMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSIM
11. S.dehaani.CUMZ00252	KYLMLGAWASMTGTALSLIIRLELSQPGSLIGDDQTYNVIVTAHAFIMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSIM
12. S.subspinipes.CUMZ00315	MYLMFGAWAAMTGTALSLIIRLELSQPGSLIGDDQTYNVVVTAHAFIMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSIT
13. S.subspinipes.IZ131452	------AWASMVGTALSLIIRLELSQPGSLIGDDQTYNVVVTAHAFIMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSIM
14. S.mutilans.NIAS18224	MYFIFGAWASLIGTALSLIIRLELSQPGSLIGDDQTYNVMVTAHAFIMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSIM
15. S.mutilans.NIAS8227	MYFIFGAWASMIGTALSLIIRLELSQPGSLIGDDQTYNVMVTAHAFIMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSIM
16. S.japonica.CUMZ00319	MYLMFGAWASMVGTALSLIIRLELSQPGSLIGDDQTYNVIVTAHAFIMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLM
17. S.japonica.SYSU:Chilo206	MYLMFGAWASMVGTALSLIIRLELSQPGSLIGDDQTYNVIVTAHAFIMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLM
18. S.subcrustalis.CEUA016MR0010	MYLIFGIWSAMVGTALSLIIRLELSQPGSLIGDDQTYNVVVTAHAFIMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLT
19. S.laeta.MCZ.AM.IZ130696	MYLIFGTWSAMIGTALSLIIRLELSQPGSFIGDDQTYNVVVTAHAFIMIFFMVMPIMIGGFGNWLIPLMLGAPDMAFPRMNNLSFWLLPPSLA
20. S.morsitans.CUMZ00344	MYLIFGAWSAMVGTALSLIIRLELSQPGSLIGDDQTYNTIVTAHAFIMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLT
21. S.morsitans.CUMZ00342	MYLIFGAWSAMVGTALSLIIRLELSQPGSLIGDDQTYNTIVTAHAFIMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLT
22. S.morsitans.SYSU:Chilo208	MYLIFGARSAMVGTALSLIIRLELSQPGSIIGDDQTYNTIVTAHAFIMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLT
23. S.morsitans.SYSU:Chilo005	MYLIFGAWSAMVGTALSLIIRLELSQPGSLIGDDQTYNTIVTAHAFIMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLT
24. S.pinguis.CUMZ00304	MYLIFGAWASMAGTGLSLLIRLELSQPGSLIGDDQTYNVVVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLT
25. S.pinguis.CUMZ00309	MYLMFGAWASMAGTALSLLIRLELSQPGSLIGDDQTYNVVVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLT
26. S.pinguis.CUMZ00314	MF-IFGAWASMAGTALSLLIRLELSQPGSLIGDDQTYNVVVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLT
27. S.calcarata.SYSU:Chilo112	MYLIFGAWASMAGAAFSLMIRLELSQPGSLIGDDQTYNVIVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLFPPSLM
28. S.calcarata.CUMZ00418	MYLIFGAWSSMVGTALSLLIRLELSQPGTLIGDDQTYNVVVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLS
29. S.calcarata.NHMUK010305528	MYLIFGAWSSMVGTALSLLIRLELSQPGTLIGDDQTYNVVVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLS
30. S.multidens.SYSU:Chilo118	MYLIFGAWSSMVGTALSLIIRLELSQPGSLIGDDQTYNVIVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLM
31. S.dawydoffi.CUMZ00294.1	MYLMFGAWSSMAGTALSLIIRLELSQPGSLIGDDQTYNVIVTAHAFIMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLM
32. S.dawydoffi.CUMZ00294.2	MYLMFGAWSSMAGTALSLIIRLELSQPGSLIGDDQTYNVIVTAHAFIMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLM
33. S.cingulata.ZFMKMYR01593	-----GACSSIVGTAVSXIIXLELSQPGKLIGDDQTYNVIVTAHAFIMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSIM
34. S.cingulata.ZFMKMYR01561	-----GAWSSMVGTALSLIIRLELSQPGSLIGDDQTYNVIVTAHSFIMI-FMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSIM
35. S.cingulata.ZFMKSco01	-----GAWSSMVGTALSLIIRLELSQPGSLIGDDQTYNVIVTAHAFIMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSIM
36. S.oraniensis.ZFMKMYR00568	-----GAWAAMAGTALSLIIRLELSQPGSLIGDDQTYNVVVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLM
37. S.alternans.IZ130689	MYLIFGAWSSMIGTGLSLLIRLELSQPGSLIGDDQTYNVIVTAHAFIMIFFMVMPIMMGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLM
38. S.polymorpha.IZ130561	MYLIFGAWASMVGTGLSLLIRLELSQPGSLIGDDQTYNVIVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLM
39. S.viridis.IZ130727	MYLLFGAWSAMVGTGLSLLIRLELSQPGSLIGDDQTYNVVVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLI
40. S.hardwickei.FIMNShardwickei	------AWASMAGTALSLIIRLELSXPGSLIGDDQTYNVIVTAHAFIMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLT
41. S.afer.IZ130688	MYLCFGVWASMVGTGLSLLIRLELSQPGSLIGDDHIYNVIVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNLSFWLLPPSLM

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Species/Abbrv △
1. S.paradoxa.CEUA017.MR0000      LLLSSALVENGAGTGWTVYPPLAGNITHAGPSVDMTIFSLHLAGASSILGAINFITTIINMRSEGMVMERIPLFVWAVKITAILLLLSLPVLAGA
2. S.paradoxa.CEUA016-Mr0006      LLLSSALVENGAGTGWTVYPPLAGNITHAGPSVDMTIFSLHLAGASSILGAINFITTIINMRSEGMVMERIPLFVWAVKITAILLLLSLPVLAGA
3. S.paradoxa.CEUA016-Mr0007      LLLSSALVENGAGTGWTVYPPLAGNITHAGPSVDMTIFSLHLAGASSILGAINFITTIINMRSEGMVMERIPLFVWAVKITAILLLLSLPVLAGA
4. S.paradoxa.CEUA016-Mr0008      LLLSSALVENGAGTGWTVYPPLAGNITHAGPSVDMTIFSLHLAGASSILGAINFITTIINMRSEGMVMERIPLFVWAVKITAILLLLSLPVLAGA
5. S.spinosissima.CEUA016.MR0009  LLMSSALVESGAGTGWTVYPPLAGNITHAGPSVDMTIFSLHLAGASSILGAINFITTIINMRSSGMVMERIPLFVWAVKITAILLLLSLPVLAGA
6. S.cataracta.CUMZ00316          LLLTSALVESGAGTGWTVYPPLASNITHSGPSVDMTIFSLHLAGASSILGAINFITTIINMRSSGMVMERMPLFVWAVKITAILLLLSLPVLAGA
7. S.cataracta.CUMZ00317          LLLSSALVESGAGTGWTVYPPLASNITHAGPSVDMTIFSLHLAGASSILGAINFITTIINMRSSGMIMERMPLFVWAVKITAILLLLSLPVLAGA
8. S.dehaani.CUMZ00333            LLLASALVENGAGTGWTVYPPLASNIAHAGPSVDMTIFSLHLAGASSILGAINFITTIINMRSSGMVMERMPLFVWAVMITAILLLLSLPVLAGA
9. S.dehaani.CUMZ00325            LLLASALVENGAGTGWTVYPPLASNIAHAGPSVDMTIFSLHLAGASSILGAINFITTIINMRSSGMVMERMPLFVWAVMITAILLLLSLPVLAGA
10. S.dehaani.CUMZ00331           LLLASALVENGAGTGWTVYPPLASNIAHAGPSVDMTIFSLHLAGASSILGAINFITTIINMRSSGMVMERMPLFVWAVMITAILLLLSLPVLAGA
11. S.dehaani.CUMZ00252           LLLASALVENGAGTGWTVYPPLASNIAHAGPSVDMTIFSLHLAGASSILGAINFITTIINMRSSGMVMERMPLFVWAVMITAILLLLSLPVLAGA
12. S.subspinipes.CUMZ00315       LLLASTLMENGAGTGWTVYPPLSSNITHAGPSVDMTIFSLHLAGMSSILGAINFITTIINMRSSGMVMERMPLFVWAVKITAILLLLSLPVLAGA
13. S.subspinipes.IZ131452        LLLTSALVENGAGTGWTVYPPLASNITHAGPSVDMTIFSLHLAGASSILGAINFITTIINMRSSGMVLERMPLFVWAVKITAILLLLSLPVLAGA
14. S.mutilans.NIAS18224          LLLTSALIENGAGTGWTVYPPLASNITQAGPSVDMTIFSLHLAGASSILGSINFITTIMNMRSSGMLMERTPLFVWSVLITTILLLLSLPVLAGA
15. S.mutilans.NIAS8227           LLLTSALIENGAGTGWTVYPPLASNITHAGPSVDMTIFSLHLAGASSILGSINFITTIMNMRSSGMLMERTPLFVWSVLITTILLLLSLPVLAGA
16. S.japonica.CUMZ00319          LLLSSAMVENGAGTGWTVYPPLASNVTHAGPSVDMTIFSLHLAGASSILGAINFITTIINMRSSGMILERMPLFVWSVKITAILLLLSLPVLAGA
17. S.japonica.SYSU:Chilo206      LLLSSAMVENGAGTGWTVYPPLASNVTHAGPSVDMTIFSLHLAGASSILGAINFITTIINMRSSGMILERMPLFVWSVKITAILLLLSLPVLAGA
18. S.subcrustalis.CEUA016MR0010  LLLASALVENGAGTGWTIYPPLASNITHAGPSVDMTIFSLHLAGASSILGAINFITTIINMRSSGMVMERMPLFVWAVFITAILLLLSLPVLAGA
19. S.laeta.MCZ.AM.IZ130696       LLLASAMVESGAGTGWTVYPPLASNITHAGPSVDMTIFSLHLAGVSSILGAINFITTIINMRSSGMVMERMPLFVWAVFITAILLLFSLPVLAGA
20. S.morsitans.CUMZ00344         LLLSSAMVENGAGTGWTVYPPLAGNLTHAGPSVDMTIFSLHLAGASSILGAINFITTIINMRSNGMVMERMPLFAWAVFITAILLLLSLPVLAGA
21. S.morsitans.CUMZ00342         LLLSSAMVENGAGTGWTVYPPLAGNLTHAGPSVDMTIFSLHLAGASSILGAINFITTIINMRSNGMVMERMPLFAWAVFITAILLLLSLPVLAGA
22. S.morsitans.SYSU:Chilo208     LLLSSAMVENGAGTGWTVYPPLAGNLTHAGPSVDMTIFSLHLAGASSILGAINFITTIINMRSNGMVMERMPLFVWAVFITAILLLLSLPVLAGA
23. S.morsitans.SYSU:Chilo005     LLLSSAMVENGAGTGWTVYPPLAGNLTHAGPSVDMTIFSLHLAGASSILGAINFITTIINMRSNGMVMERMPLFVWAVFITAILLLLSLPVLAGA
24. S.pinguis.CUMZ00304           LLLASAMLESGAGTGWTVYPPLASNITHAGPSVDMTIFSLHLAGASSILGAINFITTIINMRTSGMMMERIPLFVWAVLITAILLLLSLPVLAGA
25. S.pinguis.CUMZ00309           LLMTSAMMENGAGTGWTVYPPLASNITHAGPSVDMTIFSLHLAGASSILGAINFITTIINMRTSGMMMERIPLFVWAVLITAILLLLSLPVLAGA
26. S.pinguis.CUMZ00314           LLLASAVLESGAGTGWTVYPPLASNITHAGPSVDMTIFSLHLAGASSILGAINFITTIINMRTSGMVMERIPLFVWAVLITAILLLLSLPVLAGA
27. S.calcarata.SYSU:Chilo112     LLLSSAMLESGASTGWTVYPPLASNLTHAGPSVDMSIFSLHVAGASSILGAINFITTVINMRPSWMMMERIPLFVWAVLITTILLLLSLPVFAGA
28. S.calcarata.CUMZ00418         LLLASAALESGAGTGWTVYPPLASNLTHAGASVDMTIFSLHLAGASSILGAINFITTIINMRTSGMMMERIPLFVWAVLITAILLLLSLPVLAGA
29. S.calcarata.NHMUK010305528    LLLASAALESGAGTGWTVYPPLASNLTHAGASVDMTIFSLHLAGASSILGAINFITTIINMRTSGMMMERIPLFVWAVLITAILLLLSLPVLAGA
30. S.multidens.SYSU:Chilo118     LLLSSALVENGAGTGWTVYPPLASNVSHAGSSVDMTIFSLHLAGASSILGAINFITTIINMRSSGMVMERMPLFVWAVKITAILLLLSLPVLAGA
31. S.dawydoffi.CUMZ00294.1       LLLSSALVENGAGTGWTVYPPLASNVSHAGPSVDMTIFSLHLAGASSILGAINFITTIINMRSSGMVMERMPLFVWAVKITAILLLLSLPVLAGA
32. S.dawydoffi.CUMZ00294.2       LLLSSALVENGAGTGWTVYPPLASNVSHAGPSVDMTIFSLHLAGASSILGAINFITTIINMRSSGMVMERMPLFVWAVKITAILLLLSLPVLAGA
33. S.cingulata.ZFMKMYR01593      LLLSSALVENGAGTGWTVYPPLAGNITHAGPSVDMNIFSLHLAGASSILGAINFITTIINMRSSGMVMERXPLFVGSVKITAILLLLSLPVLAGX
34. S.cingulata.ZFMKMYR01561      LLLSSALVENGAGTGWTVYPPLASNITHAGPSVDMTIFSLHLAGASSILGAINFITTIINMRSSGMVMERIPLFVWSVKITAILLLLSLPVLAGA
35. S.cingulata.ZFMKSco01         LLLSSALVENGAGTGWTVYPPLASNITHAGPSVDMTIFSLHLAGASSILGAINFITTIINMRSSGMVMERMPLFVWSVKITAILLLLSLPVLAGA
36. S.oraniensis.ZFMKMYR00568     LLMSSALVESGAGTGWTVYPPLASNITHAGPSVDMTIFSLHLAGASSILGAINFITTIINMRTSGMVMERVPLFVWAVKITTILLLLSLPVLAGA
37. S.alternans.IZ130689          LLLSSGLVESGAGTGWTVYPPLAGNITHGGPSVDMTIFSLHLAGASSILGAINFITTIINMRTSGMVMERIPLFVWAVKITAILLLLSLPVLAGA
38. S.polymorpha.IZ130561         LLLSSALVESGAGTGWTVYPPLAGNITHAGPSVDMTIFSLHLAGASSILGSINFITTIINMRSSGMVMERMPLFVWAVKITTILLLLSLPVLAGA
39. S.viridis.IZ130727            LLLSSAMVENGAGTGWTVYPPLASNMTHAGPSVDMTIFSLHLAGASSILGSINFITTIINMRSSGMIMERMPLFVWAVKITTILLLLSLPVLAGA
40. S.hardwickei.FIMNShardwickei  LLMASAMVESGAGTGWTVYPPLASNITHAGPSVDLAIFSLHLAGASSILGAINFITTIINMRSSGMVMERMPLFVWAVKITAILLLLSLPVLAGA
41. S.afer.IZ130688               MLMGSSIVESGAGTGWTVYPPLAGNITHAGASVDMTIFSLHLAGVSSILGSINIITTIINMRSSGMVMERVPLFVWAVGITTVLLLLSLPVLAGA
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728

Species/Abbrv △	*	*	*	*	*		*			*	*	*	*	*	*	*			*	*	*	*											
1. S.paradoxa.CEUA017.MR0000	I	T	M	L	L	T	D	R	N	F	N	T	S	F	F	D	P	A	G	G	G	D	P	I	L	Y	Q	H	L	F	W	F	F	G
2. S.paradoxa.CEUA016-Mr0006	I	T	M	L	L	T	D	R	N	F	N	T	S	F	F	D	P	A	G	G	G	D	P	I	L	Y	Q	H	L	F	W	F	F	G
3. S.paradoxa.CEUA016-Mr0007	I	T	M	L	L	T	D	R	N	F	N	T	S	F	F	D	P	A	G	G	G	D	P	I	L	Y	Q	H	L	F	W	F	F	G
4. S.paradoxa.CEUA016-Mr0008	I	T	M	L	L	T	D	R	N	F	N	T	S	F	F	D	P	A	G	G	G	D	P	I	L	Y	Q	H	L	F	W	F	F	G
5. S.spinosissima.CEUA016.MR0009	I	T	M	L	L	T	D	R	N	F	N	T	S	F	F	D	P	A	G	G	G	D	P	I	L	Y	Q	H	L	F	W	F	F	G
6. S.cataracta.CUMZ00316	I	T	M	L	L	T	D	R	N	F	N	T	S	F	F	D	P	A	G	G	G	D	P	I	L	Y	Q	H	L	F	W	F	F	G
7. S.cataracta.CUMZ00317	I	T	M	L	L	T	D	R	N	F	N	T	S	F	F	D	P	A	G	G	G	D	P	I	L	Y	Q	H	L	F	W	F	F	G
8. S.dehaani.CUMZ00333	I	T	M	L	L	T	D	R	N	F	N	T	S	F	F	D	P	A	G	G	G	D	P	I	L	Y	Q	H	L	F	W	F	F	G
9. S.dehaani.CUMZ00325	I	T	M	L	L	T	D	R	N	F	N	T	S	F	F	D	P	A	G	G	G	D	P	I	L	Y	Q	H	L	F	W	F	F	G
10. S.dehaani.CUMZ00331	I	T	M	L	L	T	D	R	N	F	N	T	S	F	F	D	P	A	G	G	G	D	P	I	L	Y	Q	H	L	F	W	F	F	G
11. S.dehaani.CUMZ00252	I	T	M	L	L	T	D	R	N	F	N	T	S	F	F	D	P	A	G	G	G	D	P	I	L	Y	Q	H	L	F	W	F	F	G
12. S.subspinipes.CUMZ00315	I	T	M	L	L	T	D	R	N	F	N	T	S	F	F	D	P	A	G	G	G	D	P	I	L	Y	Q	H	L	F	W	F	F	G
13. S.subspinipes.IZ131452	I	T	M	L	L	T	D	R	N	F	N	T	S	F	F	D	P	A	G	G	G	D	P	I	L	Y	Q	H	L	F	W	F	F	G
14. S.mutilans.NIAS18224	I	T	M	L	L	T	D	R	N	F	N	T	S	F	F	D	P	A	G	G	G	D	P	I	L	Y	Q	H	L	F	-	-	-	-
15. S.mutilans.NIAS8227	I	T	M	L	L	T	D	R	N	F	N	T	S	F	F	D	P	A	G	G	G	D	P	I	L	Y	Q	H	L	F	-	-	-	-
16. S.japonica.CUMZ00319	I	T	M	L	L	T	D	R	N	F	N	T	S	F	F	D	P	A	G	G	G	D	P	I	L	Y	Q	H	L	F	W	F	F	G
17. S.japonica.SYSU:Chilo206	I	T	M	L	L	T	D	R	N	F	N	T	S	F	F	D	P	A	G	G	G	D	P	I	L	Y	Q	H	L	F	-	-	-	-
18. S.subcrustalis.CEUA016MR0010	I	T	M	L	L	T	D	R	N	F	N	T	S	F	F	D	P	T	G	G	G	D	P	I	L	Y	Q	H	L	F	W	F	F	G
19. S.laeta.MCZ.AM.IZ130696	I	T	M	L	L	T	D	R	N	F	N	T	S	F	F	D	P	T	G	G	G	D	P	I	L	Y	Q	H	L	F	W	F	F	G
20. S.morsitans.CUMZ00344	I	T	M	L	L	T	D	R	N	F	N	T	S	F	F	D	P	A	G	G	G	D	P	I	L	Y	Q	H	L	F	W	F	F	G
21. S.morsitans.CUMZ00342	I	T	M	L	L	T	D	R	N	F	N	T	S	F	F	D	P	A	G	G	G	D	P	I	L	Y	Q	H	L	F	W	F	F	G
22. S.morsitans.SYSU:Chilo208	I	T	M	L	L	T	D	R	N	F	N	T	S	F	F	D	P	A	G	G	G	D	P	I	L	Y	Q	H	L	F	-	-	-	-
23. S.morsitans.SYSU:Chilo005	I	T	M	L	L	T	D	R	N	F	N	T	S	F	F	D	P	A	G	G	G	D	P	I	L	Y	Q	H	L	F	-	-	-	-
24. S.pinguis.CUMZ00304	I	T	M	L	L	T	D	R	N	F	N	T	S	F	F	D	P	A	G	G	G	D	P	V	L	Y	Q	H	L	F	W	F	F	G
25. S.pinguis.CUMZ00309	I	T	M	L	L	T	D	R	N	F	N	T	S	F	F	D	P	A	G	G	G	D	P	I	L	Y	Q	H	L	F	W	F	F	G
26. S.pinguis.CUMZ00314	I	T	M	L	L	T	D	R	N	F	N	T	S	F	F	D	P	A	G	G	G	D	P	I	L	Y	Q	H	L	F	W	F	F	G
27. S.calcarata.SYSU:Chilo112	I	T	M	L	L	T	D	P	N	F	N	T	S	F	F	D	P	A	G	G	G	D	P	I	L	Y	Q	H	M	F	-	-	-	-
28. S.calcarata.CUMZ00418	I	T	M	L	L	T	D	R	N	F	N	T	S	F	F	D	P	A	G	G	G	D	P	I	L	Y	Q	H	L	F	W	F	F	G
29. S.calcarata.NHMUK010305528	I	T	M	L	L	T	D	R	N	F	N	T	S	F	F	D	P	A	G	G	G	D	P	I	L	Y	Q	H	L	F	W	F	F	G
30. S.multidens.SYSU:Chilo118	I	T	M	L	L	T	D	R	N	F	N	T	S	F	F	D	P	A	G	G	G	D	P	I	L	Y	Q	H	L	F	-	-	-	-
31. S.dawydoffi.CUMZ00294.1	I	T	M	L	L	T	D	R	N	F	N	T	S	F	F	D	P	A	G	G	G	D	P	I	L	Y	Q	H	L	F	W	F	F	G
32. S.dawydoffi.CUMZ00294.2	I	T	M	L	L	T	D	R	N	F	N	T	S	F	F	D	P	A	G	G	G	D	P	I	L	Y	Q	H	L	F	W	F	F	G
33. S.cingulata.ZFMKMYR01593	I	T	M	L	L	K	D	R	N	F	N	T	S	F	F	D	P	A	G	G	G	D	P	I	L	Y	H	H	L	Y	W	X	-	-
34. S.cingulata.ZFMKMYR01561	I	T	M	L	L	T	D	R	N	F	N	T	S	F	F	D	P	A	G	G	G	D	-	-	-	-	-	-	-	-	-	-	-	-
35. S.cingulata.ZFMKSco01	I	T	M	L	L	T	D	R	N	F	N	T	S	F	F	D	P	A	G	G	G	D	P	I	L	Y	Q	H	L	F	W	-	-	-
36. S.oraniensis.ZFMKMYR00568	I	T	M	L	L	T	D	R	N	F	N	T	S	F	F	D	P	A	G	G	G	D	P	I	L	Y	Q	H	L	F	W	F	-	-
37. S.alternans.IZ130689	I	T	M	L	L	T	D	R	N	F	N	T	S	F	F	D	P	A	G	G	G	D	P	I	L	Y	Q	H	L	F	W	F	F	G
38. S.polymorpha.IZ130561	I	T	M	L	L	T	D	R	N	F	N	T	S	F	F	D	P	A	G	G	G	D	P	I	L	Y	Q	H	L	F	W	F	F	G
39. S.viridis.IZ130727	I	T	M	L	L	T	D	R	N	F	N	T	S	F	F	D	P	A	G	G	G	D	P	I	L	Y	Q	H	L	F	W	F	F	G
40. S.hardwickei.FIMNShardwickei	I	T	M	L	L	T	D	R	N	F	N	T	S	F	F	D	P	A	G	G	G	D	P	-	-	-	-	-	-	-	-	-	-	-
41. S.afer.IZ130688	I	T	M	L	L	T	D	R	N	F	N	T	S	F	F	D	P	A	G	G	G	D	P	I	L	Y	X	H	L	F	W	F	F	G

Supplementary file 4. Divergence table obtained from 45 amino acid sequences of the partial COI gene belonging to 22 different species of the genus *Scolopendra*. Note that out of 820 alignments, only 14 exceptions to the five amino acids rule were found, eight of which correspond to sequences from the same species.

	S.paradoxa.CEUA017.MR0000	S.paradoxa.CEUA016-Mr0006	S.paradoxa.CEUA016-Mr0007	S.paradoxa.CEUA016-Mr0008	S.spinosissima.CEUA016.MR0009	S.hainanensis.CMMI20190702020	S.hainanensis.CMMI20180922009	Scolopendra.sp.china.CMMI20190702007	Scolopendra.sp.china.CMMI20190418008
S.paradoxa.CEUA017.MR0000									
S.paradoxa.CEUA016-Mr0006	0,000								
S.paradoxa.CEUA016-Mr0007	0,000	0,000							
S.paradoxa.CEUA016-Mr0008	0,000	0,000	0,000						
S.spinosissima.CEUA016.MR0009	0,023	0,023	0,023	0,023					
S.hainanensis.CMMI20190702020	0,062	0,062	0,062	0,062	0,052				
S.hainanensis.CMMI20180922009	0,062	0,062	0,062	0,062	0,052	0,000			
Scolopendra.sp.china.CMMI20190702007	0,067	0,067	0,067	0,067	0,062	0,014	0,014		
Scolopendra.sp.china.CMMI20190418008	0,067	0,067	0,067	0,067	0,062	0,014	0,014	0,000	
S.cataracta.CUMZ00316	0,057	0,057	0,057	0,057	0,048	0,024	0,024	0,038	0,038
S.cataracta.CUMZ00317	0,048	0,048	0,048	0,048	0,038	0,019	0,019	0,028	0,028
S.dehaani.CUMZ00333	0,053	0,053	0,053	0,053	0,053	0,028	0,028	0,043	0,043
S.dehaani.CUMZ00325	0,053	0,053	0,053	0,053	0,053	0,028	0,028	0,043	0,043
S.dehaani.CUMZ00331	0,053	0,053	0,053	0,053	0,053	0,028	0,028	0,043	0,043
S.dehaani.CMMI20180922002	0,052	0,052	0,052	0,052	0,052	0,033	0,033	0,047	0,047
S.subspinipes.CUMZ00315	0,067	0,067	0,067	0,067	0,072	0,053	0,053	0,057	0,057
S.subspinipes.IZ131452	0,049	0,049	0,049	0,049	0,049	0,029	0,029	0,044	0,044
S.mutilans.NIAS18224	0,093	0,093	0,093	0,093	0,093	0,087	0,087	0,093	0,093
S.mutilans.NIAS8227	0,082	0,082	0,082	0,082	0,082	0,077	0,077	0,082	0,082
S.mutilans.CMMI20190331001	0,082	0,082	0,082	0,082	0,082	0,082	0,082	0,087	0,087
S.alcyona.MST02	0,048	0,048	0,048	0,048	0,048	0,053	0,053	0,062	0,062
S.alcyona.MST03	0,057	0,057	0,057	0,057	0,057	0,048	0,048	0,057	0,057
S.japonica.CUMZ00319	0,057	0,057	0,057	0,057	0,057	0,043	0,043	0,053	0,053
S.japonica.SYSU:Chilo206	0,058	0,058	0,058	0,058	0,058	0,043	0,043	0,053	0,053
S.lufengia.CMMI20181030005	0,033	0,033	0,033	0,033	0,033	0,047	0,047	0,062	0,062
S.lufengia.CMMI20181228010	0,047	0,047	0,047	0,047	0,047	0,057	0,057	0,072	0,072
S.subcrustalis.CEUA016MR0010	0,056	0,056	0,056	0,056	0,066	0,067	0,067	0,072	0,072
S.laeta.MCZ.AM.IZ130696	0,082	0,082	0,082	0,082	0,082	0,077	0,077	0,087	0,087
S.morsitans.CUMZ00344	0,053	0,053	0,053	0,053	0,062	0,072	0,072	0,077	0,077
S.morsitans.CUMZ00342	0,053	0,053	0,053	0,053	0,062	0,072	0,072	0,077	0,077
S.morsitans.SYSU:Chilo208	0,058	0,058	0,058	0,058	0,068	0,078	0,078	0,083	0,083
S.morsitans.SYSU:Chilo005	0,048	0,048	0,048	0,048	0,058	0,068	0,068	0,073	0,073
S.pinguis.CUMZ00304	0,072	0,072	0,072	0,072	0,067	0,077	0,077	0,077	0,077
S.pinguis.CUMZ00309	0,067	0,067	0,067	0,067	0,062	0,077	0,077	0,077	0,077
S.pinguis.CUMZ00314	0,068	0,068	0,068	0,068	0,063	0,073	0,073	0,078	0,078
S.calcarata.SYSU:Chilo112	0,119	0,119	0,119	0,119	0,108	0,124	0,124	0,135	0,135
S.calcarata.CUMZ00418	0,091	0,091	0,091	0,091	0,076	0,082	0,082	0,087	0,087
S.calcarata.NHMUK010305528	0,091	0,091	0,091	0,091	0,076	0,082	0,082	0,087	0,087
S.multidens.SYSU:Chilo118	0,053	0,053	0,053	0,053	0,053	0,053	0,053	0,068	0,068
S.dawydoffi.CUMZ00294.1	0,043	0,043	0,043	0,043	0,043	0,043	0,043	0,057	0,057
S.dawydoffi.CUMZ00294.2	0,043	0,043	0,043	0,043	0,043	0,043	0,043	0,057	0,057
S.cingulata.ZFMKMYR01593	0,071	0,071	0,071	0,071	0,071	0,066	0,066	0,081	0,081
S.cingulata.ZFMKMYR01561	0,045	0,045	0,045	0,045	0,045	0,040	0,040	0,055	0,055
S.cingulata.ZFMKSco01	0,044	0,044	0,044	0,044	0,044	0,029	0,029	0,044	0,044
S.oraniensis.ZFMKMYR00568	0,059	0,059	0,059	0,059	0,039	0,059	0,059	0,074	0,074
S.alternans.IZ130689	0,057	0,057	0,057	0,057	0,048	0,072	0,072	0,087	0,087
S.polymorpha.IZ130561	0,053	0,053	0,053	0,053	0,043	0,053	0,053	0,067	0,067
S.viridis.IZ130727	0,088	0,088	0,088	0,088	0,088	0,083	0,083	0,088	0,088
S.hardwickei.FIMNShardwickei	0,050	0,050	0,050	0,050	0,035	0,040	0,040	0,045	0,045
S.afer.IZ130688	0,118	0,118	0,118	0,118	0,103	0,113	0,113	0,129	0,129

	S.cataracta.CUMZ00316	S.cataracta.CUMZ00317	S.dehaani.CUMZ00333	S.dehaani.CUMZ00325	S.dehaani.CUMZ00331	S.dehaani.CMMI20180922002	S.subspinipes.CUMZ00315	S.subspinipes.IZ131452	S.mutilans.NIAS18224	S.mutilans.NIAS8227	S.mutilans.CMMI20190331001	S.alcyona.MST02	S.alcyona.MST03	S.japonica.CUMZ00319	S.japonica.SYSU:Chilo206
S.cataracta.CUMZ00316															
S.cataracta.CUMZ00317	0,019														
S.dehaani.CUMZ00333	0,033	0,028													
S.dehaani.CUMZ00325	0,033	0,028	0,000												
S.dehaani.CUMZ00331	0,033	0,028	0,000	0,000											
S.dehaani.CMMI20180922002	0,033	0,028	0,000	0,000	0,000										
S.subspinipes.CUMZ00315	0,057	0,053	0,043	0,043	0,043	0,043									
S.subspinipes.IZ131452	0,024	0,024	0,029	0,029	0,029	0,029	0,044								
S.mutilans.NIAS18224	0,082	0,077	0,077	0,077	0,077	0,077	0,098	0,065							
S.mutilans.NIAS8227	0,072	0,067	0,067	0,067	0,067	0,067	0,087	0,054	0,009						
S.mutilans.CMMI20190331001	0,072	0,067	0,067	0,067	0,067	0,067	0,087	0,054	0,009	0,000					
S.alcyona.MST02	0,043	0,043	0,053	0,053	0,053	0,053	0,072	0,044	0,087	0,077	0,077				
S.alcyona.MST03	0,043	0,043	0,053	0,053	0,053	0,053	0,072	0,044	0,087	0,077	0,077	0,009			
S.japonica.CUMZ00319	0,048	0,028	0,048	0,048	0,048	0,048	0,072	0,034	0,087	0,077	0,077	0,048	0,038		
S.japonica.SYSU:Chilo206	0,048	0,028	0,048	0,048	0,048	0,048	0,073	0,034	0,088	0,078	0,078	0,048	0,038	0,000	
S.cataracta.CUMZ00316	0,033	0,033	0,038	0,038	0,038	0,038	0,062	0,034	0,082	0,072	0,072	0,028	0,028	0,038	0,038
S.cataracta.CUMZ00317	0,043	0,043	0,043	0,043	0,043	0,043	0,067	0,044	0,082	0,082	0,082	0,038	0,038	0,048	0,048
S.dehaani.CUMZ00333	0,057	0,062	0,057	0,057	0,057	0,057	0,057	0,049	0,103	0,093	0,092	0,062	0,062	0,072	0,073
S.dehaani.CUMZ00325	0,077	0,082	0,082	0,082	0,082	0,082	0,082	0,080	0,124	0,113	0,113	0,093	0,082	0,093	0,093
S.dehaani.CUMZ00331	0,067	0,067	0,072	0,072	0,072	0,072	0,082	0,070	0,118	0,108	0,108	0,053	0,053	0,062	0,063
S.dehaani.CMMI20180922002	0,067	0,067	0,072	0,072	0,072	0,072	0,082	0,070	0,118	0,108	0,108	0,053	0,053	0,062	0,063
S.subspinipes.CUMZ00315	0,073	0,073	0,078	0,078	0,078	0,078	0,088	0,075	0,124	0,114	0,114	0,058	0,058	0,068	0,068
S.subspinipes.IZ131452	0,063	0,063	0,068	0,068	0,068	0,068	0,078	0,065	0,114	0,103	0,103	0,048	0,048	0,058	0,058
S.mutilans.NIAS18224	0,087	0,077	0,082	0,082	0,082	0,082	0,087	0,080	0,108	0,098	0,098	0,103	0,093	0,087	0,088
S.mutilans.NIAS8227	0,077	0,072	0,072	0,072	0,072	0,072	0,072	0,065	0,098	0,087	0,087	0,098	0,087	0,072	0,073
S.mutilans.CMMI20190331001	0,083	0,078	0,078	0,078	0,078	0,078	0,083	0,065	0,103	0,093	0,093	0,098	0,093	0,093	0,088
S.alcyona.MST02	0,130	0,114	0,130	0,130	0,130	0,130	0,156	0,134	0,146	0,135	0,135	0,140	0,130	0,119	0,119
S.alcyona.MST03	0,082	0,082	0,093	0,093	0,093	0,092	0,103	0,085	0,118	0,108	0,107	0,093	0,087	0,093	0,093
S.japonica.CUMZ00319	0,082	0,082	0,093	0,093	0,093	0,092	0,103	0,085	0,118	0,108	0,107	0,093	0,087	0,093	0,093
S.japonica.SYSU:Chilo206	0,043	0,043	0,048	0,048	0,048	0,048	0,078	0,044	0,098	0,088	0,088	0,043	0,043	0,043	0,043
S.cataracta.CUMZ00316	0,033	0,033	0,033	0,033	0,033	0,033	0,062	0,039	0,093	0,082	0,082	0,043	0,043	0,033	0,033
S.cataracta.CUMZ00317	0,033	0,033	0,033	0,033	0,033	0,033	0,062	0,039	0,093	0,082	0,082	0,043	0,043	0,033	0,033
S.dehaani.CUMZ00333	0,060	0,060	0,071	0,071	0,071	0,071	0,097	0,066	0,097	0,092	0,092	0,050	0,060	0,071	0,071
S.dehaani.CUMZ00325	0,030	0,030	0,040	0,040	0,040	0,040	0,066	0,035	0,071	0,061	0,061	0,030	0,030	0,040	0,040
S.dehaani.CUMZ00331	0,019	0,019	0,029	0,029	0,029	0,029	0,054	0,024	0,064	0,054	0,054	0,019	0,019	0,029	0,029
S.dehaani.CMMI20180922002	0,059	0,049	0,064	0,064	0,064	0,064	0,069	0,054	0,090	0,080	0,080	0,080	0,080	0,069	0,069
S.subspinipes.CUMZ00315	0,057	0,062	0,077	0,077	0,077	0,077	0,098	0,075	0,113	0,103	0,103	0,067	0,077	0,082	0,083
S.subspinipes.IZ131452	0,053	0,043	0,062	0,062	0,062	0,062	0,087	0,054	0,087	0,077	0,077	0,057	0,067	0,062	0,063
S.mutilans.NIAS18224	0,078	0,068	0,088	0,088	0,088	0,088	0,088	0,070	0,108	0,098	0,098	0,073	0,063	0,063	0,063
S.mutilans.NIAS8227	0,050	0,045	0,050	0,050	0,050	0,050	0,066	0,055	0,103	0,092	0,092	0,071	0,061	0,055	0,055
S.mutilans.CMMI20190331001	0,118	0,113	0,124	0,124	0,124	0,124	0,145	0,123	0,150	0,140	0,140	0,124	0,129	0,129	0,130

S.lufengia.CMMI20181030005	0,014													
S.lufengia.CMMI20181228010	0,047	0,057												
S.subcrustalis.CEUA016MR0010	0,072	0,082	0,048											
S.laeta.MCZ.AM.IZ130696	0,048	0,057	0,053	0,077										
S.morsitans.CUMZ00344	0,048	0,057	0,053	0,077	0,000									
S.morsitans.CUMZ00342	0,053	0,063	0,058	0,078	0,014	0,014								
S.morsitans.SYSU:Chilo208	0,043	0,053	0,048	0,073	0,005	0,005	0,009							
S.morsitans.SYSU:Chilo005	0,077	0,093	0,082	0,093	0,098	0,098	0,103	0,093						
S.pinguis.CUMZ00304	0,072	0,087	0,082	0,103	0,093	0,093	0,098	0,088	0,033					
S.pinguis.CUMZ00309	0,073	0,088	0,078	0,093	0,098	0,098	0,099	0,088	0,028	0,043				
S.pinguis.CUMZ00314	0,108	0,124	0,151	0,156	0,135	0,135	0,140	0,130	0,093	0,103	0,099			
S.calcarata.SYSU:Chilo112	0,077	0,087	0,081	0,098	0,093	0,093	0,098	0,088	0,043	0,057	0,048	0,103		
S.calcarata.CUMZ00418	0,077	0,087	0,081	0,098	0,093	0,093	0,098	0,088	0,043	0,057	0,048	0,103	0,000	
S.calcarata.NHMUK010305528	0,024	0,033	0,058	0,088	0,058	0,058	0,063	0,053	0,088	0,083	0,078	0,119	0,073	0,073
S.multidens.SYSU:Chilo118	0,014	0,028	0,057	0,082	0,057	0,057	0,063	0,053	0,087	0,072	0,083	0,119	0,087	0,087	0,019
	0,014	0,028	0,057	0,082	0,057	0,057	0,063	0,053	0,087	0,072	0,083	0,119	0,087	0,087	0,019
	0,060	0,071	0,092	0,125	0,087	0,087	0,087	0,081	0,130	0,119	0,114	0,158	0,119	0,119	0,071
	0,030	0,040	0,061	0,092	0,066	0,066	0,071	0,061	0,087	0,082	0,076	0,131	0,087	0,087	0,040
	0,019	0,029	0,049	0,080	0,054	0,054	0,059	0,049	0,090	0,080	0,074	0,127	0,085	0,085	0,029
	0,049	0,064	0,069	0,085	0,085	0,085	0,090	0,080	0,064	0,054	0,049	0,106	0,080	0,080	0,059
	0,053	0,062	0,087	0,098	0,082	0,082	0,088	0,078	0,072	0,087	0,078	0,130	0,082	0,082	0,068
	0,048	0,057	0,077	0,098	0,072	0,072	0,078	0,068	0,067	0,082	0,073	0,108	0,082	0,082	0,048
	0,058	0,068	0,073	0,093	0,073	0,073	0,078	0,068	0,078	0,078	0,088	0,130	0,083	0,083	0,063
	0,045	0,061	0,066	0,076	0,071	0,071	0,076	0,066	0,061	0,061	0,055	0,120	0,092	0,092	0,066
	0,124	0,134	0,140	0,145	0,140	0,140	0,146	0,135	0,124	0,124	0,130	0,173	0,129	0,129	0,119

S.dawydoffi.CUMZ00294.1	0,000									
S.dawydoffi.CUMZ00294.2	0,066	0,066								
S.cingulata.ZFMKMYR01593	0,035	0,035	0,046							
S.cingulata.ZFMKMYR01561	0,024	0,024	0,040	0,010						
S.cingulata.ZFMKSco01	0,054	0,054	0,097	0,066	0,059					
S.oraniensis.ZFMKMYR00568	0,062	0,062	0,087	0,061	0,059	0,069				
S.alternans.IZ130689	0,057	0,057	0,081	0,061	0,049	0,049	0,048			
S.polymorpha.IZ130561	0,068	0,068	0,108	0,076	0,064	0,064	0,088	0,048		
S.viridis.IZ130727	0,050	0,050	0,094	0,067	0,055	0,055	0,082	0,066	0,087	
S.hardwickei.FIMNShardwickei	0,129	0,129	0,153	0,131	0,122	0,101	0,113	0,072	0,114	0,120
S.afer.IZ130688										