Morphological and molecular study of Symphyla from Colombia

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Abstract
The symphylans are a poorly studied group. In Colombia the number of symphylan species is unknown with only Scutigerella immaculata (Symphyla: Scutigerellidae) being reported previously. The aim of this research was to collect and identify the symphylan pests of flower crops in Colombia. Morphological descriptions showed that our specimens shared more than one of the characters that define different genera within Scutigerellidae. The COI barcode haplotype showed interspecific level genetic divergence with S. causeyae (at least 23%) and Hanseniella sp. (22%). Furthermore, our Colombian symphylans shared the same COI haplotype as some Symphyla found in Cameroon indicating a wide geographical distribution of this taxon. Our results suggest the presence of a new genus or subgenus in the class Symphyla.

Keywords
Scutigerella immaculata, Colombia, COI barcode, ITS2, morphology

Introduction
The symphylans (Arthropoda: Symphyla) are ancestral arthropods dating back to the early Silurian approximately 430 million years ago (Edgecombe 2004, Shear and Edgecombe 2010). Symphylans are a phylogenetic enigma within arthropods as they

http://zoobank.org/30CBCC77-D556-4BEE-B1CB-144214760CA4
Citation: Salazar-Moncada DA, Calle-Osorno J, Ruiz-Lopez F (2015) Morphological and molecular study of Symphyla from Colombia. ZooKeys 484: 121–130. doi: 10.3897/zookeys.484.8363

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have been proposed as sister taxa to different groups (Domínguez 2009). Symphyla is comprised of two families: Scutigerellidae (five genera and approximately 128 species) and Scolopendrellidae (nine genera and approximately 73 species) (Domínguez 2009). Symphylan species are morphologically determined mainly based on the chaetotaxy of the head, antennae size and shape of the scuta margins (Domínguez 1992, Edwards 1959a, b, Scheller 1961).

Only two genera in the family Scutigerellidae are considered to be pests in a wide range of crops: Scutigerella Ryder, 1882 and Hanseniella Bagnal, 1913 (Michelbacher 1938). Scutigerella immaculata Newport, 1845 is the only reported symphylan in Colombia where it is regarded as a pest of pineapple (Agredo 1988) and flower crops (Durán 1982, Navarro and Gaviria 2001). However, in these reports the authors did not describe how they identified S. immaculata. Questions are raised regarding the presence of S. immaculata in tropical Colombia. Domínguez (2009) only reports Scutigera genus in northern temperate zones. In northern Brazil, bordering Colombia and Peru, de Morais and da Silva (2009) report the presence of Hanseniella and Symphylella (Scolopendrellidae). The distribution of the family Scutigerellidae is: Scutigerella mainly in northern temperate zones; Hanseniella in tropical and warm temperate zones; Millotellina in Africa, Madagascar, Réunion, Sri Lanka, New Guinea and Australia; Scolopendrelloides in South-East Asia and Australia; and Scopoliella in North America only (Domínguez 2009).

Mitochondrial DNA Cytochrome Oxidase I (COI) barcode region (Hebert et al. 2003, Smith et al. 2005) and the ribosomal nuclear Internal Transcribed Spacer 2 (ITS2) are used as molecular markers for arthropod species identification (Hebert et al. 2003, Ruiz et al. 2005, Wiemers et al. 2009). Barcoding is a fast and accurate method for species delimitation using the Kimura Two-Parameter model (K2P) (Padial and De la Riva 2007). There are few reports using these molecular makers in symphylans (Mallatt et al. 2004, Podsiadlowski et al. 2007, Spelda et al. 2011, Stoev et al. 2010, 2013) and none characterising Colombian symphylans.

Symphylan pests in Colombia are commonly identified as S. immaculata by the presence of a single morphological feature, a U-shape groove in the scuta of the last abdominal segment. The aim of this study was to capture symphylans in two departments of Colombia and describe these using multiple morphological characters and molecular markers.

Methods

Symphylan collection and examination

Symphylans were collected from two flower companies: Flores Esmeralda S.A.S C.I. in Antioquia (6°1’0”N, 75°25’0”W, 2180 m.a.s.l.) and Flexport and CIA.S.A.C.I. in Cundinamarca (4°45’4.10”N, 74°13’30.87”W, 2548 m.a.s.l.). Symphylan collection used a modified method of Umble et al. (2006); beet slices instead of potato baits covered with
black plastic to block the passage of light were set overnight for 12 hours on flowerbeds. The next morning, the symphylans were collected from the beets and soil around the baits and transported in Petri dishes – 20 individuals per dish, each dish 9 cm in diameter, containing 17 g of soil (previously sterilized at 121 °C) and beet as a food source – to the Bio-control and Microbiology Laboratory (BIOMA), University of Antioquia, Medellín, Colombia. Symphylans were identified by morphology (N = 30) using the descriptions and keys of Domínguez (2009, 2010), Halliday (2004), and Naumann and Scheller (1977). A total of 15 specimens from Antioquia (N = 10) and Cundinamarca (N = 5) were imaged using the Scanning Electron Microscope (SEM, Hitachi S-510) methodology of A. Acevedo (unpublished). In short, specimens were first fixed in 2% glutaraldehyde and then subsequently fixed in 1% osmium tetraxide. Each sample was dehydrated in up to 100% ethanol, critical-point dried and sputter coated with gold. Vouchers specimens are stored in BIOMA laboratory, University of Antioquia.

Molecular characterisation

DNAs of ten symphylans from Antioquia were extracted using DNeasy Blood and Tissue Kit (QIAgen®, USA). The COI barcode region was amplified by polymerase chain reaction (PCR) using the primers developed by Folmer et al. (1994) and following the protocol of Ruiz et al. (2010). The rDNA ITS2 PCR was carried out using the primers of Collins and Paskewitz (1996) following the protocol of Linton et al. (2001).

Bi-directional sequencing used the Big Dye Terminator Kit® on an ABI3730 automated sequencer (PE Applied BioSystems, Warrington, England). Raw sequence chromatograms were edited using Sequencher™ v. 4.8 (Genes Codes Corporation, Ann Arbor, MI), aligned automatically in MAFFT v. 7 (ITS2) (Katoh et al. 2002) or manually (COI) using MacClade v. 4.06 (Maddison and Maddison 2003). Sequence similarities were compared with those available (October 14, 2014) in GenBank using Basic Local Alignment Search Tool (BLAST) (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and Barcoding of Life Data Systems (BOLD Systems) (http://www.barcodinglife.com/).

Results

A total of 210 symphylans were collected from Antioquia (N = 180) and Cundinamarca (N = 30) and some were used for morphological and molecular studies.

Morphology

Morphometrics from the SEM images of 15 symphylans showed the following characters. Size: average symphylan 3.9 mm (range 2.9–4.75 mm excluding antennae). Head: somewhat heart-shaped, central rod had a knob before arriving to
the posterior point of the head. Tömosvary organ was clearly defined with a hole in the centre (Figure 1A, B). **Antennae:** between 22 and 31 segments covered with setae (Figure 1A). **Abdomen:** scutes with pubescent cuticles, convex anterior tergites and
last scuta margins with a U-shaped groove covered with thin dorsal setae, and long ventral and lateral setae (Figure 1C). **Legs:** presence of sternal appendages behind the 3rd to 9th coxal sacs (Figure 2) (Table 1).

**Molecular analysis**

Two out of ten symphyllans captured from Antioquia were successfully characterised at COI (658 bp) and ITS2 (358 bp) and both specimens shared the same unique
haplotypes for each marker. An open reading frame was read for COI indicating the sequence likely represented a functional protein-coding gene not a pseudogene. GenBank sequence accession numbers: KP696390-91 (COI) and KP696392-93 (ITS2).

A comparison of our COI symphylan haplotype with sequences deposited in GenBank showed low homology with: *S. causeyae* (77%, query cover 99%, GenBank DQ666065) and *Hanseniella* n. sp. (78%, query cover 92%, GenBank AF370839). Using BOLD Systems database, 100% sequence homology was found with six specimens from Cameroon, described as Phylum Arthropoda, class Symphyla, status private, 77% homology with *Scutigerella* sp. (N = 2) from Bavaria (status private), 77% with *S. causeyae* (N = 2) source locality unknown (status private) and 76% with *S. causeyae* from Austria, Salzburg (status private).

The ITS2 haplotype characterised from our symphylans showed low homology with a sequence of *Scutigerella* sp. (95%, query cover 62%, GenBank DQ666184) and *Hanseniella* sp. (91%, query cover 70%, GenBank AY210821). The ITS2 haplotype could not be compared using BOLD Systems as this database does not collect sequences for this molecular marker.

**Discussion**

The taxonomy of the class Symphyla is unclear, a consequence of few published studies: two morphological keys for European (Edwards 1959a, b, Domínguez 2010) and one key for Neotropical (Scheller and Adis 1996) species. There are no published morphological descriptions or keys for Colombian Symphyla, therefore the exact number of genera and species is unknown. The only symphylan recorded in Colombia is *S. immaculata* (Agredo et al. 1988, Peña 1998, Corredor 1999), however, this species lacks formal morphological description and both the type specimen and the type locality (London, United Kingdom) have been destroyed and no redescription has been made (Scheller pers. comm.).

Our Colombian symphylans showed genus-level morphological ambiguity (Table 1). We observed a U-shaped groove in the anterior most scuta the character identifying *Scutigerella* (Halliday 2004), but paired sternal appendages behind the 3rd to 9th coxal sacs of the legs (Figure 2) that are unique to *Millotellina* (Naumann and Scheller 1977). Naumann and Scheller (1977) describe the sternal appendages in two subgenera of *Millotellina*, *Millotellina* with unpaired appendages between legs 5 and 10 and *Diplomillotellina* with pairs between legs 5 and 9. However, our symphylans presented paired appendages between legs 3 and 9, which could suggest the existence of a new subgenus within *Millotellina*.

According to Hebert et al. (2003) the threshold of genetic divergence for species delimitation is 3%. However, recent studies have shown that there is no single universal threshold for species’ delimitation using the barcode region, which can differ according to the group studied (Rach et al. 2008). For example, Ruiz et al. (2010, 2013) reported in mosquitoes of South America a lower interspecific threshold between 2 and 2.5%. To our knowledge only three papers have used COI barcoding within the subphylum Myriapoda, to which class Symphyla belongs. Spelda et al. (2011) showed for class Chilopoda
a mean interspecific genetic distance of 18.3%: range 12.0% between congeneric species to 25% between genera or families. Stoev et al. also for class Chilopoda showed mean interspecific genetic distances between 5 (2010) and 12 (2013) species of *Eupolybothrus* genus that ranged between 16.1–24.0% and 10.7–24.5%, respectively.

Our Colombian Symphyla COI haplotype showed genetic divergence with sequences of *S. causeyae* of at least 23% and *Hanseniella* n. sp. of 22%, similar to the congeneric ranges observed by Spelda et al. (2011) and Stoev et al. (2010, 2013). Unfortunately there are no published sequences of *S. immaculata* or a formal description of this species. As our specimens showed a mixture of morphological characters of *S. immaculata* and *Millotellina* genus, which has never before been reported in the literature, we speculate that Colombian symphylans belong to a new taxon. It is therefore necessary that a formal redescription of *S. immaculata* be published before the taxonomic status of these Colombian symphylans can be made.

It is interesting that our COI barcode shared the same haplotype as six Symphyla specimens found in Cameroon. This demonstrates that this taxon is not restricted to South America, it has a wide geographical distribution and therefore can be a widespread agricultural pest. We have two hypotheses to explain this taxon’s distribution: 1. That the specimens found in Colombia are a “tramp species”, which was introduced inadvertently by human commerce from Africa to the Americas or vice versa. 2. This taxon is native to Colombia, but due to the lack of specialists on this group along with the lack of morphological keys, this taxon has remained unrecognised.

**Conclusion**

We demonstrate for class Symphyla that the parallel use of DNA barcoding with morphological descriptions can contribute to the taxonomic resolution of this understudied group. Our specimens presented not only the morphological characters of the only symphylan species reported in Colombia, *S. immaculata*, but also the character identifying species within *Millotellina* genus whose distribution has not been recorded in the Americas (Table 1). Furthermore, we showed the same Symphyla COI haplotype in both South America and Africa. This research highlights the need for further studies of morphology and molecular phylogenies that include type material to determine the worldwide taxonomic status of class Symphyla.

**Acknowledgments**

The authors are grateful to: Ministerio de Agricultura y Desarrollo Rural (MDRE) of the Republic of Colombia for its sponsorship, Flores Esmeralda S.A.S.C.I. for its collaboration in the collection of biological specimens, Dr. Miguel Domínguez for the specimen review, and both Dr. Miguel Domínguez and Dr. Shazia Mahamdallie for their comments during the preparation of this manuscript.
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