Systematics and Biodiversity (2020), **0(0)**: 1–13

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Research Article

Genome-informed integrative taxonomic description of three cryptic species in the earthworm genus *Carpetania* (Oligochaeta, Hormogastridae)

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(Received 24 July 2019; accepted 17 December 2019)

Research on cryptic species complexes has reached a consensus on the necessity of integrating multiple sources of evidence. Reduced representation library techniques such as Genotyping-by-Sequencing (GBS) have proven useful to study these groups. Both integrative taxonomy and genome-wide single nucleotide polymorphism (SNP) data remain to be widely applied to earthworms, an animal group with widespread presence of cryptic diversity. The genus *Carpetania* (formerly the *Hormogaster elisae* species complex) was found to contain six deeply divergent genetic lineages and some inconspicuous morphological differentiation based on a handful of Sanger-sequenced markers. Marchán et al. (submitted) delimited three well-supported species-level clades on the basis of a genome-wide SNP dataset and geometric morphometric analyses, highlighting the necessity of a formal taxonomic description of these taxa. In this work, further analyses are applied to the SNP data and a thorough morphological study is performed in order to provide an integrative description of two new species and to redescribe *Carpetania elisae*. Species-specific SNPs are identified and used as diagnostic characters, and genome-wide and cytochrome oxidase C subunit 1 (COI) genetic distances are compared finding a strong correlation between them. The taxonomic description of these three cryptic species provides a useful tool to include them effectively in ecological studies and biodiversity conservation actions.

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www.zoobank.org/urn:lsid:zoobank.org:pub:2797FDDF-EA3D-4347-96C6-E632DC26CDA3

Key words: cryptic species, earthworms, Genotyping By Sequencing, integrative taxonomy, soil fauna

Introduction

Since the advent of molecular phylogenetic techniques, discovery of cryptic species complexes showed a surge in scientific interest (Bickford et al., 2007; Pfenninger & Schwenk, 2007; Trontelj & Fišer, 2009; León, de León, & Nadler, 2010; Nygren, 2014), followed by a stage of novelties in species delimitation methodologies including different algorithms, genetic markers, and integrative approaches (Pons et al., 2006; Yang & Rannala, 2010; Puillandre, Lambert, Brouillet, & Achaz, 2012; Zhang, Kapli, Pavlidis, & Stamatakis, 2013; Yang, 2015). Two main lessons can be gathered from those studies: to obtain robust species delimitation hypotheses, several information sources (molecular, morphological, ecological, ethological) must be integrated (Queiroz & De Queiroz, 2007); and nuclear molecular markers are necessary to rule out the possibility of confounding deep mitochondrial lineages (and the effect of Incomplete Lineage Sorting) with proper cryptic species (Dupont, Porco, Symondson, & Roy, 2016). Recent debate has brought into the spotlight the necessity of refining the terminology related to the cryptic speciation phenomenon, as well as the validity of the concept itself (Heethoff et al., 2018; Struck et al.,

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ISSN 1477-2000 print / 1478-0933 online

https://dx.doi.org/10.1080/14772000.2020.1730474

Published online 14 Apr 2020

2018; Chenuil, Cahill, Délemontev, du Luc & Fanton, 2019; Korshunova et al., 2019).

Some works have already shown the potential of genome-wide single nucleotide polymorphism datasets (either generated by RADseq or GBS – Genotyping By Sequencing) to provide rich phylogeographic and species delimitation information for cryptic species complexes (Garg et al., 2016; Brunet et al., 2017, Rancilhac et al., 2019), but have only been applied to earthworms in the *Lumbricus rubellus* complex (Giska, Sechi, & Babik, 2015; Anderson, Cunha, Sechi, Kille, & Spurgeon, 2017). Giska et al. (2015) found no differentiation between sympatric cryptic lineages based on RAD-seq data, which was interpreted as these lineages not corresponding to biological species. However, Anderson et al. (2017) performed equivalent analyses on different lineages of the same cryptic complex and found strong differentiation between them.

Pervasive cryptic diversity has been found in earthworms across different families (Lumbricidae – King, Andrew King, Tibble, & Symondson, 2008; Fernández, Almodóvar, Novo, Simancas, & Díaz Cosín, 2012; Shekhovtsov, Golovanova, & Peltek, 2013; Porco et al., 2018; Hormogastridae – Novo, Almodóvar, Fernández, Trigo, & Díaz Cosín, 2010; Megascolecidae – Chang, Lin, & Chen, 2008; Buckley et al., 2011, Moniligastridae – Ganin & Atopkin, 2018). Integrative taxonomy has yet to be widely employed in these complexes (but see Taheri et al., 2018).

One of the most studied cryptic species groups among these animals is the former *Hormogaster elisae* Álvarez, 1977, recognized as the genus *Carpetania* after Marchán et al. (2018). Six highly divergent cryptic lineages were identified using Sanger-sequenced mitochondrial and nuclear markers (Marchán, Fernández, de Sosa, Díaz Cosín, & Novo, 2017), but their description was precluded by the absence of clear-cut limits between the putative species. Quantitative differences in the distal end of genital chaetae were discovered between those cryptic lineages through geometric morphometrics (Marchán, Novo, et al., 2016), hinting a possible pseudocryptic status for the identified lineages. Pseudocryptic species are those classified as cryptic due to the 'inadequacy of the morphological analysis' (Knowlton, 1993) and can usually be distinguished after careful morphological analysis together with molecular data (Lajus, Sukhikh, & Alekseev, 2015).

Recently, a genome-wide SNP dataset was obtained through GBS for 17 populations and 85 individuals of *Carpetania* by Marchán et al. (2020), with the main objective of studying selection signatures and local adaptation in the cryptic complex. The authors applied different approaches to genetic structure identification and species delimitation (see Materials and methods) together with geometric morphometrics analysis (Fig. 1), finding congruent support for three species-level genetic clusters (hereafter clusters A, B and C), which comprised one or more of the six previously identified lineages as described in Marchán et al. (2017).

The well-supported putative species identified in *Carpetania* demanded a full, detailed taxonomic description: several authors have stressed the necessity of going a step further from cryptic species identification (Jörger & Schrödl, 2013; Wang et al., 2016; Fišer, Robinson & Malard, 2018; Chenuil, Cahill, Délémontey, Du Luc, & Fanton, 2019). To this end, this work will build upon the framework provided by Marchán et al. (2020), with the following objectives: (i) identify genome-wide nucleotidic positions to be used as diagnostic characters in species description (molecular taxonomy); (ii) explore further morphometric and internal anatomic characters to reinforce species description; (iii) formally describe the species within *Carpetania*; (iv) compare genomic and barcode genetic distances to validate the widespread use of the latter.

Materials and methods

Molecular data

Background: SNP data comprising the geographic distribution and internal lineages of *Carpetania* was generated in Marchán et al. (2020). A brief summary of the methodology used to generate and analyse said data is presented below.

GBS libraries were generated using the restriction enzyme *PstI* following the GBS protocol from Elshire et al. (2011) and sequenced (in NextSeq500 Illumina platform) for 17 populations with five individuals from each totalling 85 individuals. Different datasets were generated with STACKS2 (Rochette, Rivera-Colón, & Catchen, n.d.), in order to compare the performance of the different analyses and due to the specific requirements of some of the analyses.

– 'de novo-all SNPs', obtained by *de novo* assembly. This dataset contained the highest number of SNPS (26,240).

– 'de novo-one SNP', obtained after selecting a random SNP per locus through the function *populations* in STACKS2. This dataset contained 4,767 SNPs, and allowed the use of Bayesian clustering in STRUCTURE 2.3.4 (Pritchard, Stephens, & Donnelly, 2000). Another added advantage of the removal of linked SNPs within the same loci was to avoid the inflation of outlier loci in selection analyses (PCAdapt – Duforet-Frebourg, Bazin, & Blum, 2014; Fsthet – Flanagan & Jones, 2017).

– 'reference-one SNP', 3,181 SNPs, obtained by mapping the reads against a reference transcriptome of *Carpetania elisae* lineage 1. Transcriptome reads can be accessed at NCBI Short Read Archive project: PRJNA196484, and the assembly (obtained with Trinity v. r2013-08-14 (Haas et al.,

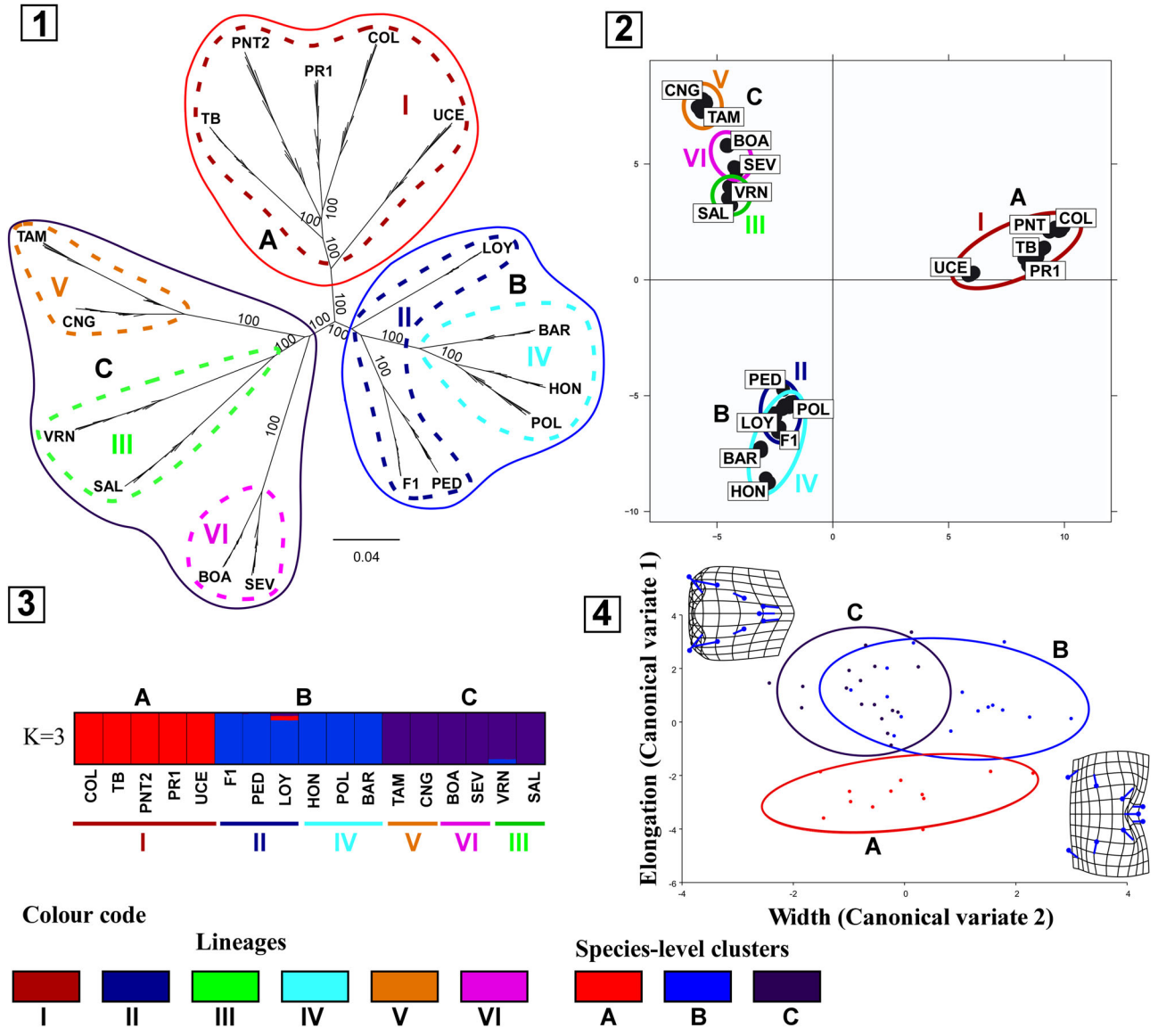

Fig. 1. Different approaches to genetic structure identification and species delimitation in the *Carpetania* complex (modified from Marchán et al., 2020). Previously defined cryptic lineages (Marchán et al., 2017) are represented by a colour and roman number. Colour codes and nomenclature are kept throughout the manuscript. (1) Maximum likelihood inference of the phylogenetic relationships of the studied populations of *Carpetania*. Solid outlines indicate the species-level genetic clusters (A, B, C) found by the other analyses. (2) Principal Component Analysis (PCA). (3) Barplot of STRUCTURE analysis. Each colour shows percentage of assignment to a cluster or ancestral population. (4) Canonical variate analysis of the shape of the genital chaetae of *Carpetania* main genetic clusters.

2013)) can be accessed in the Harvard Dataverse repository https://doi.org/10.7910/DVN/RVMQND.

Three different methodologies were applied to the SNP datasets to recover the genetic structure and phylogenomic relationships: Maximum likelihood (ML) phylogenetic inference, Principal component analysis (PCA), and Bayesian clustering.

A ML approach was applied to the concatenated sequences of SNPs datasets as implemented in RAXML-HPC v8 (Stamatakis, 2014) in Cipres Science Gateway (https://www.phylo.org/) with default parameters (GTRCAT model, ascertainment bias correction (Lewis, 2001), 1,000 rapid bootstrap inferences). PCA was performed (function *glPCA*, R package adegenet v2.0.1 (Jombart, 2008; Jombart & Ahmed, 2011) for the 'de novo-one SNP' dataset. Bayesian clustering was performed in STRUCTURE 2.3.4 (Pritchard et al., 2000). Ten separate runs were performed for each number of genetic clusters K (1–7). The Evanno's method (ΔK criterion) (Evanno, Regnaut, & Goudet, 2005)

implemented in Structure Harvester (Earl & vonHoldt, 2012) identified K = 3 as the optimal number of clusters for the 'de novo-one SNP' dataset.

New molecular analyses: The datasets 'de novo-all SNPs' and 'reference-one SNP' were further analysed in this work to estimate genetic diversity and to identify species-specific diagnostic positions, respectively.

Genetic diversity of the studied populations was described through identity by state (IBS) genetic distance within and between populations and fixation index (F_{ST}). These parameters were obtained from the *populations* function (STACKS2 package) summary files. Correlation between SNP-based genetic distances and F_{ST} and the same parameters obtained from cytochrome C oxidase 1 sequences (Marchán et al., 2017) was tested through a Mantel test in the R package vegan (Dixon, 2003).

Diagnostic SNP positions were identified using function *nucDiag* in the R package spider (Brown et al., 2012) and parsed to the corresponding contig using the *Carpetania elisae* transcriptome after it was functionally annotated with eggNOG-mapper (Huerta-Cepas et al., 2017). Diagnostic nucleotidic positions were also identified in the molecular markers COI, 16S-tRNAs, 28S and H3 (retrieved from Marchán et al., 2017) following the same method.

Morphological data

Background: Geometric morphometric data of the genital chaetae of *Carpetania* comprising its geographic distribution and internal lineages was generated in Marchán et al. (2020). A brief summary of the methodology used to generate and analyse said data is presented below.

Thirteen populations (from a total of 17 analysed for GBS) were chosen for genital chaetae extraction and preparation for scanning electron microscopy. Geometric morphometrics analyses included acquisition of landmarks, Canonical Variate Analysis (CVA) and Discriminant Function Analysis (DFA). Landmarks established in Marchán, Novo, et al. (2016) were chosen for the analysis. Genital chaetae were grouped by population and populations were subsequently grouped in the three species-level clusters recovered by the molecular analyses.

External anatomy and morphometric characters. External anatomy characters commonly used in earthworm taxonomy were studied (type of prostomium, chaetae disposition, position of female and male pores, position of clitellum and *tubercula pubertatis*), with special attention to average weight and number of segments (found to have strong phylogenetic signal in Hormogastridae Marchán, Novo, et al., 2016). These were measured in at least five mature individuals per population in a total of 22 populations: 9 populations from Cluster A, 6 populations from Cluster B, and 7 populations from Cluster C. Statistical significance of differences was evaluated using ANOVA, Fisher's LSD and Kruskal–Wallis tests in Statgraphics 18.

Internal anatomy. Internal anatomy characters commonly used in earthworm taxonomy were studied (number and position of gizzards, number of typhlosole lamellae, number and position of spermathecae, type of nephridial bladders), with special attention to the only variable internal character in the *Carpetania* species complex, relative position of septum 9/10 and spermathecae. In this genus, septum 9/10 can appear displaced backwards (to 10/11) in its dorsal insertion, resulting in the spermathecae of segments 9 and 10 belonging functionally to the same segment, or show an unmodified disposition separating both pairs of spermathecae. This character was studied in 22 populations as well.

Results and discussion

Genomic divergence and diagnostic positions

Genetic divergence parameters (average IBS distances and F_{ST} values) are presented in Fig. 2 (values can be found in Table S2). IBS distances between populations within the main clusters ranged from 0.09 to 0.15 (mean = 0.12) in both clusters A and B, while they ranged from 0.07 to 0.22 (mean = 0.17) in cluster C. IBS distances between populations of different clusters ranged between 0.15–0.24 (mean = 0.20) for clusters A and B, while they ranged between 0.18–0.24 (mean = 0.21) for cluster C *vs* A and B.

F_{ST} values between populations within the main clusters were lower for cluster A (0.30–0.60, mean = 0.46) than for the other two clusters (B: 0.31–0.76, mean = 0.59; C: 0.30–0.75, mean = 0.63). F_{ST} values between populations of different clusters showed similar ranges for all of them (A: 0.51–0.82, mean = 0.65; B: 0.51–0.84, mean = 0.68; C: 0.55–0.79, mean = 0.67). Clustering analysis of both IBS distances and F_{ST} values (Fig. 2) recovered the same clusters as the phylogenetic inference and subsequent analyses.

Mantel test for genomic IBS distances and uncorrected pairwise COI distances showed a strong, statistically significant correlation between both sets of values ($r = 0.7956$, $P = 0.001$). Mantel test for genomic and COI-based F_{ST} values showed a weaker, statistically significant correlation between them ($r = 0.4801$, $P = 0.002$).

These results suggest that divergence in COI sequence in the *Carpetania* species complex reflects to a

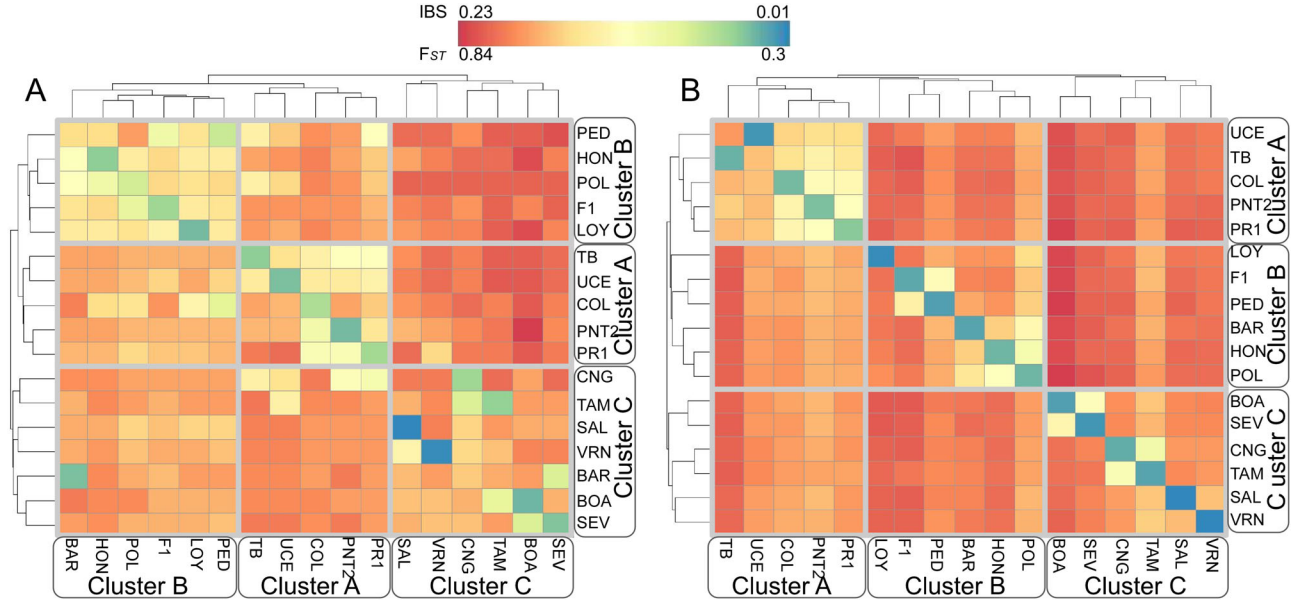

Fig. 2. Heatmap and clustering graphs displaying F_{ST} values (left) and Identity By State (IBS) distances (right) between populations of *Carpetania* obtained from genome-wide SNPs.

significant extent genetic divergence across the whole genome, supporting the use of this molecular marker as a proxy for the identification of the cryptic species. COI barcoding (Hebert, Ratnasingham, & de Waard, 2003) has been widely accepted by the scientific community as a fast, simple, and standardized method to identify, classify, and delimit species (Decaëns, Porco, Rougerie, Brown, & James, 2013). It is worth noting that lineages identified on the basis of COI distance alone must be supported with additional molecular (nuclear markers), morphological, ecological, ethological, and biogeographic evidence (Rougerie et al., 2009).

Several diagnostic SNP positions were identified: 23 for Cluster A, four for Cluster B and eight for Cluster C (Table S1). Twenty-eight diagnostic SNP positions were assigned to the putative protein coded by the surrounding region of the variant nucleotide (Table S1). Diagnostic SNP positions were found in genes with different biological functions, as regulation, transport, response to stimuli, developmental processes, cellular processes, multicellular organismal processes, and metabolism among others.

A single diagnostic nucleotidic position was found for the 28S molecular marker, distinguishing Cluster C from the other two. No diagnostic positions were identified for the rest of the Sanger-sequenced molecular markers.

Species-diagnostic SNPs have been successfully identified in plants (Cullingham, Cooke, Dang, & Coltman, 2013) and fishes (Hand et al., 2015). Even though they may appear more difficult to use for species identification than diagnostic positions in traditional Sanger-sequenced molecular markers, the cost of GBS and RAD-sequencing has lowered significantly making their use in taxonomic studies more viable. Another alternative is the development of SNP arrays based on the previously established (through GBS/RADseq) diagnostic SNPs, as an inexpensive, automatized approach.

Morphological analyses

Studied Cluster A and Cluster B populations showed significantly different average body weights (3.03 grams *vs* 4.98 grams) according to the different statistical tests. However, Cluster C average body weight (3.89 grams) was statistically indistinguishable from the other two.

No significant differences were found between the average number of segments of the studied populations of Clusters A, B, and C, even though Cluster B showed the smallest average (248 segments) and C showed the highest (273 segments).

The difference in average values of morphometric characters is reminiscent of the ones found between *Lumbricus terrestris* and its sibling species *Lumbricus herculeus* (James et al., 2010). As found here, differences in weight and number of segments were not clear-cut, with overlapping distributions. This precludes the use of these characters in the diagnosis of the cryptic species, yet they are valuable for preliminary assignment in the field.

Character states for the relative position of spermathecae and septum 9/10 (separated/not separated by septum 9/10) showed high consistency for individuals from each population. However, those character states were not shared by all populations within the clusters

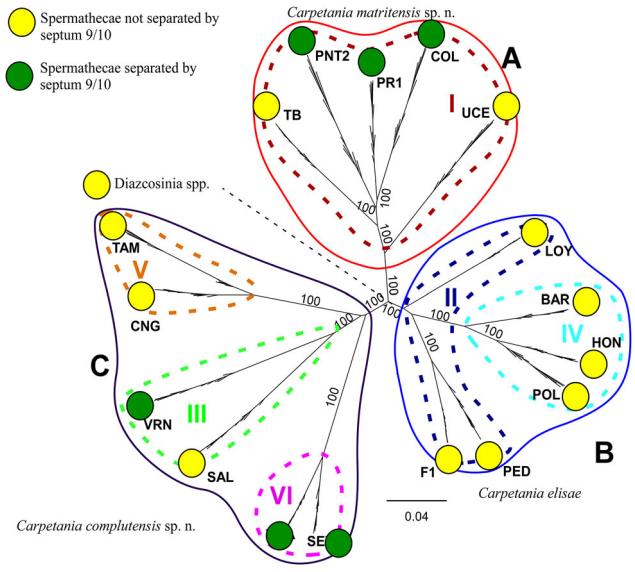

Fig. 3. Relative position of spermathecae and septum 9/10 for the populations included in the phylogenetic analysis. A hypothetical position of the sister genus *Diazcosinia* (not included in the phylogenetic reconstruction) and its character state are shown by a dashed line. Lineages from Marchán et al. (2017) are shown with the same code and colours (roman numbers, dashed outlines). Species-level clusters are represented by solid outlines and letters A, B, C.

(Fig. 3), with the exception of cluster B – where all studied populations showed spermathecae not separated by septum 9/10. Cluster A populations showed separated spermathecae, but the most basal populations within the clade showed not separated spermathecae. For cluster C this character showed no clear pattern, with two of its internal lineages showing constant states and the third containing populations with either character state. Both described species in the sister genus *Diazcosinia* possess spermathecae not separated by septum 9/10, suggesting this could be the ancestral character state for *Carpetania*.

The complex evolution of this trait disallows its use as a diagnostic taxonomic character. However, it is an interesting example of multiple independent events of regression of a character state. Spermathecae not separated by septum 9/10 due to the backward displacement of the septum appears as a derived character state present in the common ancestor of *Carpetania* and *Diazcosinia*, while spermathecae separated by septum 9/10 is the most common disposition in other species of Hormogastridae and Lumbricidae. In order to disentangle the evolutionary pressures behind these changes, it would be necessary to understand the biological advantage of both character states. Even when the mechanisms of filling and release of sperm in earthworm spermathecae is not fully known, it is likely that contraction (and subsequent increased coelomic pressure) of each segment should have a role in the process. Under this assumption, spermathecae separated by a septum would function independently, while spermathecae belonging functionally to the same segment would work coordinately. This would be relevant in the context of sexual selection and hermaphroditic sexual conflict: the capability of controlling which spermathecae stores sperm from a different mate is favourable to the female part, while diminished control over this uptake would be favourable to the male part. Novo, Almodóvar, Fernández, Gutiérrez, and Díaz Cosín (2010) found no evidence of differential sperm storage from different partners in each of the four spermathecae in *Carpetania* specimens from El Molar, which according to our hypothesis should have the ability to control front and back spermathecae separately. On the other hand, different sperm storage in front and back spermathecae has been observed in other earthworms (*Lumbricus terrestris* – Koene, Pförtner, & Michiels, 2005; *Eisenia andrei* – Porto, 2014). Further research would be needed to test this hypothesis and to propose other alternatives.

Taxonomic implications

The genetic structuring of *Carpetania* populations into three clearly separated clusters, characterized by significant genome-wide divergence, inconspicuous but detectable morphological differences and with no admixture between them calls for the formal taxonomic description of those clusters as species. Cluster B includes the type locality of *Hormogaster* (*Carpetania*) *elisae*, hence the original description is assigned to this clade. Meanwhile, Clusters A and C are described below as new species.

Phylum Annelida Lamarck, 1802
Subphylum Clitellata Michaelsen, 1919
Class Oligochaeta Grube, 1850
Superorder Megadrili Benham, 1890
Order Haplotaxida Michaelsen, 1900
Family Hormogastridae Michaelsen, 1900
Genus *Carpetania* Marchán, Fernández, Díaz Cosín & Novo, 2018

Amended description: External characters – Average number of segments from 203–323. Average weight from 1.22–7.6 grams. Clitellum in segments (12)13–27. Tubercula pubertatis in segments 22–25. Pigmentation absent, colour: greyish-fleshy. Cephalic keels present, moderately developed. No lateral expansions of the clitellum. Chaetae disposition geminate. No genital papillae in cd. Posterior genital papillae constrained within

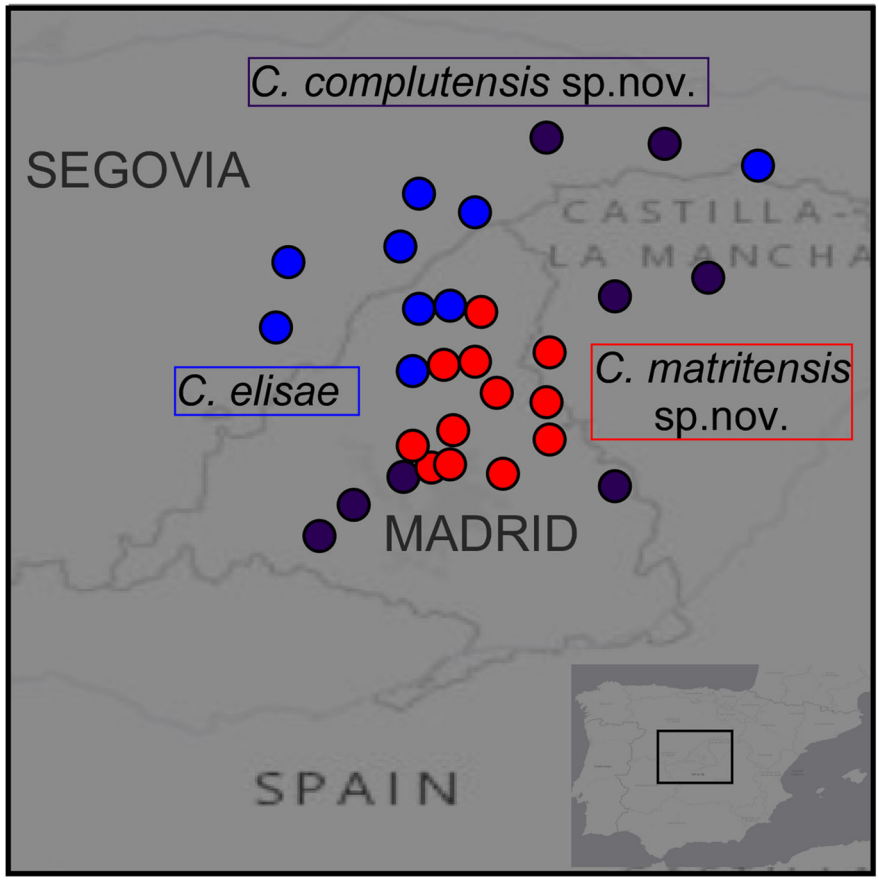

Fig. 4. Known distribution of the three species of *Carpetania*.

the extension of the clitellum. Cephalic segments not imbricated.

Internal characters – First thickened septum in 6/7. Last thickened septum in 9/10. Backward displacement of dorsal insertion of septum 9/10, one or two segments. No forward displacement of dorsal insertion of septa 7/8, 8/9. Spermathecal pores in intersegments 9/10, 10/11. Spermathecae tubular, the first pair smaller, with no repetition. Seven pairs of clearly developed hearts. Three oesophageal gizzards in segments 6, 7, and 8. Five typhlosole lamellae. Genital chaetae lanceolate, with strong dorsoventral differentiation and tip ornamentation; pore present, teeth present, dorsal depression absent, ventral groves absent. First nephridia with caeca between segments 10 and 12.

Carpetania elisae (Álvarez, 1977)

Type material: Holotype and paratypes – 12 adult and subadult individuals collected in Siguero, Segovia, deposited by J. Álvarez in the Spanish Entomology Institute collection with numbers 4661–4669 and 46610–46612. Topotypes: 8 adults (UCMLT 00368-00375), 41.185 – 3.6186, from a meadow in the outskirts of the village of Siguero, Segovia (Spain), collectors Darío J. Díaz Cosín, Marta Novo, Dolores Trigo.

Distribution: Northernmost community of Madrid, Southern Segovia, Southern Soria (Fig. 4). Full list of known localities is shown in Table S3.

Phylogenetic definition: Includes the common ancestor of all the populations assigned in Marchán et al. (2020) to the cluster B, and all its descendants. Known populations are shown in Table S3.

Reference sequences: COI – accession EF653893.1; 16S-tRNAs – accession GQ409710.1; 28S – accession GQ409654.1; H3 – accession HQ622033.1.

Amended description: External and internal morphological characters match the description of the genus *Carpetania* in all aspects but the following.

Average weight from 1.87–7.44 (mean = 4.98). Average number of segments from 231–288

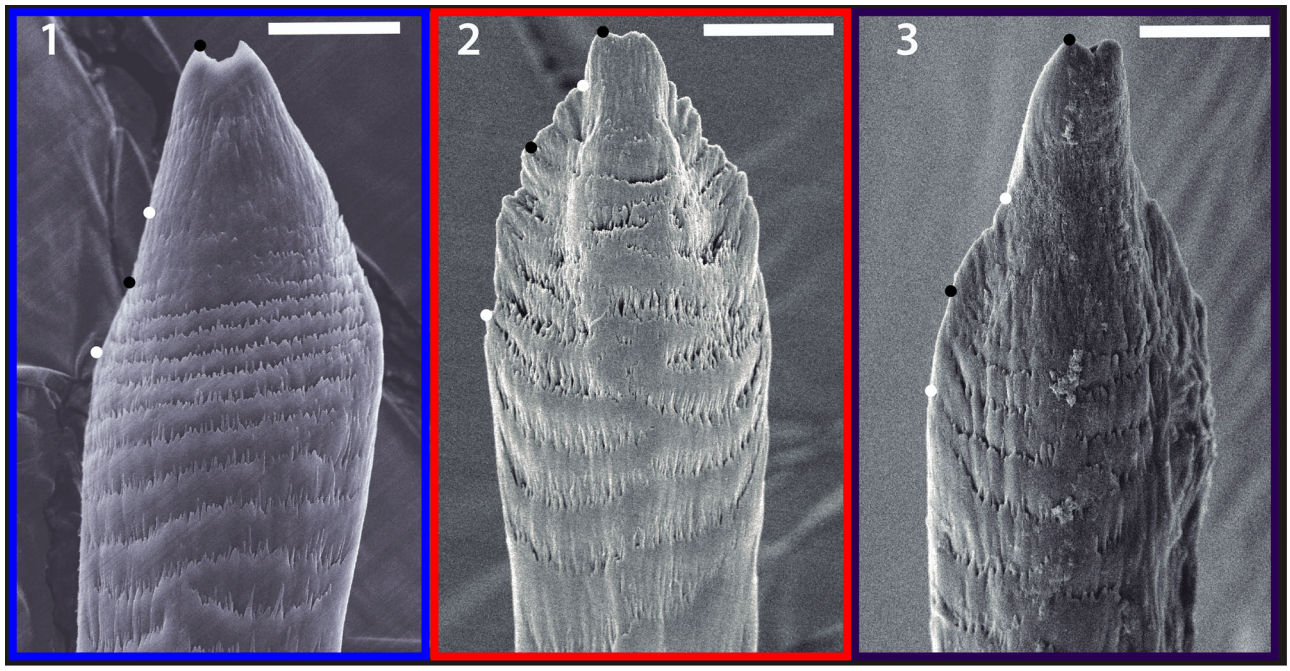

Fig. 5. Genital chaetae distal end from representatives of the three species in *Carpetania*: (1) *Carpetania elisae*, (2) *Carpetania matritensis*, (3) *Carpetania complutensis*. Black and white dots show the landmarks used in geometric morphometrics analyses for reference. Coloured boxes correspond to Fig. 4. Scale bar: 10 µm.

(mean = 248). Generally heavier and shorter than the other species: 'stout' appearance after fixation. Both pairs of spermathecae not separated by septum 9/10.

Diagnosis: Genital chaetae with an elongated, wide tip (Fig. 5). Diagnostic nucleotidic positions shown in Table S1.

Remarks: This species includes specimens from Siguero, topotypes of the holotype of *Hormogaster elisae*. Thus, the whole species must carry this name and be the type species of the genus *Carpetania*.

Carpetania matritensis sp. nov.

Type material: Holotype. Adult (UCMLT 00386), 40.7394 – 3.5647, from a meadow in the outskirts of the village of El Molar, Madrid (Spain), collectors Marta Novo, Darío J. Díaz Cosín. Paratypes. 7 adults (UCMLT 00387-00393), with the same collection data of the holotype.

Distribution: Northern and Central Community of Madrid, Madrid-Guadalajara border (Fig. 4). Full list of known localities are shown in Table S3.

Phylogenetic definition: Includes the common ancestor of all the populations assigned in Marchán et al. (2020) to the cluster A, and all its descendants. Known populations are shown in Table S3.

Reference sequences: COI – accession EF653876.1; 16S-tRNAs – accession JN209295.1; 28S – accession GQ409653.1; H3 – accession JN209636.1.

Description: External and internal morphological characters match the description of the genus *Carpetania* in all aspects but the following.

Average weight from 1.22–4.66 (mean = 3.10). Average number of segments from 203–285 (mean = 253). 'Slender' appearance after fixation. Both pairs of spermathecae separated by septum 9/10 in most populations (except in basal clades).

Diagnosis: Genital chaetae with a shortened tip and serrated lateral ridges (Fig. 5). Diagnostic nucleotidic positions shown in Table S1.

Etymology: The name of this species refers to Community of Madrid, the region to which known populations of *C. matritensis* are restricted.

Carpetania complutensis sp. nov.

Type material: Holotype. Adult (UCMLT 00376), 40.4306 – 3.925, from a open holm oak woodland in the

outskirts of the village of Boadilla del Monte, Madrid (Spain), collectors Marta Novo, Darío J. Díaz Cosín. Paratypes. 9 adults (UCMLT 00377-00385) with the same collection data of the holotype.

Distribution: Southern and Central Community of Madrid, North-eastern Segovia, South-western Soria, North-western Guadalajara (Fig. 4). Full list of known localities are shown in Table S3.

Phylogenetic definition: Includes the common ancestor of all the populations assigned in Marchán et al. (2020) to the cluster C, and all its descendants. Known populations are shown in Table S3.

Reference sequences: COI – accession GQ409664.1; 16S-tRNAs – accession GQ409704.1; 28S – accession GQ409656.1; H3 – accession HQ622004.1.

Description: External and internal morphological characters match the description of the genus *Carpetania* in all aspects but the following.

Average weight from 1.41–5.60 (mean = 3.9). Average number of segments from 204–323 (mean = 273). Generally longer than the other species: 'slender' appearance after fixation. Both pairs of spermathecae separated (or not) by septum 9/10, with variation between its internal lineages.

Diagnosis: Genital chaetae with elongated, narrow tip (Fig. 5). Diagnostic nucleotidic positions shown in Table S1.

Etymology: The name of this species refers to the Universidad Complutense de Madrid, the university in which the genus *Carpetania* was studied for close to three decades.

Remarks: This species includes three deeply divergent lineages. Further research on their range, ecology, and other characters could merit their recognition as subspecies.

Recommendations for species identification within *Carpetania*

Morphological study of traits and character states included in the descriptions and diagnoses of the different *Carpetania* species allow a preliminary approximation to assignment of individuals. However, there is overlap between the range of morphometric characters, and scanning electron microscopy imaging of genital chaetae is demanding for untrained researchers.

The most straightforward and effective approach to species identification would consist of sequencing one or more of the proposed reference molecular markers (COI, 16S-tRNAs, 28S, H3) and their comparison with the reference sequences provided in the descriptions. This would provide a reliable assignment to one of the species.

This method does not require expertise in earthworm taxonomy and should facilitate the inclusion of these species into community and soil ecology studies. Considering existing evidence of different ecological preferences between *Carpetania* species (personal communication), it is necessary that they are not bunched together in such analyses. It is also a requisite to evaluate the conservation status of the pseudocryptic species and to preserve the diversity of this endemic genus through conservation actions.

Carpetania within the context of the current 'cryptic species' debate

Several aspects of the accumulated knowledge on the *Carpetania* species complex can be discussed in the light of the recent works delving deeper in the evolutionary and conceptual meaning of cryptic species.

Fišer, Robinson, and Malard (2018) explored the relevance of recent divergence between cryptic species, finding it could explain only a small percentage of studied morphologically similar species. In the case of *Carpetania*, the three currently described species were estimated to have diverged more than 35 mya by Marchán et al. (2017): this can be considered a remarkably ancient divergence when compared with other species in the same family (most of them falling within the 10–15 mya range).

Struck et al. (2018) proposed the comparison of evolutionary rates between different species pairs within a lineage as a test for higher degrees of phenotypic similarity: in the case of the family Hormogastridae this was already explored in Novo et al. (2012) and Marchán, Novo, et al. (Marchán et al., 2016). Morphological disparity in some of the most frequently used characters in earthworm taxonomy (position of clitellum and tubercula pubertatis; position, number and type of spermathecae, number of typhlosole lamellae) was very high for other clades/genera (with a similar divergence time) when compared with *Carpetania*. This would lead to the acceptance of the species within *Carpetania* as cryptic according to the criterium proposed by Struck et al. (2018), even in the presence of some statistical morphological differences and scarce morphological diagnostic characters.

Korshunova et al. (2019) suggested that cryptic species would be expected to show high genetic similarity across the genome, with significant similarity in developmental genes explaining morphological similarity. Even though

this statement concerned recently diverged species, the evidence from *Carpetania* (with ancient diversification) contradicts that prediction according to Marchán et al. (2020) and this work. Widespread genomic divergence was found between its species, including more than 800 single nucleotid polymorphisms (several of them within developmental genes) with selection signatures and 35 diagnostic mutations of genes of diverse functions.

Another interesting conundrum presented by Korshunova et al. (2019) is the misuse or vague use of the cryptic species term for different degrees of morphological similarity *vs* genetic distinctiveness. In their study case (*Trinchesia caerulea* complex) morphological differences were identified *a priori* to molecular data (contradicting the concept of cryptic species being morphologically indistinguishable), but technical limitations and taxonomic framework ("lumper" mindset) at the time of original description precluded their identification as non-cryptic species. In *Carpetania*, its status as a cryptic/pseudo-cryptic/non-cryptic is also complex. The first work on *Carpetania* genetic diversity (Novo, Almodóvar, & Díaz Cosín, 2009) followed the *a priori* observation of differences in body size between the different populations. Due to incomplete sampling across the range and limited molecular marker data, these statistical differences could not be unequivocally assigned to putative species. At the time, another *a priori* hint of morphological differentiation was already available: the relative position of spermathecae and septum 9/10, but it was overlooked. It was after the implementation of scanning electron microscopy to the study of genital chaetae (Marchán, Novo, et al., 2016) that a truly diagnostic morphological character could be identified (Marchán et al., 2020).

To help with the inconsistent concept and terminology of cryptic speciation, Chenuil et al. (2019) proposed a framework for identification of cryptic species together with a practical and straightforward classification. This classification relies on the crossing of two criteria: genetic isolation and morphological differentiation.

In the case of *Carpetania*, genetic isolation was strongly supported by high genomic divergence and F_{ST}, Bayesian clustering and different phylogenetic methods. The next stage, biological species status, has not been formally demonstrated, but Marchán et al. (2017) performed a preliminary cross-breeding experiment between *Carpetania elisae* and *Carpetania matritensis* and found strong hints of pre- and post-copulatory reproductive isolation.

Regarding morphological differentiation, both statistical (average weight and number of segments) and diagnostic (shape of distal tip of genital chaetae) morphological differences have been described for the three species of *Carpetania*.

Crossing both criteria, *Carpetania* species would be considered cryptic species *sensu lato*, in need of taxonomic revision as new species. Thus, this work is in agreement with the framework of Chenuil et al. (2019), which appears as a promising guide for the systematized research and evaluation of putative cryptic diversity that has been shown to be pervasive across the animal kingdom.

Acknowledgements

This work was supported by Universidad Complutense de Madrid and Santander Group under grant Proyecto de Investigación Santander/Complutense PR41/17-21027; Systematics Research Fund (SRF) and Xunta de Galicia. Consellería de Cultura, Educación e Ordenación Universitaria. Secretaria Xeral de Universidades under grant ED431B 2019/038.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Supplemental data

Supplemental data for this article can be accessed here: https://dx.doi.org/10.1080/14772000.2020.1730474.

Funding

RF was funded by a Marie Skłodowska-Curie Fellowship (747607). DF was funded by a Juan de La Cierva-Formación grant (FJCI-2017-32895) from the Spanish Ministry of Sciences, Innovation and Universities. MN was funded by a Postdoctoral Fellowship UCM.

References

Anderson, C., Cunha, L., Sechi, P., Kille, P., & Spurgeon, D. (2017). Genetic variation in populations of the earthworm, *Lumbricus rubellus,* across contaminated mine sites. *BioMedCentral Genetics*, *18*, 97. doi:10.1186/s12863-017-0557-8

Álvarez J. (1977). El género Hormogaster en España. *Publicaciones del Centro Pirenaico de Biología Experimental*, *9*, 27–35.

Bickford, D., Lohman, D. J., Sodhi, N. S., Ng, P. K. L., Meier, R., Winker, K., … Das, I. (2007). Cryptic species as a window on diversity and conservation. *Trends in Ecology & Evolution*, *22*, 148–155. doi:10.1016/j.tree.2006.11.004

Brown, S. D. J., Collins, R. A., Boyer, S., Lefort, M.-C., Malumbres-Olarte, J., Vink, C. J., & Cruickshank, R. H. (2012). Spider: An R package for the analysis of species identity and evolution, with particular reference to DNA barcoding. *Molecular Ecology Resources*, *12*, 562–565. doi: 10.1111/j.1755-0998.2011.03108.x

Brunet, B. M. T., Blackburn, G. S., Muirhead, K., Lumley, L. M., Boyle, B., Lévesque, R. C., … Sperling, F. A. H. (2017). Two's company, three's a crowd: new insights on spruce budworm species boundaries using genotyping-by-sequencing in an integrative species assessment (Lepidoptera: Tortricidae). *Systematic Entomology*, *42*, 317–328. doi:10.1111/syen.12211

Buckley, T. R., James, S., Allwood, J., Bartlam, S., Howitt, R., & Prada, D. (2011). Phylogenetic analysis of New Zealand earthworms (Oligochaeta: Megascolecidae) reveals ancient clades and cryptic taxonomic diversity. *Molecular Phylogenetics and Evolution*, *58*, 85–96. doi:10.1016/j.ympev.2010.09.024

Chang, C.-H., Lin, S.-M., & Chen, J.-H. (2008). Molecular systematics and phylogeography of the gigantic earthworms of the *Metaphire formosae* species group (Clitellata, Megascolecidae). *Molecular Phylogenetics and Evolution*, *49*, 958–968. doi:10.1016/j.ympev.2008.08.025

Chenuil, A., Cahill, A. E., Délémontey, N., Du Luc, E. D. S., & Fanton, H. (2019). Problems and Questions Posed by Cryptic Species. A Framework to Guide Future Studies. In *From Assessing to Conserving Biodiversity* (pp. 77–106). Cham: Springer.

Cullingham, C. I., Cooke, J. E. K., Dang, S., & Coltman, D. W. (2013). A species-diagnostic SNP panel for discriminating lodgepole pine, jack pine, and their interspecific hybrids. *Tree Genetics & Genomes*, *9*, 1119–1127. doi:10.1007/s11295-013-0608-x

Decaëns, T., Porco, D., Rougerie, R., Brown, G. G., & James, S. W. (2013). Potential of DNA barcoding for earthworm research in taxonomy and ecology. *Applied Soil Ecology*, *65*, 35–42. doi:10.1016/j.apsoil.2013.01.001

Dixon, P. (2003). VEGAN, a package of R functions for community ecology. *Journal of Vegetation Science*, *14*, 927–930. doi:10.1111/j.1654-1103.2003.tb02228.x

Duforet-Frebourg, N., Bazin, E., & Blum, M. G. B. (2014). Genome scans for detecting footprints of local adaptation using a Bayesian factor model. *Molecular Biology and Evolution*, *31*, 2483–2495. doi:10.1093/molbev/msu182

Dupont, L., Porco, D., Symondson, W. O. C., & Roy, V. (2016). Hybridization relics complicate barcode-based identification of species in earthworms. *Molecular Ecology Resources*, *16*, 883–894. doi:10.1111/1755-0998.12517

Earl, D. A., & vonHoldt, B. M. (2012). STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, *4*, 359–361. doi: 10.1007/s12686-011-9548-7

Elshire, R. J., Glaubitz, J. C., Sun, Q., Poland, J. A., Kawamoto, K., Buckler, E. S., & Mitchell, S. E. (2011). A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. *Plos One*, *6*, e19379. doi:10.1371/journal.pone.0019379

Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology*, *14*, 2611–2620. doi:10.1111/j.1365-294X.2005.02553.x

Fernández, R., Almodóvar, A., Novo, M., Simancas, B., & Díaz Cosín, D. J. (2012). Adding complexity to the complex: new insights into the phylogeny, diversification and origin of parthenogenesis in the *Aporrectodea caliginosa* species complex (Oligochaeta, Lumbricidae). *Molecular Phylogenetics and Evolution*, *64*, 368–379. doi: 10.1016/j.ympev.2012.04.011

Fišer, C., Robinson, C. T., & Malard, F. (2018). Cryptic species as a window into the paradigm shift of the species concept. *Molecular Ecology*, *27*, 613–635. doi:10.1111/mec.14486

Flanagan, S. P., & Jones, A. G. (2017). Constraints on the FST–Heterozygosity Outlier Approach. *Journal of Heredity*, *108*, 561–573. doi:10.1093/jhered/esx048

Ganin, G. N., & Atopkin, D. M. (2018). Molecular differentiation of epigeic and anceic forms of *Drawida ghilarovi* Gates, 1969 (Moniligastridae, Clitellata) in the Russian Far East: Sequence data of two mitochondrial genes. *European Journal of Soil Biology*, *86*, 1–7. doi:10.1016/j.ejsobi.2018.02.004

Garg, K. M., Tizard, R., Ng, N. S. R., Cros, E., Dejtaradol, A., Chattopadhyay, B., … Rheindt, F. E. (2016). Genome-wide data help identify an avian species-level lineage that is morphologically and vocally cryptic. *Molecular Phylogenetics and Evolution*, *102*, 97–103. doi:10.1016/j.ympev.2016.05.028

Giska, I., Sechi, P., & Babik, W. (2015). Deeply divergent sympatric mitochondrial lineages of the earthworm *Lumbricus rubellus* are not reproductively isolated. *BioMed Central Evolutionary Biology*, *15*, 217. doi:10.1186/s12862-015-0488-9

Haas, B. J., Papanicolaou, A., Yassour, M., Grabherr, M., Blood, P. D., Bowden, J., … Regev, A. (2013). De novo transcript sequence reconstruction from RNA-seq using the Trinity platform for reference generation and analysis. *Nature Protocols*, *8*, 1494–1512. doi:10.1038/nprot.2013.084

Hand, B. K., Hether, T. D., Kovach, R. P., Muhlfeld, C. C., Amish, S. J., Boyer, M. C., … Luikart, G. (2015). Genomics and introgression: Discovery and mapping of thousands of species-diagnostic SNPs using RAD sequencing. *Current Zoology*, *61*, 146–154. doi:10.1093/czoolo/61.1.146

Hebert, P. D. N., Ratnasingham, S., & de Waard, J. R. (2003). Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *270*, 96–99. doi:10.1098/rsbl.2003.0025

Heethoff, M. (2018). Cryptic species – Conceptual or terminological chaos? A response to Struck et al. *Trends in Ecology & Evolution*, *33*, 310. doi:10.1016/j.tree.2018.02.006

Huerta-Cepas, J., Forslund, K., Coelho, L. P., Szklarczyk, D., Jensen, L. J., von Mering, C., & Bork, P. (2017). Fast Genome-Wide Functional Annotation through Orthology Assignment by eggNOG-Mapper. *Molecular Biology and Evolution*, *34*, 2115–2122. doi:10.1093/molbev/msx148

James, S. W., Porco, D., Decaëns, T., Richard, B., Rougerie, R., & Erséus, C. (2010). DNA barcoding reveals cryptic diversity in *Lumbricus terrestris* L., 1758 (Clitellata): resurrection of L. herculeus (Savigny, 1826). *Public Library of Science One*, *5*, e15629. doi:10.1371/journal.pone.0015629

Jombart, T. (2008). adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics*, *24*, 1403–1405. doi:10.1093/bioinformatics/btn129

Jombart, T., & Ahmed, I. (2011). adegenet 1.3-1: new tools for the analysis of genome-wide SNP data.

Bioinformatics. *Bioinformatics*, *27*, 3070–3071. doi:10.1093/bioinformatics/btr521

Jörger, K. M., & Schrödl, M. (2013). How to describe a cryptic species? Practical challenges of molecular taxonomy. *Frontiers in Zoology*, *10*, 59. doi:10.1186/1742-9994-10-59

King, R. A., Andrew King, R., Tibble, A. L., & Symondson, W. O. C. (2008). Opening a can of worms: unprecedented sympatric cryptic diversity within British lumbricid earthworms. *Molecular Ecology*, *17*, 4684–4698. doi:10.1111/j.1365-294X.2008.03931.x

Knowlton, N. (1993). Sibling Species in the Sea. *Annual Review of Ecology and Systematics*, *24*, 189–216. doi:10.1146/annurev.es.24.110193.001201

Koene, J. M., Pförtner, T., & Michiels, N. K. (2005). Piercing the partner's skin influences sperm uptake in the earthworm *Lumbricus terrestris*. *Behavioral Ecology and Sociobiology*, *59*, 243–249. doi:10.1007/s00265-005-0030-y

Korshunova, T., Picton, B., Furfaro, G., Mariottini, P., Pontes, M., Prkić, J., … Martynov, A. (2019). Multilevel fine-scale diversity challenges the 'cryptic species' concept. *Scientific Reports*, *9*, 6732. doi:10.1038/s41598-019-42297-5

Lajus, D., Sukhikh, N., & Alekseev, V. (2015). Cryptic or pseudocryptic: can morphological methods inform copepod taxonomy? An analysis of publications and a case study of theEurytemora affinisspecies complex. *Ecology and Evolution*, *5*, 2374–2385. doi:10.1002/ece3.1521

León, G. P.-P D., de León, G. P.-P., & Nadler, S. A. (2010). What We Don't Recognize Can Hurt Us: A Plea for Awareness About Cryptic Species. *Journal of Parasitology*, *96*, 453–464. doi:10.1645/GE-2260.1

Lewis, P. O. (2001). A likelihood approach to estimating phylogeny from discrete morphological character data. *Systematic Biology*, *50*, 913–925. doi:10.1080/106351501753462876

Marchán, D. F., Fernández, R., de Sosa, I., Díaz Cosín, D. J., & Novo, M. (2017). Pinpointing cryptic borders: Fine-scale phylogeography and genetic landscape analysis of the *Hormogaster elisae* complex (Oligochaeta, Hormogastridae). *Molecular Phylogenetics and Evolution*, *112*, 185–193. doi:10.1016/j.ympev.2017.05.005

Marchán, D. F., Fernández, R., de Sosa, I., Sánchez, N., Díaz Cosín, D. J., & Novo, M. (2018). Integrative systematic revision of a Mediterranean earthworm family: Hormogastridae (Annelida, Oligochaeta). *Invertebrate Systematics*, *32*, 652. doi:10.1071/IS17048

Marchán, D. F., Novo, M., Fernández, R., de Sosa, I., Trigo, D., & Díaz Cosín, D. J. (2016). Evaluating evolutionary pressures and phylogenetic signal in earthworms: a case study - the number of typhlosole lamellae in Hormogastridae (Annelida, Oligochaeta). *Zoological Journal of the Linnean Society*, *178*, 4–14. doi:10.1111/zoj.12410

Marchán, D. F., Novo, M., Sánchez, N., Domínguez, J., Díaz Cosín, D. J., & Fernández, R. (2020). Local adaptation fuels cryptic speciation in terrestrial annelids. *Molecular phylogenetics and evolution*, *146*, 106767. doi:10.1016/j.ympev.2020.106767

Marchán, D. F., Sánchez, N., Novo, M., Fernández, R., Pardos, F., & Díaz Cosín, D. J. (2016). Cryptic characters for cryptic taxa: On the taxonomic utility of the genital chaetae in earthworms (Oligochaeta, Hormogastridae). *Zoologischer Anzeiger - A Journal of Comparative Zoology*, *264*, 17–28. doi:10.1016/j.jcz.2016.06.008

Novo, M., Almodóvar, A., & Díaz Cosín, D. J. (2009). High genetic divergence of hormogastrid earthworms (Annelida, Oligochaeta) in the central Iberian Peninsula: evolutionary and demographic implications. *Zoologica Scripta*, *38*, 537–552. doi:10.1111/j.1463-6409.2009.00389.x

Novo, M., Almodóvar, A., Fernández, R. M., Gutiérrez, M., & Díaz Cosín, D. J. (2010). Mate choice of an endogeic earthworm revealed by microsatellite markers. *Pedobiologia*, *53*, 375–379. doi:10.1016/j.pedobi.2010.07.002

Novo, M., Almodóvar, A., Fernández, R., Trigo, D., & Díaz Cosín, D. J. (2010). Cryptic speciation of hormogastrid earthworms revealed by mitochondrial and nuclear data. *Molecular Phylogenetics and Evolution*, *56*, 507–512. doi:10.1016/j.ympev.2010.04.010

Novo, M., Almodóvar, A., Fernández, R., Trigo, D., Díaz Cosín, D. J., & Giribet, G. (2012). Appearances can be deceptive: different diversification patterns within a group of Mediterranean earthworms (Oligochaeta, Hormogastridae). *Molecular Ecology*, *21*, 3776–3793. doi:10.1111/j.1365-294X.2012.05648.x

Nygren, A. (2014). Cryptic polychaete diversity: a review. *Zoologica Scripta*, *43*, 172–183. doi:10.1111/zsc.12044

Pfenninger, M., & Schwenk, K. (2007). Cryptic animal species are homogeneously distributed among taxa and biogeographical regions. BioMedCentral Evolutionary Biology, *7*, 121doi:10.1186/1471-2148-7-121

Pons, J., Barraclough, T. G., Gomez-Zurita, J., Cardoso, A., Duran, D. P., Hazell, S., … Vogler, A. P. (2006). Sequence-based species delimitation for the DNA taxonomy of undescribed insects. *Systematic Biology*, *55*, 595–609. doi:10.1080/10635150600852011

Porco, D., Chang, C.-H., Dupont, L., James, S., Richard, B., & Decaëns, T. (2018). A reference library of DNA barcodes for the earthworms from Upper Normandy: Biodiversity assessment, new records, potential cases of cryptic diversity and ongoing speciation. *Applied Soil Ecology*, *124*, 362–371. doi:10.1016/j.apsoil.2017.11.001

Porto, P. G. (2014). La selección sexual post-cópula en la lombriz roja (Eisenia andrei Bouché, 1972). (Doctoral dissertation), Universidade de Vigo.

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, *155*, 945–959.

Puillandre, N., Lambert, A., Brouillet, S., & Achaz, G. (2012). ABGD, Automatic Barcode Gap Discovery for primary species delimitation. *Molecular Ecology*, *21*, 1864–1877. doi:10.1111/j.1365-294X.2011.05239.x

Queiroz, K. D., & De Queiroz, K. (2007). Species Concepts and Species Delimitation. *Systematic Biology*, *56*, 879–886. doi:10.1080/10635150701701083

Rancilhac, L., Goudarzi, F., Gehara, M., Hemami, M.-R., Elmer, K. R., Vences, M., & Steinfarz, S. (2019). Phylogeny and species delimitation of near Eastern Neurergus newts (Salamandridae) based on genome-wide RADseq data analysis. *Molecular Phylogenetics and Evolution*, *133*, 189–197. doi:10.1016/j.ympev.2019.01.003

Rochette, N. C., Rivera-Colón, A. G., & Catchen, J. M. (n.d). *Stacks 2: Analytical Methods for Paired-end Sequencing Improve RADseq-based Population Genomics*. doi:10.1101/615385

Rougerie, R., Decaëns, T., Deharveng, L., Porco, D., James, S. W., Chang, C.-H., … Hebert, P. D. N. (2009). DNA barcodes for soil animal taxonomy. *Pesquisa Agropecuária Brasileira*, *44*, 789–802. doi:10.1590/S0100-204X2009000800002

Shekhovtsov, S. V., Golovanova, E. V., & Peltek, S. E. (2013). Cryptic diversity within the Nordenskiold's earthworm, *Eisenia nordenskioldi* subsp. *nordenskioldi* (Lumbricidae, Annelida). *European Journal of Soil Biology*, *58*, 13–18. doi:10.1016/j.ejsobi.2013.05.004

Stamatakis, A. (2014). RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, *30*, 1312–1313. doi:10.1093/bioinformatics/btu033

Struck, T. H., Feder, J. L., Bendiksby, M., Birkeland, S., Cerca, J., Gusarov, V. I., … Stedje, B. (2018). Cryptic species–more than terminological chaos: a reply to Heethoff. *Trends in Ecology & Evolution*, *33*, 310–312.

Taheri, S., James, S., Roy, V., Decaëns, T., Williams, B. W., Anderson, F., … Dupont, L. (2018). Complex taxonomy of the "brush tail" peregrine earthworm Pontoscolex corethrurus. *Molecular Phylogenetics and Evolution*, *124*, 60–70.

Trontelj, P., & Fišer, C. (2009). Perspectives: Cryptic species diversity should not be trivialised. *Systematics and Biodiversity*, *7*, 1–3. doi:10.1017/S1477200008002909

Wang, Y., Zhou, Q.-S., Qiao, H.-J., Zhang, A.-B., Yu, F., Wang, X.-B., … Zhang, Y.-Z. (2016). Formal nomenclature and description of cryptic species of the *Encyrtus sasakii* complex (Hymenoptera: Encyrtidae). *Scientific Reports*, *6*, 34372. doi:10.1038/srep34372

Yang, Z. (2015). The BPP program for species tree estimation and species delimitation. *Current Zoology*, *61*, 854–865. doi:10.1093/czoolo/61.5.854

Yang, Z., & Rannala, B. (2010). Bayesian species delimitation using multilocus sequence data. *Proceedings of the National Academy of Sciences*, *107*, 9264–9269. doi:10.1073/pnas.0913022107

Zhang, J., Kapli, P., Pavlidis, P., & Stamatakis, A. (2013). A general species delimitation method with applications to phylogenetic placements. *Bioinformatics*, *29*, 2869–2876. doi:10.1093/bioinformatics/btt499

Associate Editor: Christoph Bleidorn