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SHORT COMMUNICATION

Diet study of geckos reveals the first records of pseudoscorpions on Desertas Islands (Cabo Verde)

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Abstract. Pseudoscorpions are known worldwide and yet are poorly studied mainly due to the difficulty of detecting them. Among their predators are ground-dwelling taxa, such as arthropods, amphibians, birds, and reptiles. Only four pseudoscorpion species are known to occur in the Cabo Verde Archipelago, and none in the Desertas Islands, located in the northwest of the country. In this study, we record the first two species for the Desertas Islands. We used molecular and morphological methods to taxonomically identify the specimens retrieved from reptile faecal pellets and pitfall traps. We identified the presence of *Garypus* cf. *saxicola* on Raso Islet, *Olpium pallipes* (Lucas, 1849) on Raso and Santa Luzia Island, and a putative new species of *Olpium* L. Koch, 1873 on Branco Islet. This study emphasizes how an indirect measure of biodiversity and ecological interactions via potential predators, using non-invasive sampling combined with metabarcoding and morphological studies, can be used to uncover unknown biodiversity, particularly of cryptic groups from highly inaccessible locations. Likewise, this study highlights the lack of 16S genetic resources for pseudoscorpions in online reference databases.

Keywords: Arachnida, genetics, morphology, Pseudoscorpiones, Macaronesia

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Pseudoscorpions are small invertebrates (frequently 0.5–5 mm length) that usually occur in low numbers in cryptic habitats, which makes them hard to detect. The ca. 3,700 described species occur worldwide, except in Antarctica, but are more diverse in the tropics and subtropics (Dunlop & Penney 2012; Harvey 2013; Zaragoza 2017). While found in nearly all terrestrial habitats, they are more common in cracks, leaf litter, soil, moss, under stones, in tree hollows, in caves or in nests of birds and mammals (Harvey 1988). Among their predators are mainly ground-dwelling taxa, such as arthropods, amphibians, reptiles, and birds. Vertebrates can have 0.2–6% of their diet composed of pseudoscorpions (Zaragoza & Seva Román 1990). Small reptiles seem to prey on them in higher proportions, even positively selecting them in their diet (Zaragoza & Seva Román 1990).

The Cabo Verde Archipelago (Republic of Cabo Verde), located in the Atlantic Ocean about 500 km off the African coast, comprises ten main oceanic islands and several islets of volcanic origin (Mitchell-Thomé 1976). The Desertas Islands (Santa Luzia, Branco and Raso) are located on the northwest alignment of the archipelago (Fig. 1A). In Cabo Verde, only four pseudoscorpion species of three families, all considered native, have been recorded, in five islands (Table 1, Fig. 1A): *Paratemnoides feai* (Ellingsen, 1906), *Withius simoni* (Balzan, 1892), *Olpium pallipes* (Lucas, 1849), and *Parolpium minor* (Ellingsen, 1910) (*P. minor* not shown in Fig. 1A; see Table 1) (Dippenaar-Schoeman & Harvey 2000; Arechavaleta et al. 2005; Harvey 2013). An unidentified specimen was recently found on São Vicente Island (Vasconcelos et al. 2009), but until this study there were no records of pseudoscorpions on the uninhabited Desertas Islands.

Here, we present the first record of pseudoscorpions for the Desertas Islands. These specimens were obtained during a study of the diet of the Cabo Verde giant wall gecko *Tarentola gigas* (Bocage, 1875). One pseudoscorpion was found within a fresh faecal pellet from a gecko captured on Raso on 29 July 2016, out of the 62 pellets collected by performing belly massages. A previous study, carried out at São Vicente Island and focused on the diet of the São Vicente wall gecko *Tarentola substituta* (Joger, 1984), also found remains of unidentified pseudoscorpions in several samples (Vasconcelos et al. 2009), indicating they might be common in the diet of Cabo Verde endemic geckos. The remaining six pseudoscorpions were caught in pitfall traps in July 2016 (Raso) and September 2017 (Branco) that were set to build a reference collection of invertebrates from those islands (Table 1, Fig. 1). All specimens were preserved in 70% ethanol until morphological and genetic identification. Several images of the seven specimens were taken through a magnifying glass and deposited in Morphobank (M681498–M681526). The following measurements of the pedipalps were taken from the photographs, for further morphological identification after Beier (1963), using ImageJ 1.47 software (online at http://imagej.nih.gov/ij/): femur length (FL) and width (FW), patella length (PL) and width (PW), chela length with pedicel (CL), chela width (CW), and fixed finger length (FiL). DNA was extracted from the whole faecal pellet using a Stool DNA Isolation Kit, following the manufacturer's instructions, and from a portion of the individuals from the pitfalls using saline extraction. A short 16S (~110 base pairs, bp) and COI (~710 bp) mitochondrial fragments were amplified and sequenced [see details in Pinho et al. (2018)] using an Illumina MiSeq (16S) and Sanger (COI) sequencing, for the faecal pellet and pitfall individuals, respectively. A Maximum likelihood (ML) tree was built using our COI sequences and sequences from Harvey et al. (2020) available in GenBank (Fig. 1B), following Vasconcelos et al. (2016).

The barcoded 16S sequence from the pellet returned only one hit with megablast search of the GenBank database (online at https://www.ncbi.nlm.nih.gov/) with 96.97% identity match with the scorpion *Diplocentrus sagittipalpus* Santibanez-Lopez, Francke & Prendini, 2013 (Scorpiones: Diplocentrinae), but with only 30% of query cover. Blastn results were overall inconclusive, with all matches showing < 85% similarity to different arthropods classes. This could be explained by contamination or the paucity of pseudoscorpion 16S

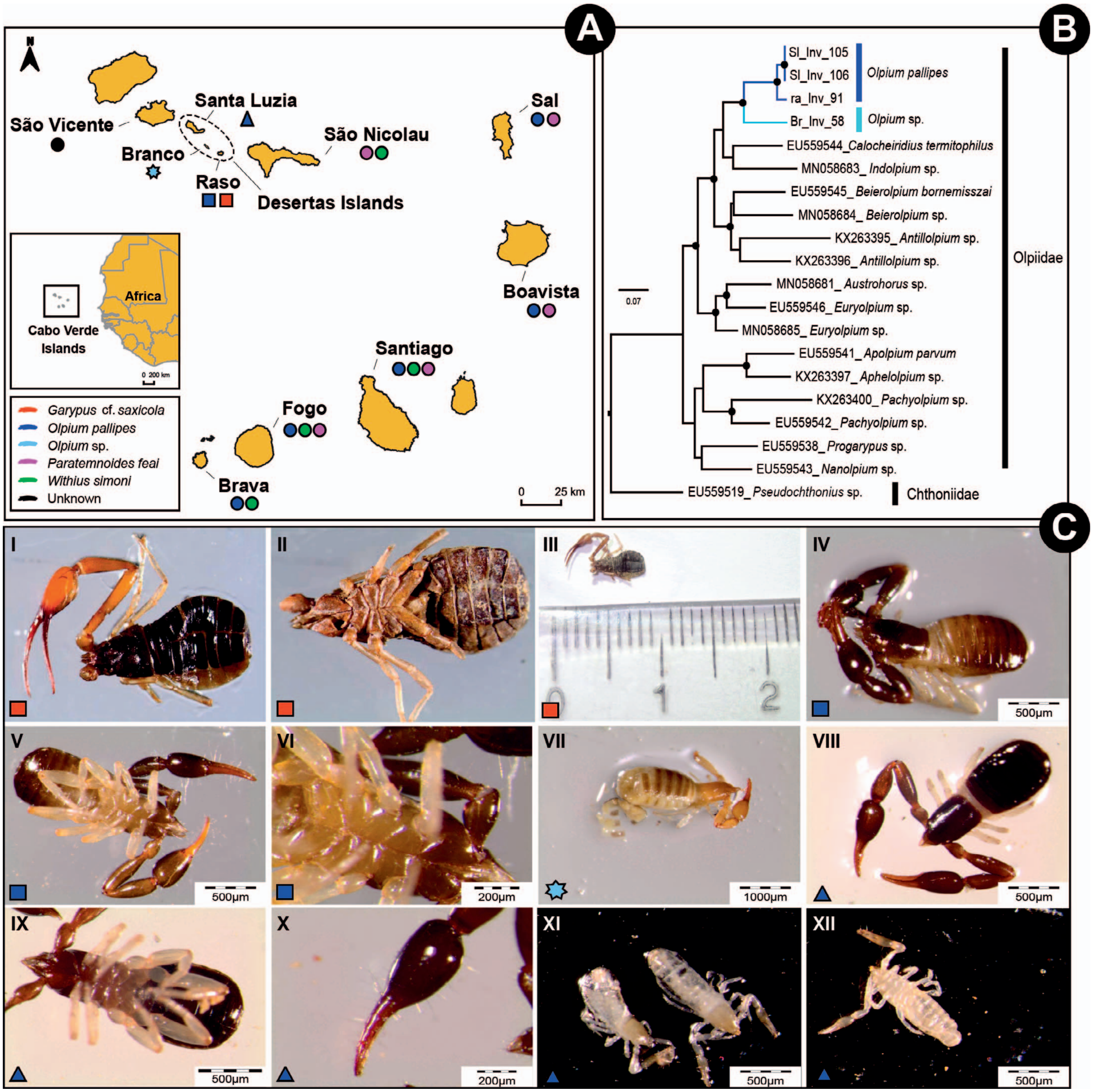

Figure 1.—Pseudoscorpion diversity of Cabo Verde. (A) Map of the archipelago showing the previously recorded (circles) and new occurrences (other symbols) of pseudoscorpions across the islands; dashed ellipse highlights our study site, the Desertas Islands; occurrences of *Parolpium minor* not shown (see Table 1); (B) Maximum likelihood phylogenetic tree (GTR+G model) of available Olpiidae sequences; black dots indicate bootstrap values $>$ 70%; (C) Photos of the pseudoscorpion specimens found on Desertas, Cabo Verde in 2016 and 2017 (colours and shapes on the left corner correspond to taxa and islands in the map): (I) *Garypus* cf. *saxicola* (found within a faecal pellet) from Raso Islet, Chã Branca (16.617723°N, 24.602239°W; specimen Tg_ra_07.80), dorsal view, (II) ventral view and (III) size detail of same specimen; (IV) *Olpium pallipes* from Raso Islet, Chã do Castelo (16.617110°N, 24.586627°W; specimen ra_Inv_91) dorsal view, (V) ventral view and (VI) pedipalp detail; (VII) *Olpium* sp. from Branco Islet, Ponta Parede – Tope Berta (16.65071°N, 24,66607°W; specimen Br_Inv_58), dorsal view; (VIII) *Olpium pallipes* from Santa Luzia Island (16.764°N, 24.743°W; SL_Inv_105), dorsal view, (IX) ventral view and (X) pedipalp detail; (XI) nymphs of *Olpium pallipes* from Santa Luzia Island (specimens SL_Inv_106 I and II), dorsal and (XII) ventral view.

Table 1.—Pseudoscorpion diversity of Cabo Verde Archipelago. Taxonomic details, distribution, values of pedipalpal femur length (FL) and width (FW), patella length (PL) and width (PW), chela length with pedicel (CL), chela width (CW), and fixed finger length (FiL) in mm, location – São Vicente (SV), Raso (Ra), Branco (Br), Santa Luzia (SL), São Nicolau (SN), Sal (S), Boavista (BV), Santiago (ST), Fogo (F), Brava (B), the mitochondrial marker sequenced and Morphobank and GenBank codes are also given. Samples marked with an (*) correspond to juveniles. Old records were retrieved from Arechavaleta et al. (2005), Vasconcelos et al. (2009), and Harvey (2013).

Superfamily	Family	Species	Sample Code	Location	FL	FW	PL	PW	CL	CW	FiL	Marker	Morphobank	GenBank
Garypoidea	Garypidae	*Garypus* cf. *saxicola*	Tg_ra_07.80	Ra	2.75	0.44	1.62	0.44	3.80	0.87	2.35	16S	M681498–M681501	-
Garypoidea	Olpiidae	*Olpium pallipes*	ra_Inv_91	Ra	0.59	0.17	0.55	0.19	0.93	0.30	0.49	COI	M681503–M681506	MW760701
Garypoidea	Olpiidae	*Olpium* sp.	Br_Inv_58	Br	0.67	0.18	0.54	0.21	1.01	0.32	0.55	COI	M681526; M693785	MW760700
Garypoidea	Olpiidae	*Olpium pallipes*	SL_Inv_105_I	SL	0.63	0.19	0.59	0.22	1.11	0.34	0.54	COI	M681507–M681511; M693786	MW760702
Garypoidea	Olpiidae	*Olpium pallipes*	SL_Inv_105_II	SL	0.63	0.17	0.57	0.20	1.01	0.30	0.53	-	M681512–M681514	-
Garypoidea	Olpiidae	*Olpium pallipes*	SL_Inv_106_I*	SL	-	-	-	-	-	-	-	COI	M681523–M681524	MW760703
Garypoidea	Olpiidae	*Olpium pallipes*	SL_Inv_106_II*	SL	-	-	-	-	-	-	-	-	M681525	-
Garypoidea	Olpiidae	*Olpium pallipes,*	-	S, BV, ST, F, B	-	-	-	-	-	-	-	-	-	-
Garypoidea	Olpiidae	*Parolpium minor*	-	Cabo Verde	-	-	-	-	-	-	-	-	-	-
Cheliferoidea	Atemnidae	*Paratemnoides feai*	-	SN, S, BV, ST, F	-	-	-	-	-	-	-	-	-	-
Cheliferoidea	Withiidae	*Withius simoni*	-	SN, ST, F, B	-	-	-	-	-	-	-	-	-	-
-	-	-	-	SV	-	-	-	-	-	-	-	-	-	-

sequences in GenBank. The exoskeleton of the specimen from the pellet was mostly intact but very dry and brittle, missing one pedipalp and some legs. Interior body content was missing, presumably due to digestion by the gecko. The success of amplification and sequencing of digested diet items depends mostly on the frequency of occurrence of each item. Therefore, the likelihood of detecting low frequency items such as pseudoscorpions in the pellets of geckos with genetic methods is rather low. However, even though its morphological identification was problematic due to its deterioration, it was tentatively identified as *Garypus* cf. *saxicola* Waterhouse, 1878 (Garypidae) (Fig. 1C: I–III), based on characteristic traits such as triangular carapace, eyes on ocular tubercles situated away from anterior margin of carapace, fingers of pedipalps without accessory teeth, femora of legs I and II longer than the patellae, short arolium, and measurements of the body and the pedipalps (Table 1). This is the first time this family is recorded in the Cabo Verde Islands. Species of Garypidae occur in a wide variety of habitats, but the genus *Garypus* L. Koch, 1873 is more commonly found close to seashores and associated with halophilic vegetation (Harvey et al. 2020; Zaragoza 2007). Due to the small size of the Desertas Islands, the seashore is a predominant habitat where halophilic vegetation thrives (Freitas et al. 2015; Lobin 2015). Altogether, 31 species of the genus *Garypus* are recognized worldwide, but only two occur on the east Atlantic coast (Harvey 2013; Harvey et al. 2020). Hence, considering that natural colonisations of the Cabo Verde Islands by pseudoscorpions would have to be through marine dispersal, using the Canary current, it is logical to assume continental Iberia and the Atlantic islands as probable source areas. *Garypus saxicola* was previously known only from Iberia (Helversen 1965; Zaragoza 2007) with two subspecies: *G. saxicola saxicola* Waterhouse, 1878 from continental Iberia and the Canary Islands, and *G. saxicola salvagensis* Helversen, 1965 from the Selvagens Islands, both North Atlantic archipelagos. It should be noted that the measurements of the pedipalps of our sample (Table 1) slightly differed from those two taxa (FL = 2.22–2.42 mm, FW = 0.50–0.53 mm, PL = 1.80–1.88 mm, PW = 0.50–0.54 mm (Beier 1963; Mahnert 2011), and FL = 2.33–2.40 mm, FW = 0.47–0.50 mm, PL = 1.76–2.00 mm, PW = 0.51–0.62 mm (Helversen 1965), respectively) and we cannot exclude the possibility of an introduction. Additionally, two other *Garypus* species are known from nearby regions: *Garypus beauvoisii* (Audouin, 1826) in the Canary Islands, and *Garypus titanius* Beier, 1961 in Ascension Island in the South Atlantic (Harvey 2013; Harvey et al. 2020). Both species have a distinctly different size of the pedipalps compared to our specimen (FL = 1.73 mm, FW = 0.40 mm, PL = 1.40 mm, PW = 0.42 mm (Beier 1963); FL = 3.20–3.35 mm, FW = 0.63–0.71 mm, PL = 2.52–2.65 mm, PW = 0.68–0.72 mm (Beier 1960), respectively; Table 1). Some pseudoscorpions exhibit phoretic behaviour, where individuals attach to other organisms such as other arachnids, insects, mammals or birds, which facilitates their fast transport to different areas (e.g., Opatova & Šťáhlavský 2018). However, within the family Garypidae, there is no evidence of phoresy (Poinar et al. 1998). Our specimen could thus represent a putative cryptic new species, since the majority of known *Garypus* species are endemic to small areas and only a few have wide distribution ranges (Harvey et al. 2020). Therefore, without a genetic comparison with the other Macaronesian specimens, it is difficult to say whether this new record represents an addition to the occurrence range of known *Garypus* species or a new taxon.

The COI sequences of specimens collected from the pitfall traps were all assigned to the family Olpiidae (superfamily Garypoidea), with a 79.0–81.9% identity match. The ML tree supported the four specimens as a monophyletic clade within Olpiidae (Fig. 1B). Even though this family has been found previously in Cabo Verde (Arechavaleta et al. 2005), this is the first record for Desertas. The morphological identification confirmed that all specimens belonged to the genus *Olpium* L. Koch, 1873, which contains 34 described species distributed across Africa, southern Europe, Asia and Australia

(Harvey 2013). The specimens from Raso (Fig. 1C: IV–VI) and Santa Luzia (Fig. 1C: VIII–X) were identified as *O. pallipes*, as they possess the smooth and bicolored pedipalps that are characteristic of this species. Moreover, measurements of the sample SL_Inv_105 (Table 1) fully corresponded to this species (Beier 1963). *Olpium pallipes* has a wide distribution range, especially in the Mediterranean region, including North Africa, and it was previously recorded in Cabo Verde (Arechavaleta et al. 2005; Harvey 2013). It should be noted that cytogenetic analysis of this species demonstrated great differences between the karyotypes of individuals from Portugal and Greece (Šťáhlavský et al. 2006). Hence, a detailed taxonomical revision of this species is necessary, as cryptic species might be present.

The sample from Branco was not possible to identify to species level (Fig. 1C: VII). This *Olpium* sp. possessed a unicolored pedipalp (yellowish-brown), with only little granulation on the small, distal part of the pedipalp femur. The measurements of the female from Branco are similar to those for specimen SL_Inv_105_I (see Table 1), however, the position of the trichobothrium *st* on the movable finger of pedipalp was distinctly nearer to *t* than to *sb*, which differs from *O. pallipes* (M693785; M693786). Thus, more individuals both from Cabo Verde and other unsampled areas, such as the West African coast, are necessary to test if that is due to intraspecific variability of *O. pallipes* or if it is a diagnostic feature of a new species.

In conclusion, since arachnid research is often focused on scorpions and spiders, it is important to bridge the gap in our knowledge on pseudoscorpions, especially on understudied islands. Our study is, to our best knowledge, the first to use faeces to indirectly sample pseudoscorpions. Whether their presence in the pellets results from active predation—as pseudoscorpions of the superfamily Garypoidea also live under the same rocks as the gecko—or from indirect consumption, is still to be clarified. Additionally, our findings demonstrated the capacity of trans-oceanic dispersal of some pseudoscorpion families that deserves further research. Finally, it emphasizes how the combination of traditional surveys with metabarcoding and morphometrics can be used to gain more insights into known taxa and to uncover unknown biodiversity, particularly of cryptic groups and from infrequently accessed locations.

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LITERATURE CITED

Arechavaleta M, Zurita N, Marrero M, Martín J. 2005. Lista preliminar de especies silvestres de Cabo Verde (hongos, plantas y animales terrestres) (Consejería de Medio Ambiente y Ordenación, Gobierno de Canarias.

Beier M. 1963. Ordnung Pseudoscorpionidea (Afterskorpione). *Bestimmungsbucher zur Bodenfauna Europas* 1:1–313.

Beier VM. 1960. Pseudoscorpione von der Insel Ascension. *Journal of Natural History* 3:593–598.

Dippenaar-Schoeman A, Harvey M. 2000. A check list of the pseudoscorpions of South Africa (Arachnida: Pseudoscorpiones). *Koedoe : African Protected Area Conservation and Science* 43:89–102.

Dunlop JA, Penney D. 2012. Fossil arachnids, Volume 2. Siri Scientific Press, Manchester, UK.

Freitas R, Hazevoet CJ, Vasconcelos R. 2015. Geography and geology. Pp. 14–36. *In* The Natural History of the Desertas Islands - Santa Luzia, Branco e Raso. (R Vasconcelos, R Freitas, CJ Hazevoet, Eds.). Sociedade Caboverdeana de Zoologia.

Harvey M. 2013. Pseudoscorpions of the World, version 3.0. Western Australian Museum, Perth. Online at http://www.museum.wa.gov.au/catalogues/pseudoscorpions, accessed on October 2020.

Harvey MS. 1988. The systematics and biology of pseudoscorpions. Pp. 75–85. *In* Australian Arachnology. (AD Austin, NW Heather, eds.). Australian Entomological Society, Brisbane, Australia.

Harvey MS, Hillyer MJ, Carvajal JI, Huey JA. 2020. Supralittoral pseudoscorpions of the genus *Garypus* (Pseudoscorpiones : Garypidae) from the Indo-West Pacific region, with a review of the subfamily classification of Garypidae. *Invertebrate Systematics* 34:34–87.

Helversen VOV. 1965. Scientific expedition to the Salvage Islands, July 1963. VI. Einige Pseudoskorpione von den Ilhas Selvagens. *Boletim do Museu Municipal do Funchal* XIX:95–103.

Lobin W. 2015. Vegetation. Pp. 120–139. *In* The Natural History of the Desertas Islands - Santa Luzia, Branco e Raso. (R Vasconcelos, R Freitas, CJ Hazevoet, Eds.). Sociedade Caboverdeana de Zoologia, Porto, Portugal.

Mahnert V. 2011. A nature's treasury: Pseudoscorpion diversity of the Canary Islands, with the description of nine new species (Pseudoscorpiones, Chthoniidae, Cheiridiidae) and new records. *Revista Ibérica de Aracnología* 19:27–45.

Mitchell-Thomé RC. 1976. Geology of the Middle Atlantic islands. Lubrecht & Cramer Ltd.

Opatova V, Šťáhlavský F. 2018. Phoretic or not? Phylogeography of the pseudoscorpion *Chernes hahnii* (Pseudoscorpiones: Chernetidae). *Journal of Arachnology* 46:104–113.

Pinho CJ, Santos B, Mata VA, Seguro M, Romeiras MM, Lopes RJ. et al. 2018. What Is the giant wall gecko having for dinner? Conservation genetics for guiding reserve management in Cabo Verde. *Genes* 9:599.

Poinar GO, Curcic BPM, Cokendolpher JC. 1998. Arthropod phoresy involving pseudoscorpions in the past and present. *Acta Arachnologica* 47:79–96.

Šťáhlavský F, Král J, Harvey MS, Haddad CR. 2006. A karyotype study on the pseudoscorpion families Geogarypidae, Garypinidae and Olpiidae (Arachnida: Pseudoscorpiones). *European Journal of Entomology* 103:277–286.

Vasconcelos R, Montero-Mendieta S, Simó-Riudalbas M, Sindaco R, Santos X, Fasola M. et al. 2016. Unexpectedly high levels of cryptic diversity uncovered by a complete DNA barcoding of reptiles of the Socotra Archipelago. *PloS One* 11:e0149985.

Vasconcelos R, Santos X, Perera A, Carretero MA, Harris DJ. 2009. Environmental impact assessment of the S. Vicente wind farm on the *Tarentola caboverdiana substituta* gecko – Final report. 32. InfraCo & CIBIO.

Zaragoza J. 2007. Catálogo de los pseudoescorpiones de la Península Ibérica e Islas Baleares (Arachnida: Pseudoscorpiones). *Revista Ibérica de Aracnología* 13:3–91.

Zaragoza JA. 2017. Checklist de Fauna Ibérica. Orden Pseudoscorpiones (Arthropoda: Arachnida) en la península ibérica e islas Baleares (edición 2017). Pp. 2 (sn) + 14. *In* Documentos Fauna Ibérica, 3. Museo Nacional de Ciencias Naturales, CSIC. (MA Ramos & M Sánchez Ruíz, Eds.). Madrid, Spain.

Zaragoza JA, Seva Román E. 1990. Sobre la presencia de pseudoscorpiones en la dieta de saurios psamófilos de la provincia de Alicante. *Mediterránea Serie de Estudios Biológicos* 59–70.

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