A New Species of *Spauligodon* (Nematoda: Oxyurida: Pharyngodonidae) in Geckos from São Nicolau Island (Cape Verde) and Its Phylogenetic Assessment

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A NEW SPECIES OF SPAULIGODON (NEMATODA: OXYURIDA: PHARYNGODONIDAE) IN GECKOS FROM SÃO NICOLAU ISLAND (CAPE VERDE) AND ITS PHYLOGENETIC ASSESSMENT

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ABSTRACT: A new nematode species, Spauligodon nicolauensis n. sp., is described from geckos Tarentola bocagei and Tarentola nicolaensis from São Nicolau Island, Cape Verde. The new nematode was found in the pellets obtained directly from the geckos in a non-invasive fashion, and its identity was assessed both at morphologic and genetic levels. The new species has morphological similarities with Spauligodon tarentolae Spaul, 1926, also parasitizing geckos from the Canary Islands. However, the male cloacal region in the new species is distinct, presenting a different shape of the caudal papillae. The overall resemblance probably resulted from colonization via descent from an ancestor of S. tarentolae carried by the ancestor of Cape Verde Tarentola. The analysis of nuclear DNA sequences confirms that the new species is phylogenetically distinct from all other Spauligodon species already analyzed, forming a group clearly separated from species parasitizing lacertid lizards. The COI genetic distance suggests that the S. nicolauensis n. sp. found in the 2 species of geckos in São Nicolau Island may have resulted from a host-switching event, when they came into contact after the unification of the island.

The Tarentola geckos from the Cape Verde archipelago originated from a single colonization, followed by radiation events between the islands (Carranza et al., 2000). Currently there are 12 accepted endemic species (Vasconcelos et al., 2012). However, despite extensive knowledge regarding these geckos, as far as we know, no studies on their helminth fauna have been performed to date. During a helminthological examination of pellets from Tarentola bocagei Vasconcelos et al., 2011, and Tarentola nicolaensis Schleich, 1984, both endemic to São Nicolau Island, an undescribed Spauligodon Skrjabin, Schikhalova and Lagodovskaja, 1960, species was found. Spauligodon includes a cosmopolitan group of nematode parasites of reptiles comprising at least 47 described species, with 20 of them occurring in the Palearctic region (Bursey and Goldberg, 2011). The recent use of molecular techniques in phylogenetic studies of Spauligodon suggests that probably there are still several undescribed species within the Palearctic (Jorge et al., 2011).

MATERIAL AND METHODS

Parasitological procedures

Nematodes were collected from pellets stored in 96% ethanol, which were obtained through spontaneous defecation or by gently massaging the abdomen of both T. bocagei and T. nicolaensis from São Nicolau Island, Cape Verde (Fig 1), in 2009. Host specimens were subsequently released after a tissue sample was taken (tail tip), which later allowed the confirmation of the host species using genetic markers (Vasconcelos et al., in press). Fifty-two pellets were collected and inspected for nematodes using a stereoscopic microscope. Nematodes were isolated, washed, and fixed in 96% ethanol. For identification, nematodes were placed onto a slide with 3% formaldehyde for 2 hours, mounted in 50% glycerol, and examined using a light microscope (Olympus CX41). Spauligodon sp. specimens were photographed using an in-built digital camera Olympus SC30 and measured with cell*B software (basic image-acquisition and archiving software, Olympus*). Following de Ley et al. (2005), a video of the male posterior end was also recorded using the same software. Illustrations were made with a drawing tube attached to the microscope. In total, 396 Spauligodon specimens were identified using a 16S rRNA gene sequence alignment. Two partial gene fragments were analyzed: the mitochondrial cytochrome oxidase subunit I (COI) and the 28S nuclear ribosomal gene. These fragments were chosen so that the results could be directly compared to those from a previous study on Spauligodon (Jorge et al., 2011). The COI fragment was amplified using the primers LCO and HCO described by Folmer et al. (1994). The 28S rDNA was amplified using the primers D1 and D2 (Crotty et al., 1998) with an additional denaturation step of 3 min at 94°C, and ending with a final extension at 72°C for 10 min. Amplified fragments were sequenced for both strands with the same primers used in the amplification process, by an international facility (Macrogen Corporation http://www.macrogen.com).

Phylogenetic analysis

For all the sequences, a contig sequence was assembled in the program CodonCode Aligner 3.6.1 (CodonCode Corporation, 2009). The contig sequences were then aligned in the program BioEdit 3.0.3 (Hall, 1999). Additional Spauligodon spp. sequences from Jorge et al. (2011), but with a slightly longer nucleotide sequence (approximately 41bp in COI and 6 bp in 28S rDNA), were added to the alignment of the 28S rDNA, for determining the relationships between new species and other Spauligodon species. Previously published (Jorge et al., 2011), but unidentified, sequences of the new species (from T. bocagei) were also included. The alignment of the COI fragment was trivial due to the absence of indels,
Table I. Nematode specimens used in the phylogenetic analysis, including their respective host species, locality, and GenBank accession numbers. Abbreviations: SN, São Nicolau; CV, Cape Verde.

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<thead>
<tr>
<th>Species</th>
<th>Specimen code</th>
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<th>Host</th>
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* Reference: Jorge et al. (2011).

while the 28S rRNA fragment was aligned using ClustalW implemented in the program MEGA 4.0.2 (Tamura et al., 2007), with default parameters. For the COI alignment all sequences were translated, confirming that all codons corresponded to amino acids. Following Jorge et al. (2011), Parapharyngodon echinatus was used as an outgroup in the 28S rRNA analyses, whereas Spauligodon lacertae was used as an outgroup in the COI analyses. The AIC criterion was implemented in jModelTest 0.1.1 (Posada, 2008) to select the model of evolution that best fit each data set. Phylogenetic inferences were estimated using 2 methods, Bayesian inference (BI) and maximum likelihood (ML), implementing the most appropriate parameters according to the estimated models. For the COI dataset the implemented model was HKY+I, while TIM+G was selected for 28S rRNA. Bayesian analyses were performed in MrBayes 3.1 (Huelsenbeck and Ronquist, 2001) and ran for 5 million generations with random starting trees, sampling every 100 generations. A 50% majority-rule consensus tree was used to summarize the trees sampled with the post burn-in trees, after verifying that stationary was reached by plotting log-likelihood values against generation time. Maximum likelihood analyses were performed in PhyML 3.0 (Guindon and Gascuel, 2003). Branch support was estimated by bootstrap analysis (Felsenstein, 1985) with 1,000 replicates. Estimates of the pairwise uncorrected differences (p-distance) for each fragment was calculated in MEGA 4.0.2 (Tamura et al., 2007). New sequences are available in GenBank (Table I).

DESCRIPTION

Spauligodon nicolauensis n. sp.
(Figs. 2–6)


Male (based on 11 mature individuals): Small, filiform, 1,111.8 ± 133.5 μm (936.6–1,329.8 μm) body length and 42.5 ± 3.7 μm (36.7–46.8 μm) wide at the level of the excretory pore (excluding lateral alae). Very narrow lateral alae, extending from anterior end behind lips to anterior border of caudal alae. Length of esophagus (excluding bulb) 146.6 ± 20.9 μm (108.7–160.1) with 15.7 ± 2.7 μm (11.1–19.7) wide; esophageal bulb 35.5 ± 5.0 μm (26.0–42.0) in length and 32.5 ± 3.9 μm (26.0–37.8) wide. Nerve ring and excretory pore located at 89.6 ± 17.8 μm (63.4–125.2) and 320.9 ± 79.7 μm (212.0–452.5), respectively, from anterior end. Posterior end extending into tail, with very slight caudal alae projected at both sides of cloaca. Three pairs of caudal papillae, first 2 enclosed by caudal alae, third pair situated at base of tail directed outward, not enclosed by caudal alae. Precloacal pair (first pair) lies in higher area of posterior end, ventrally directed, consisting of 2 spherical pedunculated papillae. Second pair consisting of 2 large papillae flattened at tip, with thick, large peduncles. Third pair resembles second pair, but smaller. Genital cone situated in mid-ventral line surrounded by membranous curtain with fingerlike extremities. Aspinose, filiform tail with 68.9 ± 9.1 μm (56.4–87.1) in length. Spicule absent.

Female (based on 10 mature individuals): Small, filiform, 3,290.0 ± 543.9 μm (2,716.2–4,441.3 μm) body length and 392.8 ± 99.7 μm (271.3–627.0) wide at mid-body level. Oral opening surrounded by 3 small, slightly bilobed lips. Length of esophagus (excluding bulb), 262.7 ± 27.5 μm (227.0–309.4) with 37.9 ± 5.6 μm (28.4–44.9) wide; esophageal bulb 108.3 ± 9.3 μm (96.2–124.7) long and 133.5 ± 11.5 μm (95.8–127.1) wide. Nerve ring located at 126.9 ± 25.7 μm (94.6–164.0) from anterior end. Excretory pore and vulva open at postbulbar level at 426.4 ± 90.6 μm (294.3–614.2) and 483.8 ± 89.4 μm (360.1–658.4), respectively, from anterior end. Thick, muscular vagina, directed posteriorly with 328.1 ± 60.3 μm (220.1–448.1) length. The ovaries are located behind the vulva, females being opistodelphic. In fully gravid females, uterus extends anterior slightly behind the vulva and posterior almost reaching the end of the body. Tail aspinose, filiform, with 387.6 ± 62.6 μm (238.6–455.5) in length. Asymmetrical eggs, with differently truncate extremities, with polar cap in 1 pole. Eggs with 138.5 ± 9.3 μm (107.5–153.1) in length and 47.0 ± 5.2 μm (36.6–56.6) wide.
The new species, *Spauligodon nicolauensis*, is both genetically and morphologically different from other species in the genus, forming a clade within the *Spauligodon* spp. phylogeny (Fig. 7). The differentiation of *S. nicolauensis* was noted in the morphology of the males, which are smaller, thinner, and with a distinct caudal extremity relative to the other described members of the genus. *Spauligodon nicolauensis* does resemble the other parasite of geckos, *S. tarentolae*, from the Canary Islands. According to phylogenetic data, the Cape Verdean *Tarentola* species originated through a colonization from the western Canary Islands (Carranza et al., 2000; Vasconcelos et al., 2010) approximately 7.73 ± 1.8 million yr ago, with São Nicolau probably being the first island colonized (Vasconcelos et al., 2010). Therefore, *S. nicolauensis* could have originated via colonization by an ancestor of *S. tarentolae* brought by the ancestral Cape Verde *Tarentola* species. However, there is still no genetic information regarding *S. tarentolae* to confirm or reject this hypothesis.

The 2 geckos, *T. bocagei* and *T. nicolauensis*, present on São Nicolau probably originated by allopatric speciation, with secondary contact occurring only after the 2 independent islands of São Nicolau became united by volcanic activity 4.7 ± 2.6 million yr ago (Duprat et al., 2007; Vasconcelos et al., 2010). The presence of *S. nicolauensis* in the 2 gecko species present in São Nicolau Island supports the hypothesis that these geckos colonized the Cape Verde archipelago through colonization from the western Canary Islands. The differentiation of these species was confirmed by the presence of a spicule, spines on the tail filament, the form of the caudal alae, and shape of the eggs.

Sequence data and phylogenetic analysis

Nucleotide sequences of the 28S rRNA fragment with an average length of 1,147 bp were obtained for 10 *S. nicolauensis* n. sp. specimens from 4 localities (Fig. 1), from the 2 different hosts, *T. bocagei* and *T. nicolauensis*. No *S. tarentolae* specimens, however, were successfully amplified for this gene. The analysis of these fragments revealed no variation across *S. nicolauensis*. Both ML and BI analyses generated the same estimate of phylogeny, with the nematode *S. nicolauensis* being quite divergent from other *Spauligodon* spp. (4.2% minimum uncorrected p-distance for the 28S rRNA; Fig. 7). The ingroup of the COI analysis consisted of 690 bp for 8 *S. nicolauensis* specimens from 3 different localities, from the 2 different host species. The analysis of the COI fragment revealed a greater differentiation within *S. nicolauensis*. Between the 2 hosts, *S. nicolauensis* presented 4.2% genetic distance, with 0.3% genetic distance within the host *T. bocagei* (uncorrected p-distance, for the COI; Fig. 8). The peptide alignment did not indicate any amino acid substitutions.

**DISCUSSION**

The new species, *Spauligodon nicolauensis*, is both genetically and morphologically different from other species in the genus, forming a clade within the *Spauligodon* spp. phylogeny (Fig. 7). The differentiation of *S. nicolauensis* was noted in the morphology of the males, which are smaller, thinner, and with a distinct caudal extremity relative to the other described members of the genus. *Spauligodon nicolauensis* does resemble the other parasite of geckos, *S. tarentolae*, from the Canary Islands. According to phylogenetic data, the Cape Verdean *Tarentola* species originated through a colonization from the western Canary Islands (Carranza et al., 2000; Vasconcelos et al., 2010) approximately 7.73 ± 1.8 million yr ago, with São Nicolau probably being the first island colonized (Vasconcelos et al., 2010). Therefore, *S. nicolauensis* could have originated via colonization by an ancestor of *S. tarentolae* brought by the ancestral Cape Verde *Tarentola* species. However, there is still no genetic information regarding *S. tarentolae* to confirm or reject this hypothesis.

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Nicolau could have evolved via 2 different events: host switching or colonization by descent. In the first case, the parasite may have switched between *T. bocagei* and *T. nicolauensis*, or vice versa, when they came into contact. In the second case, the parasite may have been already present on the 2 islands that gave rise to São Nicolau, originating from the ancestor of Cape Verdean *Tarentola* species, before the 2 host species came into contact. However, it is generally predicted that parasites exhibit more rapid evolutionary rates than their hosts (assuming that parasites have shorter generation times, larger population sizes, and higher mutation rates). Therefore, considering that the genetic distance between *S. nicolauensis* from the 2 geckos (4.2% uncorrected p-distance, for the COI) is lower than that of the hosts (9.7% uncorrected p-distance, for cyt b; Vasconcelos et al., 2012) the host-switching scenario seems the most likely, although the second hypothesis cannot be rejected.

The radiation of the *Tarentola* in Cape Verde after a single colonization event, with limited gene flow between islands (Vasconcelos et al., 2010), is suggestive of the presence of other *Spauligodon* forms in the archipelago, if the parasites successfully followed the colonization of the host. Moreover, until more data are available, the presence of other unrelated *Spauligodon* species in the Cape Verde Islands originating from the other natural

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**Figures 2–6.** *Spauligodon nicolauensis* n. sp. (2) Male, anterior end, ventral view. (3) Male, posterior end, ventral-lateral view. (4) Female, posterior end, ventral view. (5) Egg. (6) Female, anterior end, lateral view.
colonizations of reptile hosts (*Hemidactylus* geckos and *Chioninia* skinks), cannot be discarded, as in the case of the Canary Islands (Jorge et al., 2011). A more extensive study of the helminth fauna of the reptiles of the Cape Verde Islands is required to determine which species are present and the relationships and origins of the present host parasite associations.

According to the inferred *Spauligodon* spp. phylogeny (Fig. 7), the species parasitizing lacertids form a monophyletic group, an indication of host specificity. However, in the case of the species parasitizing geckos, it appears that they are paraphyletic. Whether there is host specificity at a higher taxonomic level, with the separation between lizards and geckos...
being reflected in the phylogeny of the parasite, remains to be determined.

The identification of this new species through the analysis of their host pellets is a non-invasive technique, which highlights its value not just for diet studies of the host, but also for helminth fauna studies. For this, it is recommended that pellets collected are placed in 96% ethanol to preserve the DNA of the helminth for genetic analyses. However, it should be highlighted that for morphological analysis this methodology may be problematic, yielding possible distortion of parasites from dehydration, depending on the freshness of the pellets.
ACKNOWLEDGMENTS

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LITERATURE CITED