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# A phylogenetic assessment of the colonisation patterns in *Spauligodon atlanticus* Astasio-Arbiza et al., 1987 (Nematoda: Oxyurida: Pharyngodonidae), a parasite of lizards of the genus *Gallotia* Boulenger: no simple answers

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**Abstract** Parasite taxonomy traditionally relies on morphometric and life-cycle characteristics which may not reflect complex phylogenetic relationships. However, genetic analyses can reveal cryptic species within morphologically described parasite taxa. We analysed the phylogenetic variation within the nematode *Spauligodon atlanticus* Astasio-Arbiza, Zapatero-Ramos, Ojeda-Rosas & Solera-Puertas, 1987, a parasite of the Canarian lizard genus *Gallotia* Boulenger, inferring the origin of their current association. We also attempted to determine its relationship with other *Spauligodon* spp. Three different markers, mitochondrial COI plus nuclear 18S and 28S ribosomal RNA, were used to estimate the evolutionary relationships between these nematodes. *S. atlanticus* was found to be paraphyletic, suggesting that *Gallotia* spp. were colonised by two independent lineages of *Spauligodon*. Additional analyses of other *Spauligodon* spp. are required for a more complete interpretation of the evolution of this genus from the Canarian archipelago and its closest taxa. Our results emphasise the importance of extensive sampling and phylogenetic studies at the intrageneric level, and highlight the limitations of a morphologically based taxonomy in these parasites.

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## Introduction

Our knowledge of comparative phylogenies of parasites and their hosts constitutes a key point for interpreting their association within an evolutionary context. Determining the origins of such associations (Banks & Paterson, 2005) and the events that have shaped their present distribution (Paterson et al., 2003), ultimately through a co-evolutionary pathway, has been the aim of numerous studies (Hugot et al., 2001; Johnson et al., 2003; Chilton et al., 2004; Clayton et al., 2004; Banks et al., 2006; Light & Reed, 2009). Host-parasite associations could arise through descendency, when each host species inherited the association from its ancestor, or by colonisation, where the parasite switches to a new host lineage or species other than the host ancestor (Banks & Paterson, 2005). However, several events could shape the current distribution of the parasite-host associations, resulting only rarely in a perfect match between both phylogenies (Page, 2003). Rather than tracking their host with perfect fidelity, parasites may switch lineages, speciate independently of their host, go

extinct (sorting), fail to colonise all the descendants of a host lineage, or fail to speciate when the host does. On the other hand, events other than co-speciation, such as geographical differentiation (Callejón et al., 2010), may generate "false" congruent host and parasite phylogenies (Clayton et al., 2003).

Until recently, the taxonomy of parasites had relied on the analysis of their morphological and life-cycle traits. However, parasites often present a simplified morphology, and molecular techniques are starting to show that many presumed single species actually consist of genetically different parasite lineages, suggestive of cryptic species (Blouin, 2002; Miura et al., 2005; Gutiérrez-Gutiérrez et al., 2010). The lack of morphological differentiation between related parasite species may be due to similar selective pressures promoting morphological convergence in species that are not closely related (Banks & Paterson, 2005). This event, although difficult to recognise using traditional morphological traits, is easily characterised using a molecular approach. The existence of unidentified cryptic species can result in erroneous interpretations of parasite-host associations, such as the assignment of parasites as multi-host species. Multi-host parasites may still result from other events, such as misclassified (over-split) hosts, recent host-switches, failures to speciate or incomplete host-switches (Banks & Paterson, 2005). Thus, an assessment of the genetic diversity of parasites can enlighten various aspects of host-parasite relationships.

*Spauligodon* Skrjabin, Schikhobalova & Lagodovskaja, 1960 (Oxyurida) is a cosmopolitan genus of intestinal nematodes that comprises at least 46 described species (Binh et al., 2007; Bursey et al., 2007; Monks et al., 2008), with 20 of them occurring in the Palaearctic region (Bursey et al., 2005). *S. atlanticus* Astasio-Arbiza, Zapatero-Ramos, Ojeda-Rosas & Solera-Puertas, 1987 was described as an obligate parasite specific to lacertid lizards of the genus *Gallotia* Boulenger endemic to the Canary Islands (Martín & Roca, 2005). This nematode belongs to the Family Pharyngodonidae, members of which infect reptiles (Roca, 1999; Roca et al., 2005). Since it has a monoxenus life-cycle with no free-living stages (Sánchez, 1996), parasite gene flow is strictly dependent on movements and contacts between hosts which display low dispersal abilities.

The Canary Islands are one of the most studied volcanic archipelagos in the world (Sanmartín et al., 2008). They are located approximately 100 km from the north-western coast of Africa, forming a chain of seven main islands and several islets. The archipelago was formed in an east-to-west formation during the last 20 million years (Ma) (Guillou et al., 2004; Ancochea et al., 2006; Fig. 1). Previous phylogenies also suggest that the colonisation of the archipelago by some taxa follows a stepping-stone model from older to younger islands (Sanmartín et al., 2008). However, in the case of lizards of the genus *Gallotia* this is not so clear (Cox et al., 2010). The ancestor colonised the Canary Islands in the Miocene, between 17 to 20 Ma ago (Cox et al., 2010), soon after the formation of the easternmost islands. *Gallotia* is the only endemic lacertid genus in the archipelago, with seven recognised living species (Maca-Meyer et al., 2003; Cox et al., 2010), three of which are considered threatened. The *Gallotia* spp. can be grouped in two well-differentiated size groups, one formed by small to medium-sized lizards (maximum snout-vent length, SVL, 67-136 mm) including *G. atlantica*, *G. galloti* and *G. caesaris*, and a second group of giant lizards (SVL 201-232 mm) formed by the other four species (*G. bravoana*, *G. simonyi*, *G. intermedia* and *G. stehlini*) plus the extinct *G. goliath* (see Barahona et al., 2000). In contrast to most lacertid lizards, *Gallotia* spp. are generally considered omnivorous but with a high trend towards herbivory (Martín et al., 2005; Roca et al., 2005; Carretero et al., 2006; Rodríguez et al., 2008). This trend is confirmed in their helminth communities (Roca et al., 2005). *S. atlanticus* is part of the nematode evolutionary lineage that typically parasitises carnivorous lizards (Roca, 1999; Roca et al., 2005). The prevalence of *S. atlanticus* in *Gallotia* spp. varies between 5.6% (in *G. atlantica laurae* Castroviejo, Mateo & Collado from Lanzarote) and 59.3% (in *G. galloti palmae* Boettger & Müller from La Palma) (Martín, 2005). Considering its distribution and morphological diagnosis, *S. atlanticus* appears to be a multi-host parasite, restricted to different *Gallotia* spp.

Our current knowledge, therefore, gives rise to several intriguing questions: (i) does *S. atlanticus* constitute a single evolutionary lineage or does it comprise multiple cryptic species; (ii) is there co-differentiation between the parasite and its host; and (iii) what are the relationships between *S. atlanticus* and other members of the genus. In view of these, we assessed for the first time the phylogeny of

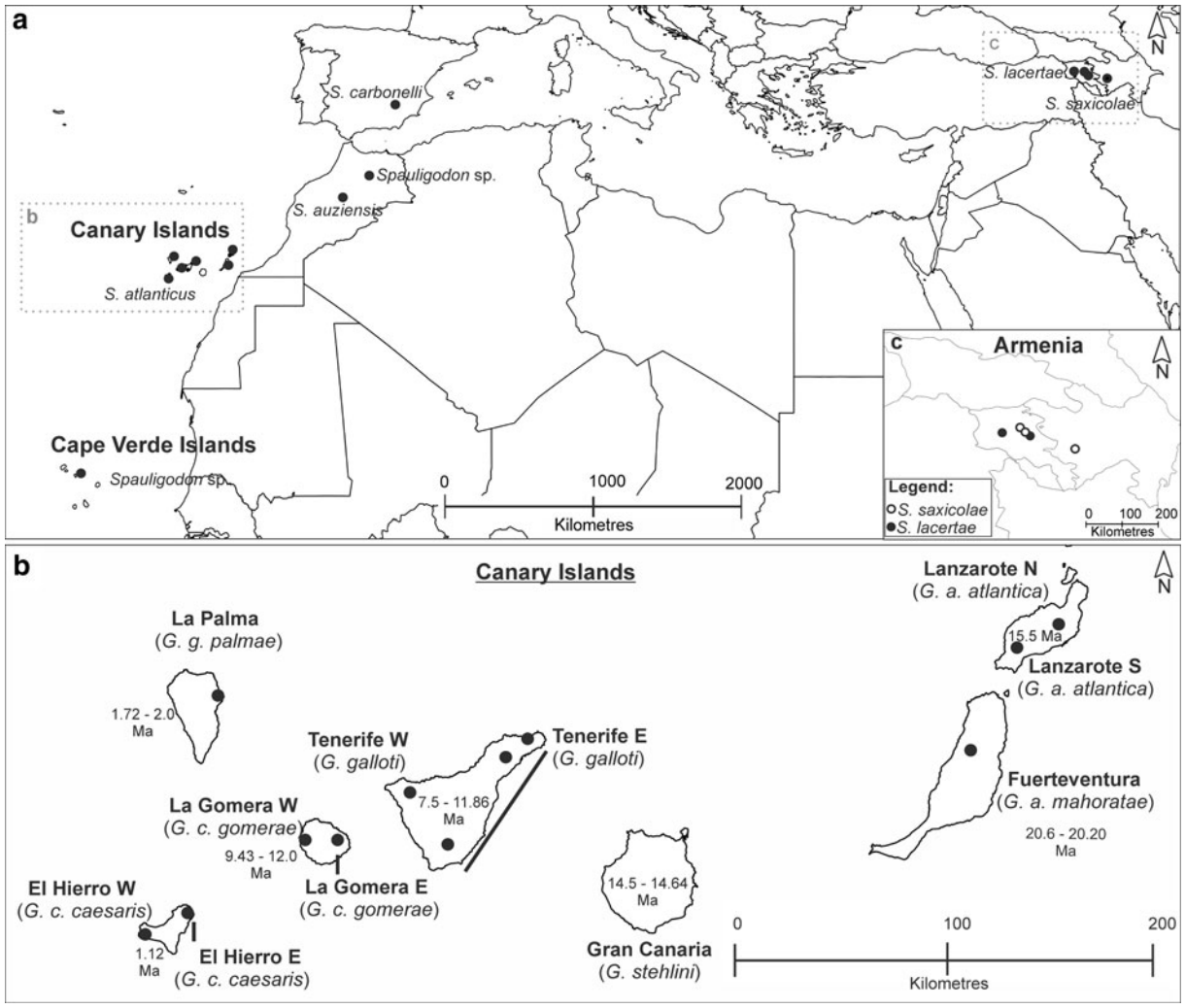


**Fig. 1** Map of the Canary Islands with the approximate ages of the islands (from Carracedo et al., 1998; Guillou et al., 2004) and the geographical localities of *Spauligodon* samples included in the analyses (See Table 1 for details). (a) South Europe, North Africa and Caucasus. (b) Canary Islands. (c) Armenia. *Abbreviation*: Ma, million years

*S. atlanticus* and related species using three different genes with different rates of evolution, the mitochondrial cytochrome oxidase subunit I and two nuclear ribosomal genes, the 18S rRNA and the 28S rRNA.

## Materials and methods

*Specimen collection*

All the seven main islands of the Canarian archipelago were sampled. Except from the three giant species, *Spauligodon atlanticus* specimens were obtained from the caecum of all lizard species from all islands (Table 1; Fig. 1). Two of them are endangered and could not be sampled due to conservation restrictions. The third, *Gallotia stehlini* Schenkel from Gran Canaria was sampled, but the nematode species could not be found. Nematodes were extracted by necropsy (lizard vouchers are deposited in the Herpetological collection of CIBIO, University of Porto, Portugal) or from fresh pellets dropped by lizards collected in the field but subsequently released. Similarly, other *Spauligodon* specimens were obtained from other lacertid genera (*Darevskia* Arribas, *Lacerta* Linnaeus and *Podarcis* Wagler) and also from geckos (*Tarentola* Gray) in order to determine the relationships

**Table 1** Nematode specimens used in the phylogenetic analyses, including their respective host species, locality and GenBank accession numbers

| Nematode species | Specimen code | Collection locality | Host | COI | 18S rRNA | 28S rRNA |
|---|---|---|---|---|---|---|
| *S. atlanticus* | 1383F | Yaiza, Lanzarote | *G. a. atlantica* | JF829273 | JF829232 | |
| *S. atlanticus* | 1341F | Yaiza, Lanzarote | *G. a. atlantica* | JF829272 | | |
| *S. atlanticus* | 1492F | Yaiza, Lanzarote | *G. a. atlantica* | JF829274 | | JF829250 |
| *S. atlanticus* | 1492F2 | Yaiza, Lanzarote | *G. a. atlantica* | JF829279 | | |
| *S. atlanticus* | 1441F | Nazaret-Teguise, Lanzarote | *G. a. atlantica* | JF829278 | | |
| *S. atlanticus* | 1407F | Nazaret-Teguise, Lanzarote | *G. a. atlantica* | JF829277 | | |
| *S. atlanticus* | 1339F | Nazaret-Teguise, Lanzarote | *G. a. atlantica* | JF829275 | | |
| *S. atlanticus* | 1339M | Nazaret-Teguise, Lanzarote | *G. a. atlantica* | JF829276 | | |
| *S. atlanticus* | 1368F | Tefia, Fuerteventura | *G. a. mahoratae* | JF829285 | JF829230 | JF829251 |
| *S. atlanticus* | 1337F1 | Tefia, Fuerteventura | *G. a. mahoratae* | JF829281 | | |
| *S. atlanticus* | 1337F2 | Tefia, Fuerteventura | *G. a. mahoratae* | JF829282 | | JF829249 |
| *S. atlanticus* | 1337M1 | Tefia, Fuerteventura | *G. a. mahoratae* | JF829283 | | |
| *S. atlanticus* | 1367F | Tefia, Fuerteventura | *G. a. mahoratae* | JF829280 | | |
| *S. atlanticus* | 1338F | Tefia, Fuerteventura | *G. a. mahoratae* | JF829284 | | |
| *S. atlanticus* | 2134F2 | La Laguna, Tenerife | *G. g. eisentrauti* | JF829312 | | JF829257 |
| *S. atlanticus* | 1277F | La Laguna, Tenerife | *G. g. eisentrauti* | JF829289 | | |
| *S. atlanticus* | 2122F | Km2 TF134, Tenerife | *G. g. eisentrauti* | JF829291 | | |
| *S. atlanticus* | 2089F | Km2 TF134, Tenerife | *G. g. eisentrauti* | JF829290 | | |
| *S. atlanticus* | 2095F2 | Km2 TF134, Tenerife | *G. g. eisentrauti* | JF829311 | | |
| *S. atlanticus* | 2124F | San Miguel, Tenerife | *G. g. galloti* | JF829292 | | JF829256 |
| *S. atlanticus* | 2107F2 | Erjos, Tenerife | *G. g. galloti* | JF829302 | | |
| *S. atlanticus* | 2123F | Erjos, Tenerife | *G. g. galloti* | | JF829231 | |
| *S. atlanticus* | 2088F | La Lomada, La Palma | *G. g. palmae* | JF829293 | JF829233 | JF829259 |
| *S. atlanticus* | 2091M | Las Casetas, La Gomera | *G. c. gomerae* | JF829294 | | |
| *S. atlanticus* | 2104M | Las Casetas, La Gomera | *G. c. gomerae* | JF829295 | | |
| *S. atlanticus* | 2513F | Las Casetas, La Gomera | *G. c. gomerae* | JF829296 | | |
| *S. atlanticus* | 2490F2 | Las Casetas, La Gomera | *G. c. gomerae* | JF829314 | | |
| *S. atlanticus* | 2490M2 | Las Casetas, La Gomera | *G. c. gomerae* | JF829315 | | |
| *S. atlanticus* | 2505F | Arure, La Gomera | *G. c. gomerae* | JF829298 | | |
| *S. atlanticus* | 2525F | Arure, La Gomera | *G. c. gomerae* | JF829299 | | |
| *S. atlanticus* | 2525M | Arure, La Gomera | *G. c. gomerae* | JF829300 | | |
| *S. atlanticus* | 2462F | Arure, La Gomera | *G. c. gomerae* | JF829301 | JF829234 | |
| *S. atlanticus* | 2504F | Arure, La Gomera | *G. c. gomerae* | JF829297 | | |
| *S. atlanticus* | 2474M1 | Valverde, El Hierro | *G. c. caesaris* | JF829305 | | |
| *S. atlanticus* | 2467F | Valverde, El Hierro | *G. c. caesaris* | JF829303 | | JF829258 |
| *S. atlanticus* | 2467M | Valverde, El Hierro | *G. c. caesaris* | JF829304 | | |
| *S. atlanticus* | 2450F1 | Ermita N$^{a}$ S$^{a}$ Reyes, El Hierro | *G. c. caesaris* | JF829308 | | JF829260 |
| *S. atlanticus* | 2450F2 | Ermita N$^{a}$ S$^{a}$ Reyes, El Hierro | *G. c. caesaris* | JF829309 | | |
| *S. atlanticus* | 2450M1 | Ermita N$^{a}$ S$^{a}$ Reyes, El Hierro | *G. c. caesaris* | JF829310 | | |
| *S. atlanticus* | 2447F | Ermita N$^{a}$ S$^{a}$ Reyes, El Hierro | *G. c. caesaris* | JF829306 | JF829235 | JF829261 |
| *S. atlanticus* | 2447M1 | Ermita N$^{a}$ S$^{a}$ Reyes, El Hierro | *G. c. caesaris* | JF829307 | | |
| *S. atlanticus* | 2458F2 | Ermita N$^{a}$ S$^{a}$ Reyes, El Hierro | *G. c. caesaris* | JF829313 | | |
| *S. lacertae* | Lm28F | Arailer, Armenia | *L. media* | JF829287 | JF829237 | JF829255 |

**Table 1** continued

| Nematode species | Specimen code | Collection locality | Host | COI | 18S rRNA | 28S rRNA |
|---|---|---|---|---|---|---|
| *S. lacertae* | Lm32FD | Arailer, Armenia | *L. media* | JF829288 | JF829236 | JF829254 |
| *S. lacertae* | 10057F | Noratuz, Armenia | *L. strigata* | JF829286 | JF829238 | JF829252 |
| *S. lacertae* | 10057M | Noratuz, Armenia | *L. strigata* | | JF829239 | JF829253 |
| *Spauligodon* sp. | 9211M | Talzemt, Morocco | *P. vaucheri* | JF829269 | JF829228 | JF829247 |
| *S. carbonelli* | Phss6F | Rambla de los Vaqueros, Spain | *P. hispanica* s.s. | JF829270 | | |
| *S. carbonelli* | Phss6M | Rambla de los Vaqueros, Spain | *P. hispanica* s.s. | JF829271 | JF829229 | JF829248 |
| *S. saxicolae* | 10171 | Ganzasar, Armenia | *D. raddei* | JF829267 | | JF829244 |
| *S. saxicolae* | 10420 | Lchaschen, Armenia | *D. nairensis* | JF829268 | | JF829245 |
| *S. saxicolae* | 9902 | Kuchak, Armenia | *D. unisexualis* | JF829266 | JF829227 | JF829246 |
| *S. auziensis* | 5057 | Ait ou Ba Allel, Morocco | *T. mauritanica* | JF829264 | JF829225 | JF829242 |
| *Spauligodon* sp. | 2597 | São Nicolau, Cape Verde | *T. darwini* | JF829265 | JF829226 | JF829243 |
| *P. echinatus* | 1328 | La Oliva, Fuerteventura | *G. a. mahoratae* | JF829262 | JF829223 | JF829240 |
| *P. echinatus* | 1345 | Lazares, Fuerteventura | *G. a. mahoratae* | JF829263 | JF829224 | JF829241 |

between *S. atlanticus* and other *Spauligodon* spp. During necropsy, the lizard caecum was removed, opened and inspected for helminths using a stereomicroscope. Nematodes were then separated and identified to the generic level under a light microscope. Specimens were placed put in a bleaching solution (Foitová et al., 2008) on a slide and covered with a coverslip, after which they were identified under a microscope to the specific level, whenever possible, based on previous descriptions (Spaul, 1926; Sharpilo, 1976; Roca, 1985; Astasio-Arbiza et al., 1987; Roca & García-Adell, 1988). All extracted specimens were photographed as a record.

*DNA extraction and sequencing*

Extractions of genomic DNA were performed on individual nematodes, using the DNeasy Blood & Tissue Kit from QIAGEN according to the manufactures protocol. Three partial fragments were tested as markers: the mitochondrial cytochrome oxidase subunit I (COI) and two nuclear ribosomal genes, the 18S rRNA and the 28S rRNA (Table 1). The COI fragment was amplified using the primers LCO and HCO previously described by Folmer et al. (1994). The set of primers Nem 18S F and Nem 18S R designed by Floyd et al. (2005) were used for amplification of the 18S rRNA fragment. For the amplification of the 28S rRNA fragment, primers 28S rD1.2a and 28S B described by Whiting (2002) were used. Polymerase chain reactions (PCR) were performed in a total volume of 20 µl, comprising: PCR buffer at 1× concentration; $MgCl_2$ at 1.5 mM; dNTPs at a concentration of 0.2 mM for each nucleotide; primers at 0.5 µM each; BSA at 0.4 µg/µl (bovine serum albumin) (Roche Applied Science); and *Taq* DNA polymerase (Invitrogen Corporation) at 0.025 units/µl; 2 µl of extracted nematode DNA template were used. The PCR reaction consisted of 35 iterations of the following cycle: 30 sec. at 94°C, 30 sec. at 45–63°C (depending on the primers used) and 1 min. at 72°C, beginning with an additional denaturation step of 3 min. at 94°C and ending with a final extension at 72°C for 10 min. Amplified products were sent for sequencing to an international facility (Macrogen Corporation http://www.macrogen.com). Sequences were obtained for both directions.

*Data analysis*

For all the sequences, a contig sequence was assembled in the program CodonCode Aligner (CodonCode Corporation, http://www.codoncode.com). The contig sequences were then aligned in the program BioEdit 3.0.3 (Hall, 1999). All sequences were uploaded into BLAST (Zhang et al., 2000) to confirm their identity as nematodes. *Parapharyngodon echinatus* (Rudolphi, 1819) from *G. atlantica mahoratae* Bischoff, was used as an outgroup. The alignment of the COI and 18S rRNA fragments was trivial due to the absence of indels, whereas the 28S rRNA fragment was aligned using ClustalW implemented in the program MEGA 4.0.2 (Tamura et al., 2007). For the COI alignment, all sequences were translated confirming that all codons

corresponded to amino acids. The AIC criterion was used in jModelTest 0.1.1 (Posada, 2008) to select the model of evolution employed for each data-set. For all the partitions the model selected was GTR+G. For the COI, a model that allows the codon positions to have different rates was also performed. Bayesian inferences were performed in MrBayes 3.1 (Huelsenbeck & Ronquist, 2001), for the three independent partitions and for the combined data-set of COI and 28S rRNA, implementing the respective inferred models. The analyses were carried out for a total of 5 million generations, using random starting trees and sampling every 100 generations. A 50% majority-rule consensus tree was used to summarise the trees sampled after stationary was reached with the post burn-in trees (after discarding 25% of the samples). The topologies of the independent partitions of COI and 28S rRNA trees were examined for conflicts involving the nodes according to the comparisons methods of Mason-Gamer & Kellogg (1996). Pairwise uncorrected differences (p-distance) for each fragment, within and between groups, were calculated in MEGA 4.0.2 (Tamura et al., 2007). New sequences were submitted to GenBank (Table 1).

## Results

### Sequences and alignment

*S. atlanticus* was identified in all *Gallotia* spp. analysed, except for *G. stehlini*. Nucleotide sequences of the 18S rRNA fragment with an average length of 858 bp were obtained for 18 specimens. The analysis of these fragments revealed low variation across the different specimens with a maximum of 1.7% uncorrected p-distance between *Spauligodon* sp. from *Tarentola darwini* Joger and *S. atlanticus* and sister taxa specimens. Due to the low variability observed, the resulting tree of 18S rRNA analysis is not presented. BLAST results confirmed that the sequences belonged to nematodes, although no closely related matches were found. Regarding the similarity with 18S rRNA sequences of *S. atlanticus*, the closest score match was 91% with members of the Order Oxyurida, to which *Spauligodon* belongs to.

Including the two outgroups, 54 and 22 nematode specimens from 13 different host species, were used in the COI and 28S rRNA analyses, respectively (Table 1). Despite being sequenced for the COI, no *S. atlanticus* from *G. c. gomerae* on La Gomera Island could be successfully sequenced for the 28S rRNA. The alignment consisted of 649 bp and 1143 bp, for the COI and 28S rRNA fragments, respectively. For the alignment of the combined COI and 28S rRNA data-set, a total of 1792 bp (from the taxa common to the two data-sets) was used for the phylogenetic analysis (with a total of 21 specimens). Within the ingroup, the COI peptide alignment consisted in 216 amino acids, from which 35 positions were variable, and of these 20 were parsimonious informative.

### Phylogenetic analysis

The Bayesian tree obtained from the analysis of COI using a partitioned model is shown in Fig. 2. Bayesian analyses of the COI and 28S rRNA (Fig. 2 and Fig. 3, respectively) produced trees that present the same topology with a high posterior probability support for the main clades, differing only in the resolution of the branches and not presenting any conflict according to the inspection and assessment of support analysis (Mason-Gamer & Kellogg, 1996). The result of the Bayesian analysis of the combined COI and 28S rRNA data-sets is shown in Fig. 4. All inferred phylogenies indicate that *S. atlanticus* is polyphyletic, with two highly divergent and well supported lineages which were not directly related, one corresponding to the eastern islands (Lanzarote and Fuerteventura) and the other to the western islands (Tenerife, La Palma, La Gomera and El Hierro). Within the eastern lineage, Fuerteventura samples (host *G. a. mahoratae* Bischoff) form a monophyletic group, whereas those from southern Lanzarote [host *G. a. atlantica* (Peters & Doria)] are paraphyletic relative to those from Fuerteventura. The western clade comprised three different lineages, one from La Gomera grouping together with the specimen from western Tenerife, another from the western area from El Hierro and a third formed by specimens from La Palma, east of El Hierro and the northern and south-eastern Tenerife. Remarkably, two different lineages were found on El Hierro [host *G. c. caesaris* (Lehrs)].

Regarding the affinities to other *Spauligodon* spp., the eastern *S. atlanticus* lineage was more closely related to *S. carbonelli* Roca & García-Adell, 1988 from the southern part of the Iberian Peninsula and *Spauligodon* sp. from Morocco, both parasitising

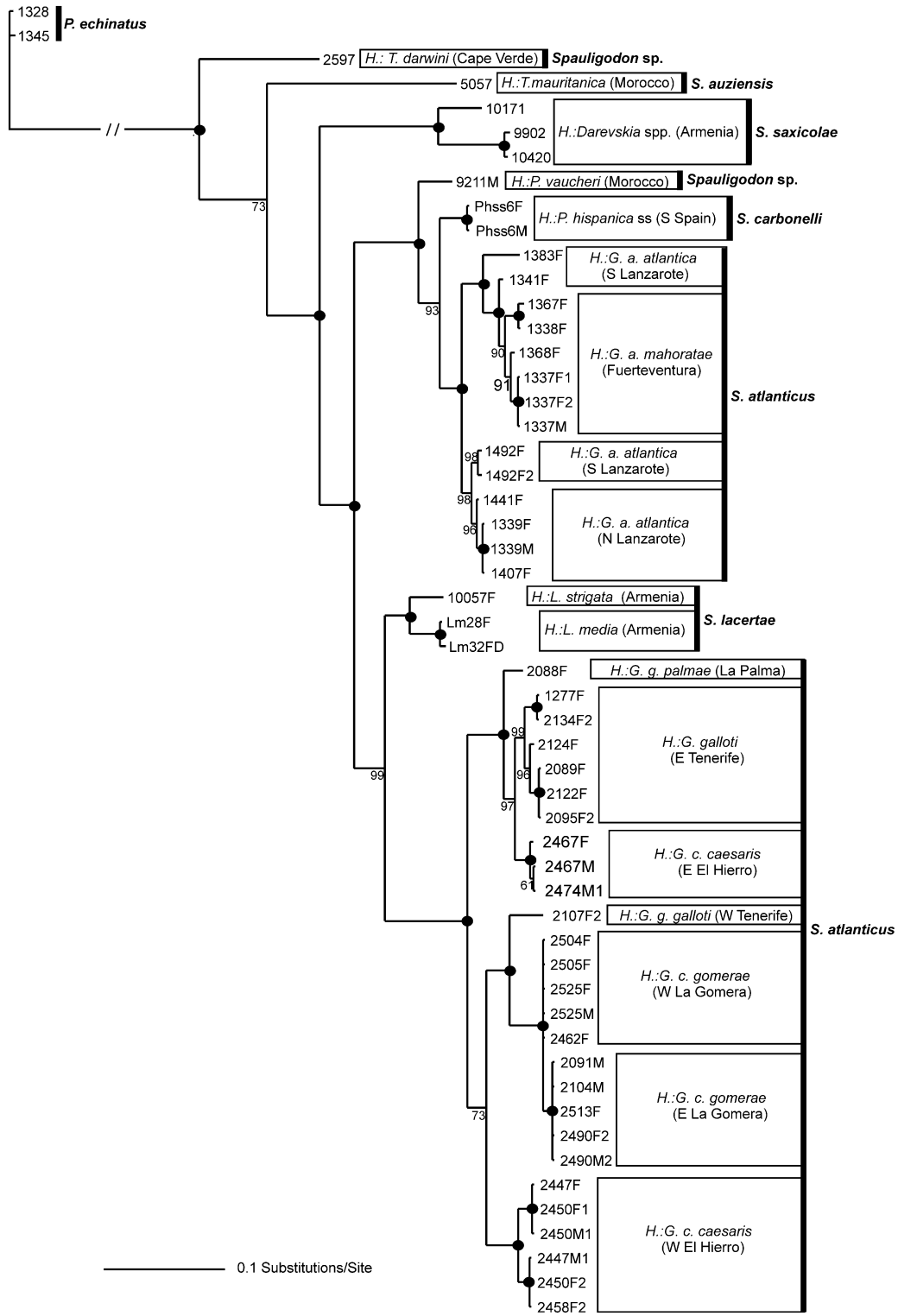


**Fig. 2** Bayesian inference tree of the COI data for the *Spauligodon* spp. analysed, with their respective host species (H) and localities. Values represent posterior probabilities. Bayesian clade credibility values of 100 are shown as a filled circle on the node. Specimen code descriptions are given in Table 1

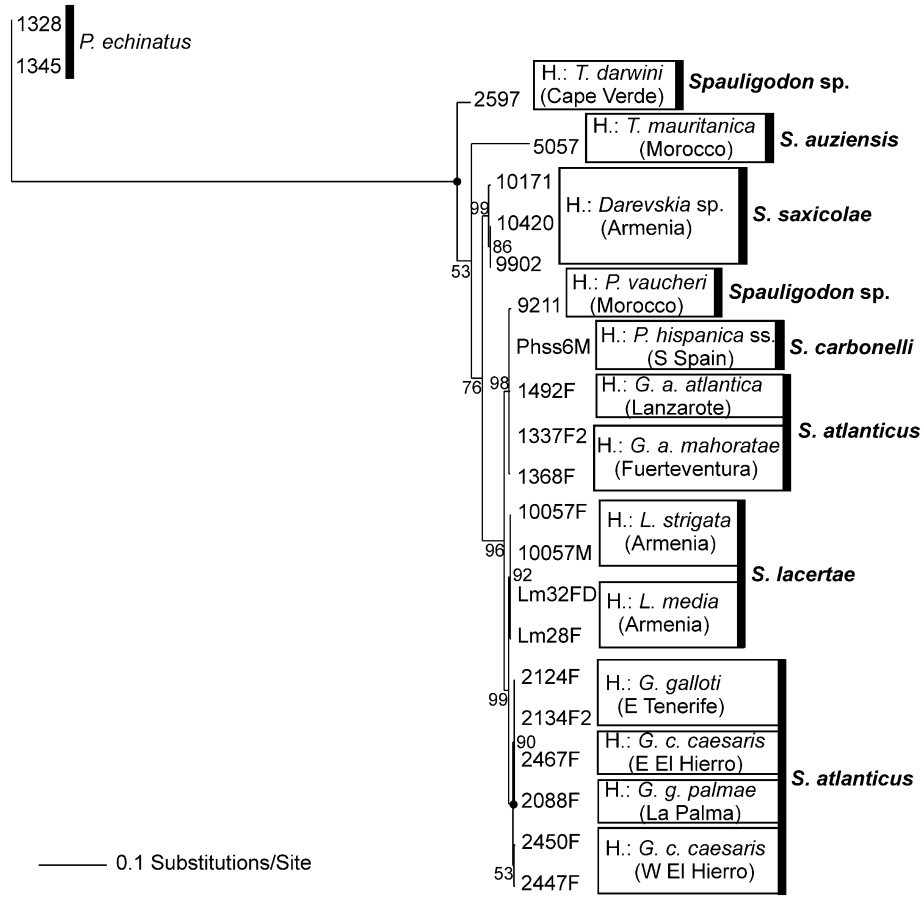


**Fig. 3** Bayesian inference tree of the 28S rRNA data for the *Spauligodon* spp. analysed, with their respective host species (H) and localities. Values represent posterior probabilities. Bayesian clade credibility values of 100 are shown as a filled circle on the node. Specimen code descriptions are given in Table 1

lizards of the genus *Podarcis*, with 100% Bayesian Posterior Probability (BPP) support (98% for the 28S rRNA analysis). In contrast, the western lineage grouped with *S. lacertae* Sharpilo, 1966 from Armenia, a parasite of green lizards *Lacerta* spp. (99% BPP for the COI and 28S analyses, and 100% BPP for the combined analysis). The remaining *Spauligodon* spp. from *Tarentola* spp. and *S. saxicolae* Sharpilo, 1961 from the Armenian rock lizards of the genus *Darevskia* are more distantly related to the *S. atlanticus* complex.

## Discussion

### Phylogeny

As already reported (Blouin, 2002; Gutiérrez-Gutiérrez et al., 2010), the 18S rRNA presents limited variation for resolving nematode relationships either between closely related species or at the intraspecific level. However, when assessing deeper relationships between *S. atlanticus* and other nematodes, this marker is very useful for an initial molecular identification. Considering the lack of overall phylogenetic information for this group, this kind of comparison was needed for the preliminary confirmation of the morphological diagnoses. No sequences of species of *Spauligodon* were previously available in GenBank, with the taxonomically closest being a sequence of *Parapharyngodon echinatus*, a member of the same family (Pharyngodonidae). In contrast, the COI proved to be useful for differentiating these closely related species of nematodes (see also Blouin, 2002; Eamsobhana et al., 2010). The 28S rRNA, a slower evolving gene than the COI, showed sufficient resolution between closely related species

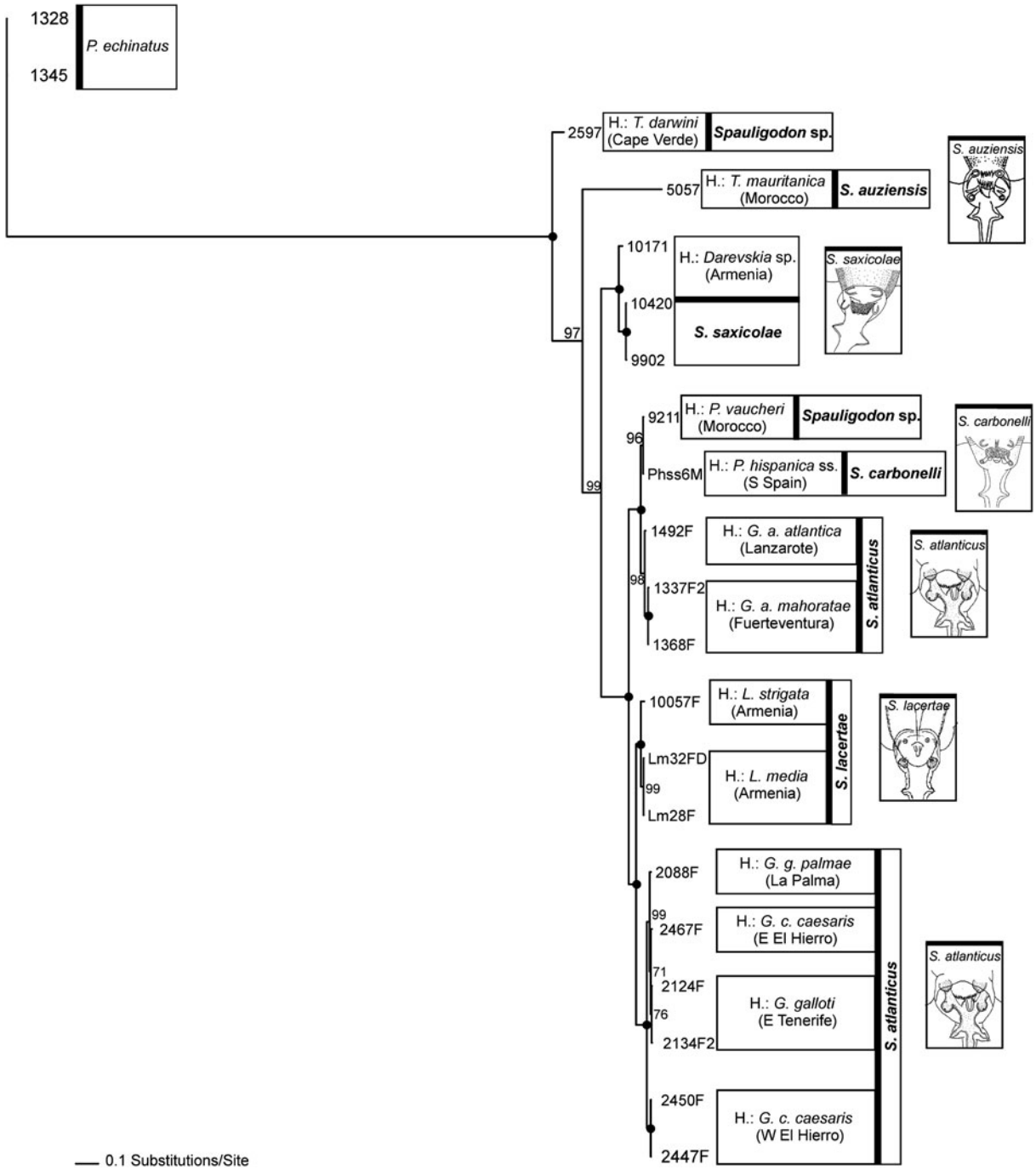


**Fig. 4** Bayesian inference tree of the combined COI and 28S rRNA data for the *Spauligodon* spp. analysed, with the respective host species (H) and localities. Values represent posterior probabilities. Bayesian clade credibility values of 100 are shown as a filled circle on the node. The male caudal extremity is shown in detail for each respective *Spauligodon* species [*Spauligodon* drawings adapted from Sharpilo (1976), Roca (1985), Astasio-Arbiza et al. (1987) and Roca & García-Adell (1988)]. Specimen code descriptions are given in Table 1

of nematodes and was also important for confirming the phylogenetic relationships based on mtDNA, which otherwise could be misleading if used as a single phylogenetic marker (Galtier et al., 2009). However, because there was no topology conflict between the two independent analyses, the combined COI and 28S rRNA data-set analysis was performed to provide a better estimate of the tree topology. The reconstruction of the phylogenetic relationships of *S. atlanticus* apparently indicates another case of cryptic speciation between nematodes. In fact, the species as currently recognised is polyphyletic, including two highly divergent and not directly related lineages, one corresponding to the eastern Canary Islands and the other to the western islands. Although further sampling from the giant *Gallotia* spp., such as *G. stehlini*, is still needed, adding their nematodes to this phylogeny would not alter this conclusion. Nevertheless, based on the latest phylogenetic analysis (Cox et al., 2010), the most basal node within the *Gallotia* phylogeny represents the divergence of *G. stehlini* (Gran Canaria) from the remaining *Gallotia* spp. situated on the eastern and western islands, so it will be interesting to determine which of the *S. atlanticus* lineages was previously detected in this lizard (Martín, 2005). The absence of *S. atlanticus* from *G. stehlini* was very surprising considering: (1) the high prevalence previously reported (51.5% from Martín, 2005); (2) the sampling was carried out in exactly the same locality (Aldea Blanca) and during the same period of the year (early spring); and (3) the high number of specimens of *G. stehlini* examined (33), including those from other localities. No plausible hypothesis to explain this dramatic drop in prevalence has previously been indicated, since this is a parasite with a direct life-cycle that does not depend on the presence of other hosts to complete its life-cycle, and the prevalences of the other species found in this study were similar to the values reported by Martín (2005). Indeed, more efforts are needed to elucidate the life-history traits of nematodes parasitising lacertid lizards.

Regarding the eastern lineage, the parasites of Fuerteventura may reflect recent lizard interchanges between this island and southern Lanzarote, probably during the marine regressions associated with the glaciations (Villalba, 1998). In fact, Lanzarote harbours much more genetic diversity than Fuerteventura (2.3% versus 1% uncorrected p-distance, respectively, for the COI). The western clade is found on the four western islands (Tenerife, La Palma, La Gomera and El Hierro), demonstrating a complex pattern. El Hierro, the westernmost island, includes two divergent lineages corresponding to the two localities sampled, one in the west and the other on the eastern part of the island, well supported in both analyses. El Hierro was formed by a single volcanic edifice and is very young (1.12 Ma, according to Carracedo et al., 1998), suggesting that divergence between the two lineages may be the result of different host colonisations to the island after its formation. Two alternative scenarios could explain this pattern. Firstly, one of these lineages originated on the other resident of the island, the unsampled and critically endangered giant, *G. simonyi*, and then host-switched to *G. c. caesaris*. Or secondly, one of these lineages could be related to the *Spauligodon* sp. found in the sympatric gecko *Tarentola boettgeri hierrensis* Joger & Bischoff (Roca et al., 1999). Regarding the first hypothesis, although the giant lizard is currently absent from the localities sampled, where only *G. caesaris* was present, there is fossil evidence that it was widespread in the recent past (Mateo, 2007), making host-switching theoretically possible. In this case, the same patterns can also occur in the islands where both *Gallotia* forms, the giants and the smaller species, coexist. Regarding the second hypothesis, preliminary analyses have shown that *S. tarentolae* Spaul, 1926 from *Tarentola delalandii* (Duméril & Bibron), as well as other *Spauligodon* spp. infecting other geckos, are genetically and morphologically distinct from *S. atlanticus* (Figs. 2–4; Jorge et al., unpublished). However, this hypothesis cannot be ruled out until *S. tarentolae* is analysed from all possible hosts of the Canary Islands. The specimens from Tenerife also present two different, less divergent lineages, one comprising the northern and south-eastern samples and the other the western samples grouping with La Gomera. However, this relationship between specimens from La Gomera and the western lineage from Tenerife has to be interpreted with caution, since only a single sample from this lineage was available. The same applies to *S. atlanticus* from *G. g. palmae* on La Palma. However, the specimen from La Palma appears to be a sister group to the specimen from eastern El Hierro and eastern Tenerife, suggesting a process of colonisation by descent, reflecting the colonisation and phylogeny of their host. This relationship could also be evidence

of a reverse colonisation of the host, followed by host-switching of the parasite.

The eastern lineage of *S. atlanticus* is more closely related to *S. carbonelli* from *Podarcis hispanica* Steindachner (*sensu stricto*), in southern Spain and *Spauligodon* sp. from *P. vaucheri* Boulenger in Morocco, than to the other *S. atlanticus* lineage. This relationship is very well supported. Furthermore, Fuerteventura and Lanzarote are the islands of the archipelago closest to the mainland, which might suggest a colonisation from the African continent mediated by other congeneric reptiles, namely species of *Tarentola* and *Chalcides*. On the other hand, the western parasite lineage appears more related to *S. lacertae* identified in the Caucasus, namely from large green lizards of the genus *Lacerta*. Given the considerable geographical distance between these specimens and the information gap in the intermediate area, the relationships between these *Spauligodon* spp. have to be considered as undetermined. Almost certainly, other *Spauligodon* spp. parasitising other reptile hosts await description. This is the case, for example, for *Spauligodon* sp. found in *T. darwini* from Cape Verde and in *P. vaucheri* from Morocco. Still, given the clear polyphyly of *S. atlanticus*, we propose the separation of the species, with the eastern lineage retaining the *S. atlanticus* designation, according to the first description of the species from *G. a. atlantica* in Lanzarote, while the western lineage should be considered as a new species, as yet to be named and formally described. In order for this to be accomplished, and to determine whether there are any diagnostic differences between these lineages, further detailed analyses of the morphology of these forms using high resolution techniques (see De Ley et al., 2005) are still required.

## *Spauligodon atlanticus* colonisation

The existence of cryptic species within *S. atlanticus* was suspected, considering the phylogeny, distribution and timing of the colonisation of the Canary Islands by their hosts, lizards of the genus *Gallotia*. However, the polyphyly of the species not tracking the monophyly of their lizard hosts was unexpected. Apparently, the two lineages of *S. atlanticus* are not sympatric, i.e. they do not occur in the same host species. Different events could be responsible for the origins and distributions of the current associations. Given the polyphyly of *S. atlanticus*, it is unlikely that both lineages colonised the Canarian archipelago by descent through ancestral forms of *Gallotia*. Interestingly, previous surveys of Ibero-Maghrebian lizards of the genus *Psammodromus*, sister taxa of *Gallotia* spp. (Cox et al., 2010), have not reported *Spauligodon* spp. (Roca, 1985). Assuming that at least one of the lineages arrived with the ancestral species *Gallotia*, the other lineage had to have originated by host-switching from a different reptile that also colonised the archipelago. Although the *Gallotia* spp. appear to have colonised the Canary Islands in a single invasion, other possible *Spauligodon* hosts, namely species of *Tarentola* and *Chalcides* also present in the archipelago, reached it in more than one colonisation event (three and two, respectively; Carranza et al., 2002; Carranza et al., 2008). Until now, only *S. tarentolae* from *Tarentola delalandii* (Tenerife) and *Spauligodon* sp. from *T. b. boettgeri* (Gran Canaria) and *T. b. hierrensis* (El Hierro) have been described in the Canary Islands (Spaul, 1926; Roca et al., 1999) in addition to *S. atlanticus*. As previously mentioned, *S. tarentolae* from *T. delalandii* is genetically distinct (Jorge et al., unpublished) from *S. atlanticus*, but there are still no molecular data regarding *Spauligodon* sp. from *T. b. boettgeri* and *T. b. hierrensis*. It is also possible that undescribed species of *Spauligodon* may be found in other species of *Tarentola* or *Chalcides* from the Canarian archipelago. A recent survey of the helminth infracommunities of *Chalcides sexlineatus* from Gran Canaria (Roca et al., 2010) failed to detect species of *Spauligodon*. Nevertheless, a more extensive study of the helminth fauna of the reptiles of the Canary Islands is needed to assess the possible origins of the current associations of *Spauligodon* spp. in this insular system.

## Taxonomic implications

The identification of nematodes based only on morphological characters can be difficult due to the lack of easily detectable, useful characters. Usually, for an accurate determination of nematode morphology both high resolution light microscopy and scanning electron microscopy are required, as well as a detailed knowledge of related forms described in the taxonomic literature (De Ley et al., 2005). In the family Pharyngodonidae, generic and specific identification is mainly based on the structure of the male

caudal extremity, female worms generally being very similar between groups. Bursey et al. (2005) selected specific characteristics for distinguishing species of *Spauligodon* on the basis of the presence or absence of a spicule, spines on the tail filament of adults, egg morphology and geographical distribution. Recent descriptions of new *Spauligodon* spp. still rely on morphology only (Binh et al., 2007; Bursey et al., 2007; Monks et al., 2008). *S. atlanticus* was also described based exclusively on morphological characters. However, despite its morphological consistency, phylogenetic analyses (using both mtDNA and nDNA markers) not only detects cryptic speciation but also reveal that this species is a polyphyletic assemblage. Interestingly, although these two different, unrelated clades of *S. atlanticus* display a similar overall morphology, they are more related to other *Spauligodon* spp. with quite different morphological characters (Fig. 4). This could be caused by morphological convergence under similar selective pressures encountered in their host, different species of *Gallotia*. Moreover, differences in the morphologies of sister taxa, mainly observed in the male genitalia, could be due to sexual selection. A different morphology may enable sexual segregation between closely related species living in sympatry, yet be inadequate for indicating phylogenetic relationships. Analyses of the vulval appendages in nematodes have also been considered as taxonomically unreliable, even at the intrageneric level, due to considerable homoplasy (Carta et al., 2009). The use of traditional taxonomy, based solely on morphology, has probably led to an underestimation of nematode diversity. In view of this, it is clear that further assessment of genes in other *Spauligodon* spp. from related reptiles is needed.

In conclusion, these results provide another example where phylogenetic analyses of a parasite reveal a more complex pattern of association between the parasite and its host than was apparent using morphological techniques. However, in the case of the association between *S. atlanticus* and *Gallotia* spp., our results reveal a complex pattern, the origin of which is still unresolved.

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