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Cryptic species unveiled: the case of the nematode *Spauligodon atlanticus*

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Abstract

The implementation of molecular tools in parasitology has led to the discovery of numerous cryptic species. However, detailed morphological studies are needed to evaluate the cryptic nature of such species, as well as to provide an appropriate and formal description. Recent phylogenetic analyses using mitochondrial and nuclear genes have revealed that the nematode *Spauligodon atlanticus*, parasite of lizards of the genus *Gallotia* endemic to the Canary Islands, consists of two highly divergent and unrelated lineages, one in the eastern islands and the other in the western ones. This study provides a detailed morphological analysis of the two *S. atlanticus* lineages characterized genetically, based on body measurements and scanning electron microscopy. This integrative approach revealed phenotypic differences between them, despite their overall morphological resemblance. As a result, the new species *Spauligodon occidentalis* sp. nov., from the formerly western lineage, is described. The morphological similarity between the two *Spauligodon* species is better explained on the basis of evolutionary convergence, since both species parasitize *Gallotia* lizards. In addition to delimiting the new nematode species, this study highlights the importance of combining genetic and morphological data with taxonomy to uncover the nature of cryptic species and decrease taxonomic uncertainty.

Key words: Canary Islands – cryptic species – morphology – nematodes – species description

Introduction

It is now relatively straightforward for taxonomists to incorporate multiple sources of information, including molecular and morphological, into a species description, an approach that strengthens both the empirical foundations of systematics and the Linnaean framework itself (Goldstein and DeSalle 2011). This integrative approach can provide taxonomists with a greater arsenal to face the realities of inventorying the actual biodiversity of the planet (Padial et al. 2010). Molecular tools offer an unprecedented opportunity to include new components in the discovery and description of biodiversity, merging contemporary technologies with the traditional morphological approaches (Nadler and Pérez-Ponce de León 2011). Such techniques are revealing a strong bias in the previous estimation of species richness, by identifying a significant number of cryptic species (Dobson et al. 2008). This is especially relevant in nematodes with microscopic structural differences, in which morphological assessment and identification of diagnostic characteristics are often difficult and require more technical and taxonomic expertise than those needed for macroscopic taxa (Abebe et al. 2011). Recently, several studies reported the discovery of cryptic species within what were considered single species, through the use of population genetics, phylogeographic or phylogenetic tools (Jorge et al. 2011; Nadler and Pérez-Ponce de León 2011; Poulin 2011a; Oliveira et al. 2012). Although several definitions of cryptic species exist (see Bickford et al. 2007; Nadler and Pérez-Ponce de León 2011), in the strict sense, they can only be considered provisionally 'cryptic', since additional morphological studies or new high-resolution microscopy techniques may unveil diagnostic structural differences that allow a rapid and practical morphological diagnosis (Fritz et al. 2006; Pérez-Ponce de León and Nadler 2010). However, despite an increase in the number of species as a consequence of the implementation of molecular tools, further morphological studies providing an appropriate and

formal description are often lacking. Consequently, there is an increase in taxonomic uncertainty that is counterproductive to research progress and synthesis in parasite systematics (Pérez-Ponce de León and Nadler 2010), considered by Littlewood (2011) as the 'cornerstone' of parasitology. Although other characteristics, such as host specificity, are relevant, morphology is still the primary source of data in parasite taxonomy, especially of metazoan parasites, although morphological characters may sometimes be misleading (Littlewood 2011; Perkins et al. 2011). Recently, there has been a dedicated effort to solve such problems in parasite systematics (Littlewood 2011) highlighting the advantages and disadvantages, promises and pitfalls of different approaches. It is now widely recognized that an integrative approach is needed to better assess parasite biodiversity, conciliating molecular tools with a traditional morphological approach that can be improved with high-resolution methods, including scanning electron microscopy and confocal microscopy. Although few such integrative studies on nematodes have been conducted (e.g. Fonseca et al. 2008; Falk et al. 2011; Razo-Mendivil and Pérez-Ponce de León 2011; Oliveira et al. 2012), they reinforce the importance and value of this approach, which should be more frequently implemented in parasitological studies.

One recent case of possible cryptic species in parasites has been observed in the nematode genus *Spauligodon*, infecting endemic lizards of the genus *Gallotia* in the Canarian archipelago (Jorge et al. 2011). *Spauligodon atlanticus* Astasio-Arbiza et al. 1987 was first described as a parasite of *Gallotia atlantica atlantica* Peters and Doria, 1882 and later identified in other host species of the same genus (Martin and Roca 2005). Despite the overall similar morphology, phylogenetic analysis revealed that *S. atlanticus* actually consists of two highly divergent lineages (12.9% uncorrected p-distance for COI). Moreover, the lineages are unrelated, suggesting that *Gallotia* spp. were colonized twice independently by *Spauligodon* nematodes (Jorge et al. 2011). Given the clear polyphyly of *S. atlanticus* revealed by both mitochondrial and nuclear genes (cytochrome oxidase subunit I and 28S ribosomal RNA, respectively), Jorge et al. (2011) proposed the separation of the species, with the eastern lineage retaining the *S. atlanticus* designation, since the first description

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of the species was from *G. a. atlantica* from the easternmost island (Lanzarote), while the western lineage should be considered as a new species, which has not yet been formally described. In this study, we perform a detailed morphological analysis of the two *S. atlanticus* lineages, to determine the putative phenotypic differences between them and, if possible, to detect diagnostic characters. Subsequently, we formally describe a new species, corresponding to the western lineage, and redescribe *Spauligodon atlanticus*, comparing previously phylogenetic evidences with morphological and morphometric characteristics obtained by means of light and scanning electron microscopy.

Material and Methods

Nematode isolation and vouchering

In 2009, nematodes of the genus *Spauligodon* were collected from six of the seven main islands of the Canarian archipelago (Fig. 1) preserved in 96% ethanol and analysed phylogenetically by Jorge et al. (2011) (Fig. 2). In this study, male and female specimens from the same localities and when possible from the same host specimen of the ones phylogenetically assessed were subjected to a detailed morphological analysis. Nematodes included in the previous genetic analysis (Jorge et al. 2011) could not be used in the morphological analysis due to limitations of the equipment used at the time. In addition, specimens collected in a previous expedition (Martin and Roca 2005) from the same localities and preserved in the same conditions (96% ethanol) were also included in the data set for morphological analysis. *Spauligodon* specimens were mounted on temporary slides with a bleaching solution (Foitová et al. 2008) and observed at different magnifications using a light microscope (Olympus CX41). All specimens were photographed using a digital camera Olympus DP25 and measured with the DP2-BSW software (Olympus®). Following De Ley et al. (2005), voucher videos were also assembled using several magnifications in different focal planes. Subsequent to the measurements, subsets of specimens were selected for scanning electron micrographs (SEM) and as vouchers to be deposited in museum (28 and 19, respectively). For scanning electron microscopy analysis, specimens were hydrated in an ethanol series followed by distilled water. They were then postfixed in 1% OsO_4, dehydrated through ethanol series and then dried to a critical point. The specimens were coated with AuPd to 10 nm thickness and examined with a Cambridge Instruments S360 scanning electron microscope fitted with Dindima Image Slave frame grabber and with Zeiss Sigma VP FEG scanning electron microscope fitted with the HKL INCA Premium Synergy Integrated ED/EBSD system (the latter was only used for 10 specimens). Description photographs and videos have been deposited in MorphoBank (http://www.morphobank.org.Type vouchers and type specimens were deposited in the Natural History Museum, London. Additional specimens and DNA extractions are available upon request to the authors.
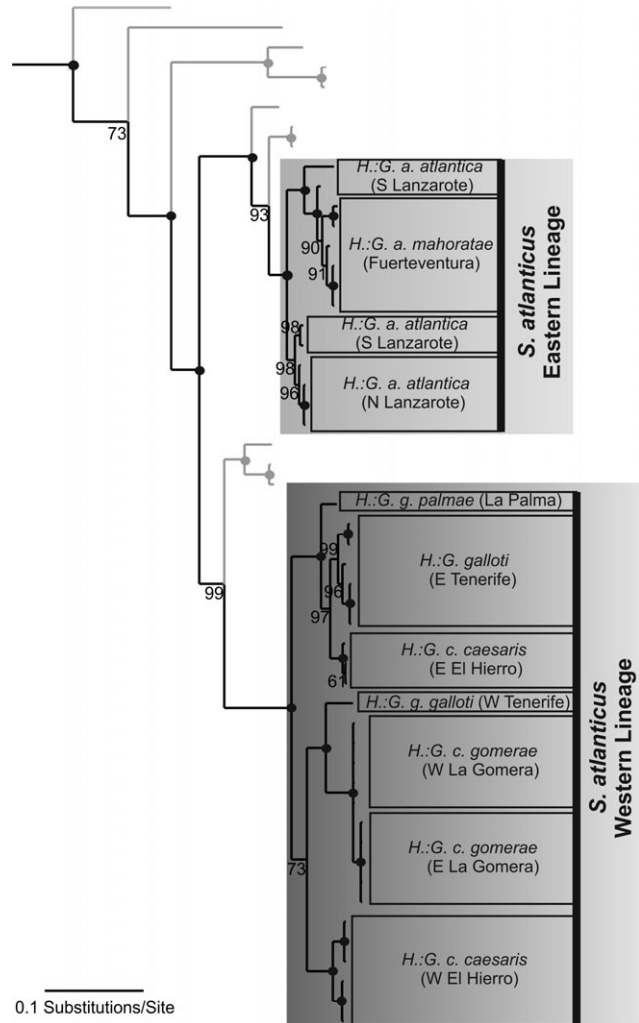

Fig. 2. Bayesian inference tree of the COI data for the *Spauligodon* spp. analysed in Jorge et al. (2011) with their respective host species (H) and localities. Values represent posterior probabilities. Bayesian clade credibility values of 100 are shown as a filled circle on the node. Adapted from Jorge et al. (2011)

Morphology

Prior to the morphometric study, SEM were taken from fifteen *Spauligodon* specimens belonging to the two existing lineages (six specimens from the western and nine from the eastern lineage) in search of possible
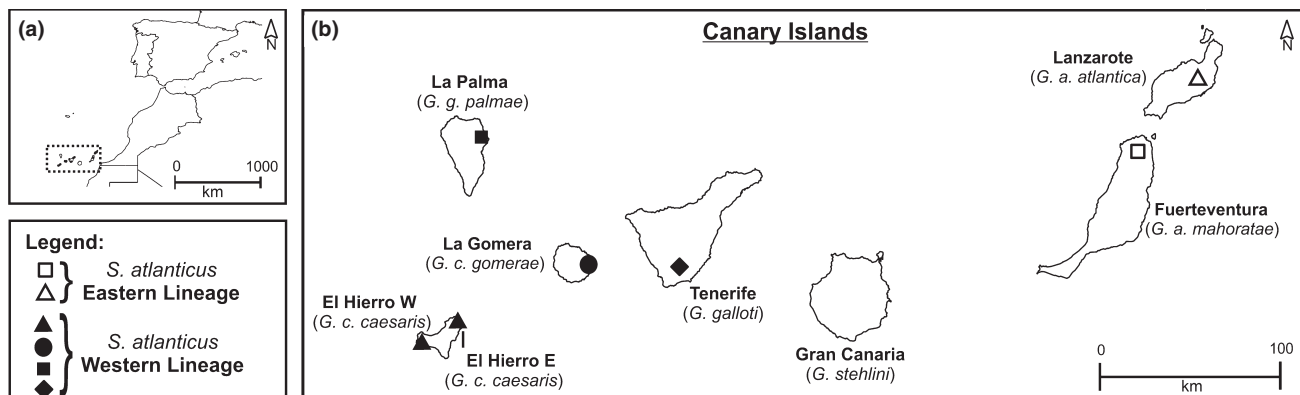

Fig. 1. Map of the Canary Islands showing the geographical location of *Spauligodon atlanticus* samples included in the morphological analyses. a, geographical location of the Canary archipelago; b, Canary Islands

diagnostic morphometric characters. Preliminary micrographs suggested differentiation in the posterior region of the male, namely with regard to the size of the papillae. After this, seventeen characters in males (seven of them concerning the caudal extremity) and 14 in females were measured under a light microscope (Table 1, Fig. 3), for a total of 63 males and 54 females from seven localities. No females from La Palma were included in the analyses because of the limited number of specimens in good conditions. Representatives from all localities included in this study had been previously analysed genetically by Jorge et al. (2011) (Figs 1 and 2). All linear measurements (Fig. 3) were recorded with the same equipment (camera/software/microscope) by the same person (FJ). Body length (BL) was measured from the anterior edge of the lips to the posterior edge of the body in females and to the posterior edge of the third pair of papillae in males. Body width (BW) was recorded at the middle body level in females and at the level of the excretory pore (excluding lateral alae) in males. Oesophagus length (OL) and width (OW) were recorded from the anterior border to the posterior margin that connects to the bulb, and at the level of the last third of the oesophagus, respectively. Length of oesophageal bulb (OBL) was recorded from the anterior border that connects with the oesophagus to the posterior margin, and oesophagus bulb width (OBW), at its widest point. Positions of the nerve ring (NR) and excretory pore (ExP) were recorded from the anterior edge to the nerve ring and excretory pore, respectively. Tail length (TL) was measured from insertion (at the base of the third pair of papillae in

Table 1. Descriptive statistics for all the linear measurements of adult specimens of the different lineages (eastern and western) taxa included in the multivariate analysis (in µm)

Eastern lineage

	Males (N = 18)		Females (N = 18)	
Character	Mean ± SD	Range	Mean ± SD	Range
BL	843.51 ± 119.01	659.40–1075.10	2846.45 ± 536.13	1835.28–3796.86
BW	136.19 ± 21.29	94.58–170.48	368.24 ± 60.43	273.35–473.09
OL	148.61 ± 19.04	106.04–183.34	261.57 ± 19.78	222.32–297.76
OW	23.46 ± 2.56	18.47–27.50	33.90 ± 4.27	25.10–41.76
OBL	54.09 ± 6.05	42.93–68.51	83.76 ± 8.52	63.42–96.11
OBW	58.92 ± 5.43	46.36–66.76	96.94 ± 7.29	84.14–111.46
NR	75.57 ± 14.68	40.42–99.17	107.45 ± 15.18	80.97–150.00
ExP	253.30 ± 31.88	205.20–328.44	235.34 ± 69.59	132.67–374.99
TL	219.89 ± 49.29	110.17–278.29	481.80 ± 31.41	430.08–539.26
LA	53.44 ± 11.38	39.44–68.39	–	
CT1	20.14 ± 1.55	17.64–22.80	–	
CT2	12.12 ± 1.26	9.25–13.82	–	
TW	12.84 ± 1.30	9.34–14.90	–	
3p1	5.39 ± 0.64	3.85–6.71	–	
3p2	4.00 ± 0.67	3.01–5.61	–	
3p3	8.67 ± 1.20	6.52–10.38	–	
3pl	8.33 ± 1.12	6.12–10.88	–	
Vu	–		282.56 ± 72.90	162.13–413.76
Va	–		448.14 ± 73.43	303.33–591.01
Weggm	–		36.12 ± 3.90	28.01–47.60
Leggm	–		121.35 ± 5.50	103.94–133.33
Spines	0		7.64 ± 1.03	6–9

Western lineage

	Males (N = 45)		Females (N = 36)	
Character	Mean ± SD	Range	Mean ± SD	Range
BL	1317.45 ± 228.77	915.56–1749.14	3117.09 ± 535.11	2323.64–4457.37
BW	160.51 ± 28.68	98.93–219.02	382.60 ± 53.05	295.02–516.24
OL	264.04 ± 25.43	202.06–316.87	382.88 ± 47.51	156.41–449.30
OW	25.82 ± 3.04	20.13–32.83	35.93 ± 3.44	29.59–44.32
OBL	68.09 ± 9.23	49.61–90.10	103.04 ± 7.01	87.16–115.73
OBW	75.34 ± 10.15	51.96–98.21	117.05 ± 9.48	92.96–141.33
NR	119.75 ± 17.54	70.57–153.73	123.78 ± 10.00	104.01–142.97
ExP	395.65 ± 53.53	288.07–557.85	302.50 ± 67.29	200.08–417.03
TL	128.10 ± 16.95	98.95–165.66	389.70 ± 56.79	267.97–510.33
LA	93.12 ± 26.95	55.07–129.44	–	
CT1	30.59 ± 2.87	23.83–35.71	–	
CT2	16.58 ± 2.78	11.90–24.42	–	
TW	12.32 ± 1.26	10.21–15.30	–	
3p1	8.48 ± 1.10	5.25–11.03	–	
3p2	5.48 ± 1.24	2.22–8.06	–	
3p3	9.75 ± 1.20	7.63–13.23	–	
3pl	12.06 ± 1.54	9.14–14.95	–	
Vu	–		361.30 ± 70.33	239.28–475.61
Va	–		532.12 ± 73.21	390.63–727.45
Weggm	–		40.14 ± 3.49	33.71–51.45
Leggm	–		132.25 ± 6.41	115.97–152.27
Spines	0		7.31 ± 1.33	5–11

For each variable, mean ± standard deviation (SD), range and sample size (N) are given.
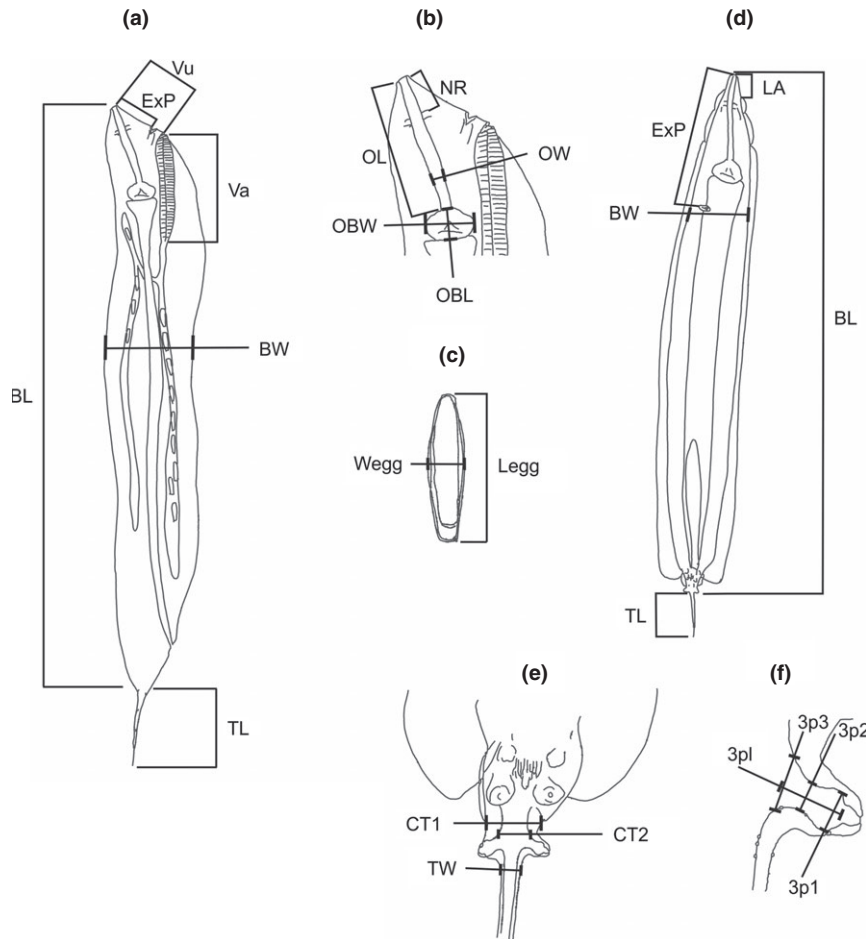
Fig. 3. Linear measurements that were recorded for morphological analyses with their respective designation, for females (a-c) and males (d-f). See Material and methods for variables abbreviations

males) to its tip (broken tails were not included) and the tail width (TW) at its widest point. Lateral alae (LA) were measured from the anterior edge to the anterior beginning of the lateral alae (only measured in males). In males, measurements at the posterior end of the caudal papillae were also recorded (Figs 3e and f). Caudal trunk width was measured at its widest point (CT1) and narrowest point (CT2), at the insertion of the third pair of caudal papillae (Fig. 3e). The width of one of the papilla of the third pair of caudal papillae was also measured, at the tip (3p1), middle (3p2) and insertion point (3p3). For the same papillae, the length (3pl) was also measured. Bilateral measurements were taken from the same side of the nematode whenever possible. In females, vulva (Vu) position was also recorded from the anterior edge. Vagina (Va) was measured from the vulva until the posterior border of the vagina. Egg length (Legg) and egg width (Wegg) were also measured from the longest and widest parts, respectively, for a total of four eggs per female, and average egg length (Leggm) and width (Weggm) were calculated per individual. Spines were counted as the total number of cuticular spines present in tail.

Statistical analyses

Due to accentuated sexual dimorphism in *Spauligodon*, statistical analyses were conducted on males and females separately. Morphometric analyses were performed for a total of 117 specimens (63 males and 54 females; Data S1), corresponding to seven localities (Fig. 1) including 16 morphological variables in males (LA was not included) and 14 in females. Lateral alae (LA) were not included because data were missing for the majority of the specimens.

Prior to analysis, measurements were log-transformed and checked for homoscedasticity (Bartlett test) and normality (Shapiro–Wilk test) using

the functions *bartlett.test* and *shapiro.test* of the R package, respectively (R Development Core Team 2011). Since several variables did not meet the assumptions, a nonparametric approach was followed.

To determine whether to include body length (BL) as a covariate in the subsequent analyses, nonparametric Spearman correlations between BL and remaining body measurements were performed, using the function *cor* of the R package (R Development Core Team 2011). Morphological differences between lineages were analysed using a permutational (multi)variate analysis of covariance (M)ANCOVA. This procedure is a good alternative to sum-of-squares-based (M)ANCOVAs, in cases where data do not meet normality and homoscedasticity assumptions (Anderson 2001). Permutational (M)ANCOVAs based on 1000 permutations were calculated using the function *adonis* implemented in the package *Vegan* (Oksanen et al. 2012) of the R software (R Development Core Team 2011). A full model including the main effects of the factors LINEAGE, ISLAND considered as nested in LINEAGE, body length (BL) and their interaction was tested using a sequential sum of squares. The interaction factor was used to test the assumption of slope homogeneity (Engqvist 2005). Least squares means (LS means, adjusted means) and 95% confidence intervals were represented graphically using the software STATISTICA 7.1 (StatSoft Inc 2005).

To summarize the main sources of variation, principal component analysis (PCA) including all body measurements was performed for males and females separately. We used the *prcomp* function implemented in the package R (R Development Core Team 2011).

Results

Descriptive statistics of the biometric variables for all specimens are given in Table 1.

Males

BL was correlated with most of the body measurements, with the exception of TW ($r = -0.13$, $p = 0.292$). Correlations were positive in all cases, except in TL, which was negatively correlated with BL ($r = -0.40$, $p < 0.001$).

Body length (BL) and several other body measurements differed between lineages (Table 2, Fig. 4). Regarding BL, individuals from the western *S. atlanticus* lineage were larger than the eastern ones (Fig. 4). Specifically, males from El Hierro and La Palma (see Fig. 1) were the largest, while the ones from Fuerteventura and Lanzarote were the smallest (Fig. 4). However, despite the generally smaller size, individuals from the eastern lineage (Lanzarote and Fuerteventura) had comparatively longer tails (TL) (Table 2, Fig. 4). Body width (BW) and tail width (TW) were similar in both lineages (Table 2, Fig. 4). Caudal trunk (CT1 and CT2) was also larger in the western than in the eastern lineage (Table 2, Fig. 4). Regarding the digestive tube, there were no differences in the oesophagus bulb size (nor length, OBL or width, OBW), although the western lineage had a longer (OL) but not wider (OW) oesophagus than the eastern lineage (Table 2, Fig. 4). Both the nerve ring (NR) and excretory pore (ExP) were, in general, in a more posterior position in the western than in the eastern lineage (Table 2, Fig. 4). Finally, the size of the third pair of caudal papillae also showed some differences; the western lineage had a larger and wider third pair of papillae (3pl and 3p1; see Fig. 3 for details) but similar width of the peduncle (3p2 and 3p3; Table 2).

We also identified island differentiation within each lineage (Table 2, Fig. 4). Within the eastern islands, individuals from Lanzarote were larger (BL) and had longer tails (TL), shorter oesophagus (OL), more anterior nerve ring position (NR) and wider insertion of the third papillae (3p3; Fig. 4) than those from Fuerteventura. Regarding the western *S. atlanticus* lineage, individuals from La Palma and El Hierro were larger than the rest. In addition, specimens from La Palma were comparatively thinner (BW) and had a wider caudal trunk (CT2) and a wider base of the papillae (3p3) than the ones from the other western islands (Fig. 4).

These differences were reflected in a good separation of the two lineages across the first two axes of the principal component analysis, encompassing 66.8% of the total morphological variance (Fig. 5). The first component (PC1) explained 56.8% of the total variance; body size and most of the remaining variables related to body size have similar contribution and sign across the PC1, with the exception of tail length (TL) and tail width (TW)

Table 2. Results of the permutational analysis of covariance on the males of *Spauligodon* showing the effects of the factors lineage, island nested in lineage, and their interaction, on body measurements using body length as covariate. For each variable, sequential sum of squares (SS), F statistic (F), R-squared values (R^2) and p-values (p) are shown. Significant results ($p < 0.05$) are in bold

	BL				Lineage				Lineage:island			
	SS	F	R^2	p	SS	F	R^2	p	SS	F	R^2	p
BL					0.470	192.173	0.587	**0.001**	0.191	19.627	0.239	**0.001**
MANCOVA	3.970	93.688	0.484	**0.001**	1.179	27.829	0.144	**0.001**	0.531	3.132	0.065	**0.004**
BW	0.140	54.423	0.329	**0.001**	0.003	1.275	0.008	0.264	0.133	12.986	0.314	**0.001**
OL	0.677	557.106	0.717	**0.001**	0.176	145.123	0.187	**0.001**	0.011	2.292	0.012	0.083
OW	0.069	40.569	0.394	**0.001**	0.007	3.998	0.039	0.057	0.004	0.528	0.021	0.718
OBL	0.195	92.778	0.618	**0.001**	0.001	0.258	0.002	0.599	0.001	0.068	0.002	0.989
OBW	0.205	115.359	0.623	**0.001**	0.002	1.045	0.006	0.302	0.002	0.211	0.005	0.943
NR	0.292	61.378	0.331	**0.001**	0.244	51.287	0.276	**0.001**	0.059	3.089	0.067	**0.031**
ExP	0.546	326.417	0.801	**0.001**	0.040	24.078	0.059	**0.001**	0.006	0.852	0.008	0.490
TL	0.333	77.394	0.324	**0.001**	0.330	76.692	0.321	**0.001**	0.127	7.386	0.124	**0.001**
CT1	0.343	328.406	0.667	**0.001**	0.097	92.575	0.188	**0.001**	0.008	1.832	0.015	0.131
CT2	0.231	75.380	0.474	**0.001**	0.028	9.019	0.057	**0.004**	0.043	3.472	0.087	**0.012**
TW	0.001	0.456	0.007	0.495	0.004	2.331	0.034	0.147	0.010	1.424	0.083	0.252
3p1	0.329	99.713	0.473	**0.001**	0.167	50.777	0.241	**0.001**	0.015	1.172	0.022	0.338
3p2	0.289	37.453	0.342	**0.001**	0.006	0.791	0.007	0.404	0.062	2.008	0.073	0.108
3p3	0.037	15.088	0.168	**0.002**	0.003	1.385	0.015	0.262	0.040	4.001	0.178	**0.006**
3pl	0.282	99.224	0.538	**0.001**	0.070	24.669	0.134	**0.001**	0.011	0.989	0.021	0.439

|	BL*Lineage				BL*Lineage:island			
	SS	F	R^2	p	SS	F	R^2	p
BL								
MANCOVA	0.069	1.634	0.008	0.170	0.285	1.680	0.035	0.081
BW	0.009	3.619	0.022	0.067	0.008	0.769	0.019	0.539
OL	0.001	0.598	0.001	0.447	0.017	3.567	0.018	**0.011**
OW	0.000	0.003	0.000	0.964	0.009	1.312	0.051	0.278
OBL	0.010	4.854	0.032	**0.021**	0.002	0.259	0.007	0.904
OBW	0.008	4.221	0.023	0.050	0.022	3.138	0.068	**0.027**
NR	0.007	1.441	0.008	0.251	0.038	2.011	0.043	0.131
ExP	0.000	0.009	0.000	0.923	0.005	0.704	0.007	0.586
TL	0.006	1.298	0.005	0.266	0.012	0.698	0.012	0.602
CT1	0.004	3.380	0.007	0.067	0.010	2.344	0.019	0.060
CT2	0.019	6.288	0.040	**0.019**	0.010	0.827	0.021	0.509
TW	0.001	0.344	0.005	0.548	0.015	2.124	0.124	0.088
3p1	0.001	0.155	0.001	0.656	0.015	1.157	0.022	0.331
3p2	0.000	0.009	0.000	0.938	0.094	3.053	0.112	**0.027**
3p3	0.002	0.835	0.009	0.377	0.014	1.367	0.061	0.239
3pl	0.003	1.037	0.006	0.317	0.013	1.100	0.024	0.380
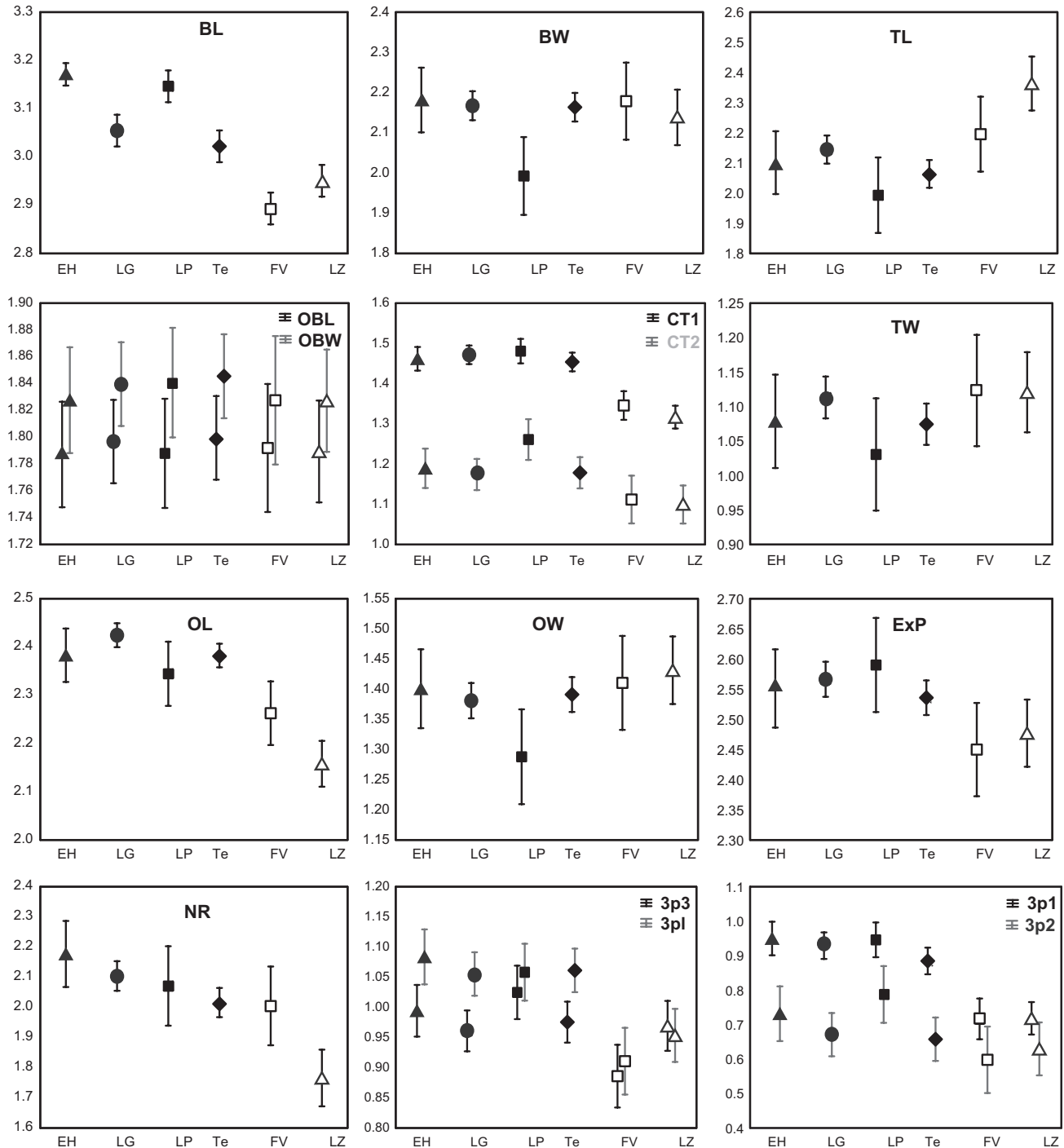
Fig. 4. Representation of the adjusted means (Least square means, LS) and confidence intervals (95%) by island for all body measurements of *Spauligodon* male individuals included in this study. Mean body size (covariate) value used to compute males LS was 3.017. See Material and methods for variables abbreviations. Island abbreviations: El Hierro (EH), La Gomera (LG), La Palma (LP), Tenerife (Te), Fuerteventura (FV) and Lanzarote (LZ). Black symbols represent the western islands and white ones the eastern ones

that have different signs (Table 3). PC1 showed a clear differentiation of the individuals from the eastern (Fuerteventura and Lanzarote) and western (remaining islands) lineages (Fig. 5). The second principal component (PC2) accounted for 10% of the total variation, with the variables contributing the most being body width (BW) and oesophagus width (OW) (Table 3). PC3 explained less than 7% of the variation. The variables contributing the most across this axis were 3p2 and 3p3 of the third pair of papillae (Table 3), although in this case, there was no clear differentiation of any specific population.

Females

All body measurements were correlated with body size (Spearman correlations, p < 0.05), with the exception of tail length (TL), number of spines in the tail (Spine) and egg width (Weggm) (in these cases, p > 0.05). In females, individuals from the western lineage were larger than those from the eastern one (BL, p < 0.05; Tables 1 and 4). However, as observed in males, western *S. atlanticus* had comparatively shorter tails than the eastern ones (Table 4, Fig. 6), although both had similar number

Table 3. Variable loadings (eigenvalues) extracted from the three-first principal components (PC) of the principal component analysis (PCA) on males (left) and females (right). For each principal component, eigenvalues and % variance are shown

|	Males				Females		
	PC1	PC2	PC3		PC1	PC2	PC3
BL	0.306	−0.131	0.059	BL	0.249	0.431	−0.039
BW	0.182	−0.446	0.298	BW	0.210	0.429	−0.303
OL	0.305	0.078	0.241	OL	0.359	−0.236	−0.056
OW	0.189	−0.528	−0.119	OW	0.214	0.342	−0.049
OBL	0.271	−0.289	−0.063	OBL	0.374	−0.035	−0.087
OBW	0.277	−0.303	−0.148	OBW	0.331	−0.079	−0.331
NR	0.240	0.294	0.244	NR	0.280	−0.078	−0.072
ExP	0.298	0.017	0.118	ExP	0.295	0.085	0.554
TL	−0.234	−0.321	−0.150	TL	−0.171	0.423	0.095
CT1	0.309	0.087	0.102	Vu	0.304	0.099	0.518
CT2	0.254	0.216	−0.076	Va	0.297	0.094	−0.111
TW	−0.053	−0.001	0.356	Spines	−0.012	−0.012	0.424
3p1	0.280	0.215	−0.079	Leggm	0.249	−0.229	−0.023
3p2	0.221	0.133	−0.453	Weggm	0.183	−0.432	0.028
3p3	0.173	0.088	−0.584				
3pl	0.272	0.082	0.134				
Eigenvalues	9.095	1.600	1.101	Eigenvalues	5.863	2.280	1.278
%variance	56.85	10.00	6.88	%variance	41.880	16.280	9.130
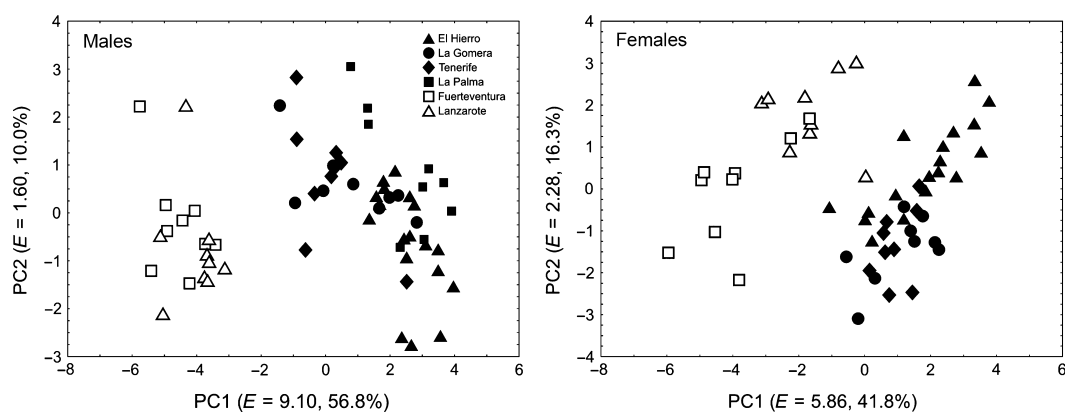
Fig. 5. Representation of the distribution of the individuals across the first two principal component axes. For each axis, eigenvalues (E) and% contribution of each axis to the total variance are detailed

of spines in the tail and similar body width (Table 4). Regarding the digestive system, there were no differences between lineages in oesophagus width (OW), although individuals from the western lineages had longer oesophagus (OL) and bigger oesophagus bulb (OBL and OBW, Table 4; Fig. 6). The excretory pore (Exp), nerve ring (NR) and vulva (Vu) had, in general, a more posterior position in individuals from the western than in those from the eastern lineage. The vagina (Va) was also larger than in those from the eastern lineage. Regarding eggs, they were bigger (both in length and width) in the western lineage (Table 4; Fig. 6).

We also found island variation within each lineage. Within the eastern lineage, individuals from Fuerteventura had a smaller body size (BL and BW) and digestive system (oesophagus length and width, OL, OW and oesophagus bulb length and width: OBL and OBW, Table 4, Fig. 6) than individuals from Lanzarote. In addition, individuals from Fuerteventura had a more posterior excretory pore (ExP) and vulva (Vu) than those from Lanzarote. They also had a smaller vagina (Va), egg size, especially egg width (Weggm) and a lower number of spines in the tail (Spines; Fig. 6). Regarding the western lineage, individuals

from El Hierro were the largest, but had the shortest tails. They also had a smaller oesophagus bulb size (OBL, OBW). Individuals from La Gomera had the longest and thinnest digestive tubes (Table 4).

These morphometric differences were reflected in a good separation of the lineages in the multivariate analysis. The first principal component (PC1) explained 42% of the total variation. The variables contributing the most were body length (BL) and most of the other body measurements, all of them with similar contribution and positive sign in the first component, with the exception of tail length (TL) and to a lesser extent the number of spines (Spines) that also contributed to variation across the first axis, but with a negative sign (Table 3). These differences were responsible for the separation of the two lineages across the first axis (Fig. 5). Regarding the second component (PC2), it explained 16% of the variation, with body length and width (BL and BW), oesophagus length and width (OL and OW), tail length (TL) and egg size (Weggm and Leggm) being the most important variables (Table 3). Finally, the third component (PC3) explained 9% of the total variation, with body width (BW), oesophagus bulb width (OBW), position of the excretory pore

Table 4. Results of the permutational analysis of covariance on the females of *Spauligodon* showing the effects of the factors lineage, island nested in lineage and their interaction, on body measurements using body length as covariate. For each variable, sequential sum of squares (SS), F statistic (F), R-squared values (R2) and p-values (p) are shown. Significant results ($p < 0.05$) are in bold

	BL				Lineage				Lineage:island			
	SS	*F*	R^2	p	SS	*F*	R^2	p	SS	*F*	R^2	p
BL					0.020	7.712	0.062	**0.008**	0.179	22.597	0.544	**0.001**
MANCOVA	0.656	19.343	0.191	**0.001**	0.890	26.245	0.259	**0.001**	0.162	1.590	0.047	0.109
BW	0.105	51.995	0.486	**0.001**	0.000	0.157	0.001	0.705	0.007	1.129	0.032	0.337
OL	0.048	58.909	0.119	**0.001**	0.311	378.510	0.763	**0.001**	0.009	3.508	0.021	**0.025**
OW	0.037	25.917	0.300	**0.001**	0.002	1.513	0.018	0.228	0.016	3.842	0.134	**0.011**
OBL	0.039	46.934	0.232	**0.001**	0.076	91.350	0.451	**0.001**	0.013	5.115	0.076	**0.005**
OBW	0.016	18.737	0.114	**0.001**	0.067	76.289	0.466	**0.001**	0.011	4.139	0.076	**0.014**
NR	0.021	10.954	0.138	**0.002**	0.037	19.003	0.240	**0.001**	0.002	0.310	0.012	0.809
ExP	0.162	17.224	0.215	**0.001**	0.096	10.233	0.128	**0.001**	0.017	0.586	0.022	0.625
TL	0.007	3.680	0.029	0.061	0.122	65.778	0.514	**0.001**	0.018	3.239	0.076	**0.032**
Vu	0.151	22.127	0.244	**0.001**	0.090	13.231	0.146	**0.001**	0.013	0.653	0.022	0.592
Va	0.066	20.746	0.236	**0.001**	0.042	13.385	0.152	**0.001**	0.001	0.088	0.003	0.959
Weggm	0.001	1.319	0.016	0.269	0.031	31.598	0.385	**0.001**	0.001	0.405	0.015	0.723
Leggm	0.001	5.838	0.034	**0.023**	0.016	82.919	0.478	**0.001**	0.007	12.788	0.221	**0.001**
Spines	0.002	0.582	0.009	0.459	0.000	0.001	0.000	0.976	0.047	4.335	0.205	**0.009**

	BL*Lineage				BL*Lineage:island			
	SS	*F*	R^2	p	SS	*F*	R^2	p
BL								
MANCOVA	0.121	3.566	0.035	**0.014**	0.118	1.162	0.034	0.308
BW	0.014	6.731	0.063	**0.011**	0.002	0.249	0.007	0.870
OL	0.002	2.685	0.005	0.121	0.001	0.393	0.002	0.771
OW	0.002	1.126	0.013	0.300	0.003	0.746	0.026	0.530
OBL	0.000	0.001	0.000	0.975	0.004	1.580	0.023	0.215
OBW	0.004	4.291	0.026	**0.045**	0.007	2.680	0.049	0.062
NR	0.001	0.350	0.004	0.553	0.008	1.330	0.050	0.278
ExP	0.029	3.129	0.039	0.088	0.036	1.267	0.047	0.299
TL	0.004	2.084	0.016	0.186	0.005	0.905	0.021	0.441
Vu	0.033	4.884	0.054	**0.023**	0.030	1.468	0.049	0.244
Va	0.023	7.109	0.081	**0.013**	0.008	0.852	0.029	0.459
Weggm	0.003	2.708	0.033	0.089	0.001	0.384	0.014	0.762
Leggm	0.000	0.029	0.000	0.862	0.000	0.748	0.013	0.523
Spines	0.007	2.036	0.032	0.155	0.014	1.238	0.059	0.296

(ExP) and the Vulva (Vu) and number of spines (Spine), being the most influential variables. PC3 did not separate clearly any population.

Integrative results and taxonomic summary

Altogether, the results presented here clearly demonstrate that the two genetic lineages retrieved by Jorge et al. (2011) are morphologically distinct and support their formal description as full species. In the subsequent paragraphs, the western lineage is described as a new species, whereas the eastern one is restricted to the original description of *S. atlanticus*.

Order Oxyurida Weinland, 1858
Family Pharyngodonidae Travassos, 1919
Genus *Spauligodon* Skrjabin, Schikhobalova and Lagodovskaja, 1960
***Spauligodon occidentalis* sp. nov**
MorphoBank M148036-M148105 and M148674-M148680 (Figs. 7-8)

Diagnosis
Spauligodon occidentalis sp. nov. closely resembles *S. atlanticus* presenting on average larger males and females. Excretory pore and nerve ring in both males and females as well as vulva in females have a more posterior position. Females of the new species also possess a larger vagina. Males have a larger caudal extremity end (CT1 and CT2), and the third pair of caudal papillae consists of two large prominent papillae and larger peduncles. However, these morphological characters show overlap between the two species. The characters that unambiguously separate the two species are the molecular characters. *S. occidentalis* and *S. atlanticus* consisted of two genetically different, unrelated species, presenting 12.9% and 1.4% (uncorrected p-distance) of divergence for the COI and 28S rRNA, respectively.

Specimens examined
Eighty-one (45 males, 36 females; Table 1 and Data S1).

Type material
Holotype: adult male (NHMUK 2012.9.13.1; MorphoBank M148057–M148058); Allotype: adult female (NHMUK 2012.9.13.2; MorphoBank M148055–M148056); and Paratypes: four males and four females (NHMUK 2012.9.13.3–10), from El Hierro Island (Canary Islands), Valverde (27.81798°N, 17.90859°W).

Etymology
The species epithet *occidentalis* alludes to the geographical distribution of the species, which is present in the western islands of the Canary archipelago.
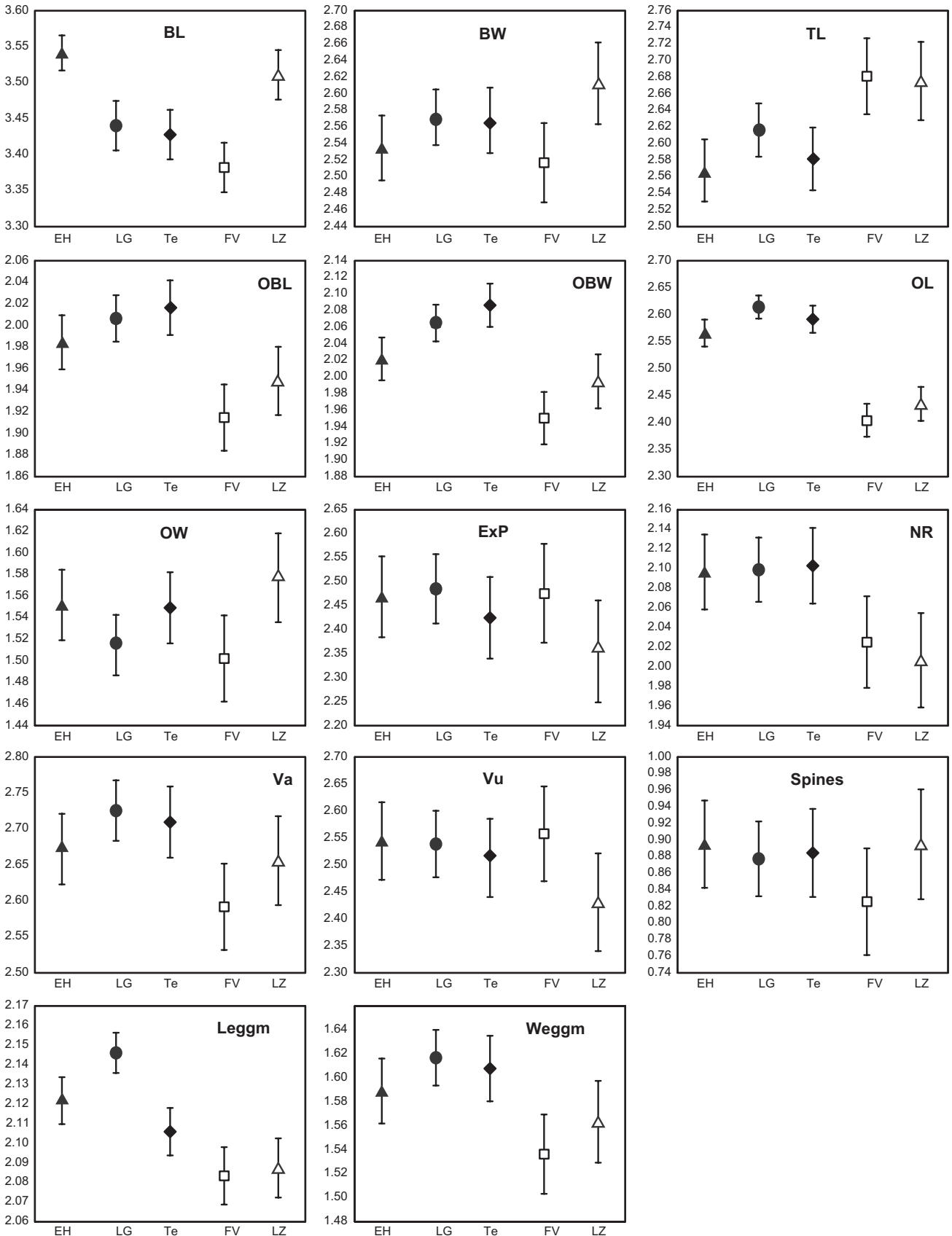
Fig. 6. Representation of the adjusted means (Least square means, LS) and confidence intervals (95%) by island of all body measurements of *Spauligodon* females individuals included in this study. Mean body size (covariate) value used to compute females LS was 3.461. See Material and methods for variables abbreviations. Island abbreviations: El Hierro (EH), La Gomera (LG), La Palma (LP), Tenerife (Te), Fuerteventura (FV) and Lanzarote (LZ). Black symbols represent the western islands and white ones the eastern ones
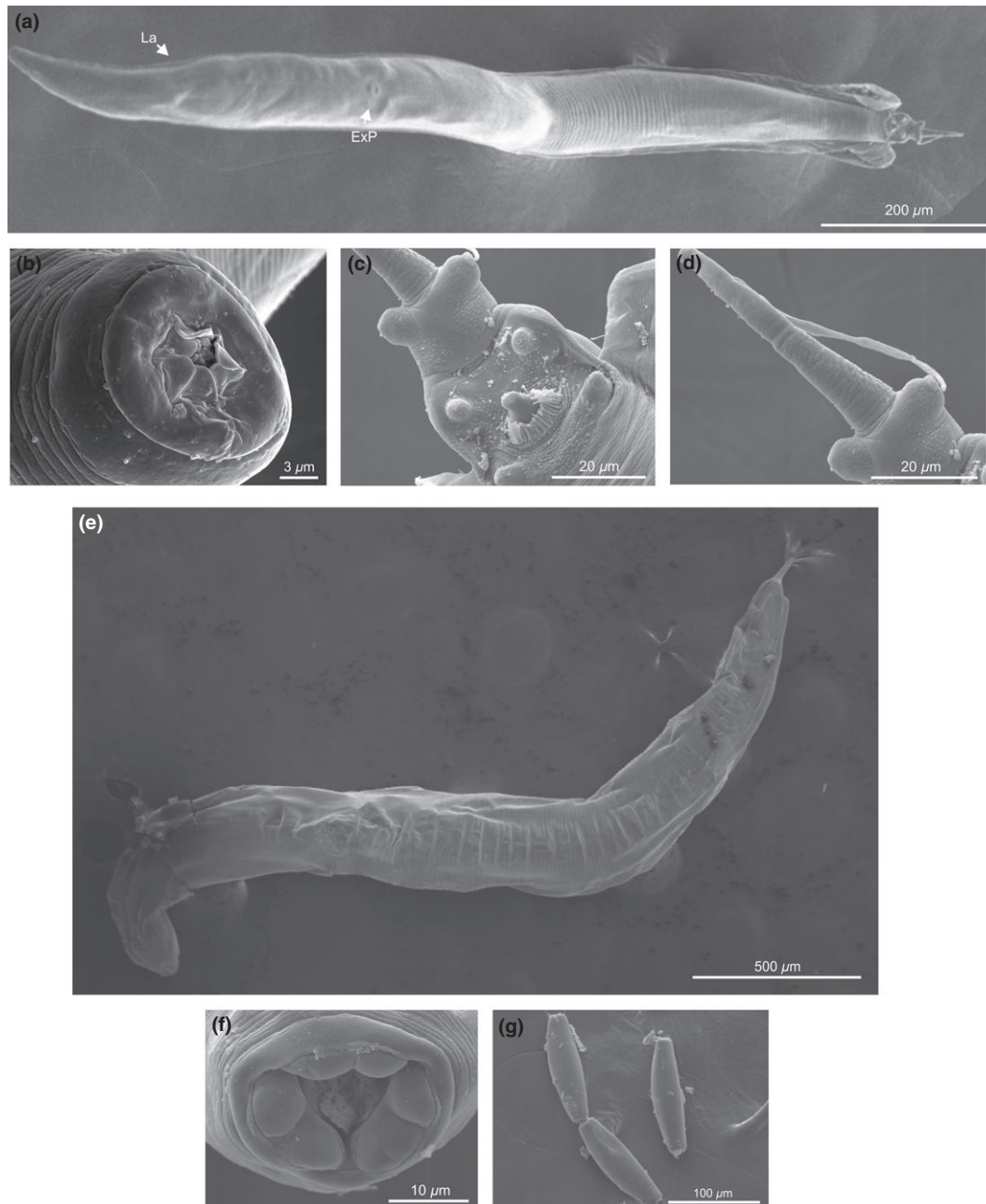
Fig. 7. Scanning electron micrographs of *Spauligodon occidentalis* sp. nov. male (a–d) and female (e–g). a, general view of the male; b, cephalic end of the male; c, ventral view of the caudal extremity; d, ventral view of the third pair of caudal papillae; e, general view of the female; f, cephalic end of the female; g, eggs. La, lateral alae; ExP, excretory pore

Description

Small-sized nematodes with cylindrical body with tapering anterior extremities and ending with a posterior filiform tail. Cuticle with distinct transversal striations more marked in males, starting after the lips and extending until the posterior extremity. Single lateral alae in males and discrete double lateral alae in females. Mouth opening triangular, enclosed by six labial plates in males and three bilobed lips in females. Short, straight oesophagus that ends in subspherical bulb.

Male: Small, filiform nematodes. Cuticle with distinct transversal striations, starting after the circumoral ring until the posterior extremity. Mouth opening triangular, enclosed by six equal overlapping labial plates, surrounded by a circumoral ring, which bears two amphids located on opposite sides. Excretory pore at the end of the first third of the body, surrounded by robust cuticular ring. Very narrow lateral alae at its start, but progressively extending along the body, reaching its maximum width with auricular shape, projected on both sides of the cloaca. Three pairs of mammiliform caudal papillae, first two enclosed by caudal alae, third pair situated at the base of the tail directed outward, not enclosed by caudal alae. Precloacal pair (first pair) lies in higher area of posterior end, directed outward, consisting of two middle-sized spherical pedunculated papillae. Second pair, postanal, resembles first pair, but larger and elongated. Third pair consisting of two large prominent papillae, with thick, large peduncles. Genital cone situated in mid-ventral line, with an enlarged cuticularized proconus with double papillae and two lateral side pieces, surrounded by a pleated membranous curtain with coiled edges. Caudal extremity ends large and robust. Posterior end extending into aspinose, filiform tail. Spicule absent.
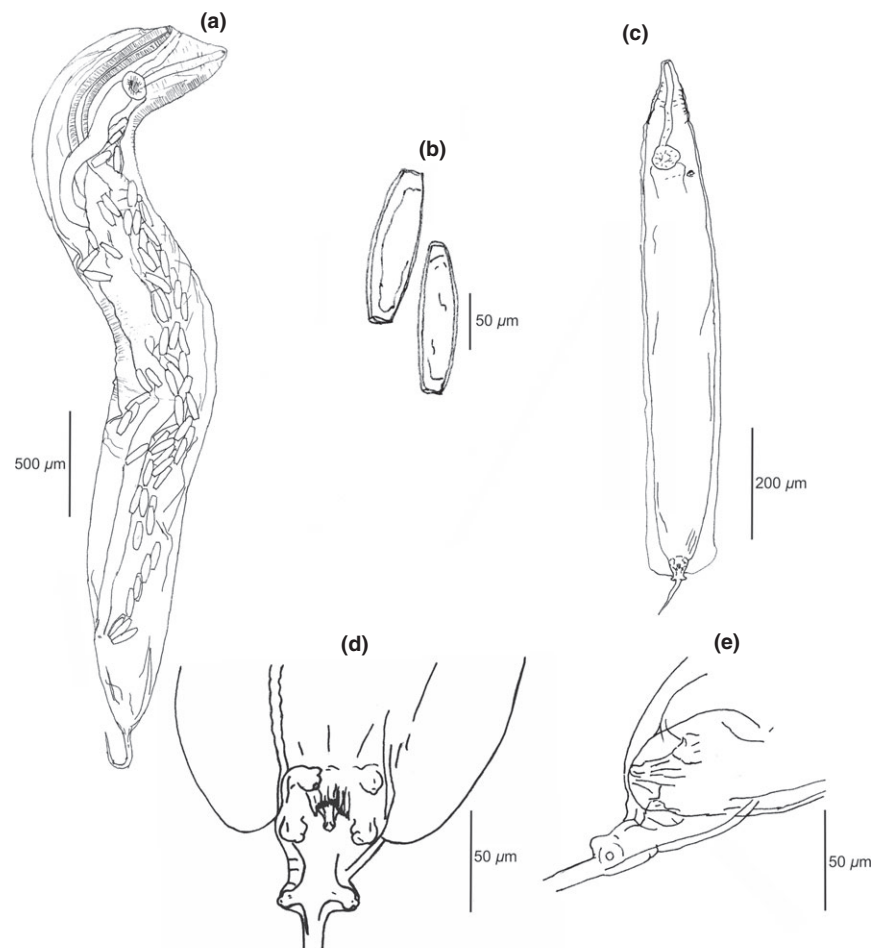
Fig. 8. Drawings of female (a–b) and male (c–e) of *Spauligodon occidentalis* sp. nov. a, general view of the female; b, eggs; c, general view of the male; d, ventral view of the caudal extremity; e, lateral view of the caudal extremity

Female: Filiform nematodes, larger than conspecific males (42% larger on average). Oral opening surrounded by three bilobed lips and with two opposite amphids. Cuticle with transversal striations, starting after the lips until the posterior extremity. Very tenuous, almost imperceptible double lateral alae, extending along the body. Excretory pore and vulva open at prebulbar level, surrounded by robust cuticular rings. Long, thick, muscular vagina, directed posteriorly. Ovaries located behind the vulva, females being opistodelphic. In fully gravid females, uterus extends anteriorly slightly past the vulva and posteriorly almost reaching the end of the body. Filiform tail, with five to nine cuticular spines. Asymmetrical eggs, with truncated extremities and a polar cap in each pole.

Distribution
El Hierro, La Gomera, La Palma and Tenerife from Canary Islands, Spain.

Host species
This species has been identified from the intestine of the lizards *Gallotia caesaris caesaris* (Lehrs, 1914), *G. c. gomerae* (Boettger and Müller, 1914), *Gallotia galloti galloti* (Oudart, 1839), *G. g. eisentrauti* Bischoff, 1982 and *G. g. palmae* (Boettger and Müller, 1914).

Genetic and phylogeographic remarks
Spauligodon occidentalis sp. nov. is a highly divergent clade from *Spauligodon atlanticus* (12.9% uncorrected p-distance for the COI, Jorge et al. 2011; Fig. 2). Although these species were previously considered conspecific, they apparently are not sister taxa (Jorge et al. 2011). *Spauligodon occidentalis* sp. nov. appears more closely related to *S. lacertae* identified in lizards from the subfamily Lacertinae than to *S. atlanticus*. *Spauligodon occidentalis* sp. nov. harbours greater genetic diversity than *S. atlanticus* (5.5% versus 2.7% uncorrected p-distance for COI, respectively; Jorge et al. 2011). The new species is present in the western, more recent islands of the Canarian archipelago (see Distribution). GenBank accession numbers: JF829231, JF829233-JF829235 (18S rRNA), JF829256–JF829261 (28S rRNA), JF829289–JF829315, and KC588965 (COI).

Order Oxyurida Weinland, 1858
Family Pharyngodonidae Travassos, 1919
Genus *Spauligodon* Skrjabin, Schikhobalova and Lagodovskaja, 1960
***Spauligodon atlanticus* Astasio-Arbiza, Zapatero-Ramos, Ojeda-Rosas and Solera-Puertas, 1987.**
MorphoBank M148005-M148035 and M148671-M148673 (Figs 9–10)

Diagnosis
Spauligodon atlanticus is morphologically similar to *S. occidentalis* sp. nov. but has overall smaller size, except for the tail, which is larger (Table 1). In males, the third pair of caudal papillae is smaller with a thinner tip (and a shorter peduncle (Table 1). Females with well-defined double lateral alae.
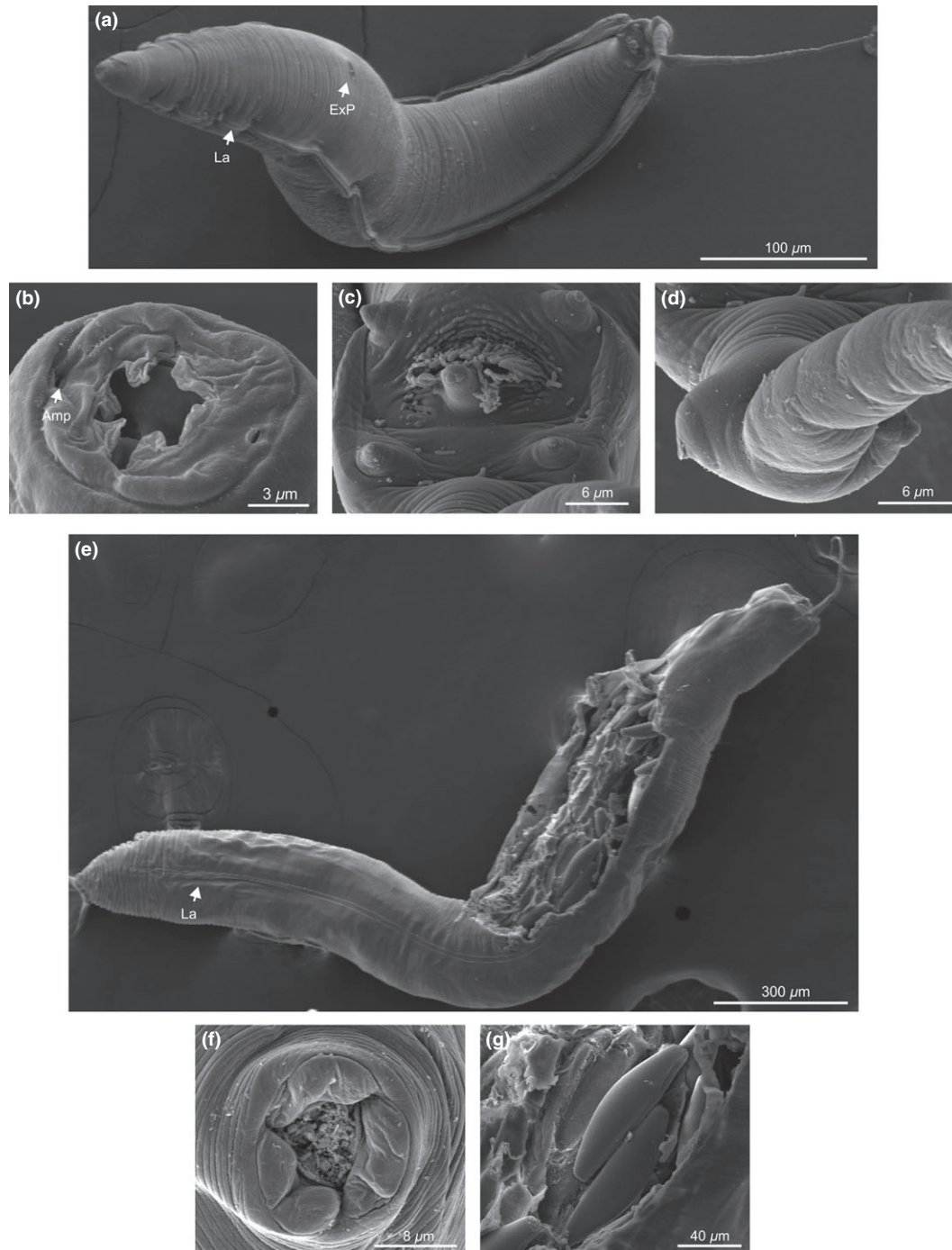
Fig. 9. Scanning electron micrographs of *Spauligodon atlanticus* male (a-d) and female (e - g). a, general view of the male; b, cephalic end of the male; c, ventral view of the caudal extremity; d, ventral view of the third pair of caudal papillae; e, general view of the female; f, cephalic end of the female; g, eggs. La, lateral alae; ExP, excretory pore; Amp, amphid

However, the majority of the morphological characters show overlap between the two species. The characters that unambiguously separate the two species are the molecular characters (see Diagnosis and Genetic and phylogeographic remarks of *S. occidentalis*).

Specimens examined
Thirty-six (18 males, 18 females; Table 1 and Data S1).

Type material
Vouchers: four males and five females (NHMUK 2012.9.13.11–19; MorphoBank M148021–M148024, for only two of the males), from Lanzarote Island (Canary Islands), Nazaret (29.04646°N, 13.56206°W).

Re-description
Small-sized nematodes with cylindrical body with tapering anterior extremities and ending with a posterior filiform tail. Sexually dimorphic, with males approximately one-third the size of gravid females. Cuticle with distinct transversal striations more marked in males, starting after the lips until the posterior extremity. Single lateral alae in males and double lateral alae in females. Mouth opening triangular, enclosed by six labial plates in males and three slightly bilobed lips in females.
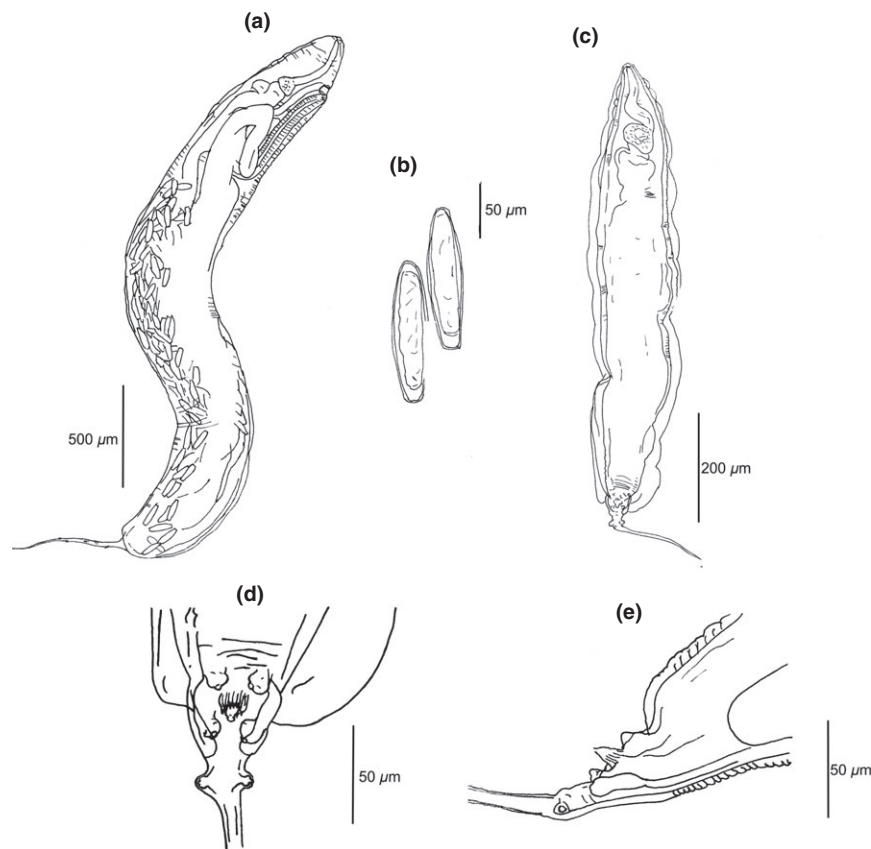
Fig. 10. Drawings of female (a–b) and male (c–e) *Spauligodon atlanticus*. a, General view of the female; b, eggs; c, general view of the male; d, ventral view of the caudal extremity; e, lateral view of the caudal extremity

Male: Small, filiform nematodes. Cuticle with distinct transversal striations, starting after the circumoral ring until the posterior extremity. Mouth opening triangular, enclosed by six equal overlapping labial plates and surrounded by a circumoral ring with two amphids located in opposite sites. Excretory pore at the end of the first third of the body, surrounded by a robust cuticular ring. Very narrow lateral alae at its start, but progressively extending along the body, reaching its maximum width with auricular shape, projected on both sides of the cloaca. Three pairs of small mammiliform caudal papillae, first two enclosed by caudal alae, third pair situated at base of tail directed outward, not enclosed by caudal alae. First pair, pre-anal, lies in higher area of posterior end, consisting of two small spherical papillae, postero-laterally directed. Second pair, postanal, be similar to first pair, but slightly larger. Third pair consisting of two larger prominent and pedunculated papillae. Genital cone situated in the mid-ventral line, with an enlarged cuticularized proconus with double papillae and with two lateral side pieces associated with the proconus, surrounded by a pleated membranous curtain with coiled edges. Posterior end extending into an aspinose, filiform tail. Spicule absent.

Female: Filiform nematodes, twice the size of conspecific males. Oral opening surrounded by three slightly bilobed lips with two amphids and no visible labial papillae. Cuticle with transversal striations, starting after the lips until the posterior extremity. Very thin, but well-defined double lateral alae extending along the body. Excretory pore and vulva opening at prebulbar level, surrounded by robust cuticular rings. Long, thick, muscular vagina, directed posteriorly. Opistodelphic females, with ovaries located behind the vulva. In fully gravid females, uterus extends anteriorly slightly past the vulva and posteriorly almost reaching the end of the body. Tail filiform, with six to nine cuticular spines. Asymmetrical eggs, with truncated extremities and with polar cap in each pole.

Distribution
Lanzarote and Fuerteventura from Canary Islands, Spain.

Host species
This species has been identified from the intestine of the lizards *Gallotia atlantica atlantica* (Peters and Doria, 1882), *G. a. laurae* Castroviejo et al., 1985 and *G. a. mahoratae* Bischoff, 1985.

Genetic and phylogeographic remarks
Spauligodon atlanticus is a monophyletic clade (Fig. 2), not directly related to *Spauligodon occidentalis* sp. nov. (Jorge et al. 2011). *S. atlanticus* is phylogenetically more closely related to *Spauligodon* sp. from the southern part of the Iberian Peninsula and from Morocco, both parasitizing lizards of the genus *Podarcis* Wagler, 1830 (Jorge et al. 2011). This species is present in the eastern, older islands of the Canarian archipelago (see distribution). GenBank accession numbers: JF829230, JF829232 (18S rRNA), JF829249–JF829251 (28S rRNA), and JF829272–JF829285 (COI).

Discussion

Previous phylogenetic analyses showed that what was described as *Spauligodon atlanticus*, actually consisted of two genetically different, unrelated species (Jorge et al. 2011) with the overall morphological similarity between the specimens analysed
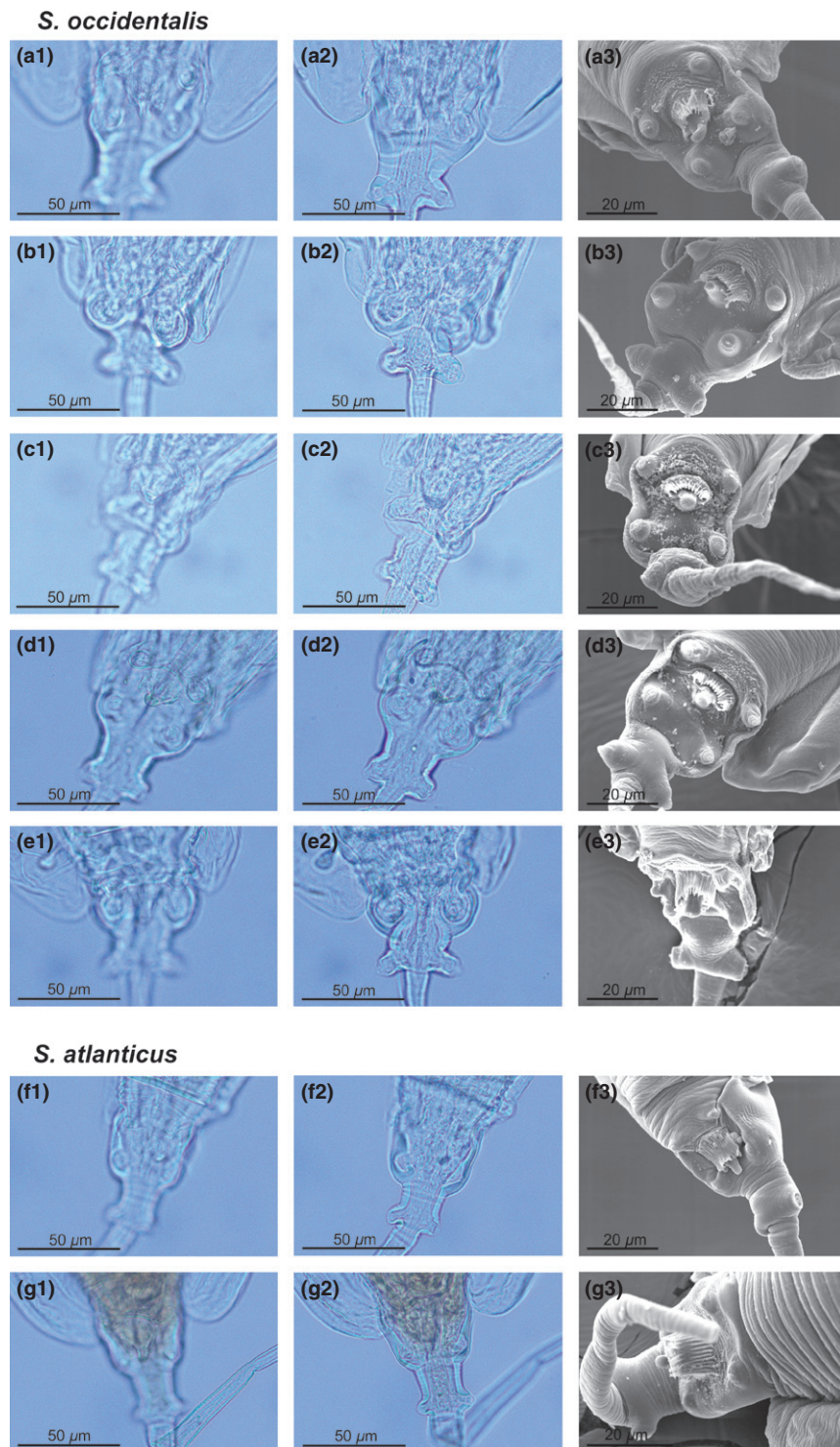
Fig. 11. Light microscope micrographs (1–2) of the ventral view of the caudal extremity and their respective scanning electron micrograph (3) from *Spauligodon* males from all populations analysed from the western and eastern lineages. a, El Hierro east; b, El Hierro west; c, La Gomera; d, La Palma; e, Tenerife; f, Lanzarote; g, Fuerteventura; 1, focus on genital cone and first and second pair of caudal papillae; 2, focus on the third pair of caudal papillae. See Fig. 1 for more details on the localities

suggesting cryptic species. In the original description by Astasio-Arbiza et al. (1987), only specimens found in *G. a. atlantica* from Lanzarote, in the eastern Canary Islands, were analysed. Later studies identified *Spauligodon* specimens present in the gut of other species of *Gallotia* as *S. atlanticus sensu lato* (Martin and Roca 2005; Jorge et al. 2011), with no attempt to analyse possible intraspecific morphological variation between the

original description and the specimens found in the new host species. The overall similarity between *S. occidentalis* sp. nov. and *S. atlanticus sensu stricto* can be observed both in females and in males. For example, in both species, females have the vagina opening past the excretory pore at prebulbar level, filiform tails with cuticular spines and asymmetrical eggs with truncated extremities, while males have no spicule and show

aspinose tails, lateral alae with auricular shape at the posterior end, spherical genital papillae and a genital cone with an enlarged cuticularized proconus with two side pieces, surrounded by a pleated membranous curtain. However, the combination of morphometrics and SEM allowed us to detect phenotypic differences between the two lineages. For this differentiation, the majority of the linear measurements were shown to be important. *Spauligodon occidentalis* sp. nov. is generally larger than *S. atlanticus*, in both males and females and with several morphological features presenting a more posterior position. Inclusion of measurements of the caudal extremity was also found to be important in the morphometric analysis and should be added to the features traditionally measured for these nematodes. According to our results, *Spauligodon occidentalis* sp. nov. males have a comparatively larger caudal extremity end (CT1 and CT2) and a larger third pair of caudal papillae, when compared to *S. atlanticus sensu stricto* (Fig. 11). Regarding females, they also presented a comparatively smaller tail and more discrete double lateral alae than the *S. atlanticus sensu stricto* females. Local morphological differentiation was also detected for both males and females within each species, but this was less pronounced than the differentiation detected between the two species. Although several mechanisms including drift or isolation might account for such intraspecific variation, current evidence does not allow reaching further conclusions.

The combination of different methodologies allowed us to distinguish between what was first identified as probable cryptic species. However, the overall similarity between these two species is notable, particularly given their genetic distinctiveness. Although *S. atlanticus sensu stricto* appears as more closely related to *Spauligodon* sp. from the southern part of the Iberian Peninsula and from Morocco (parasites of *Podarcis hispanica sensu stricto* and *P. vaucheri*, respectively; Jorge et al. 2011), the lateral alae are wider at the posterior end with an auricular shape in *S. atlanticus*, with the second pair of caudal papillae having a different shape and differing also from *Spauligodon* sp. of *P. hispanica sensu stricto* by a smaller but wider genital cone. However, these later nematodes may represent an undescribed species, which still lacks a detailed morphological study and eventually a formal description. In the case of *Spauligodon occidentalis* sp. nov., it differs from *S. lacertae* in the presence of tails with cuticular spines in females, which are not present in *S. lacertae* females, and in the different shape of the second pair of caudal papillae and lateral alae with a narrower width in males. However, given the considerable geographical distance between them, they are probably not the closest species for comparison. It must be stated that to determine morphological evolutionary patterns (ancestral versus derived character states) in these nematodes, their phylogeny needs first to be resolved, which will require the identification of all closely related species. The morphological resemblance between *S. occidentalis* sp. nov. and *S. atlanticus* could be the result of morphological stasis or evolutionary convergence. This issue only could be fully accessed by analysing the morphology of a wide data set of species placed in a more complete phylogeny of the genus, which is out of the scope of this study. Nevertheless, given that both species infecting *Gallotia* lizards are not sister taxa (Jorge et al. 2011 and Fig. 2) and that the genetically closest species (*S. lacertae* and *Spauligodon* sp., Jorge et al. 2011) are morphologically different, we here favour evolutionary convergence as the most parsimonious scenario. The host genus *Gallotia* arrived to the Canary islands between 17 to 20 Myr (Cox et al. 2010) and presents a number of unique characteristics (e.g. large body size, karyological $2n = 40$ chromosomes, strong trend to herbivory) that separate them from other members of the family Lacertidae

(Arnold 1989; Arnold et al. 2007). Poulin (2011b) argued that parasite evolution has often been shaped by convergence. Convergent morphologies among divergent parasite species may be expected, due to adaptations to functionally similar internal or external environments of many host species (Perkins et al. 2011). The characteristics of the host species, the *Gallotia* lizards, could be the common factor responsible for the morphological similarity between *S. atlanticus* and *S. occidentalis* sp. nov. In this respect, convergence is indeed a critical issue in systematics, since it can potentially mislead phylogenetic reconstruction methods based on morphological characters, for example, by causing the analyses to group distantly related organisms that share similar habitats (Wiens et al. 2003). On the other hand, traditional parasite descriptions often rely only on specimens found in a single host species (or even a single host specimen) from one locality neglecting intraspecific variation. Several *Spauligodon* species have been described in recent years (e.g. Bursey and Goldberg 2011, 2012) based exclusively on morphological characters, but cannot be easily placed in a phylogenetic framework. Furthermore, descriptive parasitological studies that only rely on morphology to identify species may underestimate the true diversity, which can be uncovered with a molecular approach. Nevertheless, we must remain cautious about how easy it is to detect new species by means of molecular tools. Incorporation of all relevant information into species descriptions will not only strengthen parasite systematics, but also contribute towards a better knowledge of host-parasite interactions.

Résumé

Especies crípticas al descubierto: el caso del nematodo Spauligodon atlanticus La incorporación de los métodos moleculares a la parasitología esta conduciendo al descubrimiento de numerosas especies crípticas. No obstante, se necesitan estudios detallados para evaluar la naturaleza críptica de tales especies, así como una apropiada descripción formal de las mismas. Análisis filogenéticos recientes basados en genes mitocondriales y nucleares han revelado que el nematodo *Spauligodon atlanticus*, parásito de los lagartos del género *Gallotia*, endémico de las Islas Canarias, comprende dos linajes fuertemente divergentes y no relacionados, uno en las islas orientales y otro en las occidentales. Este estudio lleva a cabo un análisis morfológico pormenorizado de ambos linajes de *S. atlanticus* caracterizados genéticamente, que se basa en medidas corporales y microscopía electrónica de barrido. Esta aproximación integrada revela que, a pesar de su similitud morfológica global, existen diferencias fenotípicas entre ambos. Como consecuencia, se describe una nueva especie, *Spauligodon occidentalis* sp. nov., para el anterior linaje occidental. La semejanza morfológica entre las dos especies se explica mejor por una convergencia evolutiva ya que ambas parasitan a los lagartos del género *Gallotia*. Además de delimitar las nuevas especies de nematodos, este estudio subraya la importancia de combinar datos genéticos y morfológicos en taxonomía para poner de manifiesto la naturaleza de las especies crípticas y disminuir la incertidumbre taxonómica.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Descriptive statistics for all the linear measurements of adult specimens from the different localities included in this study.