

Parasites in Spanish populations of *Psammodromus algirus* (Algerian sand lizard, lagartija colilarga) and *Psammodromus occidentalis* (Western sand lizard, lagarto de arena occidental) (Squamata, Lacertidae, Gallotiinae)

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Psammodromus algirus from Madrid, Ávila, and Cádiz provinces, Spain, and *P. occidentalis* from Cádiz province were examined for the presence of external and internal parasites. Among those parasites represented were: *Ixodes inopinatus* (Arthropoda, Arachnida, Acari, Ixodidae); *Haemaphysalis punctata* (Arthropoda, Arachnida, Acari, Ixodidae); *Skrjabinelazia* cf. *taurica* (Nematoda, Secernentea, Ascaridida, Seuratidae); *Spauligodon carbonelli* (Nematoda: Secernentea, Oxyurida, Pharyngodonidae); *Parapharyngodon psammodromi* (Nematoda, Secernentea, Oxyurida, Pharyngodonidae); *Abbreviata abbreviata* (Nematoda, Secernentea, Physalopteroidea, Physalopteridae); *Mesocestoides* sp. (Platyhelminthes, Cestoda, Cyclophyllidea, Mesocestoididae); and *Oochoristica* cf. *tuberculata* (Platyhelminthes, Cestoda, Cyclophyllidea, Davaineidae). Details regarding localities from which host species were collected, number of parasites and sites of attachment, and estimates of prevalence and intensities of infection are presented. Nematode diversity, along with parasite prevalence, parasitaemia, and relationship to elevation are also discussed. A table of *Psammodromus* parasites in Spain is also included.

Key words: Ávila Province; Cádiz Province; cestodes; *Iberolacerta*; Madrid Province; nematodes; *Psammodromus*; Spain; ticks.

Distributional data regarding parasites, when coupled with habitat information regarding vertebrate hosts, can be instructive with respect to ecological conditions experienced by both parasites and their hosts. External parasites may not be retained or identified, and internal parasites are rarely discovered, during field surveys of living individuals; large samples of museum specimens from well-referenced localities can be



Figure 1: *Psammodromus algirus*, Cádiz, vicinity of Facinas (note tick scar, axil area), (left); *P. occidentalis* (CM 54808), Cádiz; Facinas, 7.3 km (airline) NE (N36.14809 W5.62074, 174m), (right).

difficult to locate and access.

Although nematode and cestode parasites of Spanish *Psammodromus algirus* and *P. occidentalis* have been subject of previous studies (ROCA *et al.*, 1986; ROCA & LLUCH, 1986, 1988; BUSACK & BURSEY, 2016), and known parasites were summarized in SALVADOR (2014), detailed distributional data, useful to parasitologists, herpetologists, ecologists, and conservationists remain difficult to locate and access. Our limited data from Ávila Province, considered along with recent data for *Iberolacerta cyreni* provided by ROCA (2017a), provide an enhanced picture of nematode infection in lacertid lizards from the Sierra de Gredos. Previously unpublished data regarding identification and distribution of internal and external parasites from three populations of *Psammodromus algirus*, and one population of *P. occidentalis*, (Fig. 1), based on museum specimens from Cádiz Province, Spain, are presented below; prevalence, intensity of infection or infestation, and abundance data are considered in the discussion. A summary of parasites known to be hosted

by lizards of the genus *Psammodromus* from the Iberian Peninsula is provided in Supplementary Material S1.

MATERIALS AND METHODS

Specimens examined

While examining historical (Cádiz, 1969 -1971, Madrid and Ávila, 1975) collections of *P. algirus* and *P. occidentalis* housed at Carnegie Museum of Natural History (CM), Pittsburgh, PA, and the U.S. National Museum of Natural History (USNM), Washington D.C., parasites were removed and identified. Internal parasites are deposited in the H. W. Manter Laboratory of Parasitology (HWML), Lincoln, Nebraska, U.S.A., and ticks are deposited at the U.S. National Tick Collection (USNM ENT), located and curated at Georgia Southern University, Statesboro, GA, U.S.A.

Data analysis

Snout-vent length (SVL in mm) of lizard specimens were recorded after several years of storage in 70% ethanol. Helminth identification was facilitated by utilizing descriptions and illustrations in

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ANDERSON *et al.* (2009) for genera, and BURSEY & GOLDBERG (2015), LHERMITTE *et al.* (2008), and MORGAN (1945) for species; cestode identification was confirmed following RAUSCH (1994), McALLISTER & BURSEY (2017), and McALLISTER *et al.* (2013); ESTRADA-PEÑA *et al.* (2014, 2017) were consulted for tick identification. Identification guides generally illustrate only male *Skrjabinelazia* and we found only females in our samples; those females, however, most closely resembled illustrations of *S. taurica*. In *Parapharyngodon*, females in our samples most closely resembled illustrations of *P. psammodromi*.

To ensure accuracy of species identification from scar tissue resulting from previous tick infestations in specimens no longer carrying representative adults, we compared previously-infested tissue with tissue from specimens continuing to carry larval *Ixodes inopinatus* (Arthropoda, Acari, Ixodidae) at the same locality (see ESTRADA-PEÑA *et al.*, 2014 for developmental data regarding *Ixodes inopinatus*).

Original locality data were transcribed from field notes (SDB, unpubl.) and presented as modern locality data using *Trip and Waypoint Manager v 5* and the *City Navigator Europe NT 2014.4* update in *Map Source* version 6.16.3 (<https://www.garmin.com/en-US/maps/outdoor>); airline distances and elevations are from Google Earth™, Spearman's Rank Correlation (Rho) was calculated using the on-line program provided by Social Science Statistics (<https://www.socscistatistics.com/tests/spearman/default2.aspx>).

RESULTS

From Manzanares el Real (Madrid Province; ~N40.73728 W3.85772, elev. ~982 m), one male *Psammodromus algirus* (USNM 199213), of two sampled during late August, carried scar tissue and one larva (left axil; USNM ENT 00862246) indicating prior and current tick infestation (*Haemaphysalis punctata*; Arachnida, Acari, Ixodidae).

Close to Guisando (Ávila Province; 2.7 km NE [airline, 244.9° true] ~ N40.23294 W5.11034, elev. 824 m), one male *P. algirus* (CM 60959, SVL 57.1), not otherwise parasitized during late August, 1975, was found heavily infected with *Mesocestoides* sp. (Platyhelminthes, Cestoda, Cyclophyllidea, Mesocestoididae; HWML 96281).

In the vicinity of Nogal del Barranco (N40.23145, W5.15995, elev. ~1102 m, Ávila Province), also during late August, 1975, 10 of 12 *P. algirus* (CM 60953-60956, USNM 199215 & 199217-199219) bore scar tissue consistent with prior tick infestation, and two (USNM 199216 & 199221; USNM ENT 00862246 & 00862247) carried *H. punctata* larvae, in axillary regions. Three of these 10 (USNM 199216-199218) individuals also carried five, three, and two *Skrjabinelazia* cf. *taurica* (Secernentea, Ascaridida, Seuratidae; HWML 96279) in the posterior stomach and/or anterior small intestine, and one (USNM 199219), in addition to several axillary *H. punctata* scars, was host to one male and 8 female *Spauligodon carbonelli* (HWML 96280; Secernentea, Oxyurida, Pharyngodonidae) in her anterior small intestine. Two *P. algirus*, apparently not parasitized by ticks (CM 60957, USNM

Table 1. Estimates for nematode intensities of infection arranged by elevation within Spain.

Host species	Elevation (m)	Helminth species	Intensity	Authority
<i>Iberolacerta cyreni</i>	1780	<i>Spauligodon carbonelli</i>	3.3 ± 6.9	Roca (2017a)
		<i>Skrjabinelazia</i> cf. <i>hoffmanni</i>	1.0 ± 0	Roca (2017a)
<i>Psammodromus algirus</i>	~1102	<i>Spauligodon carbonelli</i>	0.8 ± 0.8	This study
		<i>Skrjabinelazia</i> cf. <i>taurica</i>	2.6 ± 0.8	This study
	80-440	<i>Skrjabinelazia</i> cf. <i>taurica</i>	4.2 ± 1.3	This study
		<i>Abbreviata abbreviata</i>	1.1 ± 0.2	This study

199221), carried one and two *S. cf. taurica* in the anterior small intestine or posterior stomach.

Haemaphysalis punctata prevalence at the Nogal del Barranco locality was 83% (95% confidence limits [CL]: 59% - 100%; see also BUSH *et al*, 1997). For internal parasites the nematode *S. cf. taurica* was found to be most prevalent (42%; CL: 9% - 74%), with an estimated mean intensity of 2.6 ± 0.8 for the number of parasites expected per individual; the prevalence of *S. carbonelli* was 8% (CL: 0% - 27%), and its estimated mean intensity was 0.8 ± 0.8 at this locality. Nematode diversity (Brillouin's Index, $H = 0.369$; POOLE, 1974) at this locality, based on 22 worms supported by six of 12 *P. algirus*, is apparently the highest diversity yet reported from the Iberian Peninsula (*vide* ROCA 2017a: Table 2).

Of 198 *P. algirus* examined from localities within Cádiz Province (Fig. 2a, circles), 85 (43%) were female and 113 (57%) were male, suggesting that the sex ratio in the population may be narrowly skewed toward males ($p = 0.05$); some specimens at these localities were

unsuitable for inclusion in our analysis, however, and the precise degree of parasite representation across the province cannot be determined using these data. Six *P. occidentalis* (Fig. 2a, triangles) were also examined.

Number and precise geographical localities (Fig. 2b) for lizard hosts, their SVL, sex, and month of discovery; the number, sex, and developmental stage of individual parasites encountered; and the host's site of infection/infestation are provided below. Museum catalogue numbers for hosts and parasites are also provided:

1. *Parapharyngodon psammodromi* (Nematoda, Secernentea, Oxyurida, Pharyngodonidae)

La Algaida (N36.85791 W6.30826, elev. 2 m)

Three male *P. algirus* (CM 54874 [no letter], SVL 67.1 [June]; CM 54874I, SVL 49.5 [June]; and CM 53403, SVL 61.2 [August]) each carried one *P. psammodromi* (HWML 64802 & 94159) involving stomach, small intestine, or body wall as infection sites (see also BUSACK & BURSEY, 2016).

Two female *P. algirus* (CM 53247, SVL

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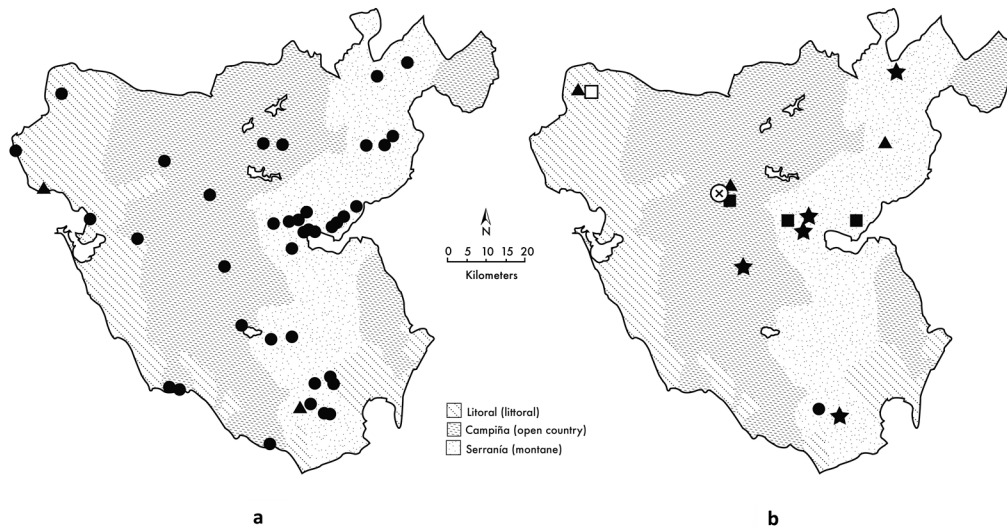


Figure 2. *Psammodromus* specimens examined (a) ● *Psammodromus algirus*, ▲ *P. occidentalis*; (b) ■ *Skrjabinelazia* cf. *taurica*, □ *Parapharyngodon psammodromi*, ▲ *Abbreviata abbreviata*, ⊗ *Oochoristica* cf. *tuberculata*, ★ *Ixodes inopinatus* (*P. algirus*), and ● *Ixodes inopinatus* (*P. occidentalis*). Agricultural areas indicated are defined in *Mapas provinciales de suelos*. Cádiz (Instituto Nacional de investigaciones Agronomicas, 1971).

54.1 [May] and CM 54677G, SVL 63.6 [April] were each parasitized by a single *P. psammodromi* (HWML 64802 & 94159). In one specimen, the nematode was attached to the body wall, in the other it was found in the lower portion of the large intestine.

2. *Skrjabinelazia* cf. *taurica* (Nematoda, Ascaridida, Secernentea, Seuratidae; lot catalogued as HWML 64803 & 94157)

La Barca de la Florida, ~3.8 km E. on CA-503 (N36.62981 W5.86369, elev. 80 m)

Four male *P. algirus* (CM 53139, SVL 59.5 [April]; CM 51934, SVL 61.6 [September]; CM 53306, SVL 63.5 [June]; and CM 51977, SVL 68.4 [September]) carried one, 10, 12, and two female *S. cf. taurica*, respectively, within the rear intestinal tract and bladder.

Four female *P. algirus* (CM 51333, SVL 55.9 [August]; CM 51332, SVL 56.0 [August]; CM 53140, SVL 57.8 [April]; and CM 51089, SVL 59.0 [July]) carried five, one, one, and six female *S. cf. taurica* respectively within the caecum, small intestine, or attached to the ventral body wall.

Montes Propios de Jerez, various sites along logging roads in the vicinity of Galis (N36.55958 W5.60101, elev. 440 m)

One male *P. algirus* (CM 54687F, SVL 70.2) collected ~9.7 km WNW of Galis (277° true, N36.5706 W5.7080, elev. 244 m) during May, carried two female *S. cf. taurica* (HWML 94157) within its intestinal tract, and one female *P. algirus* (CM 55442, SVL 72.4) collected ~6.4 km ENE of Galis (73° true, N36.5768 W5.5314, elev. 594 m) during August, carried two female *S. cf.*

taurica (HWML 94157), one within the posterior large intestine, the other within the anterior stomach.

3. *Abbreviata abbreviata* (Nematoda, Secernentea, Physalopteroidea, Physalopteridae; lot catalogued as HWML 64801 or 94158)

La Algaida

During June two male *P. algirus* (CM 53291, SVL 54.2; CM 53353, SVL 65.9) were found hosting *A. abbreviata*; one male parasite was attached to the ventral abdominal wall of CM 53291, and a female parasite was found in testicular tissue of CM 53353. During August another male (CM 53426D, SVL 64.8) was host to an immature *A. abbreviata* found unattached within the anterior abdomen, and during October a female *P. algirus* (CM 55662, SVL 59.8) was discovered with a female *A. abbreviata* attached to the external wall of her stomach.

La Barca de la Florida

During March, two female *A. abbreviata* were found on the anterior stomach wall of a 56.4 mm male *P. algirus* (CM 53092); during April, two male *P. algirus* (CM 53139, SVL 59.5; CM 53045D, SVL 61.0) hosted one and two female *A. abbreviata*, respectively, in the abdominal cavity. CM 53045D also carried one unidentified parasite (HWML 64801). During July a female *P. algirus* (CM 51106, SVL 59) carried a female *A. abbreviata* in its small intestine, and in September one female *P. algirus* (CM 51935, SVL 60.1) was discovered with an immature *A. abbreviata* in its stomach.

Benamahoma, 1.6 km N on A-372 (N36.7558 W5.4548, 636 m) or Ubrique, 8.4 km SE (airline, 208° true; N36.6114 W5.4911, 579 m)

Assignment to the original locality for

each of these four *P. algirus* (one male, three females; CM 55456 - CM 55459) collected in September is not possible, and the following data are included here and within composite analyses for the province. The only parasitized specimen within the series (a male, CM 55456, SVL 69.6) carried two female *A. abbreviata* at the junction of his stomach with the intestine.

4. *Oochoristica cf. tuberculata* (Platyhelminthes, Cestoda, Cyclophyllidea, Davaineidae)

La Barca de la Florida

Six pieces conforming to the remains of a cestode (cf. *Oochoristica*) were found in the intestinal tract of CM 53045B, a 40.1 mm SVL male *P. algirus* collected during April. *Oochoristica cf. tuberculata* parasitizing *Acanthodactylus erythrurus* at La Algaida have been deposited in the U.S. National Helminthological Collection [USNM 74378], and with the Laboratoire de Zoologie (Vers), Muséum National d'Histoire Naturelle, Paris [MNHN 153 Hb-164 Hb, Bocal C91] (BUSACK & JAKSIĆ, 1982).

5. *Ixodes inopinatus* (Arachnida, Acari, Ixodidae)

Alcalá de los Gazules, 8.7 km W on "old" C-440 (N36.46835 W5.84245, 62 m)

A female *P. algirus* (CM 51084, SVL 62) collected in July was parasitized in the right axil by one nymph and one larva (USNM ENT 00862190).

Facinas, 7.3 km (airline) NE (N36.14809 W5.62074, 174 m)

One female *P. occidentalis* (CM 54808, SVL 41.7) hosted a nymph (USNM ENT 00862245) in the right axil during May.

Los Barrios (benchmark), ca. 8.2 km SW (230° true), (N36.13894 W5.56441, 259 m)

A male *P. algirus* (CM 53197, SVL 43.4)

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was found with two larvae attached in the right axil, and one larva was found attached directly over the heart (USNM ENT 00862191) during May.

Algodonales, 3.2 km W on N-342, then 6.1 km N on C-339, (N36.91652 W5.42705, 549 m)

Two nymphs and one larva (USNM ENT 00862192) were found in the right axil of a male *P. algirus* (CM 53311, SVL 63.3) during June.

Montes Propios de Jerez, various sites along logging roads in the vicinity of Galis (N36.55958 W5.60101, 440 m)

Psammodromus algirus were collected and "lot catalogued" from five sites (N36.59800 W5.61980 [226 m], N36.58187 W5.64094 [130 m], N36.57800 W5.66500 [517 m], N36.57738 W5.66582 [474 m], and N36.57060 W5.70800 [238 m]) during May in what is now the Parque Natural de Los Alcornocales. *Ixodes inopinatus* was encountered on three individuals from these five sites, but cannot be associated with any specific site. Two male *P. algirus* (CM 54687B and CM 54687C, SVL 72.6 and 76.6, respectively) hosted a total of seven nymphs (USNM ENT 00862193-00862194) in right and left axillae. The left axil of a female (CM 54687D, SVL 67.4) carried three nymphs and one larva, the right axil carried two nymphs, and one larva was recovered from the mid-lateral portion of the abdomen (USNM ENT 00862221).

6. *Ixodes inopinatus* (Arthropoda, Arachnida, Acari, Ixodidae)

Facinas, 7.3 km (airline) NE (266.2°; N36.14809 W5.62074, 71 m)

One female *P. occidentalis* (CM 54808, SVL 41) hosted a nymph (USNM ENT 00862245) in the right axil during May.

Considering only nematode diversity from within provincial borders of Cádiz, only one specimen (CM 53139), a male from La Barca de la Florida, carried more than one nematode species (*S. cf. taurica* and *A. abbreviata*). Brillouin's Diversity index for this *P. algirus* sample ($H = 0.1104$) is also higher than those summarized by ROCA (2017a: Table 2) for the Iberian Peninsula.

DISCUSSION

The tick *Ixodes inopinatus* has been recorded in Austria, Germany, Romania, Portugal, Spain, Morocco, and Tunisia in the Western Palearctic region, largely from regions not inhabited by the morphologically similar *Ixodes ricinus*, (ESTRADA-PEÑA *et al.*, 2017). Although immature stages (larvae and nymphs) parasitize several lizard species, adults parasitize mammals (especially red fox [*Vulpes vulpes*]; ESTRADA-PEÑA *et al.*, 2017), but this tick is not recognized as a vector of any pathogen (ESTRADA-PEÑA *et al.*, 2017). Immature stages of *Haemaphysalis punctata* parasitize a wide variety of lizards, birds, and small mammals, whereas adults parasitize mainly ungulates across much of the Southern Palearctic region (BARANDIKA *et al.*, 2011; ESTRADA-PEÑA *et al.*, 2017). In contrast with *I. inopinatus*, *H. punctata* serves as a vector for several pathogens, including arboviruses, rickettsial bacteria and protozoa (including *Babesia* spp., an apicomplexan parasite that infects red blood cells) (ESTRADA-PEÑA *et al.*, 2017).

Tapeworms in the genus *Mesocestoides* have a complex three stage life cycle that apparently includes *Psammodromus* among its secondary hosts.

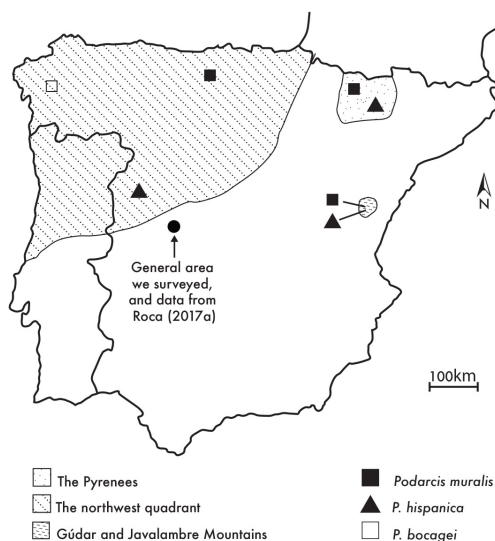


Figure 3. Map redrawn from Roca *et al.* (1990) providing comparison among survey localities.

LITERÁK *et al.* (2006) discuss morphometric and ribosomal DNA in isolates of *M. litteratus* from red fox (*Vulpes vulpes*) originating in the Czech Republic, Slovakia, and Spain (Solsona, Lleida Province). Their results suggest that 18S rDNA sequences can be used to differentiate between *M. litteratus* and *M. lineatus* that, along with *M. ambiguus*, have been reported from Spain and are known to parasitize humans (FUENTES *et al.*, 2003; IBERFAUNA, 2005; & BOWMAN, 2014).

The correlations between estimates of prevalence (52% vs 8%), and intensity of infection (3.3 ± 6.9 vs 0.8 ± 0.75), regarding *Spauligodon carbonelli* parasitism of *Iberolacerta cyreni* at ~1780 m and *Psammodromus algirus* at ~1102 m of elevation are positive (*I. cyreni* data from Roca, 2017a: Table 1). Whether these results are best explained by a paucity of

suitable hosts at higher elevations, a seasonally-reduced developmental period, by unrecognized issues precipitated by physiogeographic evolution of the Sistema Central of Spain, or simply demonstrate that these two data sets alone cannot provide a sufficient basis for an appropriate generalization of correlations between parasite populations and elevations (see discussion in Roca [2017a]) cannot, as yet, be determined.

Positive identification of female nematodes in the genus *Skrjabinelazia* requires access to male specimens, and no males were represented in our sample, or in the sample examined by Roca (2017a); consequently, the tentative species assignments differ between samples. We considered our specimens to represent *S. cf. taurica* because the females most closely resembled available illustrations of male morphology; Roca (2017a) considered a sample he examined from a nearby locality to represent *S. cf. hoffmanni* because this was "the species usually found parasitizing other lizards in this area (Roca *et al.*, 1990)". We have redrawn the map referenced in Roca *et al.* (1990), and on it we have placed a symbol locating our joint area of investigation (Fig. 3).

There are two refuges in the area, the Refugio de Reguero Llano (closest for vehicle access [40°16.550'N, 5°14.225'W, 1907 m]), and the Refugio Laguna Grande de Gredos (40°15.040'N, 5°16.791'W, 1950 m), whose locations can be used to create a triangle that likely includes the general area from which Roca's specimens were collected ("40°15'N, 5°13'W"). Elevations between ~1780 meters and ~1950 meters,

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coupled with the terrain and its general difficulty of access, make it reasonable to assume that previous investigators were operating within this triangle, and that *Iberolacerta cyreni* samples were collected in proximity to one, or both, of these refuges.

Skrjabinelazia hoffmanni has recently (ROCA, 2017b) been reported parasitizing *P. algirus* in the area of La Alberca (Salamanca Province, 40°29.350'N 6°6.667'W, 1050 m), approximately 86 km northwest from ROCA's (2017a) Sierra de Gredos locality, and on the western side of the Sierra de Gredos. Elevations within a circular area of 6 km diameter centered on La Alberca range between 980 m and 1350 m, placing it within the elevational range (824 m - 1100 m) for the *S. cf. taurica* samples we sampled from the eastern side of the Sierra de Gredos, rather than in the much higher elevational range (1780 m - 1950 m) for the *Iberolacerta cyreni* reported by ROCA (2017a). ROCA (2017b) further states "...se confirma la gran variabilidad intraespecifica de esta especie de nematodo en la peninsula ibérica...", a point made previously in ROCA *et al.* (1990), as he explains why assignment to subspecies is not advisable for the La Alberca specimens.

Skrjabinelazia hoffmanni is diheteroxenic and *poecilogynous*; its life cycle includes two intermediate hosts, one of which is a coprophagous insect, and females are of two types. Few third stage larvae develop in the uterus of viviparous young females, providing for parasite multiplication directly inside the host, and old females are oviparous, their eggs containing a third stage infective larva that requires "secondary ingestion". "These

eggs, after being ingested by field-cricket, hatch and without further development remain as larvae in the insect. An ..."entomophagous lizard host, which... has no opportunity to ingest the eggs, is infested by eating insects...", and lizard parasitism remains low (CHABAUD *et al.*, 1988).

Intermediate hosts may differ among *Skrjabinelazia* species, and may include arthropods and gastropods among *Podarcis* species studied by ROCA (2006). Citing ORTEGA-MORA & ROJO-VÁZQUEZ (2007), ROCA (2015) infers that ecological factors may favor encounters between insect hosts and the lizards that feed upon them, thereby facilitating completion of the parasite life cycle. As neither low host specificity (eurixeny), nor high host specificity (estenoxeny) require "long contact time in the [co-evolution] of parasite and host", if placed on a continuum *S. hoffmanni* falls close to the low end, and far from the high end.

Iberolacerta cyreni, a lizard inhabiting rocky areas of limited vegetative diversity at relatively high elevations (1700 m - 2500 m [with occasional variation at the lower end] in the Gredos Mountains), is active from late March until early October as temperatures and weather conditions allow maintenance of suitable body temperatures (18.4°C - 37.5°C, \bar{x} = 29.4; ARRIBAS, 2014). At these high elevations population densities of poikilothermic species potentially acting as intermediate parasite hosts generally occur in lower densities, and may be active at lower body temperatures, than suitable hosts at lower elevations (SDB, pers. obs.), and nematodes with life histories tolerating

lower-density hosts or lower developmental temperatures will likely benefit from less competition from nematode species not well adapted to conditions at these high elevations.

The landscape separating our *Psammodromus algirus* from Roca's *Iberolacerta cyreni* presents a rugged topography of varying elevations. Crossing the approximately 5.2 km (airline) distance from our site at ~1100 m to Roca's site will traverse rugged terrain at 1597 m, then 1955 m, and then 1657 m before reaching the approximately 1780 m locality supporting *I. cyreni*. That the lacertid lizard species representing this lower elevation is generally active at body temperatures between 32.0°C and 38.6°C ($\bar{x} = 35.2 \pm [SE] 0.5^\circ\text{C}$, $n = 15$; see also BUSACK, 1976), suggests that different species of *Skrjabinelazia*, along with differing species of invertebrate carriers, may be represented in each of these areas in the Sierra de Gredos.

BOOTH-REA *et al.* (2018) present a scenario explaining geological and biological observations regarding the exchange of terrestrial biota between Iberia and Africa before (~7 Ma – 3 Ma), during (10 Ma – 6.2 Ma), and after the Mediterranean Salinity Crisis (MSC). Fig. 1 and 2 in BOOTH-REA *et al.* (2018) illustrate how the eastern Alboran Basin's well-differentiated (14 km–17 km thick) volcanic arc, with sufficient buoyancy to create an archipelago and support a filter bridge, may serve to explain these phenomena. Progressive build-up and later subsidence of that filter bridge can explain the extended exchange of terrestrial biota between Iberia and Africa, and agrees with patterns of biota speciation and terrestrial

fossil distribution before the MSC.

Extensive morphological and phylogenetic analyses provide confirmation of eastern and western clade development within *Psammodromus algirus* (BUSACK *et al.*, 2006; CARRANZA *et al.*, 2006: Fig. 2; VERDÚ-RICOY *et al.*, 2010: Fig. 1) during the Pliocene (approximately 3.6 Ma). Between western assemblages, Iberian and North African specimens represent two, weakly-supported, monophyletic groups that appear to have developed approximately 1.9 Ma, around the beginning of the Pleistocene (see Fig. 1 in CARRANZA *et al.*, 2006).

Two of three localities supporting both *P. algirus* and nematode species in the genus *Skrjabinelazia* under consideration are positioned, respectively, at similar elevational ranges on eastern and western sides of the Sierra de Gredos. The third locality is at a considerably higher elevation, and supports both a different lacertid lizard species (*I. cyreni*), and representatives of the nematode genus *Skrjabinelazia*. Unfortunately, only La Alberca, western-most of the three localities, provided any male parasite specimens required for positive species identification, thereby reducing experienced parasitologists to providing provisional identifications of species involved.

What we now understand regarding physiogeographic changes in Iberia throughout the phylogenetic, and natural history of the genus *Psammodromus*, provides at least a theoretical pathway to understanding why *P. algirus* may host different species of the nematode genus *Skrjabinelazia* within similar elevation

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ranges on each side of this mountain range. The Pleistocene "split" referenced by CARRANZA *et al.* (2006) may have been precipitated, and later facilitated, by northward expansion within the western clade of *P. algirus*. This, and the fact that representatives of western and eastern clades of *P. algirus* may be represented on lower elevation areas of the western and eastern slopes of the Sierra de Gredos, suggests that different species of *Skrjabinelazia* may also be represented on these lower and higher elevation areas of the Sierra de Gredos. Is the *Skrjabinelazia* species discovered at the highest elevation likely to represent *S. hoffmanni*? Or does it, too, represent yet a separate species? As there are considerable intraspecific differences found among populations representing several areas of Spain (ROCA *et al.*, 1990; ROCA, 2017b; Fig. 3), it is our opinion that additional research is necessary before defensible generalizations regarding parasitization by *Skrjabinelazia* species within the Iberian Peninsula may be presented.

In Cádiz Province, at elevations varying between 80 m and 440 m, 15 *Skrjabinelazia* cf. *taurica* were found distributed as five, one, one, six, and two nematodes within five female *P. algirus*, and 27 *S. cf. taurica* were found distributed as one, 10, 12, two, and two nematodes within five male *P. algirus*. Mean parasite abundance and intensity was 3.0 ± 1.1 nematodes per female host, and 5.4 ± 2.3 nematodes per male host. When sex of the host is not considered, mean abundance and intensity for *S. cf. taurica* infection was 4.2 ± 1.3 nematodes

per individual *P. algirus*.

Prevalence of *S. cf. taurica* infection in male *P. algirus* may be significantly greater than that in females ($p = 0.03$), but further analysis suggests no association between number of parasites and SVL for either males ($n = 5$, Spearman rank [r_s] = 0.15; $p = 0.80$), or females ($n = 5$, $r_s = 0.15$; $p = 0.80$); neither is there a statistical association between parasite load and individual SVL, when sex is not considered as a variable ($n = 10$, $r_s = 0.19$; $p = 0.59$). If, however, we consider cumulative body size and parasite number within sexes to be the more important comparison, in this particular population sample a total of 27 *S. cf. taurica* were identified within 323.2 mm of cumulative male body length and a total of 15 *S. cf. taurica* were identified within 301.1 mm of cumulative female body length. Viewed from this perspective, infected males carry a parasite load ~1.6 times that of infected females (0.08 parasites/mm versus 0.05 parasites/mm of SVL).

Proportions of female (0.46) and male (0.54) *Psammmodromus algirus* hosting *A. abbreviata* ($n = 13$) within Cádiz Province were representative of a 50:50 relationship ($p = 0.78$). When considering abundance data from three different localities within the province to be representative of the province as a whole, 100% of males carried these parasites, but only 50% of females did. Mean helminth-to-host intensity estimates for infection, however, overlap broadly between females (1.1 ± 0.2) and males (1.4 ± 0.2). Only during August and September were immature parasites found, but insufficient sample sizes preclude any meaningful comparison

between parasite abundance and SVL for either sex; neither is there sufficient variability in this relationship when sex is not considered a variable.

An on-going discussion regarding the relationship between parasitism and elevation has offered both negative and positive correlations regarding this relationship; ROCHA *et al.* (2003) attributed low helminth diversity within *Aspronema dorsivittatum* (Scincidae; see HEDGES & CONN [2012] regarding this taxonomic change) not to an elevation-diversity effect, but to the "relative isolation (insularity effect)" of their study area at 2460 m in Brazil's Mantiqueira Mountains. VAN SLUYS *et al.* (1997) similarly suggested habitat isolation as an explanation for low nematode diversity in "restinga" habitat within southeastern Brazil.

DOBSON *et al.* (1992) found no relationship between elevation and parasite density or abundance for *Anolis* in the northern Lesser Antilles; MAIA *et al.* (2016) did not detect a correlation between parasitaemia and elevation but found individual *Pristrurus rupestris* (Gekkota, Sphaerodactylidae) to be less-infected with haplotype 5 haemogregarines at higher elevations than at lower elevations in Oman. CARBAYO *et al.* (2019), working in Madrid Province, Spain, found the prevalence of infection by intra-erythrocytic parasites (haemococcidia) assigned to *Schellackia* sp. (Conoidasida: Eucoccidiorida; Lankesterellidae) significantly lower at a higher elevation (48.3% at 1200 m; Navacerrada, 40°44'N, 4° 02'W) than at a lower elevation (90.3% at 850 m; Colmenar Viejo, 40°41'N, 3°49'W), but infestation by tick nymphs tentatively

assigned to *Ixodes* sp. was significantly lower at Colmenar than at Navacerrada (1.6% infected vs 91.6% infected).

Data for the relationship between *Skrjabinelazia* prevalence and parasitaemia, and elevation within Spain, for example, are not easily generalized. At higher elevations in the Sierra de Gredos (ca. 1780 m) *Iberolacerta cyreni* was found to host *S. cf. hoffmanni* at a lower level (one parasite/individual) than a sample of *Psammodromus algirus* was found to host *S. cf. taurica* in the same general geographical area but at a lower elevation (ca. 873 m - 1098 m; one-five parasites/individual). At a significantly lower elevation in Cádiz Province (ca. 440 m), one male and one female *P. algirus* each carried few (two) *S. cf. taurica*, but at an even lower (76 m) elevation four males and four females hosted between 1-12 and one-six *S. cf. taurica*, respectively (see RESULTS - Cádiz, Section 2).

In summary, we note that infection intensity for *Skrjabinelazia* sp. at 1780 meters (1.0 ± 0) increases to 2.6 ± 0.8 as you descend to 1102 m, and increases further to 4.2 ± 1.3 as sea level is approached (between 440 m and 80 m). In the same relatively-high-elevation localities, infection intensity for *Spauligodon carbonelli* decreases greatly between 1780 m (3.3 ± 6.9) and 1102 meters (0.8 ± 0.8). Overall helminth species diversity, on the other hand, appears highest ($H = 0.369$) at localities around 1102 m and lowest (0.110) at localities between 80 m and 440 m in Cádiz Province.

Larger samples are necessary before a generalized relationship between elevation and parasite intensities of infection can be

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supported (see Table 1), at least for these nematodes in Spain.

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