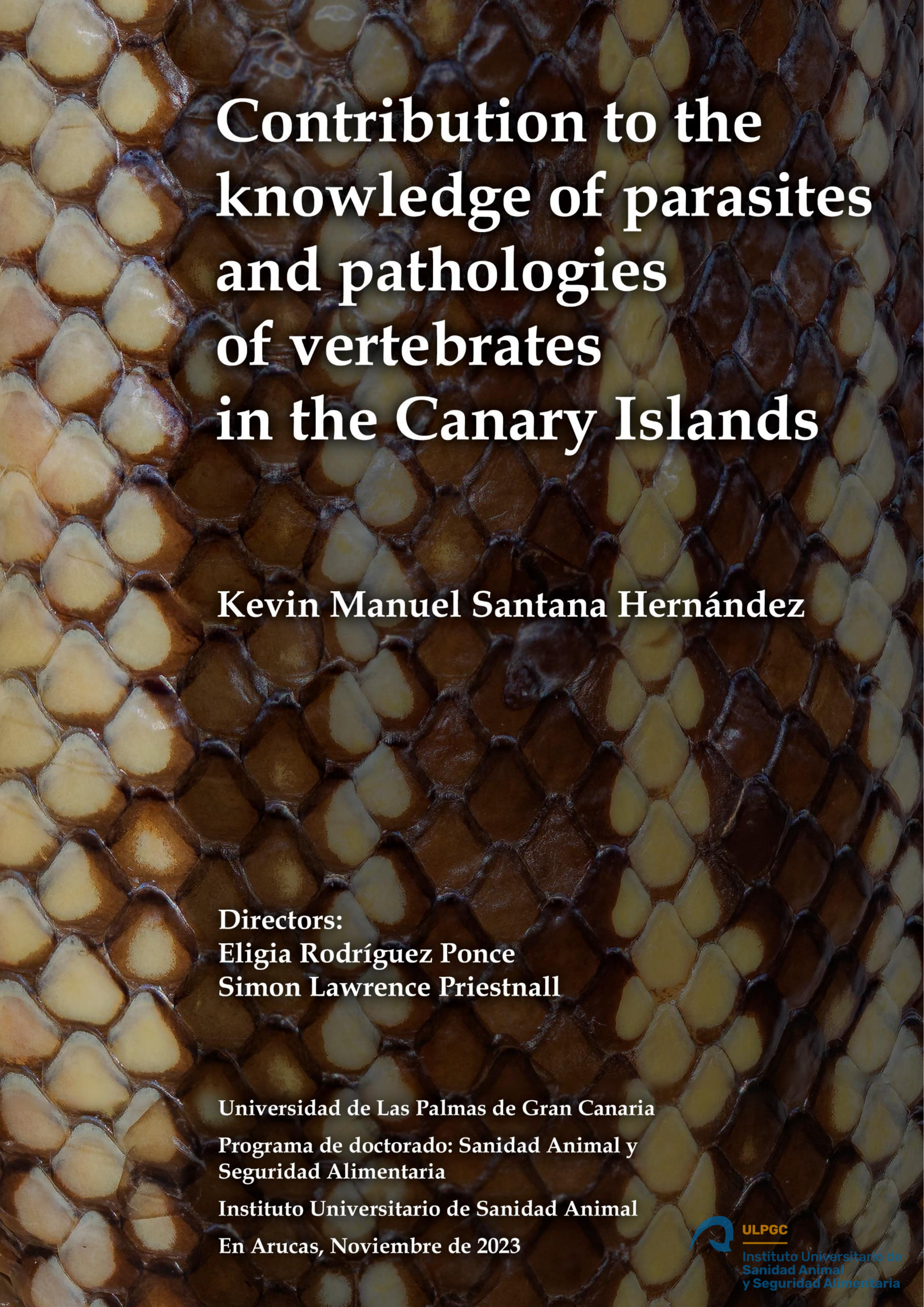

Contribution to the knowledge of parasites and pathologies of vertebrates in the Canary Islands

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To my Granny,
who never understood what I was doing,
or why I was working for free,
but she always supported me in every way possible.
I still love you,
wherever you are…

A mi Abuelita,
Que nunca supo qué hacía,
O por qué trabajaba gratis,
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Te sigo queriendo,
Donde sea que estés…

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This seems to be the end of an era; however, it is just the beginning. It's been quite a long time since the day I discovered what even a parasite was. My love for these little creepy creatures started long before I even entered vet school. Since I was a kid, I have loved bugs. Every time we were out, I took the custom to collect whatever beetle, worm, or snail I came across. Hence… Imagine when I knew that I had bugs living on my head! The day my mother discovered that I was heavily infested with lice, she had to chase me along the corridor and around the rooms to treat me. I started running and hiding while shouting "Leave my bugs be!". Therefore, you must blame **my parents** for allowing me to write this thesis.

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Index

Abbreviations and terminology considerations

sp. – Species, singular.

spp. – Species, plural.

Syn. – Synonyms

Different Latin names that a certain species has received over time

CI – Canary Islands

Spanish archipelago located off the north-eastern coast of Africa.

CK – California kingsnake

Lampropeltis californiae. Colubrid snake native to southwestern North America,

MN – Main nucleus (eastern)

Invasion cluster of California kingsnakes in Gran Canaria. Discovered first.

SN – Secondary nucleus (northern)

Invasion cluster of California kingsnakes in Gran Canaria. Discovered second

TN (in text) – Third nucleus (southern)

Invasion cluster of California kingsnakes in Gran Canaria. Discovered third

FN – Fourth nucleus (North-eastern)

Invasion cluster of California kingsnakes in Gran Canaria. Discovered fourth

BIOTA – Bank of Data on Biodiversity of Canary Islands

An online project to gather all information on biodiversity of the Canary Islands. It is the online successor of the "List of wild species of Canary Islands".

C/O – Courtesy of

Co-introduced A species introduced to a non-native geographical zone usually within a host.

Co-invasive (Lymbery *et al*. 2014): An introduced species which is documented switching its host in its new geographical range. In the case of co-introduced parasites with indirect life cycles, the author of this thesis considered them as co-invasive species since by definition they have switched from their original intermediate hosts, whether or not they are able to reach adult stage and reproduce in native hosts.

Resumen

Pese a su pequeño tamaño, las Islas Canarias poseen una rica variedad de climas. A lo largo de los milenios estos han demarcado diferentes ecosistemas, agrupados generalmente en base a su vegetación y zonas climáticas. Este hecho refuerza la idea del uso de islas como Gran Canaria a modo de "pequeños laboratorios" para la realización de estudios en ecología y biodiversidad. Al mismo tiempo, estas marcadas zonas climáticas hacen a estos ecosistemas vulnerables a invasiones biológicas, ya que normalmente están habitados por cientos de seres vivos con nichos ecológicos muy especializados. Desgraciadamente, los estudios sobre la ecología de este último fenómeno suelen centrarse en la investigación de especies de vida libre, que desencadenan labores de control o conservación de aquellas que tienen valor o causan impacto socioeconómico. Con lo cual, acaban excluyendo por definición, a una de las formas de vida más comunes del planeta: Los parásitos.

Las formas de vida parasitarias juegan un importante papel como reguladores de los ecosistemas, siendo en algunos casos incluso mas diversos que sus propios hospedadores. De hecho, en las Islas Canarias existen más especies de parásitos únicamente afectando al lagarto gigante de Gran Canaria (*Gallotia stehlini*) (n=17) que especies de reptiles endémicos en todo el archipiélago (n=15). Sin embargo, todos ellos no son exclusivos de este hospedador, han sido registrados también en otros reptiles del archipiélago. En tiempos geológicos, estos parásitos cambiaron de hospedadores, adaptándose a ellos y produciendo fenómenos de "spillover"/"spillback", convirtiéndose a su vez en especies co-invasivas. Hoy en día, parte de estos parásitos como el nematodo *Spauligodon* spp. son considerados especies endémicas de pleno derecho, al igual que sus hospedadores.

Por lo tanto, los parásitos de las especies invasoras tienen capital importancia a la hora de inferir cómo de exitosa puede ser una invasión, ya que la simple ausencia de estos se considera uno de los factores mas cruciales en el desarrollo de una invasión biológica (teoría del "enemy release").

En el caso de Canarias, varios hospedadores introducidos en tiempo históricos (gatos, ratas, ratones…) han ejercido de "caballos de troya" dispersando sus propios parásitos. No se conocen las consecuencias reales de esta co-invasión silenciosa, al igual que los potenciales parásitos introducidos más recientemente, como el caso de la culebra real Californiana (*Lampropeltis californiae*).

Por ejemplo, algunas formas larvarias de parásitos de roedores se pueden encontrar infestando invertebrados endémicos, con especial interés en la presencia de nematodos zoonóticos como *Angiostrongylus* cantonensis. A su vez,

larvas de este último nematodo junto a otros metastrongilidos de gatos perros y erizos (*Atelerix* algirus) han sido encontrados en el lagarto tizón (*Gallotia galloti*). En lo que a gatos respecta, cisticercoides de sus especies de cestodos se han hallado en reptiles endémicos (*Tarentola* spp.) y pequeños mamíferos. Este es un ínfimo ejemplo, considerando las 67 especies introducidas de vertebrados en Canarias, de cuán compleja es la ecología de estos animales, sus parásitos y el potencial de convertirse en especies co-invasoras.

Anteriores a esta tesis, solo existen dos publicaciones referenciando parásitos (todos Apicomplexa) en menos de 11 culebras reales de California en total (*Isospora crotali, Cryptosporidium serpentis, Eimeria* sp. y *Haemogregarina* sp.). No obstante, de estos cuatro parásitos únicamente *C. serpentis* está correctamente descrito, siendo *I. crotali* sinónima de *Sarcocystis* sp., sin retener su epíteto específico, una descrita solo a nivel de género, y *Haemogregarina* usado incorrectamente como un nombre genérico para referirse de manera amplia a Haemogregarinidae. Estos hechos evidencian lo infra estudiada que se encuentra la fauna parásita de esta popular especie de mascota exótica.

En Gran Canaria únicamente se menciona encontrar una garrapata (probablemente género *Hyalomma*) en uno de los ejemplares capturados durante las labores de erradicación. Esta falta de información hace necesario estudiar no únicamente su biología como especie norteamericana invasora sino también sus potenciales parásitos, algunos de ellos, zoonóticos (Pentastomida, *Entamoeba* spp., *Ophionyssus* spp.).

Para diagnosticar la comunidad parasitaria de estas serpientes, se inspeccionaron cadáveres y heces en diferentes periodos entre 2016 y 2019. En total, se testaron para parásitos 108 cadáveres y 121 muestras de heces (44 congeladas y 77 frescas)

Como era esperable en invasores exitosos, se encontraron parásitos metazoos adultos únicamente en dos individuos, una infestada con *Serpentirhabdias* sp. (nematodo pulmonar) y otra con *Ophionyssus natricis* (ácaro de la piel con potencial zoonótico). En las muestras de heces se encontraron algunos Apicomplexa propios de la serpiente (*Sarcocystis* sp. (10/121), *Caryospora* sp. (1/121)) así como un parásito de invertebrados (*Adelina tribolii* (3/121)).

Para confirmar que la serpiente era el hospedador definitivo de los *Sarcocystis* sp. y no una contaminación de parásitos de presas, se secuenciaron algunas de las muestras fecales positivas y se compararon con quistes tisulares de la cola de dos lagartos gigantes de Gran Canaria así como se hicieron cortes histológicos seriados del intestino de las serpientes infectadas. Se encontraron estadíos evolutivos de *Sarcocystis* en el intestino de las serpientes. El material secuenciado difirió de las secuencias publicadas en NCBI incluida la usada como comparación. Por lo tanto, esta especie de *Sarcocystis* es indudablemente propia de la culebra y se ha adaptado perfectamente como co-invasora (spillover). El hospedador intermediario actualmente es desconocido, sin embargo la mayoría de especies de *Sarcocystis* relacionadas con serpientes usan pequeños mamíferos. Es difícil que este parásito se haya transmitido en colecciones privadas de serpientes, lo que significaría que la población fundadora de culebras mascota fue importada directamente a la isla desde su hábitat natural en Norte América.

Sorpresivamente, casi todas las serpientes estaban parasitadas por larvas de parásitos locales (spillback): Al menos tres especies de nematodos del orden Spirurida y dos especies de cestodos del género *Diplopylidium* (*D. acanthotetra* y *D. nölleri*). Es probable que los Spirurida aquí encontrados pertenezcan como mínimo a tres taxones diferentes: parásitos de roedores (Spiruroidea, Spirocercidae), aves (Acuarioidea, Acuaridae) y un último grupo que afecta tanto a mamíferos como aves (Pysalopteroidea). Los potenciales hospedadores para estos dos últimos grupos de parásitos son demasiado amplios como para analizarlos en detalle en esta tesis. No obstante, los otros parásitos (Spirocercidae y *Diplopylidium* spp.) representan especies co-invasivas de hospedadores invasores introducidos en tiempos históricos: Ratones (*Mus musculus*) (después del siglo I y antes del siglo XII), gatos (XV), rata negra (*Rattus rattus*) (XV) y rata marrón (*Rattus norvegicus*) (XVIII).

Para acabar en la serpiente, estos parásitos requieren de un hospedador intermediario y de la participación de uno o varios hospedadores de transporte que formen parte de la dieta de *Lampropeltis.* Para poder inferir este último eslabón, se han inspeccionado cadáveres de vertebrados misceláneos desde 2014 hasta la fecha de defensa de esta tesis. La única especie en la que se han encontrado gran parte de las formas larvarias diagnosticadas en las serpientes ha sido el perenquén (*Tarentola boettgeri*). En el caso de otros animales, se hallaron larvas de Spiroceridae en lisas de Gran Canaria (*Chalcides sexlineatus*) y Acuarioidea en musarañas (*Crocidura russula*). Otras especies de vertebrados (hurones y varias aves) que no se consideran presas de la serpiente fueron positivas a larvas de Spirocercidae.

Además de *Adelina tribolii* en heces de tres serpientes, con estudios posteriores usando muestras fecales de diversos animales (n=476) se reveló otra especie, *Adelina picei* en 1 de 298 gatos (4 de 476 en total). Este género de parásitos tiene un ciclo vital muy peculiar, ya que la reproducción y esporulación ocurre dentro de la cavidad corporal del invertebrado, dejando como única opción natural de diseminación la muerte de su hospedador. Normalmente debido a insectivorismo, los ooquistes esporulados se liberan en el estómago del predador, siendo finalmente expulsados en sus heces. Pese a la baja cantidad de heces positivas, son suficientes para ser conscientes de la presencia de estos parásitos en las Islas Canarias para futuros muestreos y estudios moleculares.

Siendo los gatos el hospedador definitivo para ambas especies de *Diplopylidium* diagnosticadas en la culebra real californiana, se hicieron dos estudios separados en parásitos de gatos. Se recolectaron muestras de distintas colonias felinas (n=290) en todos los municipios de Gran Canaria. A su vez, los datos se testaron para identificar cambios de prevalencia relativos a las estaciones y las condiciones climáticas. El segundo estudio consistió en analizar los contenidos intestinales de cadáveres (2008, 2015-2019) buscando específicamente especies de cestodos.

En el caso de las muestras fecales, solo fue posible identificar los huevos de *Diplopylidium* spp. a nivel de familia, ya que comparten morfología con *Joyeuxiella* spp. Estos parásitos fueron estadísticamente mas prevalentes en las zonas climáticas mas cálidas y secas de la isla, en concordancia con la distribución natural de los perenquenes endémicos. Estos parásitos no exhibieron patrones de estacionalidad.

Las inspecciones *post-mortem,* indicaron claramente que los cestodos en gatos asilvestrados son más prevalentes que lo diagnosticado en las muestras fecales, estando presentes en 62 de 78 gatos. Los cestodos se deterioran muy rápido una vez muerto su hospedador, por lo que no fue posible identificar la especie de todos los especímenes recuperados. El género *Diplopylidium* fue el segundo más prevalente (23/78) registrando 9 gatos positivos a *D. acanthotetra* y 8 *D. nölleri*. Conjuntamente a este parásito, el cestodo más común en infestación mixta fue *Joyeuxiella,* de su misma familia y compartiendo hospedador de transporte: los perenquenes.

Se requerirían futuros estudios epidemiológicos y moleculares para revelar el papel definitivo que la culebra real de California representa en la fascinante ecología de los parásitos de la vida silvestre en Gran Canaria.

Actualmente estamos trabajando en la identificación molecular de las especies de *Diplopylidium* spp. and larval spirocercids en distintos hospedadores (definitivos, intermediarios y de transporte). En lo que respecta a los otros dos grupos de nematodos en serpientes (Acuarioidea y Physalopteroidea) se está recabando lentamente material proveniente de aves (*Synhimantus* spp. y *Physaloptera* spp.) para futuros análisis moleculares.

Objetivos

1. Teniendo en cuenta estudios previos sobre especies co-introducidas y co-invasoras, es objetivo de esta tesis revisar la potencial fauna parasitaria del género *Lampropeltis* reuniendo todas las referencias posibles. A su vez, se realizará un estudio epidemiológico en las poblaciones de Gran Canaria mediante el uso de coproscopía y disecciones parasitológicas, en busca de parásitos metazoarios.

2. Como se refleja en previa literatura, algunas especies parásitas de gatos se han establecido como co-invasoras y pudieran también afectar a la culebra. Por lo tanto, el segundo objetivo de esta tesis consiste en ahondar en el conocimiento de las especies parásitas en gatos asilvestrados de Gran Canaria, como hospedadores históricos de cestodos co-invasores afectando a fauna nativa canaria.

3. Del mismo modo que los parásitos de los gatos, las formas larvarias de otras especies parásitas podrían infectar a la culebra. Con lo cual, dentro del marco de esta tesis, se realizará un análisis de los parásitos de vertebrados que estén registrados en el Banco de Datos de Biodiversidad de Canarias (BIOTA). De encontrarse otras formas larvarias, se deberán realizar futuras prospecciones parasitológicas para recabar material y hacer las debidas comparaciones moleculares.

Conclusiones

Artículo 1:

“Parasitological findings in the invasive California kingsnake (*Lampropeltis californiae*) in Gran Canaria, Spain.”

1. Se encontró una baja prevalencia de parásitos adultos en las serpientes, sin embargo, casi toda la muestra (98,4%) estaba infestada con larvas de helmintos.

2. La mayoría de parásitos encontrados en la culebra no serán capaces de alcanzar sus respectivos hospedadores definitivos, disrumpiendo el ciclo natural de dichos parásitos en la isla.

3. Estos invasores contribuirán no solamente a la extinción de los perenquenes y otros reptiles endémicos, sino también de sus respectivos parásitos, algunos de los cuales son endémicos para las Islas Canarias (*Thelandros filiformis, Alaeuris stehlini, Sarcocystis stehlini, Ophionyssus setosus…*).

4. Este supone el primer registro de dos especies de *Diplopylidium* y tres tipos de larvas de nematodos en la culebra real de California, así como el primer registro de *Diplopylidium* en una serpiente norteamericana.

5. Independientemente de la especie, la carga parasitaria y prevalencia encontradas en Gran Canaria es considerablemente superior a las reportadas para parásitos en fase larvaria en otros reptiles en España y Norte América.

6. Las serpientes probablemente presentan una respuesta inmune mayor frente a los cestodos debido a la falta de co-evolución.

7. *Ophionyssus natricis* es un ácaro que representa potencial zoonótico.

8. El posible “spillover” de estos ácaros, así como de *Serpentirhabdias* sp. requiere futura investigación.

Artículo 2:

“Dispersion of adeleid oocysts by vertebrates in Gran Canaria, Spain: report and literature review.”

1. Pese a encontrarse una baja prevalencia, esta constituye los primeros datos de base para el estudio de la patología de invertebrados en las Islas Canarias.

2. Se necesitan futuros estudios epidemiológicos en parásitos de invertebrados en las islas para determinar el hospedador (nativo o exótico) de estos parásitos así como los insectívoros que intervienen el ciclo natural de *Adelina* spp.

3. El entendimiento del rol que cumple este parásito en las dinámicas poblacionales sería de particular importancia en una Isla, donde la mayor parte de la fauna es nativa/endémica, y en ocasiones en peligro.

4. Las Canarias, y otras islas similares podrían ser utilizadas como sistemas modelo para el estudio de los parásitos de artrópodos.

5. Considerando la morfología, los ooquistes aquí descritos son cercanos a varias especies, pero existen diferencias importantes en el número de esporocistos.

6. Por lo tanto, se requeriría mayor cantidad de material para estudiar su status taxonómico definitivo, sobre todo considerando la variabilidad que presenta *A. tribolii.*

7. Con el apropiado muestreo molecular de Adeleidos en invertebrados, los predadores podrían servir como especies centinela para la detección y estudio de este parásito en Canarias y otros lugares del mundo.

Artículo 3:

"*Sarcocystis* sp. infection (Apicomplexa: Sarcocystidae) in invasive California kingsnake *Lampropeltis californiae* (Serpentes: Colubridae) in Gran Canaria."

1. Todos los animales positivos fueron encontrados en los núcleos en el este de la Isla (núcleos principal 7/61; y cuarto 3/8)

2. Las secuencias de ADN de *Sarcocystis* en serpientes invasoras y las de los lagartos endémicos difirieron significativamente, demarcándose en dos clados diferentes.

3. Al contrario, el *Sarcocystis* de *Gallotia stehlini* fue muy similar a *S. gallotiae* (>99 identity) de *G. galloti* en Tenerife

4. Es probable que el *Sarcocystis* de la culebra real Californiana se trate de una especie serpiente-específica que se ha establecido como co-invasiva en Gran Canaria.

5. El hospedador intermediario para esta especie de *Sarcocystis* es hasta ahora desconocido, En futuros estudios, se deberían dirigir a investigar especies tanto nativas (lisas, perenquenes y pequeñas aves) como invasoras (roedores) que habitan en los núcleos poblacionales de *Lampropeltis*.

6. La presencia de *Sarcocystis* en culebras invasoras en gran canaria sugiere que la población fundadora de serpientes se estableció probablemente con individuos extraídos directamente de individuos silvestres del sureste de los Estados Unidos y norte de México, más que por serpientes nacidas en cautividad.

7. La clarificación del ciclo vital de este sarcosporidio de serpientes en Gran Canaria es necesaria para responder cuestiones relativas al posible impacto de este parásito sobre la fauna endémica en peligro de extinción de Gran Canaria.

Artículo 4:

"Small islands as potential model ecosystems for parasitology: climatic influence on parasites of feral cats."

1. Se han diagnosticado algunas especies zoonóticas en este trabajo como *Ancylostoma* spp. *T. cati, Giardia* spp. y *T. gondii*.

2. La ecología de *T. gondii* en Gran Canaria es un claro ejemplo de cómo las colonias de gatos asilvestrados afectan no solo a los humanos, sino también a los animales de granja y silvestres, existiendo una baja prevalencia en gatos, pero un 60% o más en el resto.

3. La gran prevalencia de *Ancylostoma* spp. y *T. cati* descrita en este estudio representa un claro problema de salud pública que debe ser atajado, particularmente considerando la probable diseminación dentro de las casas por gatos domésticos contaminados.

4. Estos datos sugieren que las condiciones climáticas locales parecen ser mas importantes que las estaciones para la mayoría de parásitos con excepción de los Apicomplexa (*C. felis* y *Giardia* spp.).

5. Las prevalencias más altas de cestodos Dipylidiidae (*Diplopylidium* spp. y *Joyeuxiella* spp.) se dieron en áreas DD, coincidiendo con las zonas de mayor densidad poblacional de perenquenes.

6. El ciclo de vida de *Diplopylidium* spp. y *Joyeuxiella* spp. recalcan la urgente necesidad de controlar especies invasoras como las colonias de gatos y sus respectivos parásitos co-invasores para preservar la frágil biodiversidad de las Islas Canarias,

7. Islas similares a Gran Canaria pueden ser utilizadas como ecosistemas modelo para la evaluación de la influencia climática sobre las comunidades de parásitos en vida salvaje, y además, con futuras prospecciones, las potenciales consecuencias del cambio climático en la biodiversidad parasitaria.

Summary

Despite their small size, the Canary Islands have a remarkable variety of climates, forming different ecosystems usually grouped by general climatic conditions and vegetation. This climatic isolation endorses the use of islands such as Gran Canaria as miniature laboratories to study ecology and biodiversity. At the same time, these marked climatic barriers make their ecosystems vulnerable to invasive species, since they are inhabited by hundreds of living beings with very specific biological niches. The ecological studies on these phenomena are usually focused on free-living species, triggering conservation efforts toward species with socioeconomic importance and usually excluding one of the most common living beings on the planet: parasites.

Parasites play important roles as ecosystem regulators and, in some cases, are even more diverse than their hosts. In fact, in the Canary Islands, there are more species of parasites infecting just the Gran Canaria giant lizard (*Gallotia stehlini*) (n=17) than species of endemic reptiles in the whole archipelago (n=15). All these parasites are not exclusive to this host, they are shared with several other species of reptiles in the archipelago. In geological times, these parasites switched hosts and adapted to them, producing what is called "spillover" and "spillback" phenomena, stablishing themselves as co-invasive species and, in modern days, endemic parasitic species (e.g. *Spauligodon* spp.). Therefore, parasites of invasive species do matter when assessing how successful a potential invasion will be. Furthermore, the mere absence of these parasites (the enemy release theory) is one of the most common facts about invasion success.

Historic introductions of invasive hosts such as cats and rats, or the more recent California kingsnake (*Lampropeltis californiae*) to Gran Canaria, are likely to have introduced their own parasites to the ecosystems of the islands, with yet unknown consequences for local fauna.

Few rodent parasites can be found in their larval form infecting endemic invertebrates, with special interest in zoonotic nematodes such as *Angiostrongylus cantonensis*. This last nematode species, in addition to other metastrongilid nematodes from cats, dogs, and hedgehogs (*Atelerix algirus*), has been found infecting the endemic Gallot's lizard (*Gallotia galloti*). In the case of cats, their cestode species can be found as larvae in endemic reptiles (*Tarentola* spp.) and small mammals. These are just few examples, from the 67 introduced vertebrates, of how complex the ecology of introduced hosts, their parasites, and their potential to become invasive is.

For California kingsnake, even being a popular pet snake, just two reports were found prior to this thesis, in which four apicomplexans are described (*Isospora crotali*, *Cryptosporidium serpentis*, *Eimeria* sp. and *Haemogregarina* sp.) in less than 11 California kingsnakes in total. These two reports on parasitic species in the

California kingsnake, are considered inadequate to assess the parasitic biodiversity harboured by this snake species, with just one parasite described to full species (*C. serpentis*), one just to genus, one just to an incorrect generic name (*Haemogregarina* sp.), and the remainder *I. crotali* was reassigned to the genus *Sarcocystis* without retaining the specific epithet.

In Gran Canaria, there were no data regarding the parasitic species of the California kingsnake prior to this thesis, except for unidentified ticks (likely genus *Hyalomma*) in a report from the eradication plan. Therefore, it is necessary to study not only its biology as a American invasive species in Gran Canaria but also its potential role as a "Trojan horse" to introduce further co-invasive species to the environment. These could include parasites that not only pose a threat to precious endemic species on the island but also have zoonotic potential, as in the case of Pentastomida, *Entamoeba* spp., *Ophionyssus* spp.

To assess the parasitological community of kingsnakes, carcases were inspected in different periods between 2016 and 2019. In total, 108 carcasses and 121 stool samples (44 frozen, 77 fresh) were tested for parasites.

As is usual in successful invaders, California kingsnakes were "released from their enemies" with adult metazoan parasites found in just two snakes: one infected with *Serpentirhabdias* sp. (lungworm) and another with *Ophionyssus natricis* (zoonotic skin mites). A few species of apicomplexans were found: *Sarcocystis* sp. (10/121), *Caryospora* sp. (1/121), and *Eimeria* sp. (1/121)) in the snake faeces, including a parasite of invertebrates (*Adelina tribolii*) (3/121)).

To confirm the snake as the actual host for *Sarcocystis* sp., the positive faecal samples were sequenced, as were cysts from the tail muscles of two Gran Canaria giant lizards for comparison. Stages of *Sarcocystis* were found in histological sections of the snake species, and the sequenced material differed from those stored in NCBI, including the one used for comparison. Therefore, this species of *Sarcocystis* is indeed a proper snake-related apicomplexan that has successfully adapted to its new environment as a co-invasive parasite (spillover). The intermediate host is still unknown; however, most snake related *Sarcocystis* spp. use small mammals such as rodents as intermediate hosts. This parasite is also unlikely to be transmitted in snake collections, which could mean that the founder population of pet California Kingsnakes was imported to the island directly from wild-caught North American individuals.

Surprisingly, almost all snakes were parasitized by larval parasites from local species (spillback): At least three species of Spirurida nematodes and two tapeworms of the genus *Diplopylidium* (*D. acanthotetra and D. nölleri*). The Spirurid larvae are likely to belong to three different taxa: Spiruroidea: Spirocercidae (likely parasites of rats), Acuarioidea: Acuaridae (birds), and Physalopteroidea (birds and mammals). The possible definitive hosts for the last two groups of nematodes are too broad to be analysed in detail in this thesis. Still, the other parasites, which represent the spillback events of three local parasites, are in fact more complex. These parasites represent at least three co-invasive species of invasive hosts introduced in historical times: House mice (*Mus musculus*) (after the Ist century and before the XIIth), cats (XVth), black rats (*Rattus rattus*) (XVth) and brown rats (*Rattus norvegicus*) (XVIIIth).

The circulation of these parasites uses an invertebrate intermediate host and the participation of one or several paratenic hosts, which are ingested by the snake. This last link for the tapeworm species has been widely demonstrated in the literature to be the endemic Boettger's wall gecko (*Tarentola boettgeri*). In El Hierro (the westernmost island), there have been isolated nematode larvae with the same characteristics as endemic lizards (*Gallotia caesaris*).

For further knowledge on which of the snake's prey could be transmitting these larval parasites, we have inspected carcasses of miscellaneous vertebrates since 2014 to the current date of this thesis. The only vertebrate species included in the diet of the snake on the island of Gran Canaria found to be infected with larval Spirurida have been the same as the tapeworms: the Boettger's wall gecko. In addition to larval parasites from rodents, larval Acuarioidea were found in geckoes and shrews (*Crocidura russula*).

These rodent parasites seem to have a rather broad spectrum of possible paratenic hosts since they have been found in feral ferrets (*Mustela putorius*), houbara bustards (*Chlamydotis undulata*), Eurasian stone curlew (*Buhrinus oedicnemus*), Eurasian hobby (*Falco Subbuteo*) and Barbary falcon (*Falco peregrinus pelegrinoides*) from several islands.

Further studies using stool samples from multiple host species (n=476) revealed another species of *Adelina* (1 out of 298 cat faeces) *A. picei*. This genus has a very peculiar life cycle since reproduction and sporulation occur within the host's body cavity. Hence, the only natural way out is death. This usually happens by predation, releasing multiple sporulated oocysts that pass through the intestine and are expelled within the animal faeces. The positive animals were rather scarce—three snakes and one cat out of 476 samples—but enough to be aware of the presence of these parasites in the Canary Islands for further sampling and sequencing.

Since cats are the definitive hosts for both species of *Diplopylidium* from the California kingsnake, there have been two separate studies on cat parasites. Faecal samples (n=290) from cat colonies distributed in all municipalities of Gran Canaria were analysed. In addition, the data were tested for seasonal and climatic patterns. *Post-mortem* examinations of cat carcasses (2008, 2015-2019) were also performed, searching specifically for tapeworm species.

In the case of stool samples, it was only possible to identify the eggs of *Diplopylidium* spp. at the family level since they share morphology with

Joyeuxiella. These parasites were statistically more prevalent in the drier and hotter zones of the island, matching the natural distribution of endemic geckoes. These parasites did not show a seasonal pattern.

Post-mortem examinations showed that tapeworm species in feral cats were more prevalent than expected from faecal samples, being present in 62 out of the 78 cats. Tapeworm species damage themselves rather quickly with decay; thus, it was not possible to identify the species level of all the specimens. The genus *Diplopylidium* was the second most prevalent parasite (23/78) registering 9 positive cats for *D. acanthotetra* and 8 for *D. nölleri*. The most common co-existing parasites were species of *Joyeuxiella* and *Diplopylidium,* biologically linked by infecting geckoes.

Further host surveys and molecular sampling are needed to fully reveal the link that California kingsnakes represent in the fascinating ecology of wildlife parasites in Gran Canaria.

We are currently working on the molecular identification of *Diplopylidium* spp. and larval spirocercids comparing DNA from definitive, intermediate, and paratenic hosts. As far as the other two groups of larval nematodes in snakes (Acuarioidea and Physalopteroidea) are concerned, we are slowly gathering material from birds (*Synhimantus* spp. and *Physaloptera* spp.) for further taxonomic and molecular work.

Section I:
Introduction

Chapter I: Canary Islands, a hotspot of biodiversity.

Chapter II: Invasive species.

Chapter III: California kingsnake: Taxonomy, life history and tale of an invasion.

Objectives.

List of references.

Chapter I:

Canary Islands: A hotspot of biodiversity

1.1 The climatic frame: a tiny laboratory for ecology.

1.2 Biodiversity in numbers.

"Can we name all species on Earth before they go extinct?"

– Costello *et al.*, 2013.-

As alarming as it might sound, it is an uncomfortable reality. We may never establish how many species have gone extinct because of our own actions as an invasive species. We are witness to the 6th mass extinction (Wake and Vredenburg 2008), and ultimately, all political measures and international collaborations are and will likely be insufficient, or non-existent. Pollution, habitat loss, mass fishing, emerging diseases and invasive species are several of the reasons for a general biodiversity decline on our planet (Stork 2010).

Documented extinctions and biodiversity loss are usually focused on free-living organisms that have certain value to mankind, either sentimental or economic, triggering governmental conservation efforts. However, it is likely that most extinction events are happening without even being noticed, affecting invertebrate species: edaphic fauna, parasites… which are unlikely to be preserved (Strona 2015). Common interactions such as the strange mycorrhizal network (Figueiredo *et al.* 2021), with fungi allowing plants to communicate and transfer nutrients, epibiotic mites living on bird feathers that feed on their fungi and bacteria (Doña *et al.* 2019), classic "malaria" parasites of reptiles whose actual definitive hosts are free-living haematophagous invertebrates (Telford 2009) (including leeches). Every species counts, every link matters in the vast global ecology, making immediate and long-term consequences of biodiversity loss, as well as the introduction of exotic species (e.g., the arachnid mayhem caused by brown tree snakes in Guam, discussed later in this thesis (Chapter II)) unpredictable.

Biodiversity is the core of our world - the oil that greases the wheels of every single system, including those artificially created by mankind such as crops or dams. For humans, biodiversity is a matter of economic losses. We either choose to protect or eradicate what is beneficial to our economy, e.g., breeding just the useful species of bees for honey and "artificial" pollination, touristic advertisements such Pandas in China, elephants in India etc (Strona 2015) Thus, we are far from reaching a healthy equilibrium with what is best for humans and global ecosystems.

In a non-human approach, biological diversity is a sign of a healthy environment in which all its links are interconnected in one way or another, including parasites. A rich parasitological community is one of the best examples of a healthy ecosystem (Marcogliese 2005; Strona 2015), especially those with indirect life cycles. In this sense, parasites offer a natural control of invertebrate as well as vertebrate species. Without this natural control, or even with more tolerance to a given parasite, certain species can be unregulated or regulated in different ways, potentially dominating an ecosystem (Park and Frank 1950). This fact also applies to invasive species, which carry their own parasites into their new environments.

These new species of parasites can be regarded as a method to control populations of new exotic species, but they can also represent a new pathogen for wildlife, humans, and native invertebrates, or even a threat to local parasites.

Thus, studying our biological diversity and their ecological relationships is key to a further understanding and the creation of predictive models for any sort of future scenario. Unfortunately, as strange as it might sound, most studies require sampling, which in many cases implies sacrifices or landscape damage. These samples must be respected, preserved, and deposited in such a way to allow further research, and with that also open windows to the past, through museum collections. For example, these samples can reveal the prevalence of certain pathogens in the past or even spot native strains that changed in history into invasive ones (e.g., the fatal epizootic fungal disease caused by *Batrachochytrium dendrobatidis* in amphibians (see (Jairam 2020)). This utility applies to parasites as well, which can be extracted from stored specimens. Furthermore, those parasitic specimens kept for taxonomical purposes serve the same goal to enlighten problems in systematic classification, morphological changes, cryptic speciation, and synonymy etc.

Since the dawn of humanity, we have been collecting information about the living world, past or present, from a mythical or scientific approach. For example, bones collected as dragons or giants, were finally identified as belonging to dinosaurs and elephants. There are records from many cultures of specimen or item knowledge collectors, millennia before the common era, such as in ancient Egypt, Sumer or China which started to curate collections of diverse items and documents (Simmons 2018). However, our modern conception of museums as scientific institutions is based on the more extensive "cabinets of curiosities" held by wealthy members of society around the XV century. These were constructed with the sole purpose of showing off, from little drawers to rooms filled with items. However, with the death of the owners, these would be donated to public entities which would start to construct museums of different sorts (Simmons 2018). Therefore, with public exhibition and popularization to lower classes, the golden era of naturalists began, collecting specimens all around the world to be studied and preserved, and of course, exchanged for coin (Mateo *et al.* 2020).

The Canary Islands were no exception, with flora, fauna, or even mummies from local aboriginals being "exported" to important museums to this day. As a matter of fact, the natural history museums of London and Vienna hold more specimens of critically endangered endemic species of lizards from the Canary Islands (e.g., *Gallotia simonyi*) than the natural history museums in Spain (Mateo *et al.* 2020).

Therefore, "thanks" to the growing interest of naturalists in the biodiversity of the Canary Islands, it is possible to study the past, to learn about potentially new parasitic species introduced by exotic species, or even to determine if overall biodiversity loss in previous centuries has driven local parasitic species to extinction.

Further chapters beyond the scientific voyage presented in these pages (see ongoing work and final remarks), will need to use specimens from natural history collections to be completed. For example, California kingsnakes from their natural habitat, larval parasites in reptiles endemic to the Canary Islands such as lizards, skinks… nematode specimens from the upper digestive tract of birds, ectoparasitic mites of reptiles…

1.1 The climatic frame: a tiny laboratory for ecology.

The Canary Islands are a subtropical volcanic archipelago composed of 8 islands, 5 islets, and 8 important stacks. These are divided in two provinces: Santa Cruz de Tenerife (western islands) and Las Palmas (eastern islands). In the first, there are four islands (from west to east): El Hierro, La Palma, La Gomera and Tenerife, with the capital city of the province located on the latter island. The eastern islands (in the same order) are Gran Canaria, which holds the capital city; Fuerteventura, Lanzarote and the Chinijo (a local word that means tiny) archipelago. In the Chinijo archipelago, (north of Lanzarote), is the 8th populated island, La Graciosa. The western capital city takes the name of the province, while the eastern capital city adds the name of the island as Las Palmas de Gran Canaria.

The islets are in Las Palmas: the isle of "Lobos" on the northeastern coast of Fuerteventura (the name of the islet stands for the extirpated populations of monk seals, which in old days used to be called marine wolves in Spanish). The remaining four, comprise the rest of the Chinijo archipelago, called Alegranza, Montaña Clara, Roque del Este, and Roque del Oeste.

Most of the stacks are found in the province of Santa Cruz de Tenerife, with the Salmor and Bonanza off El Hierro, and Garachico, Anaga, and the Fasnia stacks off Tenerife. In Las Palmas, the stacks of Gando and Farallón de Sardina are located off Gran Canaria's coasts.

The climatic conditions in the Canary Island are famous around the globe for being warm all year round, however, that is only applicable to coastal zones, since higher altitudes range from 6-10°C or even down to -6°C at Tenerife's Teide volcano (Agencia Estatal de Meteorología de España and Instituto de Meteorologia de Portugal 2012). Important features of the Archipelago's weather are the cold northern winds (trade winds or Alisios in Spanish), the Azores anticyclone, and the cold Canary marine current (García-Herrera *et al.* 2001). This combination of meteorological phenomena maintains stable and relatively dry conditions in the

Canary Islands. Most heavy rain events and heat waves occur when some of these factors are perturbated.

Nevertheless, the trade winds play a more crucial role in the diversification of ecosystems on the islands, bringing fresh and wet air to northern and northeastern localities. This fact is notorious in places situated at higher altitudes, where this humidity condenses forming clouds below the highest point of the islands ('the cloud sea'), which also brings more humidity to these places in the form of "horizontal rain" (García-Herrera *et al.* 2001). Additionally, the Canary Islands are commonly affected by sandstorms or "calima" events originating from strong winds in the Sahara and central Africa (Cuevas *et al.* 2021). These important events affect not only the migration of several birds and invertebrates, which do not appear naturally in the Canary Islands, but also human health.

Despite their small size, the islands have a remarkable variety of climatic zones forming different ecosystems usually grouped by general climatic conditions and vegetation.

The general classification of climatic zones is usually derived from Köppen publications (Köppen 1918, 1936), using a system based on three letters (Chazarra Bernabé *et al.* 2022). The first letter describes five generic types (namely, A to E) presented from the hottest and driest to the coldest polar conditions. The second and third letter describe the overall pluviometry and temperature respectively, with exceptions.

The climates are:

A. Tropical climates: Jungle (Af), Monzonic (Am), and savanna (As) without thermal subdivision.
B. The arid climates: desert (BW) and steppe (BS) each of them subdivided in cold (k) and hot(h) (BWh, BWk, BSh, BSk).
C. Temperate climates: Dry summer (Cs), dry winter (Cw) and those with no dry season (Cf). They are subdivided by overall summer temperature, having hot (a) temperate (b) and cold (c) summers (Csa, Csb, Csc, Cwa, Cwb, Cwc, Cfa, Cfb, Cfc).
D. Cold climates: with dry summer (Ds), dry winter (Dw) or without dry season (Df). They are also divided by hot (a), temperate (b) or cold (c) summers, or very cold winters (d) (Dsa, Dsb, Dsc, Dsd, Dwa, Dwb, Dwc, Dwd, Dfa, Dfb, Dfc, Dfd).
E. Polar climates: There are only two polar climes: Tundra (ET) and Glacial (EF).

In the Canary Islands there are three of the major zones, the arid (B), temperate (C) and cold climates (D). Still, the only zones classified as cold climes (Dsb and Dsc) are located only in Tenerife's highest points around the top of Teide. In addition, temperate climates of the archipelago belong only to those with dry summers (Csa, Csb, Csc) (Chazarra Bernabé *et al.* 2022).

Despite the weather stability procured by the trade winds, the Azores anticyclone and the cold Canary current, the Canary Islands are no exception to climate change. However, this change may be more subtle than in mainland Spain, where desert and steppe have almost doubled their extension from 1950 (11% of the territory) to 2020 (21% of the territory) (Chazarra Bernabé *et al.* 2022).

In general terms, since 1950, the Canary Islands have experienced a significant desertification process, increasing from 64% to 69% of the territory. Within these arid climes, the hot desert (from 42% to 48%) and hot steppe zones (from 11% to 15%) have increased to the detriment of the cold desert (from 1% to 0.1%) and the cold steppe zones (from 10% to 6%). By contrast, the temperate climes have decreased their overall extension from 36% to 31%, losing temperate cold and mild (28% to 21%) with a light increase in temperate hot (8 to 9%) (Chazarra Bernabé *et al.* 2022).

This climatic classification was modified for research (Figure 1) on climatic influence on parasitic species by (Rodríguez-Ponce 1994; Rodríguez-Ponce *et al.* 1995). This modification enables easier use in terms of geolocation, abbreviations, and distribution of vegetation mass. They are grouped and renamed as follows: dry desert (BWh+BWk), dry steppe (BSh+BSk), temperate mild (CSa+CSb), temperate cold (CSc), and high mountain (Dsb and Dsc).

Each climatic zone below is followed by an estimation in brackets of their respective altitude ranges and the temperature fluctuations in Gran Canaria. The high mountain climate is not present on this island. These climatic zones are arranged in concentric circles, separated by altitude ranges from sea level to the highest point at the centre of the island (Pico de las Nieves, 1949 meters). Therefore, most municipalities contain several ecosystems as they ascend in altitude, each having different climatic conditions.

Following each climatic zone, is a description of the typical ecosystem associated with it. Despite the climatic constraints in some of them, they can extend to higher or lower altitudes depending on the islands and the influence of the trade winds, which, in final terms is what dictates the overall humidity and precipitation of a certain location on the islands. All the information regarding ecosystems and forests was extracted from Santos (1984).

Dry desert (0-250m): Average annual minimum temperature (Tmin) of 14-21°C. Average annual maximum temperature (Tmax) around 20-27°C.

Most of this climatic area is characterized by the halophilic coastal ring and the ‘cardonal-tabaibal’ ecosystem (Figure 2). These have xerophilic vegetation highly adapted to dry, windy and hot climates as well as to very abrupt, rocky and eroded soil.

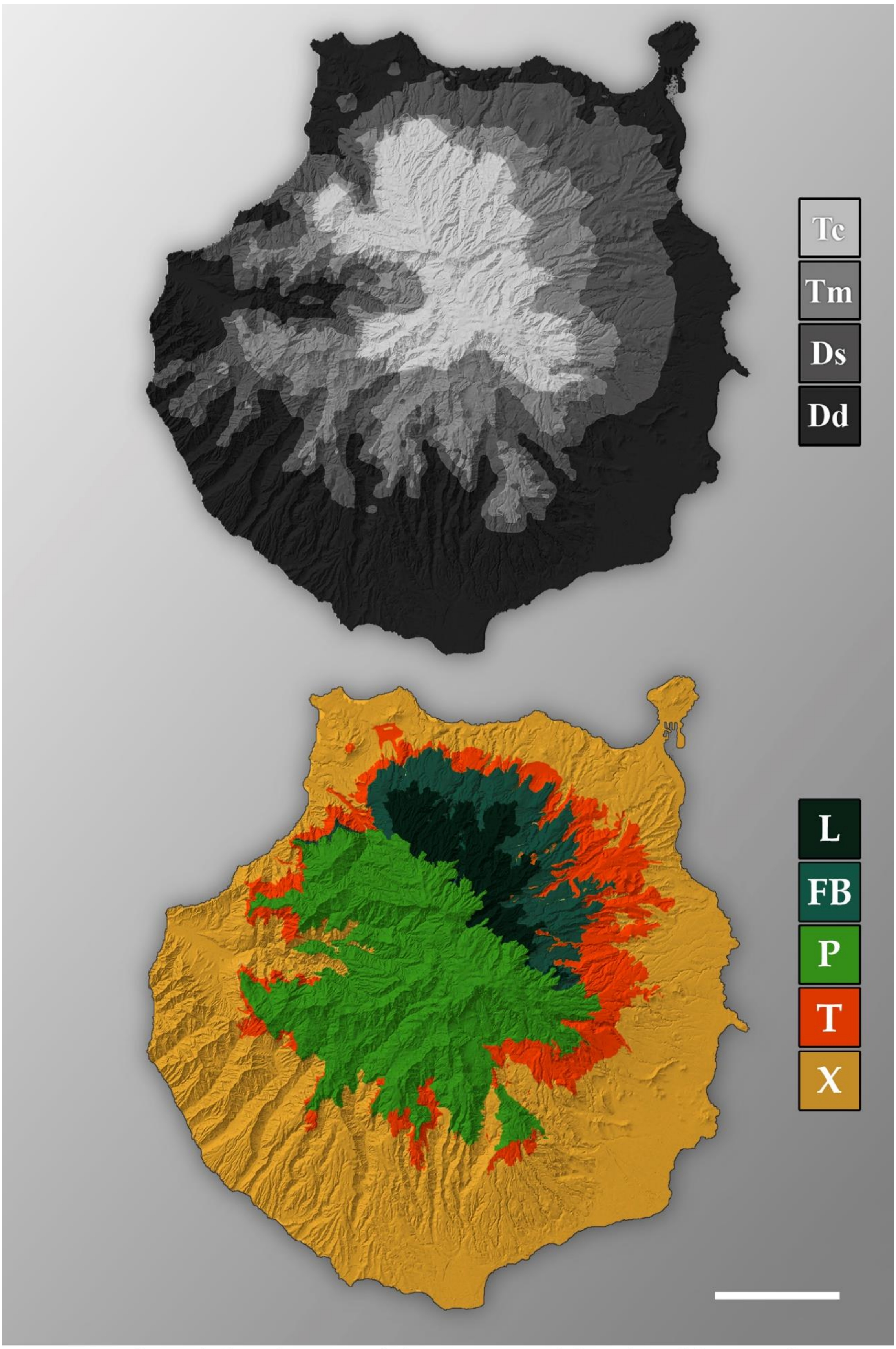


Figure 1: Adapted classification of climatic zones (above) and forests of Gran Canaria (below).**Tc**: temperate cold; **Tm**: temperate mild; **Ds**: dry steppe; **Dd**: dry desert; **L**: Laurel forest; **FB**: Fayal-brezal; **P**: Pine tree forest; **T**: Thermophile forest; **X**: Cosastal ring+Tabaibal-cardonal. Scale bar = 10 km.

The halophilic coastal ring (first 50-100m in altitude) is directly influenced by the sea; therefore, its flora is adapted to withstand saline soils (halophylic). This ecosystem can be subdivided into those with sandy soils, generally at beaches (psammophytes) and those with more distant, rocky, dry and saline soils, which also include cliffs.

Above these, where the saline influence of the sea on the ground ends, the tabaibal-cardonal ecosystem begins (up to 300-500m). It receives the name from the predominant plants of this habitat the cardón (*Euphorbia canariensis*) and the multiple tabaiba species (*Euphorbia* spp.). Still, the cardón usually grows further from the sea than the tabaiba species. Among these, the cardón has an important role in the ecosystem serving as protection for other plants that can grow between its branches. However, whenever a humid season occurs, the layout changes with the bloom of herbaceous pastures.

Dry steppe (250-650m): Tmin=12-20°C. Tmax= 18-26°C.

Depending on the zone of the island, this zone can be dominated either by cardonal-tabaibal or thermophile forest. The latter is composed mainly of palm trees *(Phoenix canariensis*), tamarisks (*Tamarix* spp.), lentisks (*Pistacia lentiscus*) among other trees and bushes (Figure 3A). As in the latter climatic zone, there are blooms of diverse plants during the humid seasons.

These forests are one of the most affected by humans, since they were predilected places for human settlement. Hence, very few examples of pure forests remain on the islands. According to the chronicles, in Gran Canaria dense palm tree forests existed; in fact, "Las Palmas" in the name of the capital city of Gran Canaria, gained its name from those relict palm forests.

Temperate mild (650-950 m): Tmin= 11-19°C. Tmax= 16-25°C.

Linked to the influence of the trade winds, which translates to higher overall humidity and rainfall, the Fayal-brezal is found in the northeast of the island. In contrast, the opposite side of the island is populated mostly by pine tree forests. In this climatic zone the highest points of thermophile forests can also appear.

Pine tree forests (Figure 3B) are characterized by a poor botanic community, highly dominated by the Canarian pine tree (*Pinus canariensis*) and other plants whose seeds can sprout after fires such as the "amagante" (*Cystus symphytifolius*). Apart from the generally dry conditions in these forests, it is believed that one of the reasons for their lack of floristic diversity is the influence of aboriginal culture and later conquest and deforestation. Canary Island aboriginals are believed to have caused cultural fires to fertilize the lands and renovate pastures for their livestock. However, these pines resist fire regimes and re-sprout if burned.

The fayal-brezal (Figure 3C) also receives its name from the two main trees of which it is composed: the fire tree (*Myrica faya*) and the tree heath (*Erica arborea*). These are also accompanied by a variety of small trees and bushes that, if the conditions are more humid, such as in the cloud sea segment, can grow taller than others at higher or lower altitudes.

Temperate cold (950-1949m): Tmin= 7-16°C. Tmax= 13-23°C.

In the same way, the ecosystems of the latter climatic zone vary from Fayal-brezal or laurel forest (Laurisilva) (Figure 4) in the humid North-east, to pine tree forests in the dry south-west.

It is interesting how the botanic species overlap and constitute different forests at different altitudes depending on the overall influence of the cloud sea (horizontal rain). Therefore, in that exact segment of the island (around 1000-1100m) in the north-east, is found what is considered a botanic gem, the laurel tree forests or "laurisilva". It is characterized by a highly diverse composition of flora and fauna, which brings surprises to the researchers, discovering new species every few years. The most iconic species are the laurel tree (*Laurus azorica)*, the canary avocado (*Persea indica*), and the white wood (*Picconia excelsa)* followed by various fern species which can grow up to one metre high. Within this forest, members of the fayal-brezal plant communities which are more resistant also grow. Therefore, above the cloud sea, the fayal-brezal plants and the pine trees continue to the top of the island.

Its abrupt landscape and all these climatic conditions allow different species to flourish, and most importantly, isolate different populations of living beings within the same island, which then contribute to the separation of species and subspecies (Suárez *et al.* 2022; Pérez-Delgado *et al.* 2022). This constitutes the Canary Islands in general as a miniature laboratory to unravel how climatic conditions and limited spatial ecology contribute to create new species and in contrast, how markedly different ecosystems could limit the spread of invasive/exotic species and diseases.

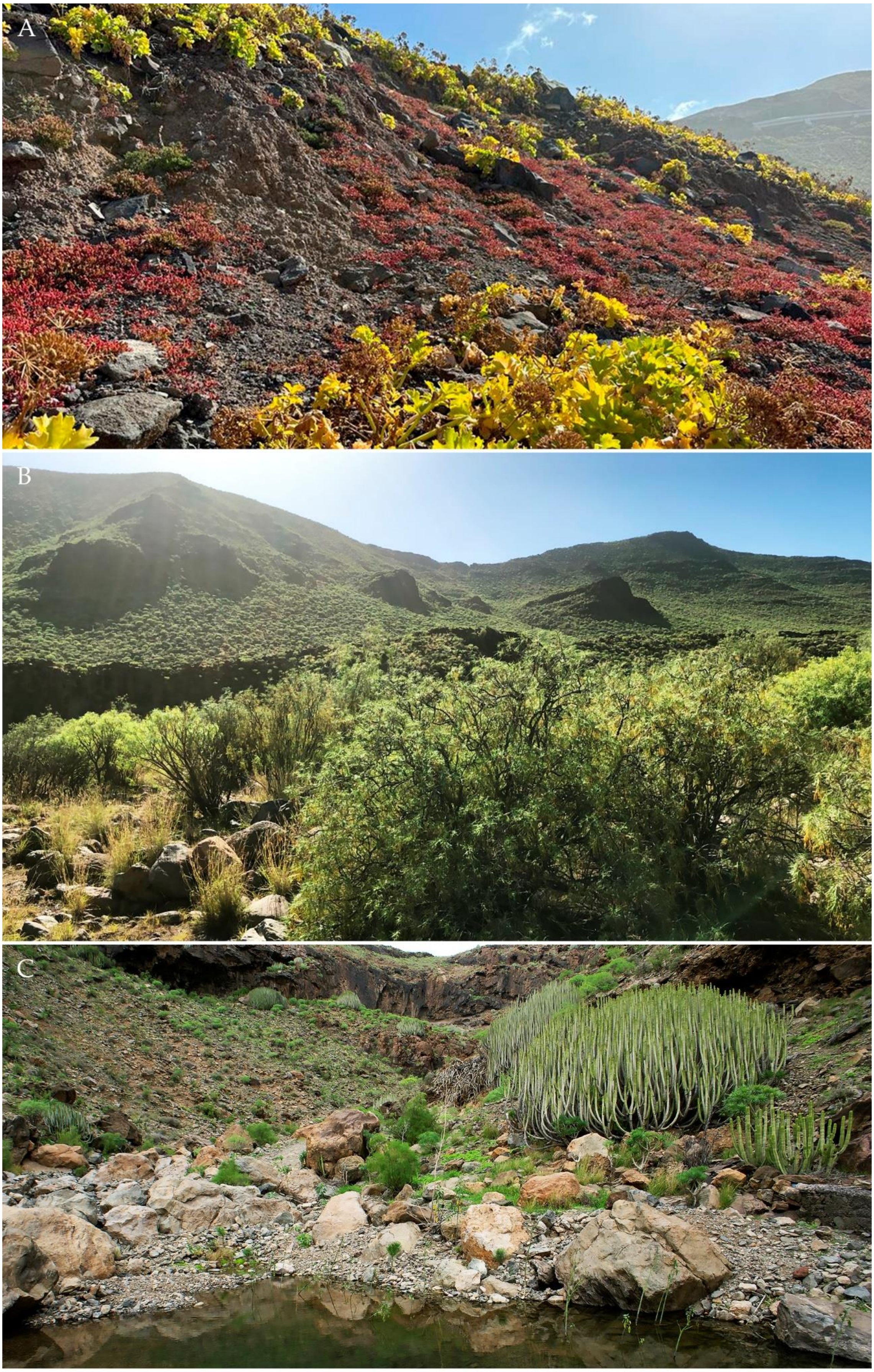


Figure 2: Ecosystems in Gran Canaria. **A**: Coastal ring; **B**: Tabaibal; **C**: Tabaibal-Cardonal. Courtesy of Mr. de Blas (A-C).

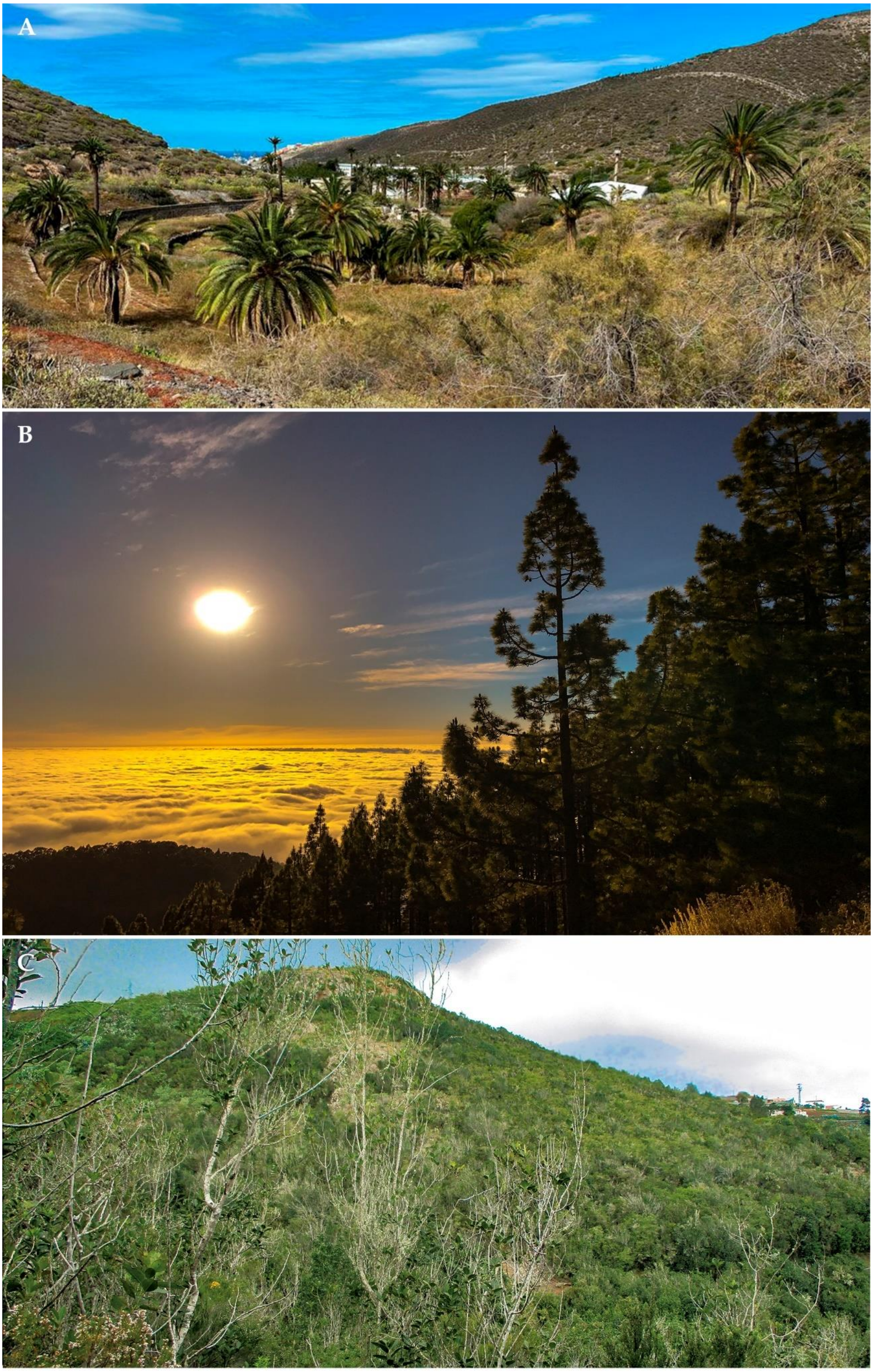


Figure 3: Ecosystems in Gran Canaria. **A**: Thermophile forest C/O Mr. de Blas; **B**: Pine tree forest C/O Mr de Blas; **C**: Fayal-Brezal C/O Daniel Suárez.

Figure 4: Ecosystems in Gran Canaria. **A-C:** Laurel forests. **C**: Forest floor. Courtesy of Kevin M. Santana (A), Raúl E. Bruno (B); Mr. de Blas (C).

1.2 Biodiversity in numbers.

> *"…Here the poor are remarkably lousy, and are not ashamed of it, for the women may be seen sitting at the doors of their houses picking the lice out of one another's heads…"* **-Captain George Glas,** (1767). –
>
> Probably the first parasite cited in the Canary Islands, *Pediculus humanus* on Spaniards**.**

According to the Bank of Data on Biodiversity of the Canary Islands (BIOTA) (https://www.biodiversidadcanarias.es/biota/), a total of 27,692 species are registered (accessed February 2022) and still today, there are new reports made every year. The most diverse group is the phylum Arthropoda (12,763 spp.) followed by the kingdom fungi (4262 spp.) and plantae (3800 spp.). The species are divided in BIOTA into marine (7,258 spp) and terrestrial (20,434 spp). In addition, according to the Spanish Inventory of terrestrial species, there are 91,000 terrestrial species recorded in Spain, which means that 22.4% of the land biodiversity of the country can be found in the archipelago.

In the case of vertebrates (Table 1), a total of 1129 extant species are recorded, of which most are fish (822 spp.) and birds (221 spp.). For endemic species, there are 46 registered endemisms, of which 14 are extinct. A total of 48 subspecies gathered in this database are regarded as endemisms.

Considering this vast biological diversity, the current data on accidental and obligate parasitic species of vertebrates was extracted from the database (Table 2), excluding parasites and associates of plants and other invertebrates. With these characteristics, 296 parasites and 46 opportunistic parasites were established (Appendices 1-3).

Dipterans were the only occasional parasites found in the Arthropoda group, with the potential to cause myiasis, and epibiotic crustaceans (Thecostraca) which can cause disease in marine vertebrates, without feeding on their "host".

There are also parasitic species classified as endemisms according to the Bank of Data on Biodiversity of the Canary Islands: 15 species of Arthropoda; namely 6 insects (5 Diptera, 1 Siphonaptera), 8 mites (6 Parasitiformes, 2 Trombidiformes) and 1 Pentastomida; and 10 species of helminths (9 nematodes, 1 trematode). There are 4 endemic species of flies that might cause myiasis. Most of these parasites can be found in Tenerife (15spp) La Palma (12 spp), La Gomera and Gran Canaria (10spp each), with the least in Fuerteventura (3spp), Lanzarote (7spp) and El Hierro (8spp). The most

	Terrestrial	**Marine**	**Total**
Actinopterygii	16*	714	730
Elasmobranchia	0	85	85
Holocephali	0	7	7
Anphibia	3	0	3
Reptilia	23	6	29
Aves	165	56**	221
Mammalia	22	32	54
Total	229	900	1129

Table 1: Vertebrates of Canary Islands. *Freshwater **Shorebirds.

Group	Total entries	Obligate parasites	Occasional parasites
Monogenea	28	28	0
Trematoda	25	25	0
Cestoda	39	39	0
Nematoda	195	73	1
Annelida	73	6	0
Arthropoda	12,763	120	45
Total	13,123	291	46

Table 2: Records on the Bank of Data on Biodiversity of Canary Islands for common parasitic groups.

widespread species is *Spauligodon atlanticus,* which inhabits endemic reptile species on the 7 main islands, followed by *Thelandros filiformis,* which shares hosts, but lives on just 5 islands.

In addition, 10 species and 1 subspecies of parasite represent individual island endemisms, with Lanzarote and Gran Canaria the islands with the most (4spp each) species living exclusively in/on their fauna. Next on the list with 2 spp is Tenerife, and finally El Hierro with 1 spp. Most of these species are parasites of endemic reptiles (9/11), especially helminths (4 nematodes, 1 trematode) and arthropods (3 mites, 1 pentastomid) which seem to have co-evolved with their hosts. However, one species of mite (*Echinonyssus molinae*), one species and subspecies of flea (*Xenopsylla guancha* and *Leptopsylla algira atlantidis*) are found in invasive/introduced rodents and shrews, which could represent either an identification error, or survivors of the extinction of the endemic rodents of Lanzarote and Gran Canaria.

In more general terms, there are 80 species of non-endemic parasites living only on one island, in the same way as island endemisms. The taxonomic composition is diverse, with 37 Arthropoda species, 27 nematodes, 13 flatworms and 3 leeches. Still, most of them are likely to occur on all islands, since their hosts are widespread in the wild or represent pests and/or domestic animals (i.e., *Dipylidium caninum* (tapeworm) and *Ancylostoma caninum* (nematode) from domestic carnivores or *Columbicola columbae* (louse) from rock pigeons (*Columba livia*) etc).

The island with the most exclusive parasitic species (see Figures 5 and 6), both endemic and non-endemic, is Tenerife (50 of their 128 species) followed by Gran Canaria (12/71) and Fuerteventura (10/43). However, expressed in percentages, Fuerteventura is in second place with 23.2%, followed by El Hierro (20.0%, 7/35), Gran Canaria (16.9%), Lanzarote (14.2%, 5/35), La Palma (9.4%, 5/53) and La Gomera (2.3%, 1/43). These data are presumably biased by the number of studies performed on each of the islands, with those more populated having more research facilities and therefore easier field work.

The only non-endemic parasites recorded on the 7 main islands are: *Toxocara canis* zoonotic nematode of dogs, *Enterobius vermicularis* nematode of humans, *Parapharyngodon micipsae* nematode of endemic reptiles, and three hematophagous dipterans (*Stomoxys calcitrans, Culex pipiens* and *Culiseta longiareolata).* However, there are 111 species recorded in BIOTA without any geographical data associated with them, 38 and 73 marine and terrestrial species respectively.

Most parasites recorded in BIOTA infest mammalian (89), avian (71) or reptilian hosts (41). After these, there appear the generalist parasites of land hosts, which include leeches and other free-living hematophagous invertebrates (40) and parasites of bony fishes (38). The least records are found among shark and ray (9) and amphibian (3) hosts. This distribution of parasites, considering the percentual composition of vertebrates on the islands, points to a clear selection bias, with more comprehensive studies regarding mammalian parasites (pests, domestic animals, and humans), and general field sampling for entomological studies.

Therefore, there is plenty of work to perform to fully discover the definitive checklist of parasitic species on the Canary Islands, their distribution, biology and potentially endemic status before they hit extinction unnoticed: A race for discovery. Moreover, it is reasonably clear, according to these data, that the Canary Islands, as most authors predict for the rest of the world, hold more species of parasites, than vertebrates to host them.

In this sense, it is important to consider the introduction of new parasitic species into the environment through new exotic species of hosts colonizing the Canary Islands, not only as potential pathogens to local species, but also as a way of increasing biodiversity. In some cases, these exotic species adapt respectfully to the environment, contributing as seed spreaders, pollinators or even as food items for predators, contributing to enrich the environment (Ruiz *et al.* 2020; Guerrero-Campos *et al.* 2023).

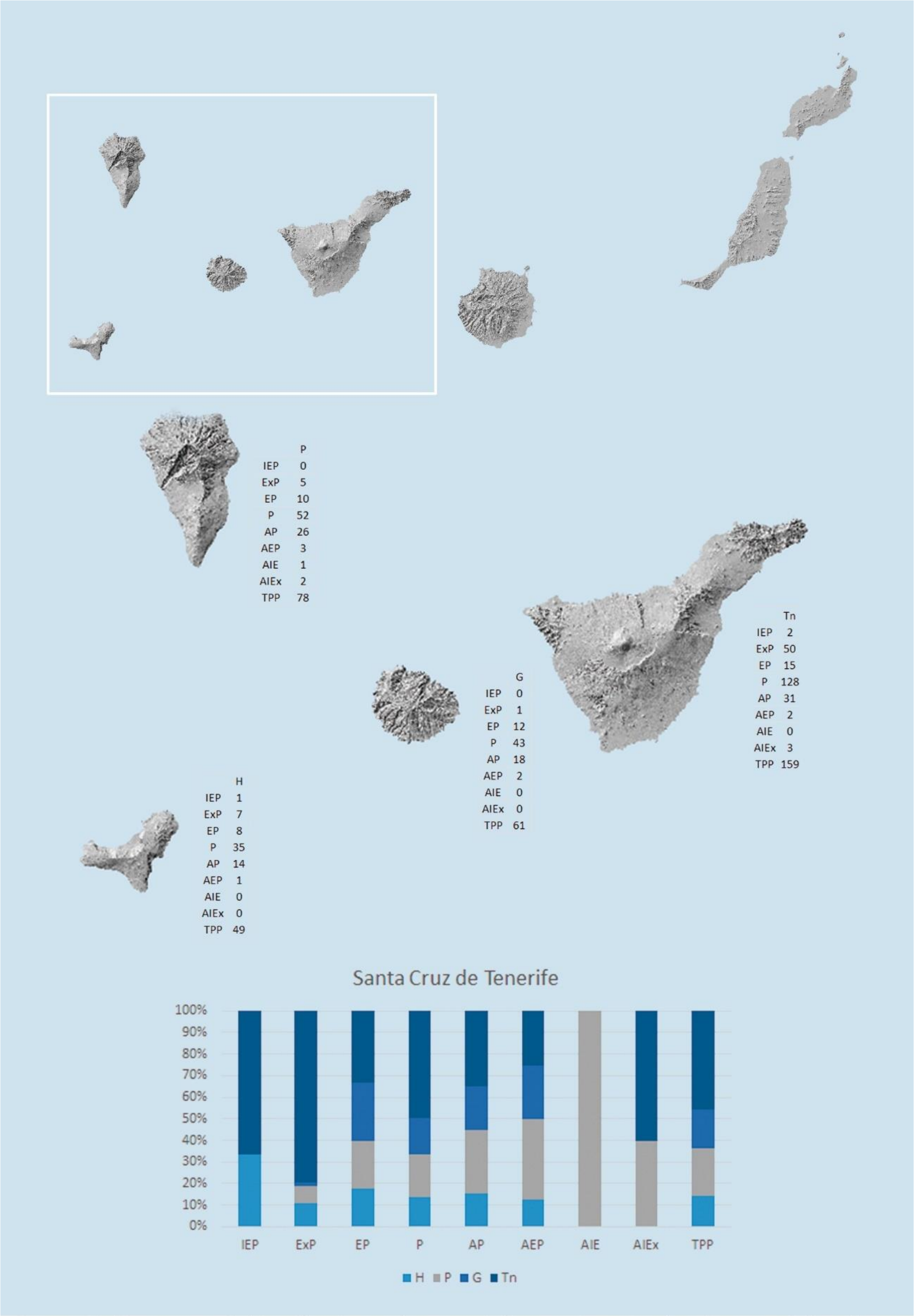
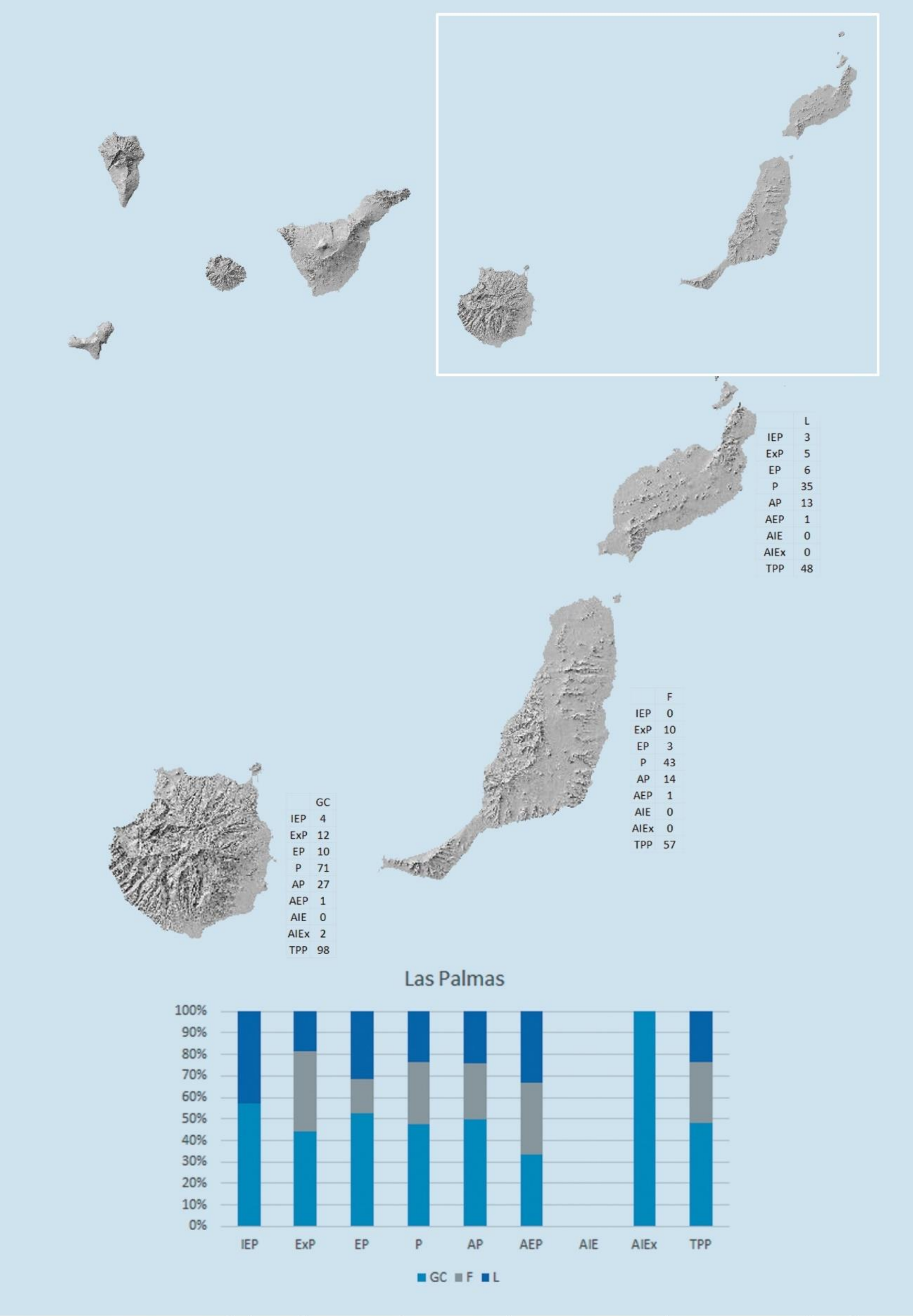


Figure 5: Percentual distribution of potential parasites in the Canary Islands: **IEP**: Island endemisms; **ExP**: Non-endemic parasites exclusive to an island; **EP**: Endemic parasites; **Pa**: Total parasitic species; **AP**: Accidental parasites; **AEP**: Accidental endemic parasites; **AIE**: Accidental island endemisms; **AIEx**: Accidental, non-endemic parasites exclusive to an island; **TPP**: Total potential parasites; **H**: El Hierro; **P**: La Palma; **G**: La Gomera; **Tn**: Tenerife.



	L
IEP	3
ExP	5
EP	6
P	35
AP	13
AEP	1
AIE	0
AIEx	0
TPP	48

	F
IEP	0
ExP	10
EP	3
P	43
AP	14
AEP	1
AIE	0
AIEx	0
TPP	57

	GC
IEP	4
ExP	12
EP	10
P	71
AP	27
AEP	1
AIE	0
AIEx	2
TPP	98

Figure 6: Percentual distribution of potential parasites in the Canary Islands: **IEP**: Island endemisms; **ExP**: Non-endemic parasites exclusive to an island; **EP**: Endemic parasites; **Pa**: Total parasitic species; **AP**: Accidental parasites; **AEP**: Accidental endemic parasites; **AIE**: Accidental island endemisms; **AIEx**: Accidental, non-endemic parasites exclusive to an island; **TPP**: Total potential parasites; **GC**: Gran Canaria; **F**: Fuerteventura; **L**: Lanzarote.

Chapter II:
Invasive species

2.1 General traits and paths to invasiveness.

2.2 Historic and current invasive species in the Canary Islands.

2.3 The strong prevail.

A living species that is translocated from its natural habitat into a new one is known as an exotic or alien species. If their populations grow, expand to new territories, and start to cause damage to local species, they are transferred to the category of invasive species (Lymbery *et al.* 2014).

Invasive species are common events in natural history. In most cases, the natural introduction of living beings to an isolated system has been responsible for the evolution of millions of endemic species around the world. Once established, a process of evolution starts, with morphological and behavioural adaptations at first and further genetic differentiation to create new full species (Sakai *et al.* 2001).

This might occur in geological terms of time if their populations are truly isolated, and no external genetic material is added to them. Nevertheless, parasitic species are prone to disappear than to evolve in an isolated setting (Moir *et al.* 2010). In fact, there exists a common biological phenomenon known as "island syndrome", in which the same species of hosts have a less diverse parasitic community on islands than those on the mainland (Nieberding *et al.* 2006). Furthermore, in those habitats in which multiple hosts of the same parasite co-exist; whether native, introduced, or migrants it ends up diversifying the genetic pool of the parasites, making them less likely to evolve.

In our modern day, invasive species pose one of the biggest challenges in species conservation, since in most cases they put under threat of extinction lots of local, native, and endemic species that are highly specialized and poorly armed against alien invaders (Sakai *et al.* 2001; Doherty *et al.* 2016). However, invasion events offer unique opportunities to observe short-term evolutionary changes, with native species starting to show morphological or behavioural differences to overcome the effect of the invaders (Sakai *et al.* 2001; Piquet *et al.* 2022).

The main hazard that invasive species pose to endemism is usually related to their trophic ecology, either by competition or predation. However, these living beings also compete for their habitat, transmit diseases, or even hybridise with local species (Sakai *et al.* 2001). Furthermore, these patterns are not only exhibited by free-living vertebrates, plants, algae etc, but also by parasitic species.

Parasites are the predominant life form on the planet; therefore, they represent both sides of the coin in this topic: Naïve species susceptible to perish along with their host, and those that can survive in other hosts and spread as invasive species (Lymbery *et al.* 2014).

2.1 General traits and paths to invasiveness.

Once any sort of living being is translocated from its natural habitat, it undergoes a dichotomic pathway into either death or survival. The total genetic pool of the founder population should be sufficient to mitigate the effects of inbreeding and allow these species to endure. In addition, the match between the general biology of a species and the vacant ecological niches in their new location will likely result in adaptation (Sakai *et al.* 2001). Moreover, a reduced number, or the lack of natural predators, competitors, or diseases (concept of enemy release) will also determine the success of the invasion.

Therefore, if a species can survive in an ecosystem, it will start an adaptation process that could end in successful reproduction, stability, or conversely demise. If the species survives and adapts, it starts to spread and conquer more territory. In some cases, the climatic conditions and fauna associated with surrounding habitats could limit its spread. Once in expansion, its invasive character will be determined by how this species interacts with the environment and its inhabitants (Sakai *et al.* 2001).

Invasive species usually compete with locals for resources, mainly by occupying territory and being able to reproduce quicker. They can become efficient predators and singlehandedly drive dozens of species to extinction. They can also transmit diseases to local species, domestic or wild animals, or even humans. They can hybridise with local species resulting the long-term disappearance of the local species (Sakai *et al.* 2001); e.g. the hybridation between the globally endangered white headed duck (*Oxyura leucocephala*) and the introduced North America ruddy duck (*Oxyura jamaicensis*) (Muñoz-Fuentes *et al.* 2007). In summary, alien species can alter and reset millenarian ecosystems within a few decades, with higher impacts on islands.

Islands are prone to worse alterations because their ecosystems are isolated and usually contain numerous specialized species (Gurevitch and Padilla 2004; Carroll 2007) Therefore, predatorial species introduced to small systems, can end in catastrophic consequences, as in the case of the brown tree snake (*Boiga irregularis*) in Guam, the 15th most damaging invasive species in the world according to the Global invasive species database (www.iucngisd.org).

The brown tree snake is an arboreal, nocturnal, venomous colubrid native to southeast Asia and Oceania. Therefore, its natural habitat is islands; however, in Guam, likely due to enemy release, its population of introduced species exploded and became a clear threat to the conservation of Guam's native fauna. It is believed that it was introduced as a stowaway in military planes around 1940 (Gallo-Barneto *et al.* 2016). Guam was devastated after World War II; thus, when they discovered how birds were disappearing from the south of the island, the first thing that came to mind were pesticides (Rodda *et al.* 1997). This lack of birds and life in general extended through the island, leaving just little areas populated by birds, which were almost completely extirpated during the 1980s. Since locals knew about the disease introduced in Hawaii (an invasive bird malaria parasite), they contacted an avian pathologist to research what was killing birds (Rodda *et al.* 1997) and as logical as it might sound, you cannot investigate a disease if you do not have carcasses. Therefore, during the late 80's, the culprit of this faunistic genocide in Guam was confirmed: the brown tree snake (Savidge 1987). The consequences are not only a matter of species conservation, the annual cost of controlling this species to avoid its escape to other nearby islands is calculated at between 1 to 4 million dollars per year (Gallo-Barneto *et al.* 2016). As a result of this loss of biodiversity, those other species in Guam predated by birds have experienced the same enemy release. Hence, native spiders have reproduce enormously covering extensive areas of forest with their webs (Rogers *et al.* 2012). These predators will likely continue the extinction cascade with the next links of Guam's food chains.

In these cases, sometimes two introduced predators might reach a curious balance, mitigating the overall effect of their niche exploitation. On the Pacific coast of Mexico, an island with this very story exists: Isla Isabel.

Isabel is a volcanic island that has important breeding colonies of blue-footed boobies (*Sula nebouxii*). These birds were predated mostly by two invasive species (feral cats, black rats (*Rattus rattus*)), and a native snake, the Atlantic Central American milk snake (*Lampropeltis polyzona*) (Ortega *et al.* 2021). Therefore, to protect the breeding colonies and for the sake of the rest of the island's biodiversity, they started to perform an eradication program for these invasive species. Thanks to this, they managed to extirpate cats and rats from the island, in that order. Once the cats were gone, there was an apparent reduction in the natural predation of snakes on eggs and chicks. However, after the removal of rats, it was noted that there was an increased number of snakes eating in the colonies. Hence, against all odds, the extirpation of these invasive species was not as good in practice as it was on paper (Ortega *et al.* 2021). These three predators reached a balance in the detrimental effect on the blue-footed boobies, apparently being more effective without cats in the picture. As shocking as it might sound, black rats do have a negative effect on snake populations. In fact, the elimination of rats on the Caribbean Island of Great Bird (Antigua and Barbuda), has helped to recover a threatened native species, the Antiguan racer snake (*Alsophis antiguae*) (Daltry 2006).

Invasiveness is not a quality only attributed to free-living species; parasites can become invasive as well. Those organisms carried by alien species go through the same selective process as their hosts, finding their own ways to endure in new ecosystems. If a parasitic species is successful in its ability to survive, it is considered co-introduced (Lymbery *et al.* 2014)**.** Then, if these living beings infect local hosts and successfully reproduce, they are considered co-invasive (Lymbery *et al.* 2014)**.** This phenomenon is known as "spillover". Therefore, those survivor parasites with indirect life cycles are here considered co-invasive. Whether or not they infect other definitive hosts, co-introduced parasites inherently must switch to local intermediate hosts. In contrast, it is considered a "spillback" in cases where an exotic species gets infected with local parasites (Lymbery *et al.* 2014)**.** From an evolutionary perspective, parasites that switch hosts, have not co-evolved with their new ones; therefore, they can be more pathogenic and virulent with higher prevalence and parasitic loads than their autochthonous counterparts (Lymbery *et al.* 2014).

In certain cases, parasitic species can be translocated to other localities without their original host and become invasive by themselves (e.g. planktonic forms of parasites in ballast water) (Lymbery *et al.* 2014). Furthermore, they can be transported in more convoluted ways, such as producing a spillback in the parasite's home range (e.g. trade of domestic animals or travelling with pets and zoonotic helminths), and then be transported with that host, to the host's prior home geographical location. This last method is believed to be responsible for introducing *Varroa destructor,* an important parasitic mite of bees (*Apis* spp.) native to Asia, to Europe through the trade of honeybees (*Apis mellifera*) (Anderson and Trueman 2000).

Each host by itself is a compendium of different ecosystems. Each parasitized organ has its own populations of each parasitic species (infrapopulation), which forms the whole community of different taxa living inside each host (infracommunity) (Bush *et al.* 1997). Hence, in the same way as their hosts, parasites can also compete for resources with their own kind and other parasites inside their host (dominance over other parasites (Bolnick *et al.* 2023)), affecting in some cases the growth of each other (crowding effect) (Roberts 2000).

In addition, in the same way that free-living species do, parasites can hybridise with close species, potentially driving native parasites to extinction. In this sense, a clear example of how a parasitic species can behave as a highly damaging invasive species is *Anguillicola crassus,* from the Japanese eel *(Anguilla japonica).*

The Japanese eel is an Asian fish very common in aquaculture, which was found to be more efficient (more resistant, faster growth, etc) than local European eels (*Anguilla anguilla*) (Heinsbroek 1991). However, with the use of these species, a species of swim bladder nematode, *Anguillicola crassus* was imported to Germany during the 1980s (Wielgoss *et al.* 2008). Shortly after, they discovered how this parasite had not only invaded European waters without its original host but was also more virulent to local eels, than to their natural host (Wurtz *et al.* 1996). Due to eel migrations and the continued importation of Japanese eels, nowadays *A. crassus* has spread to Africa and America (Dangel *et al.* 2015). Before this record, other eel and *Anguillicola* species were recorded in Lake Bracciano, in Rome, Italy. This is an almost round lake, in which locals introduced European and later New

Zealand eels (*Anguilla australis*) (Paggi *et al.* 1982). These latter fish also introduced their own species of swim bladder parasite, *Anguillicola novaezelandiae* which successfully established itself in European eels without any major disease or further spread to European waters. After the introduction of *A. crassus*, males of this parasite were recorded to be able to hybridise with females of *A. novaezelandiae* in this lake. This ended with the extirpation of this parasitic species from Lake Bracciano, with only *A. crassus* surviving (Dangel *et al.* 2015).

Therefore, the further consequences of translocated species are not the pure importation of an exotic pet or plant, but the transportation of a whole ecosystem, populated with more species (helminths, bacteria, fungi, virus, etc.) with the potential to be invasive and unleash extinction cascades, altering the dynamics of a geographical range.

2.2 Historic and current invasive species in the Canary Islands.

As with most oceanic islands, unaltered ecosystems suffer significant damage once any sort of disturbance is added, such as humans and other mammalian invasive species. The Canary Islands are no exception, with numerous records of extinct species likely due to the direct influence of either Canarian aboriginals or Spanish conquerors (de Nascimento *et al.* 2020).

It is not fully understood the exact time of arrival and the pattern that was followed by the first humans in Canary Islands, the only certain fact is that they were supposedly absent in the first century, and the oldest human archaeological remains are dated around the years 246 - 538 of the common era (CE) (Glas 1767; Hernández-Marrero et al. 2016). However, during the first century, signs of civilization, including demolished buildings, were discovered. Charcoal analysis suggests a change in fire regime 330 years before the common era (2280 calibrated years before present), which could imply that the Canary Islands were inhabited by an unknown culture long before the aboriginals documented between the XIV and XV centuries (Glas 1767; Ravazzi et al. 2021).

Aboriginal people from the Canary Islands are thought to have originated from Berber populations in central and western North Africa, who could have colonized new territory by boat (Fregel *et al.* 2019). They brought domestic animals (goats, sheep and pigs) and crops as their main sources of food, together with dogs. In addition to these animals, they potentially introduced the house mouse (*Mus musculus*) (Figure 7) to La Palma, Tenerife, Fuerteventura, and Lanzarote, according to ^{14}C data (de Nascimento *et al.* 2020) constituting the first registered invasive species in the Canary Islands.

Pollen and charcoal analysis suggest that aboriginals likely contributed to the extinction of an unknown number of botanical species. Pre-colonization pollens show different configurations of flora in the same areas where they were analysed, with a reduced number of certain pollens, while increasing the amount of charcoal. Thus, it is believed that aboriginals caused an increase in the fire regimes of the islands due to cultural customs, which likely led to the extinction of an unknown number of as yet undocumented vegetal specimens and contributed to the low abundance of certain fragile endemic flora. It is also presumed to have caused local exhaustion of wood resources, justified by changes over time in the types of charcoal from archaeological places, with plants located in further locations (de Nascimento *et al.* 2020)

The civilisation's diet consisted mostly of marine fauna, especially gastropods and fish, with records of crabs and sea urchins, as well as their domestic animals and crops, game, and gathering. Despite the fact that game is not proven to have been a huge percentage of aboriginal food items, it is believed that centuries of indiscriminate hunting and later invasive species led to the extinction of more than a dozen species. Documented wild animal remains in archaeological places with signs of butchery or human consumption are: lizards of the genus *Gallotia* (two of them, *G. goliath* and *G. simonyi auritae,* are extinct), lava and Scopoli's shearwaters (*Puffinus olsoni* (extinct) and *Calonectris borealis),* Canary Islands quail (*Coturnix gomerae,* extinct), extant birds such as the common buzzard (*Buteo buteo*), common kestrel (*Falco tinnunculus*), pigeon species (*Columba* spp.), common raven (*Corvus corax*) and the monk seals (*Monachus monachus* extirpated from the archipelago). The giant rat species (genus *Canariomys,* extinct) have been found in these places; however, there is no direct evidence pointing to their active hunting or consumption.

The Spanish conquest of the Canary Islands started in 1402 and ended in 1496 with the defeat of Tenerife after the decisive second battle of Acentejo. During this period, other important invasive species were introduced by ship: domestic cats and black rats (*Rattus rattus*) (Figure 7). A record of black rat remains in Lanzarote dated around 580-650 CE exists; however, most of the current populations of black rats in the Canary Islands were introduced during the XV century, with the introduction of the brown rat (*Rattus norvegicus*) (Figure 7) more recently in the XIX century (Nogales *et al.* 2006; Atoche Peña 2009; Rando *et al.* 2012; López *et al.* 2013). With this historic event came not only aboriginal demise but the extinction and extirpation of many endemic species.

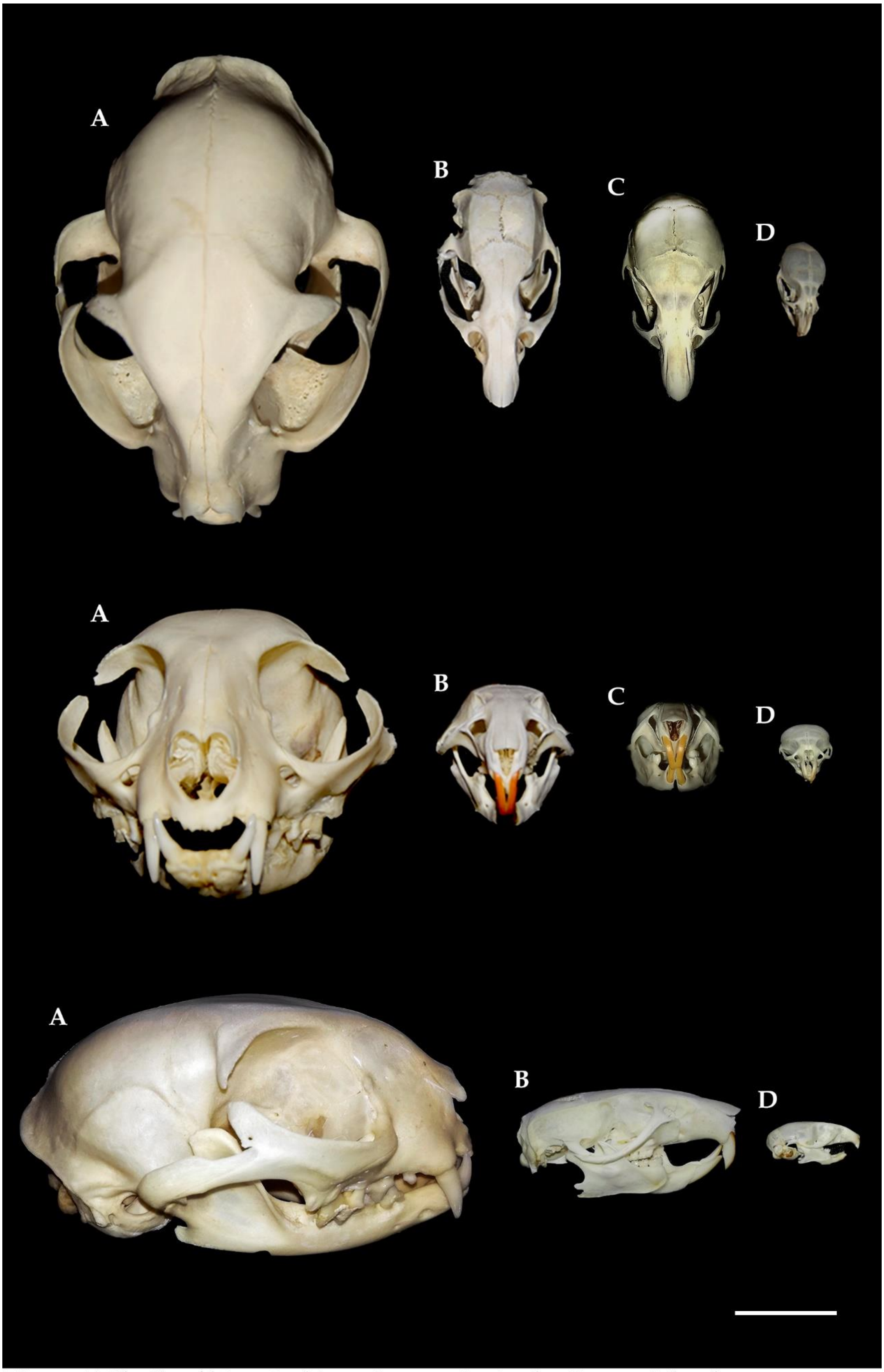


Figure 7: Skulls of historical invasive species in the Canary Islands: (**A**) domestic cat (*Felis catus*), (**B**) brown rat (*Rattus norvegicus*), (**C**) black rat (*Rattus rattus*) and (**D**) house mouse (*Mus musculus).* Scale bar = 2cm.

Dogs, pigs, goats, and sheep introduced by the locals are believed to have started the ecological pressure on endemic species, especially botanical specimens in the case of livestock since they fed on natural pastures instead of cultivated crops. In addition to dogs hunting for fun or food, local endemic vertebrates or pigs, and cultural fires damaging soil invertebrates. However, cats and black rats are a documented cause of active biodiversity loss, and extinctions, whose shadow has lasted centuries in the Canary Islands, and are still contributing to the inevitable decline of lots of endemics. Globally, invasive rodents and feral cats are directly linked to the extinction of 75 and 63 species, as well as the decline of 430 and 420 species, respectively (Doherty *et al.* 2016).

To the date of this thesis, there are 2,126 records of exotic species in the Bank of Data on Biodiversity of the Canary Islands (BIOTA) of which 241 species are considered invasive and another 96 are potentially invasive. In the case of vertebrates, 39 out of the 67 introduced species are considered invaders. Within the introduced species, BIOTA considers 91 species of parasites and 10 species of flies with the potential to cause myiasis as introduced species. There are no records of parasites of vertebrates catalogued as invasive species in BIOTA.

2.3 The strong prevail.

In reptile parasitology, there is a tendency to believe in diet-based host specificity rather than phylogenetic specificity (Roca 1999; Roca *et al.* 2005). This fact is not absolute, which could likely be linked to the poor immune system among squamates. However, reptilian oxyurids do present a clear diet-based pattern, with only a few genera found exclusively in reptiles with herbivorous habits and an undeniable predilection for tortoises.

In the Canary Islands, a discrepancy exists between the evolution of reptilian fauna and its parasitic communities. This fact was noted as well by other researchers (Zapatero *et al.* 1999) working on parasites in Gran Canaria giant lizards (*Gallotia stehlini*) which harbour massive parasitic loads (hundreds to thousands of specimens per caecum) of typical pinworms of herbivorous reptiles (*Tachygonetria* spp. and *Alaeuris* spp.) (Martín and Roca 2004). However, the genus *Gallotia* likely originated from ancestral lizards living in Northwestern Africa, a common ancestor to the Iberian lizards as well (Cox *et al.* 2010). These animals likely had an almost strict diet based on invertebrates; hence, they unlikely brought their current infracommunity in the process. Therefore, how have these vegetarian-specific parasites prevailed for millennia in ancestral insectivorous *Gallotia*? As co-invasive species.

There is fossil evidence for the existence of giant tortoises (*Centrochelys* spp. syn. *Geochelone* spp.), in the Canary Islands, likely evolving from north African species (Hutterer *et al.* 1997). There is no trace of a cause of extinction or pattern of colonization for these animals, but remains have been found in four of the seven main islands (Hutterer *et al.* 1997). Hence, the presence of tortoise-specific parasites, with thousands of these worms in almost every single Gran Canaria giant lizard, is a clear pattern of recent co-invasive nematodes that survived the extinction of their former host. These parasites can be found in other *Gallotia* species, in low ecological indices; however, there are gaps in the literature regarding the other giant lizards to draw further conclusions. Moreover, how pet tortoises kept in gardens could have introduced this parasite to the islands is another topic for future research.

Other pinworms in endemic reptiles from the Canary Islands are known to have switched hosts in geological times. *Spauligodon* spp., is believed to have invaded the islands in historic times, likely with the arrival of the ancestral skinks (currently *Chalcides* spp.) (Jorge *et al.* 2018). Along with their colonization pattern and coexistence, the ancestral *Spauligodon* lineages evolved into *S. atlanticus* in reptiles from the eastern islands (known hosts: *Gallotia atlantica* and *Tarentola angustimentalis*) and *S. occidentalis* in the western islands (*Gallotia* spp., *Chalcides* spp., *Tarentola delalandii*) (Jorge *et al.* 2018).

In this sense, historic invasive host introductions such as mice, cats, and rats, or the more recent California kingsnake, could have introduced their own parasites to the ecosystems of the Canary Islands with yet unknown consequences for local fauna. Conversely, during their introduction, house mice and rats coexisted with the endemic and extinct species of mice (on the westernmost islands) and giant rats, which could open the possibility of host switching between their parasitic species. Therefore, potential ancient endemic lineages of rodent parasites in the Canary Islands, such as *Echinonyssus molinae*, *Xenopsylla guancha* and *Leptopsylla algira atlantidis* (see Chapter 1) may have endured to the modern day.

Few rodent parasites can be found in their larval form infecting endemic invertebrates (Montoliu *et al.* 2013), with special interest in zoonotic nematodes such as *Angiostrongylus cantonensis* (Martin-Alonso *et al.* 2015). This last nematode species in addition to other metastrongilid nematodes from cats, dogs, and hedgehogs (*Atelerix algirus*) have been found infecting the endemic Gallot's lizard (*Gallotia galloti*) (Anettová *et al.* 2022; Izquierdo-Rodriguez *et al.* 2023). In the case of cats, their cestode species can be found as larvae in endemic reptiles (*Tarentola* spp.) and small mammals (Roca *et al.* 1987; Sanchez-Vicente 2013). This is just a brief example, with a few of the 67 introduced hosts, on how complex the ecology of introduced hosts, their parasites, and their potential to become invasive is.

There were no data regarding the parasitic species of California kingsnake in Gran Canaria prior to this thesis. Therefore, it is necessary to study not only its biology as an invasive species in Gran Canaria but also its potential role as a "Trojan horse" to introduce further co-invasive species to the environment. These could include parasites that not only pose a threat to the precious endemic species of the island but also have zoonotic potential, as in the case of Pentastomida.

An example of how a snake can successfully introduce a new co-invasive species is the case of Burmese pythons (*Python bivittatus*) in southern Florida. This snake was introduced either actively or passively around the start of the new millennium, establishing stable populations and spreading through the state. The presence of an old-world pentastomid (*Raillietiella orientalis*) in the pythons was originally confirmed in 2018 (Miller *et al.* 2018); however, in 2020, a survey of native snake species ratified its co-invasive nature. Thirteen species of snakes infected with this parasite were recorded, not only in the main invaded zone but also in allopatric locations (Miller *et al.* 2020). Pentastomids use vertebrates as intermediate hosts, causing granulomatous reactions in those infected organs such as the liver and lungs. Still, *in vivo* tests with *Raillietiella orientalis* showed no increased mortality compared to control lizards and insects during the time of the study (Palmisano *et al.* 2022). However, in natural conditions, with the long-term accumulation of nymphs in vital organs and other parasites and diseases, it would likely affect not only fitness but also the ability of these intermediate hosts to elude predators.

Chapter III:
California kingsnake: Taxonomy, life history and a tale of invasion

3.1 Taxonomy of the Genus *Lampropeltis.*

3.2 Taxonomy of *Lampropeltis californiae.*

3.3 Distribution and biology.

3.4 Invasion ecology.

3.5 Previous studies.

Kingsnakes (family Colubridae) are a group of ophidians classified under the genus *Lampropeltis*. Its name stands for "the royalty among snakes" or even reptiles, because of its predation of other squamates, snakes included. This is not simply a dietetic choice but an evolutionary adaptation, since they possess immunity to the venom of various species, such as rattlesnakes and cottonmouths (Weinstein *et al.* 1992). Grouped within the genus *Lampropeltis* are the milksnakes, which are mainly constituted of former subspecies of *Lampropeltis triangulum* (Ruane *et al.* 2014). They receive this name based on popular tales of snakes crawling at night to suck cows or women dry. These stories, although somewhat brutal or fantastic in nature, are widespread among cultures in different continents (Ermacora 2017). Interestingly, these were so integrated into culture during the 17th century that scientific experiments were performed exclusively to prove that snakes could not behave that way (Redi 1686).

In any case, these snakes have a similar trophic ecology to the rest of *Lampropeltis* feeding on other reptiles, eggs, mammals, or birds.

3.1 Taxonomy and life history: Genus *Lampropeltis*.

The genus *Lampropeltis* was established by Fitzinger in 1843, for the species with the most synonyms and subspecies: *Herpetodryas getulus*, currently *Lampropeltis getula*. The definition of the genus remained incomplete until other characters were taken into consideration to separate other Colubrida genera (mostly *Ophibolus* from *Coronella*), therefore establishing solid roots for further studies on king and milksnakes. This discussion will be mostly set around 1862 (Cope 1862).

Here is an adaptation of the description of the genus as synthesised by Blanchard (1921) in the review of its taxonomy:

"*Numerous color patterns (ringed, striped, or spotted in light to dark brown or black, yellow/cream to white, or red) can aid in identifying species groupings.*

Except in a few specialized or aberrant species where it is obviously broader at the temples, the head barely differs from the neck. Normal-sized eyes with a rounded pupil.

Dentition: palatines, 8 to 14, subequal; pterygoids, 12 to 23, slightly smaller than the palatines and gradually shrinking in size posteriorly; mandibular teeth, 12 to 18, decreasing in size posteriorly; maxillary teeth, 12 to 20, subequal, the last two occasionally slightly stouter and longer than the preceding, not separated from the latter by an interspace.

The body is often narrow, cylindrical above, with approximately uniform diameter, and the sides and belly meet at a visible angle. Only a few aberrant species have a longer tail than 15% of the whole length; the tail is small, swiftly tapering to a horny tip, and ranging from 0.09 to 0.18% of the total length.

The nostril is located between two nasals, and the cephalic plates, which include paired parietals, prefrontals, internasals, and a single frontal, are typical. The loreal is often present, along with one preocular, two postocular, and two, three, and four temporals in the first, second, and third rows, respectively. The lower labials are typically 8-10, with the fifth being the biggest; the upper labials are typically seven, occasionally ranging to eight.

Two parallel pairs of chin shields, the posterior of which are not always longer than the anterior and may only be half as long and separated by one or two small scales, follow the first lower labials as they come together on the median line behind the triangular mental plate at the lower jaw.

There are 17 to 27 longitudinal rows of dorsal scales on the body, each with two pits towards the posterior extremity and broader on the lowest row or two and narrower above. The ventrals are a single series of broad transverse plates on the abdomen that range in number from 152 to 254 depending on the genus. These are usually ended posteriorly by a single anal plate. The plates under the tail are generally in two rows, but some individuals have one or more of them; their numbers range from 27 to 79.

Penis rounded, bilobed, or forked at end; sulcus spermaticus single; calyces, continuous across the end of the organ or leaving there a small bare space, apical, few, with short processes which vary from 5 or 6 to 10 or 15-20, in the latter case passing into spines which increase in size gradually toward the base; no spines distinctively enlarged or separated from their fellows; basal portion of organ, below the large spines, smooth or with numerous minute spines."

Originally, the species of *Lampropeltis* were aggregated into three large groups based on colour patterns and penis characters (Blanchard 1921) which in brief could be summarised as: *Triangulum* group: those with red patterns; *Calligaster* group: spots with a dark border; *Getula* group: different from the other two groups. After decades, more snakes have clustered in other different groups, or even species complexes, in which the differentiation is almost only made by molecular methods. Thus, here (Table 3) are classified using both classical taxonomy (the three main groups of species) and current molecular classification of species complexes and clades within the previously established groups (in brackets, references to current molecular research in the taxonomy of that group).

Group	*subgroup*	*Species*
Triangulum (Ruane *et al.* 2014)	***Triangulum****	*L. abnorma*
		L. annulate
		L. elapsoides
		L. gentilis
		L. micropholis
		L. polizona
		L. triangulum
	Zonata (Myers *et al.* 2013).	*L. multifasciata*
		L. zonata
	Pyrromelana* (Burbrink *et al.* 2011)	*L. pyromelana*
		L. knoblochi
		L. webbi
	Mexicana (Bryson *et al.* 2007)	*L. mexicana*
		L. ruthveni
		L. alterna
Getula (Pyron and Burbrink 2009; Krysko *et al.* 2017)	***Getula****	*L. californiae*
		L. extenuata
		L. floridana
		L. getula
		L. holbrooki
		L. meansi
		L. nigra
		L. splendida
	No molecular data:	*L. catalinensis*
Calligaster (McKelvy and Burbrink 2017).	***Calligaster****	*L. calligaster*
		L. occipitolineata
		L. rhombomaculata

Table 3: Combined taxonomic groups and molecular complexes of *Lampropeltis.* *If no complex has been officially stated, is named after the group or the problematic species.

Despite considerable molecular research, the discussion seems far from over since *Lampropeltis* was separated again into three genera, *Lampropeltis*, *Ophibolus* and a new genus for *L. calligaster, Eksteinus,* in 2012 (Hoser 2012). Still, this classification was dismissed in 2013 (Kaiser *et al.* 2013). These movements are based on the paraphyly of the *calligaster* snakes, grouping in another branch with the genus *Cemophora* (Dahn *et al.* 2018).

3.2 Taxonomy and life history: *Lampropeltis californiae.*

The California kingsnake (*Lampropeltis californiae* Blainville 1835) has had a convoluted taxonomy over the years. Considering colour patterns (see figure 8) as valid taxonomic characters, it was believed to be (mainly) two separate species, one with longitudinal lines (*californiae*, stringed pattern) and one with the ringed or banded pattern (*boylii*). Until they were reproduced in terrariums, they realised that these patters were not of any taxonomical importance, fusing them together as a subspecies of *Lampropeltis getula* as *L. g. californiae* (Klauber 1936). However, *L. g. californiae* and its synonyms would change genera, species, and subspecies, until molecular taxonomy revealed it to be a full species (*L. californiae*), with another synonym, *L. g. nigrita* (Pyron and Burbrink 2009).

Originally, it was described as *Coluber* (*Ophis*) *californiae* in 1835; however, it would not be definitively transferred and broadly accepted to the genus *Lampropeltis* until the early 20th century.

Transcription of the original description of *Lampropeltis californiae* (Blainville 1836)**:**

"*Coluber (Ophis) californie.*

Corps de forme ordinaire et de médiocre longueur; tête assez grosse, déprimée á museau court et obtus; queue assez courte, conique et aiguë. Narines latérales, ovales, échancrant également les deux scutelles nasales et touchant la frontale extérieure. Yeux médiocres, latéraux. Scutelles céphaliques: 4 frontales, 1 loréale, 1-2 oculaires. Écailles assez petites lozangiques, imbriquées et parfaitement lisses. Couleur générale jaune, plus claire en dessous, plus foncée en dessus, avec six bandes longitudinales plus ou moins effacées, plus ou moins anastomosées, de couleur noire, les supérieures plus larges et souvent confondues. Longueur totale 0^{m}*.66, dont* 0^{m}*.095 pour la queue.*

Observ. Cette belle espèce de couleuvre véritable, appartenant à la division à laquelle Wagler donné le nom d'ophis, caractérisée par la forme en couteau, et la plus grande longueur des dents maxillaires postérieures, me semble ne pouvoir être rapprochée d'aucune des six espèces que les erpétologistes ont rapportées à cette section, ce qui nous déterminé la considérer comme nouvelle."

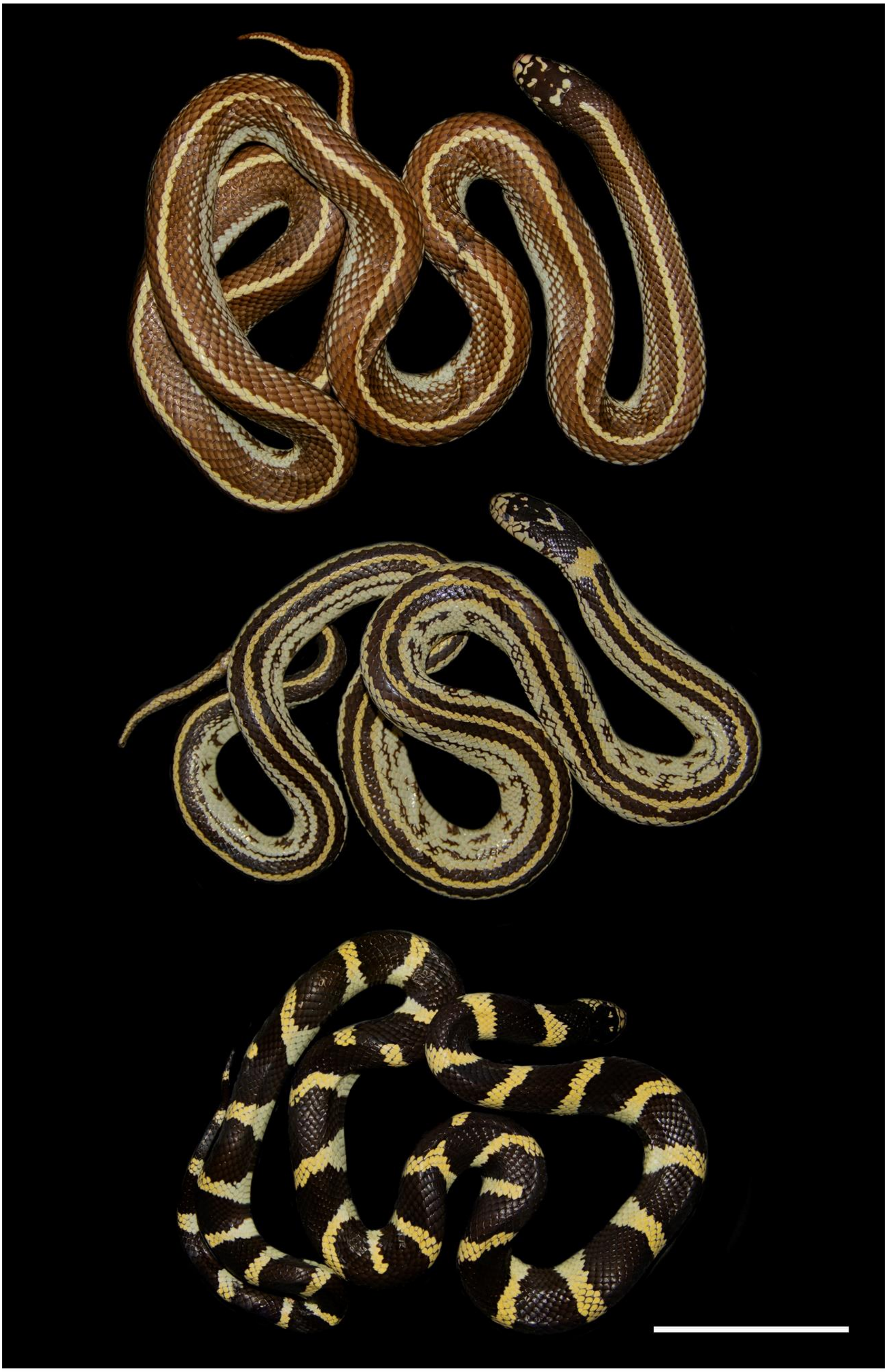


Figure 8: Colors and patterns of California Kingsnake. Light brown, linear pattern (above), dark brown, linear pattern (middle), black, banded pattern (below). Scale bar = 5cm.

Translation:

"Body of ordinary shape and of mediocre length; fairly large, depressed head with a short, obtuse muzzle; rather short, conical, and pointed tail. Lateral nostrils, oval, also indenting the two nasal scales and touching the external frontal. Mediocre, lateral eyes. Cephalic scales: 4 frontal, 1 loreal, 1-2 ocular. Scales are rather small, lozenge-like, imbricated, and perfectly smooth.

General colour yellow, lighter below, darker above, with six longitudinal bands more or less obliterated, more or less anastomosed, of black colour, the upper ones wider and often confused. Total length: 0.66m, tail 0.095m.

Remarks: This beautiful species of true snake, belonging to the division to which Wagler gave the name of *ophis*, characterized by the knife shape and the greatest length of the posterior maxillary teeth, seems to me not to be able to be compared with any of the six species that herpetologists have related to this section, which determined us to consider it as new."

Description extracted and adapted from Pyron and Burbrink (2009), based on several authors (Blanchard 1921; Blaney 1977; Hubbs 2009; Gallo-Barneto *et al.* 2016)**:**

The California Kingsnake (*L. californiae*) is a medium- to large-bodied constrictor that can grow up to 200 cm in length. The mean adult size range for this snake is 76–122 cm. The number of ventral scales in both sexes' ranges from 213-255, with males having 46–63 subcaudal scales and females having 44–57. Anal plate is solitary, with 23–25 rows of dorsal scales at midbody. Scales are smooth.

This snake can be distinguished from other *Lampropeltis* based on colour patterns; however, the combination of colours and line patterns produces more than 70 morphs, both natural and terrarium-selected, e.g., albino patterns (figures 9 and 10). These could be black or dark brown ground colour, with 21–44 broad crossbands of white or light yellow, which typically widen laterally. Others may exhibit the same dark colours with a dorsal longitudinal line from neck to tail. In addition, some Mexican populations are completely dark in colour, mostly black, without any cream-white line pattern.

A list of holding institutions and types of material can be found in appendix 4.

Figure 9: Colors and patterns of California kingsnake. Albino snakes, linear pattern (above), banded pattern (below). Scale bar = 5cm.

Figure 10: Examples of head patterns and colors of California kingsnakes. Normal (above) and albino (below). Scale bar=1cm.

3.3 Distribution and Biology.

The California kingsnake is widely distributed around the southwestern territories of the United States (Arizona, California, Colorado, Nevada, Oregon, and Utah) and Northwest Mexico (Baja California, Sonora, and some islands of the Cortés Sea), which makes this species one of the most widely distributed kingsnake in the Americas (Hubbs 2009; Gallo-Barneto *et al.* 2016; Gallo-Barneto and Mateo 2020). In addition, it has been documented as an invasive species in Gran Canaria, Spanish Macaronesia.

This snake is not environment-specific, inhabiting forests, rocky areas, deserts, meadows, coastal areas, lakes, peri-urban, and urban areas. Although it can be found from sea level up to 1800m, it lives mostly within the first 900m (Hubbs 2009; Gallo-Barneto *et al.* 2016; Gallo-Barneto and Mateo 2020)

It is considered a fossorial species, spending long periods of time in a burrow. Females tend to be more sedentary, living and hunting around their burrows. In contrast, males tend to move long distances, probably in search of food or a mate (Anguiano and Diffendorfer 2015; Gallo-Barneto *et al.* 2016).

Adults can grow from 90 to 120 centimetres long and live more than sixteen years (Hubbs 2009; Gallo-Barneto *et al.* 2016).

This snake can bear temperatures from 2-40°C, although the optimal range is 23-29°C and the minimum temperature for its normal activities is around 15.5°C (Gallo-Barneto *et al.* 2016). In Gran Canaria, climatological studies (Gallo-Barneto and Mateo 2020) have revealed that the snakes prefer going out from their burrows in the early or late hours of the day, when the temperature is around 19-20 °C.

They are opportunistic constrictors, they can feed on mostly everything that fits in their mouth, including their own kin (Jackson *et al.* 2004; Hubbs 2009; Gallo-Barneto and Mateo 2020) In their natural habitat, they have been reported predating on southern short-tailed shrews (*Blarina carolinensis),* rodents, and other small mammals in general. Further diet components are birds, including their eggs and chicks; lizards, turtles, and their eggs, specially *Chelydra serpentina* and *Trachemys scripta*. As an ophiophagous snake, it has been documented to hunt many species of snakes and their eggs, such as aquatic snakes (genera *Nerodia, Seminatrix,* and *Thamnophis*), copperheads (*Agkistrodon* spp.), rattlesnakes (*Crotalus* spp.) and many other miscellaneous snake genera such as *Coluber* and *Lampropeltis.* Finishing this long menu, other prey items reported for *Lampropeltis californiae* in its natural habitat are frogs, salamanders, and large invertebrates (Weldon and Schell 1984; Winne *et al.* 2007; Steen *et al.* 2010).

Their digestion is slow and temperature dependent. According to Jackson *et al.* (2004), most of the digestion occurs within the first 4 days and is complete within

15 days. They can consume about 25% of their body mass in prey every 12 days of the active season (Godley *et al.* 2017). They ambush their prey and kill them by suffocation, or even swallow them alive in the case of small animals (Gallo-Barneto and Mateo 2020).

Despite its fierce predator habits, it is a very calm snake, which makes it one of the easiest reptiles to handle and care for. Since the 80s the California kingsnake has been spread worldwide as one of the most popular pet reptiles. These snakes were captured mostly in San Diego County, California, and then bred in captivity (Hubbs 2009).

In their natural habitat, they are predated by numerous terrestrial carnivores and birds of prey (Wallach *et al.* 2014).

This is an oviparous snake that lays 6-29 eggs a year inside a nest (Hubbs 2009). These could also be communitarian nests, hatching eggs from several mothers. In some cases, these snakes may use rodent burrows as their own nests (O'Donnell 2004). They reach sexual maturity around 4-5 years old and are approximately 600 mm long excluding the tail.

Due to its non-specific habitat and prey, the absence of natural predators, important pathogens, fossorial life habits, and climate conditions like those of their native environment, the invasion of Gran Canaria has been successful (Gallo-Barneto *et al.* 2016).

3.4 Invasion ecology.

(If no reference is stated, it is information extracted from local press 2008-2018 or Gallo-Barneto *et al.* 2016; Gallo-Barneto and Mateo, 2020)

The first sightings were reported in the municipalities of Telde and Valsequillo, in the southeast of the island of Gran Canaria, in 1998. These remained anecdotal sightings by inhabitants of the two vicinities, however, they were increasing, with reports even in 2004 of snakes in higher municipalities (figure 11) connected by the same ravines (San Mateo and Santa Brígida) and hundreds of sightings in 2007. That year, the Department of the Environment and Territorial Organization of the Canary Island Government, in collaboration with the Cabildo of Gran Canaria, began to plan a program to control and eradicate this species. However, during 2005-2007, a total of 47 snakes were captured prior to the official eradication program, some of them in northern localities of the island.

During the subsequent years, the authorities started to realise how big the population was in the east of the island, and some snakes started to appear in more southern locations, such as Agüimes municipality. Using the geolocations of the snakes captured between 2008 (n=92) and 2009 (n=122) it was determined that the extension of this population was around 38 km^2, which represented 2.4%

of the total land area of the island. Three years later, new estimations showed that this percentage had grown to 4%. This population was named the **main nucleus (MN**).

The main nucleus at the time (2010) was comprised of four municipalities: Telde, Valsequillo, Santa Brígida, and San Mateo. This area is almost a triangular-shaped piece of land in the east of the island and is composed of most of the ecosystems and all climatic zones from coast to peaks, dry desert (DD), dry steppe (DS), temperate mild (TM) and temperate cold (TC) (see Chapter 1).

The most concentrated area is in the northwest zone of Telde, continuing with the eastern part of Valsequillo, a DS zone of mostly thermophile forest.

In this nucleus, a great number of snakes had albino patterns, which undoubtedly pointed to their pet-trade origin.

As soon as these campaigns started, it was clear that citizen collaboration would be critical for better control of this invasive species. Thanks to this, 2009 ended with 132 snakes, most of them captured by local residents.

In 2010, in addition to regular eradication campaigns, studies related to the biology of this species started, to better understand how to implement these campaigns. One of the first experiments consisted of radiotracking specimens with intracoelomic radio transmitters to investigate their movements. In the same year, a new nucleus was officially declared, the **secondary nucleus (SN)** in the north-west of the island (figure 12), in the natural monument of Amagro which belongs to the municipality of Gáldar. This nucleus is characterized mainly by DD zone with a small region of DS in the southeast. At first sight, these snakes appeared to be the product of a second introduction since albino patterns were not found among these specimens.

The study of stomach content, faeces, and regurgitated prey confirmed heavy predation of precious endemics, with the Gran Canaria giant lizard (*Gallotia stehlini*) comprising half of its prey items. The second most prominent prey varied from the Grancanarian skink (*Chalcides sexlineatus*) in the main nucleus to small mammals in the secondary nucleus. No differences have been reported in the predation on the Boettger's wall gecko (*Tarentola boettgeri boettgeri*), but it is the least common prey species in the two studied nuclei followed by small unidentified birds (Monzón-Argüello *et al.* 2015). Unfortunately, these snakes live in burrows where most of the digestion is performed. Thus, during these 4 to 15 days, they cannot be captured by hand, which enhances the crucial role of efficient trapping methods.

Three years passed since the beginning of the eradication plan, and no evident progress was noticed. Therefore, in 2010, an application for a LIFE+ (European Union) project was submitted. This was granted in September 2011 with the title

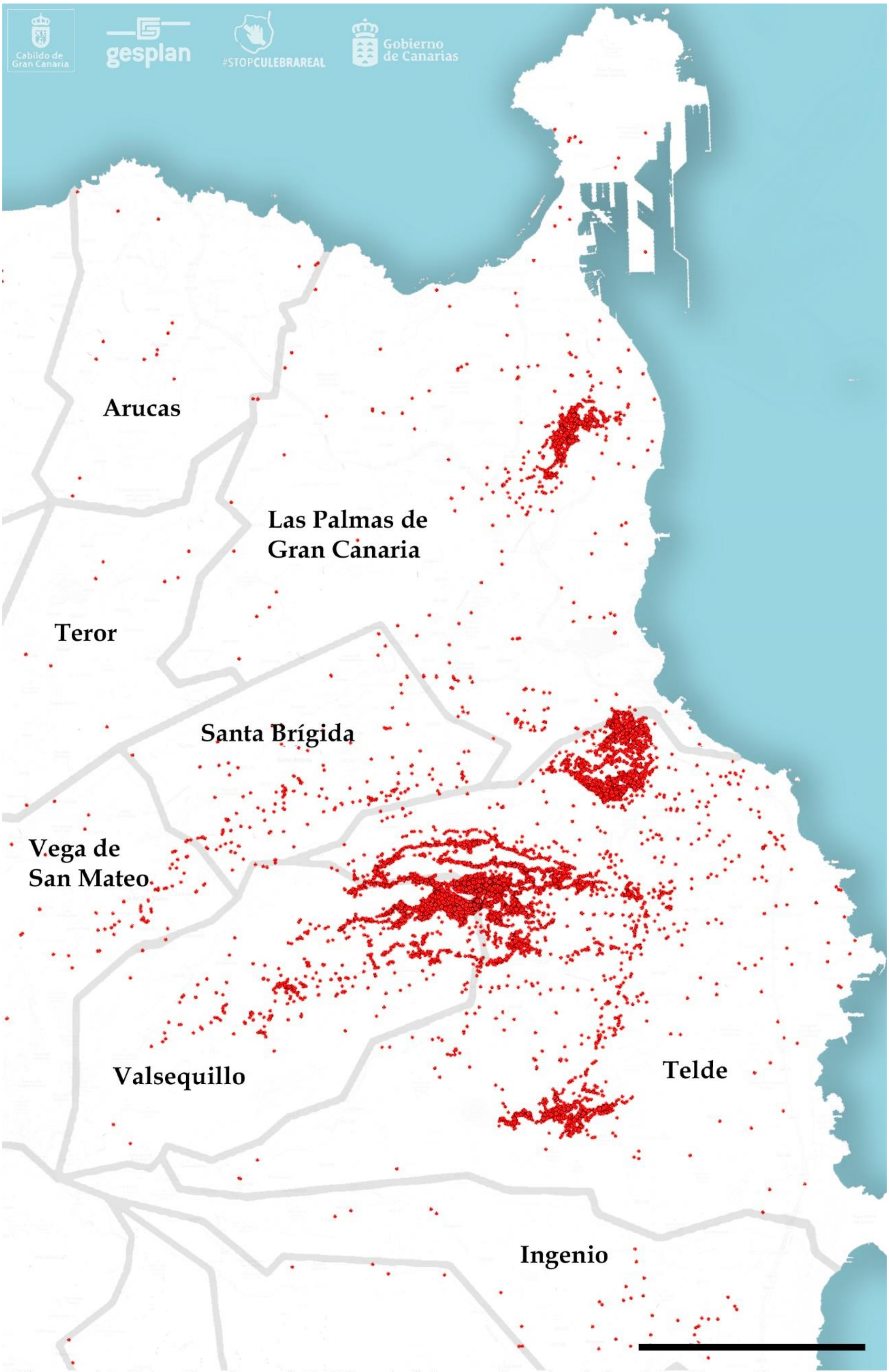


Figure 11: Distribution of California kingsnakes (red dots) in Gran Canaria, North-eastern nuclei. The fourth (Las Palmas de Gran Canaria) and the main nuclei (mostly Telde and Valsequillo). Scale bar = 5km.

“Control de la especie invasora *Lampropeltis getula californiae* en la isla de Gran Canaria - LIFE 10NAT/ES/000565” and was funded by different entities. The

European Union contributed 50% of the total of 1.025.863€, the Government of the Canary Islands with 27,29%, the Council of Gran Canaria with 20,79% and the public company GesPlan S.A. with 1,95%.

The main objective of this new program was to eradicate this species or at least reduce its population density to minimize its ecological impact on local endemic species such as the Gran Canaria giant lizard (*Gallotia stehlini*) or the Grancanarian skink (*Chalcides sexlineatus*), limiting its distribution to just the main nucleus. In addition, to create an international network of people involved in the fight against exotic or invasive species an Early Alert System would be added, which would allow citizens to better help capture tasks.

With the help of this monetary injection into the eradication program, they were able to use and test different trapping options and methods, optimize capture campaigns by studying their biology, install climatological stations to monitor local parameters and capture success, increase the number of publicization events to inform the general public, activate the Early Alert System, and fund further molecular research to estimate the effective population size and genetic variability.

Thanks to this project, in November 2011, the catalogue of invasive exotic species (Royal Decree 1628/2011) prohibited the keeping, transportation, and/or commercial transactions of living or dead individuals of all Colubridae on the Canary Islands, Ibiza, and Formentera (Balearic Islands).

Indeed, this resulted in an almost instant success, practically doubling the number of captured snakes from 2010 (n=216) to the end of 2011 (n=581). In the next few years, with few exceptions, every year more snakes will be captured until the time of this thesis (accessed in 2022).

Trapping strategies were diverse and meant a journey of testing and learning different strategies. At first and until today, the most common method has been direct catch campaigns in the field, in which many people volunteer. Then they started to use wooden planks (60x120x1.5 cm) according to the bibliography. These simulate shelter for the snakes, so the staff have to just look under them and catch the snakes by hand. Interestingly, these are particularly used by the snakes after rainy and/or cloudy days, presumably as a source of retained heat/shelter. Although it is the cheapest trap to manufacture, it still requires a lot of time in the countryside, so further strategies were implemented.

Next, double funnel traps were employed, which are pipes with a funnel at each tip, together with bait or a “diversion wall” to direct the snakes to the trap. Strange as it sounds, when these snakes tend to follow the direction of the walls,

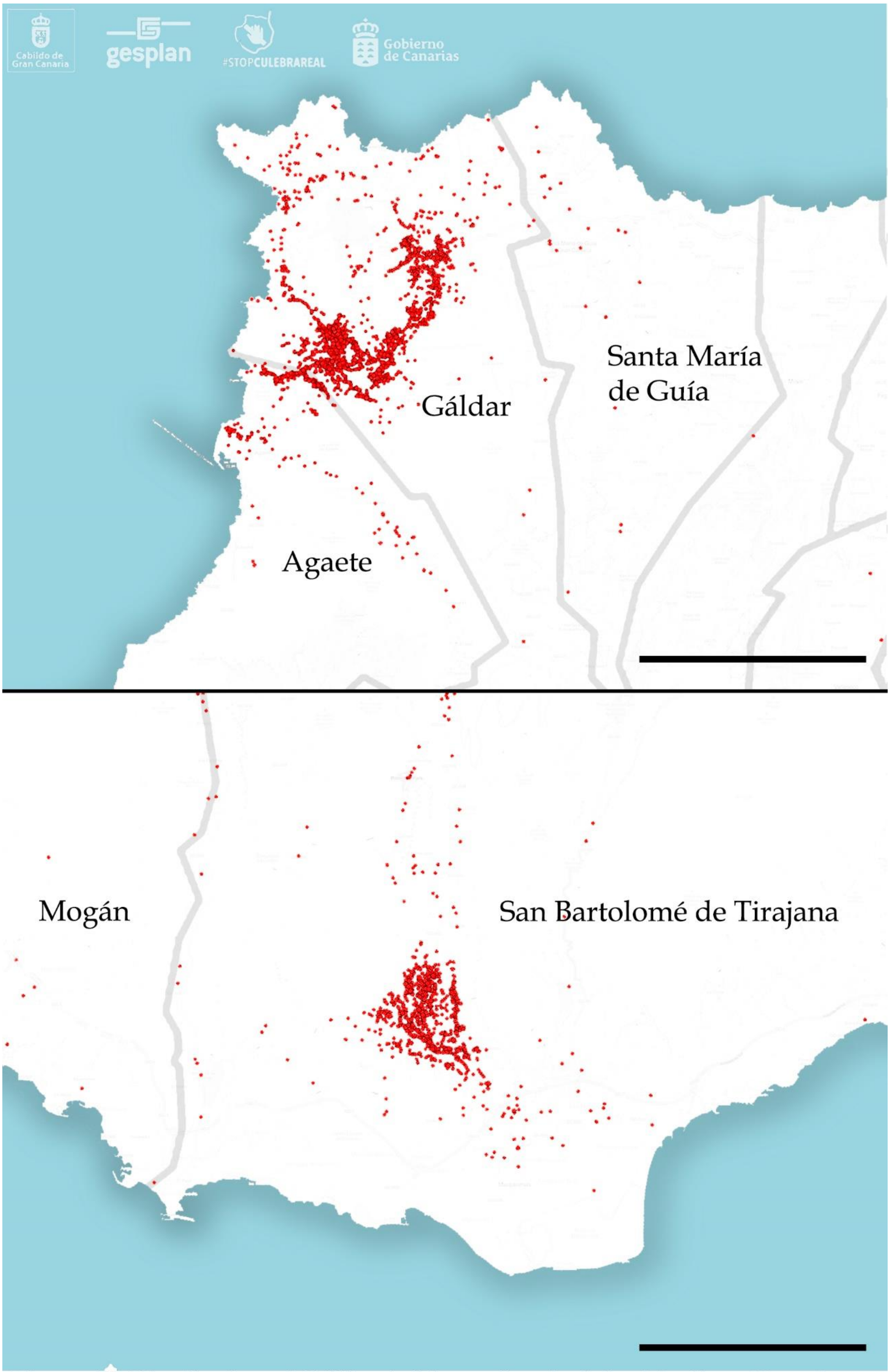


Figure 12: Distribution of California kingsnakes (red dots) in Gran Canaria, **A**: Secondary nucleus (north-west)(Guía, Gáldar and Agaete). **B**: Third nucleus (south)(San Bartolomé de Tirajana). Scale bars = 5km.

it is used as an advantage to put traps in the field. However, this trap was not as effective as plank traps.

Finally, the third type of trap was the box trap, with 4 funnels associated with diversion walls. This last trap had better results than the others and required less field work, but it was more expensive to manufacture.

In addition, a few attempts were made to train hawks and dogs to capture the snakes. They resulted in being too time-consuming with less success than trapping. The problem with training dogs letting them smell relaxed snakes since these animals secrete stinky chemicals though scent glands in the cloaca. Therefore, trained dogs may be searching for stressed snakes instead of snakes in general.

During LIFE +, with the huge help of the general public, around 260 workshops and talks were organised, the Early Alert System was started, centralizing the calls to the emergency telephone number and CECOPIN numbers, and a new mobile app called 'Lampropeltis' was created.

Thanks to the use of the *Lampropeltis* app, by collecting specimens during 2009-2011 from the main and secondary nuclei, the hypothesis and mystery of colour patterns were finally resolved. These two populations corresponded to two different introductions of individuals, which suggests that the secondary nucleus did not originate from the main one. In contrast, these data also suggest that the main nucleus is also composed of two different introductions of snake groups, with probably two release/escape events (Monzón-Argüello *et al.* 2015) .

In 2013, measurement of the population density of Gran Canaria giant lizards in the main nucleus was begun in order to evaluate further changes due to the predatory pressure of the snake.

Despite all these efforts made possible by LIFE+, its territory was inevitably expanding. Such was the increase, that in 2015 a third nucleus, the tertiary nucleus (TN) was declared in the south of the island (figure 12) around La Data Mountain occupying around 4 km^2. This and the other two nuclei are situated in zones of special conservation, according to the Official Bulletin of the Canary Islands number 60 from 15th May 2000.

This nucleus is characterized as a dry desert zone to the south of San Bartolomé de Tirajana, around La Data mountain. Currently (2022) few captures have been made in higher locations in this same municipality, which are characterised by DS and TM zones with thermophilic and pine tree forests, respectively.

In 2015, a second campaign for the Gran Canaria giant lizard was made to establish some baseline data on the predation of this endemic species by the snake (Gesplan 2013, 2015; Gallo-Barneto *et al.* 2016)**.** It showed a clear decrease in population density of *Gallotia* in the locations where the snakes were actively captured. In addition, their predation seems to be especially concentrated on small and younger lizards, with a higher percentage of adults surviving than juveniles. This could be explained by the huge strength of these animals, which are potentially capable of fighting back the snakes. Throughout this period, many snakes were found with scars and wounds, probably as a result of interactions with domestic animals and potentially full-grown *Gallotia* males. These data suggest that the *Gallotia* population is at risk and could become extinct in areas where snakes inhabit. Consequently, there may also be an indirect impact on flora, since these lizards act as seed spreaders (Olesen and Valido 2003; Valido *et al.* 2003).

After all these years of campaigns, it was possible to establish the seasonality and full life cycle of this snake: With the arrival of spring, around February, the emergence period begins. Mature, male snakes (3-4 years old) leave their burrows and begin to look for food or forage, as well as for females, usually travelling long distances. During this period, the captures reach their peak, with most of them being male snakes. After the copulation period (March-June), females begin to develop the eggs, and then, 45-65 days later (April to August), they lay an average of 16 eggs per nest. The small ophidians begin to hatch from July to October, with the brumation period beginning within the next few months.

The Life+ project ended in 2015, after it received further funding as a post-life project to continue the fight. However, three years later, despite all these efforts by Gesplan and local people, captures had increased and snakes appeared, and still appear, in new parts of the island. It is unknown whether these animals are translocated by man or by population dispersion, probably due to male copulating efforts. To this day (2018), a total of 5,126 California kingsnakes had been captured in Gran Canaria, even around the capital city. The story seems to continue, with a **fourth nucleus** in Las Palmas de Gran Canaria (figure 11), around the Guiniguada ravine. This and the latter nucleus are believed to have originated from wandering individuals of the main nucleus. This last nucleus is situated in a DD zone.

To the date of this thesis (2022), 628 traps are active, and a total of 14,381 snakes have been captured since the beginning of this story with, worryingly, one from Fuerteventura and another from Lanzarote (2017).

A more recent evaluation of endemic reptilian fauna in the invaded areas, compared to non-invaded zones, revealed a decrease in the population of Gran Canaria giant lizard, Grancanarian skink, and Boettger's wall gecko by 90%, 80%, and 50%, respectively (Piquet *et al.* 2021). These surviving populations (except for the Gran Canaria giant lizard) present significant biometrical differences with those in non-invaded municipalities, which could mean an intrinsic phenotypical adaptation or selection pressure on those characteristics (Piquet *et al.* 2022).

The analysis of biological parameters during all the years of the eradication demonstrated that sadly, the invasion has been a total success. All snakes present an optimal body condition (constant amount of accumulated fat all year round) and production of eggs, as good or higher than natural US populations (Fisher *et al.* 2019, 2021). These factors, as well as the absence of natural predators, suggest that California kingsnakes have found a paradise in Gran Canaria.

3.5 Previous parasitological studies on California kingsnakes.

Previous studies on this host and other species of Lampropeltis.

Despite its popularity as a pet snake or even as a zoo animal, there are few parasitological studies in this species or genus. Most of the reports that do exist are of parasites in small numbers of animals, or studies with larger samples as a result of joining small samples from numerous different snake species. Publications, mainly by Chris T. McAllister and collaborators, provide lists of hosts and parasites to help unify the knowledge of the parasitological fauna of American reptiles (McAllister *et al.* 1995, 2008, 2010, 2013, 2015, 2017; McAllister and Bursey 2008, 2012).

Seventy six references, exclusively citing parasites in snakes of the genus *Lampropeltis* (appendix 5) and taking into consideration different host species and different localities if the host was cited twice, were identified. There were reports of parasites in only 10 out of the 27 species of this genus. Out of these, most references belonged to *L. holbrooki* (23/76; 30.3%), *L. getula* (13/76; 17.1%), *L. calligaster* and *L. triangulum* (10/73; 13.2% each). This means that the first 3 out of 10 studied species hold more than 50% of all scientific work on the parasitic diversity of this genus.

The recorded parasites belonged to three kingdoms: Animalia (56.6%), Chromista (35.5%), and Protozoa (7.9%). The most diverse group was the kingdom animalia, with parasites from six groups (Nematoda 19/76, Trematoda 9/76, Acari 7/76, Cestode 4/76, Pentastomida 3/76, and Acanthocephala 1/76). Most descriptions were related to adult parasites (61/76; 80.2%) while others refered to parasites that use the snake as an intermediate or paratenic host (14/76;18.4%). In one case, it was unclear if the larval parasites were immature stages of the snake's *Physaloptera* spp., or if the host was acting as a transport host for other animals.

The actual location of the parasite within the host was not provided in all cases (20/76 missing data); therefore, these were inferred considering the likely infection site according to the biology of the genus and/or species. Most of the referenced parasites inhabit the digestive system (47/76; 61.8% of the references), followed by pulmonary parasites (10/76; 13.2%) and hematic parasites (9/76; 11.8%). The most unusual locations for parasites, recorded only in 1 each out of the 76 research papers, were: pleroceroids of *Spirometra mansonoides* (larval cestodes) in the subcutis of *L. holbrooki*, adult *Styphlodora horrida* (Trematoda) in the urinary tract of *L. polyzona*) and adult *Macdonaldius oschei* (Nematoda) in the mesenteric arteries of a *Lampropeltis* sp.

There were a total of 31 parasitic genera and 48 species within the 76 reports. These were composed of 21 genera and 31 species in the kingdoms Animalia, 6 gen., 12spp Chromista and 4 gen., 5spp. in Protozoa. Within these taxa, the most diverse were the phylums Nematoda (Animalia) with 7 gen. and 12spp and Miozoa (only reported phylum Chromista) composed of 6 gen. and 12 spp. The genus with the most species was the nematode *Kalicephalus* with 5 species described in *Lampropeltis* spp. followed by *Entophionyssus* (Arthropoda: Arachnida) and *Caryospora* (Miozoa: Apicomplexa) with 3 species each. However, in terms of references, the most reported parasites are the genera *Kalicephalus* and *Caryospora* with 8/76 citations each, followed by *Ochetosoma* (Platyhelminthes: Trematoda) and *Eimeria* (Miozoa: Apicomplexa) with 5/76 each.

For California kingsnakes, there were just two reports prior to this thesis (Van Peenen and Birdwell 1968; Xiao *et al.* 2004) in which they described 4 protozoan species (*Isospora crotali, Criptosporidium serpentis, Eimeria* sp. and *Haemogregarina* sp.) in less than 11 California kingsnakes in total. In addition, the presence of ticks (Likely genus *Hyalomma*) was described in the official reports of eradication projects (www.lifelampropeltis.com)

The only larval forms previously described in *Lampropeltis* snakes are the cystacanths of *Macracanthorhynchus ingens* in the coelomic cavity of *Lampropeltis getulus* from Louisiana (Elkins and Nickol 1983), subcutaneous plerocercoids of *Spirometra mansonoides* in a *L. holbrooki* from Louisiana (Corkum 1966), intracoelomic tetrathyridia of *Mesocestoides* sp. in *L. getula* and *L. pyromelana* (Goldberg and Bursey 2004; Jacobson 2007) and free third instar larvae of *Physaloptera* sp. in the stomach of *L. pyromelana* from Arizona (Goldberg *et al.* 2007).

These two reports on parasitic species in California kingsnakes (Van Peenen and Birdwell 1968; Xiao *et al.* 2004), are wholly inadequate to assess the parasitic biodiversity harboured by this snake species, with just one parasite described to full species (*C. serpentis*), one just to genus, one just to an incorrect generic name (*Haemogregarina* sp.), and the remainder, *I. crotali,* being reassigned to the genus *Sarcocystis* without retaining the specific epithet. Therefore, the taxonomy and general life history of these parasites are provided below, along with lists of potential species gathered from other snakes of the genus *Lampropeltis.*

Parasitic species described in Lampropeltis californiae.

Considering the huge overlap that exists between most species of unicellular parasites, it is now mandatory to utilize molecular methods for a more accurate diagnosis. In the case of the parasitic species previously described in the California kingsnake (*Eimeria* sp., *Sarcocystis* sp. (syn. *Isospora crotali*), *Haemogregarina* sp. and *Cryptosporidium serpentis*), their whole group of classic protozoans with veterinary importance (Apicomplexa) has been moved to the kingdom Chromista, unicellular organisms that resemble protozoa, algae, or fungi (Ruggiero *et al.* 2015; Cavalier-Smith 2018).

The current (reviewed in 2022) high classification of these parasites is: Kingdom **Chromista** Subkigndom **Harosa** Infrakingdom **Halvaria** Superphylum **Alveolata** Phylum **Miozoa** Subphylum **Myzozoa** Infraphylum **Apicomplexa** Superclass **Sporozoa**

Class **Coccidiomorphea**

Subclass **Coccidia**

Order **Eimeriida**

Family **Eimeriidae**

Eimeria sp.

The genus is a vast compendium of 2000 apicomplexans, in which few attempts at reclassification have been made. The actual taxonomic positioning of these parasites has been debated for generations, particularly the potential division of those species of *Eimeria* infecting reptiles (Megía-Palma *et al.* 2015). In the case of some reptilian *Eimeria* spp. two distinct genera are currently recognised: *Choleoeimeria* (ellipsoidal to elongated, mostly biliary parasites) and *Acroeimeria* (oval-roundish and intestinal) (Megía-Palma *et al.* 2015). This division is supported by molecular methods; however, further sampling is required to accurately classify all reptilian *Eimeria.*

Despite the number of *Eimeria*-like parasites described in snakes, their biology is partly understood only by a few, i.e. *Eimeria cascabeli* of rattlesnakes (*Crotalus* spp.) from California and Colorado (Vetterling and Widmer 1968) or *Choleoeimeria ghaffari* of Arabian sand boa (*Eryx jayakari*) from Saudi Arabia (Abdel-Baki *et al.* 2014).

These parasites have a direct life cycles: in the case of snakes, the oocysts can sporulate within the gallbladder and intestine, becoming infectious right after being expelled within the faeces (Vetterling and Widmer 1968; Abdel-Baki *et al.* 2014). The sporocysts and sporozoites are released within the gastrointestinal tract either by digestion or active movement of all the sporozoites to finally infect their target cells, which in the case of snake *Eimeria* spp. is the simple columnar epithelium of the gall bladder and extra-hepatic bile ducts. The first stage of asexual reproduction occurs with the production of meronts (at least two generations in *E. cascabeli* (Vetterling and Widmer 1968) to finally transform into feminine and masculine sexual gamonts, fecundate, form, and release the oocysts.

Adapted description from Van Peenen and Birdwell (1968) of *Eimeria* sp. in *Lampropeltis californiae*:

"Measures based on 50 oocysts the values are expressed in means followed by the ranges in brackets. Oocysts were ellipsoidal, with a single smooth, colourless wall and a yellowish membrane-like inner lining about 1 µm. Micropyle or oocyst residuum is absent, with a clear round polar granule about 3-4 µm in diameter. Oocysts measured 38.4 (35.5-40.8) x 20.9 (19.8-22.4) µm: length-width ratios ranged from 1.85 (1.69-2.24).

Sporocysts were ovoid to circular, measuring 11.8 x 10.3 (10.5-13.2 x 9.2-10.5) µm. A sporocyst residuum consisting of many clear refractile balls of different sizes was centrally clumped and measured approximately 4 µm in diameter. Stieda body is absent. Sporozoites, comma-shaped and averaged 10 µm in length; they appeared slightly folded over at the thick end, which measured up to 5 µm in width. Gametogony and schizogony are unknown."

Location: unknown, found free in faeces, likely gall bladder.

Locality: San Diego, California.

Host: *Lampropeltis californiae.*

Potential species:

Eimeria lampropeltis

Location: Unknown, described from faeces.

Locality: USA.

Host: *L. calligaster.*

Eimeria zamenis

Location: Gallbladder.

Locality: USA.

Host: *L. calligaster*, *L. holbrooki* and *L. triangulum.*

The description of both coccidians can be found in Anderson et al. (1968)

According to morphological and biological criteria, these two species could be assigned to the genus *Choeoeimeria,* which makes *E. lampropeltis* a probable biliary parasite.

Differentiation between these two coccidia can be accomplished using the oocyst shape index (1.53-2.23 *E. zamenis;* 1.69-3.53 for *E. lampropeltis*). Nevertheless, the most remarkable feature is the shape of the sporocysts of these two species, which are round in *E. zamenis* and fusiform in *E. lampropeltis.* The shape of sporocysts in *E. lampropeltis* could be misinterpreted as free sporozoites inside the oocyst, since these two stages of oocyst maturation have been demonstrated in other biliary coccidia such as *Choleoeimeria ghaffari* (Vetterling and Widmer 1968; Abdel-Baki *et al.* 2014).

Remarks:

Considering the shape of the oocysts, and the provenance of most snake *Eimeria* spp., it is likely to be another biliary coccidia of the genus *Choleoeimeria.* These morphological descriptions match almost perfectly the description of *Eimeria zamenis* produced by Anderson *et al.* (1968). The differences between both are the presence of polar granules, bigger oocysts, and thicker comma-shaped sporozoites in *Eimeria* sp. from *L. californiae,* whereas they are absent, smaller (28-33x14-19µm), and thinner (8-11x2 µm), respectively, in *Eimeria zamenis* from two *Lampropeltis* species. However, biometrical parameters seem to change between hosts in this coccidia, so further molecular sampling is necessary to clarify the taxonomical positioning of these species, whether both descriptions refer to the same parasite or a complex of a few species.

Family **Sarcocystidae**

***Sarcocystis* sp.**

Syn *Isospora crotali*

Since these parasites have two sporocysts, as *Isospora*-like parasites, more than half a dozen *Sarcocystis* species in snakes were initially described as genus *Isospora*, such as *Isospora crotali* in *L. californiae* (Upton *et al.* 1992). They were separated later, mainly due to the characteristics of the oocyst wall and its biology.

Sarcocystis is a rather thin-walled coccidia with an obligate indirect life cycle that involves a predator-prey trophic relationship. In snakes, most *Sarcocystis* species are related to a rodent-snake type of cycle; however, other reptiles and even primates, including humans, can be involved in the biology of *Sarcocystis* spp. infecting snakes, e.g. *S. stenodactylicolubris* (Modrý *et al.* 2000) or *S. nesbitii* (Mohd-Fadil *et al.* 2019).

The definitive host expelled sporulated oocysts or even released sporocysts to the environment with the faeces (Modrý *et al.* 2000; Mohd-Fadil *et al.* 2019). Infection of an intermediate host, depending on the parasitic species, then occurs. The sporozoites are released and travel via blood vessels to their target muscle bundles, where asexual reproduction takes place, forming the sarcocysts. Once the definitive host eats and digests the prey with the sarcocysts, the released protozoans infect the intestinal epithelium. As in other coccidia, they pass through a first stage of asexual reproduction, forming meronts, to then differentiate into feminine and masculine gamonts (Modrý *et al.* 2000; Mohd-Fadil *et al.* 2019). Instead of releasing the recently formed zygote or oocyst, it is transported to the lamina propria, through an unknown mechanism, where sporulation takes place. Finally, these sporulated oocysts leave the intestinal tissue, and the cycle continues. According to **Entzeroth *et al.*** (Entzeroth *et al.* 1985) three hypotheses could explain how *Sarcocystis* spp. reach the lamina propria: the infected cell containing the parasite moves to the lamina propria; it is transported by leucocytes to the lamina propria; or the zygote migrates by itself. However, Lainson and Paperna (Lainson and Paperna 2000) suggest that this is more likely to be due to parasite active movement than other host cells, since the enterocyte degenerates as the zygote matures into an oocyst. Further observation by these same authors suggests that the parasite is encapsulated in a parasitophorous vacuole inside the host cell, concluding that they cannot explain how these events evolve.

Adapted description from Van Peenen and Birdwell (1968) of *Isospora crotali* in *Lampropeltis californiae*:

"Measurements from 50 oocysts: No micropyle, oocyst residuum, or polar granule was seen. Oocysts measured 19.3 x 11.5 (18.4-19.8 x 10-5-13.2) µm. Length-width ratio: 1.69 (1.40 to 1.88). Sporocysts, ellipsoidal, with a refractile yellowish outer wall and a very thin colourless inner membrane visible only on rupture; sporocysts measured 11.8 x 10.3 (10.5-13.2 x 9.2-10.5), mean µm. A large, granular, and irregular residuum was present, clumped at one end of the sporocyst. Stieda body is absent. Sporozoites were comma-shaped, measuring approximately 2x6 µm. In histologic section, there were sporulated oocysts in the lamina propria".

Location: Intestine.

Locality: San Diego, California.

Host: *Lampropeltis californiae.*

Potential species:

Sarcocystis lampropeltii.

Syn: *S. montanensis, Cryptosporidium lampropeltis.*

Location: Faeces.

Locality: USA.

Host*: L. holbrooki and L. calligaster.*

Descriptions: Lindsay et al. 1992; McAllister et al. 1995; Duszynski and Upton 2009.

Remarks:

In this description, the two most important things that diagnose the identity of the genus *Sarcocystis* instead of *Isospora*, are the location of the oocysts within the lamina propria and the delicate nature of the oocyst wall, as illustrated in Van Peenen and Birdwell's (1968) article. In the case of *Sarcocystis* spp., a number of species have very close measurements, making morphological identification useless. In addition, these host do not share the same geographic location, which makes them more likely to be separate *Sarcocystis* species. Molecular work, transmission electron microscopy, and experimental infections to establish its biology and ultrastructure are necessary for species determination.

Order **Adeleida**

Family **Haemogregarinidae**

***Haemogregarina* sp.** (likely an *Hepatozoon* sp.)

Haemogregarines are composed of hematic parasites transmitted in either aquatic (mostly *Haemogregarina* spp.) or terrestrial (*Hepatozoon* and *Karyolysus*) environments. In the case of snakes, according to Siddal, (1995), haemogregarines from snakes should be treated as belonging to the genus *Hepatozoon.* However, few species of *Haemogregarina* have been demonstrated in snakes with amphibious habits transmitted by leeches.

These parasites have complex life cycles in which one or many intermediates (invertebrate and/or vertebrate) are involved. For many *Hepatozoon* spp., the actual natural cycle is not known; thus, here is an approach to a potential generic life cycle of *Hepatozoon* spp. It may be possible for this parasite to colonize paratenic hosts, since it is unlikely that snakes eat the invertebrate host.

It is discussed if some species of this parasite, such as *H. pictiventris, H. sauritus or H. polytopis,* infecting snakes in Florida might be transmitted by bite as well. since infective stages been found inside the mosquito proboscis (Telford 2009).

Once the snake host eats the mature infective stages in the invertebrate host, the sporozoites penetrate the intestinal wall and travel to the target organs, mostly the liver, but also the lungs. In the first asexual reproduction, macromeronts are formed, with few and big macromerozoites. These infect other hepatic or endothelial cells and produce micromeronts, bigger cysts composed of lots of smaller parasite cells, micromerozoites. This last form is the one that travels back into the bloodstream and infects erythrocytes (Telford 2009).

Once eaten in the bloodmeal by a capable invertebrate host (e.g. mites/ticks, mosquitoes/midges) the micromerozoites penetrate the gut wall (depending on the *Hepatozoon* spp) of the host and start sexual reproduction with the formation of gamonts. Finally, fecundation takes place and forms a sporogony, leading to sporulated oocysts. These forms could either be infective to the vertebrate host or would need a second host (small vertebrates such as lizards, frogs, etc.) to fulfil the life cycle (e.g. *H. sipedon* and H. *sirtalis*). To continue the cycle, the invertebrate host needs to be eaten by the vertebrate host (Telford 2009).

Adapted description of *Haemogregarina* sp. from Van Peenen and Birdwell (1968):

"Blood forms were elongated, refractile bodies occupying the length of the erythrocyte. There was crowding of the host cell nucleus but no obvious karyolytic effect. These forms, presumed to be gametocytes, were found only in red blood cells or free in serum, measuring 8-20x2-8µm. In the largest forms, dark blue caps were occasionally seen at either end.

Schizonts were not numerous in any tissue but were most frequently seen in the liver. In microscopic sections, schizonts were of two types: barrel-shaped with from two to six merozoites and more compact ellipsoidal forms with merozoite nuclei, numbering from eight to twenty-four, clustered at the edges. In the latter, the schizont appeared shrunken away from the surrounding parenchyma. The earliest identifiable forms were independent of surrounding tissue, so host cells could not be identified."

Location: Blood, liver.

Locality: San Diego, California.

Host: *Lampropeltis californiae.*

Potential species:

Hepatozoon eurytopis, H. karyilisi, H. rexi.

Location: Blood and liver.

Locality: Florida.

Host: *L. getula floridana.*

Description: Telford 2010.

Remarks:

In the same way as most chromists and protozoans, to accurately identify the parasites, it is necessary to perform molecular techniques since morphology is not sufficient. In the first approach, only tissue forms could be used. Since these are developed in the liver and the biology of its host does not include aquatic habits, as suggested by a few authors, this parasite should be treated as *Hepatozoon* sp. Considering the distant habitats of this snake and the Florida kingsnake and the potential host specificity of *Hepatozoon* spp. in natural conditions, this species is likely to be a distinct species from those described in *L. floridana.*

Class **Gregarinomorphea**

Sublass **Cryptogregarina**

Order **Cryptogregarida**

Family **Cryptosporidiidae**

Cryptosporidium serpentis (type A)

This parasite is usually regarded as a snake-specific pathogen; however, it has been retrieved from clinical lesions in other reptilian species. It is accepted to be an entity formed by two distinct genotypes that tend to infect different hosts: *C. serpentis* genotype A is commoner in snakes, while genotype B is more often isolated from non-ophidian squamates (Bogan 2019). So far, no zoonotic cases have been reported for this parasite (Zahedi *et al.* 2016); however, it is a very important parasite for pet snakes, producing chronic infections that can be lethal in many cases (Bogan 2019). In other species of *Cryptosporidium* (enteric), it is proven to be an intracellular, extra cytoplasmatic parasite, located between both layers of the cell phospholipid membrane (Bones *et al.* 2019).

Cryptosporidium spp. has a direct life cycle that ends with the production of thin or thick-walled oocysts, which can reinfect the host or be expelled within the faeces respectively. Once excysted, the sporozoites infect their target cells (stomach epithelium in the case of *C. serpentis*), forming a parasitophorous vacuole that then differentiates into the trophozoite stage (Pumipuntu and Piratae 2018). After invasion, asexual reproduction takes place, forming two different types of meronts. The first type releases 8 trophozoite-like merozoites that continue to form a second meront, and from the second type 4 merozoites emerge that reinfect host cells to form sexual gamonts. Once fecundated, the zygote evolves into either thin or thin-walled oocysts (Bogan 2019).

Morphological description:

The authors of this paper (Xiao *et al.* 2004) tested the snakes by molecular methods, sequencing *C. serpentis* type A; a morphological description was not provided. These authors measured oocysts in faeces of a desert monitor (*Varanus griseus*) identified molecularly as *C. serpentis.* This parasite produces rather small (5.9 x 5.1µm), almost spheric oocysts (shape index 1.14), with 4 free sporozoites.

Location: Stomach.

Locality: Worldwide.

Host: *Lampropeltis californiae.*

Remarks:

In addition to these parasite genotypes, it is likely other *Cryptosporidium* species from their prey and environment, such as *C. parvum, C. muris* or *C. bailey* (Xiao *et al.* 2004; Richter *et al.* 2011) can be found; thus, molecular identification of positive samples is necessary. In the case of kingsnakes, in their natural habitat, they are prone to infection since ophiophagy has been proven to be an efficient way of transmission. (Bogan 2019). In kingsnakes, another species of *Cryptosporidium, C. lampropeltis,* was described in a *L. calligaster* from Arkansas (Anderson *et al.* 1968)**,** but it was considered to be incorrectly classified and a synonym of *Sarcocystis* (Morgan *et al.* 1999), likely *S. lampropeltii.* It is not proven that *Cryptosporidium* spp. can be vertically transmitted; however, newborn snakes can be infected by their mother's cloaca or contaminated egg shells (Bogan 2019).

Aims and Objetives

1. **Aim**: Review the potential parasitic fauna of the genus *Lampropeltis.* **Objective***:* Studying and reviewing references on parasites of these snakes, and examining the invasive population on Gran Canaria with coproscopy and further parasitological dissections, searching for metazoan parasites.

2. **Aim**: Deepen the knowledge of parasitic species present in cats as historic hosts for currently spreading co-invasive parasites in native and potentially exotic fauna. **Objective**: Perform an extensive coprological survey in colonies of feral cats.

3. **Aim**: Review the parasitic fauna present on the Canary Islands to establish potential larval parasites to be found inside California kingsnakes. **Objective**: Extract all potential species of parasites affecting local vertebrates from the Bank of Data on Biodiversity of the Canary Islands.

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Section II: Published papers

Paper 1: Parasitological findings in the invasive California kingsnake (*Lampropeltis californiae*) in Gran Canaria, Spain.

Paper 2: Dispersion of adeleid oocysts by vertebrates in Gran Canaria, Spain: report and literature review.

Paper 3: *Sarcocystis* sp. infection (Apicomplexa: Sarcocystidae) in invasive California kingsnake *Lampropeltis californiae* (Serpentes: Colubridae) in Gran Canaria.

Paper 4: Small islands as potential model ecosystems for parasitology: Climatic influence on parasites of feral cats.

Paper 1:

Parasitological findings in the invasive California kingsnake (*Lampropeltis californiae*) in Gran Canaria, Spain.

Parasitology

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Parasitological findings in the invasive California kingsnake (*Lampropeltis californiae*) in Gran Canaria, Spain

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Research Article

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Abstract

The California kingsnake (*Lampropeltis californiae)*, native to North America, is a significant threat to the conservation of endemic species in the Spanish Macaronesian island of Gran Canaria. However, its role disseminating potential invasive parasites, such as zoonotic pentastomids, has not been proven. Among its parasitic fauna, only protistans have been documented, in contrast to other *Lampropeltis* spp., which are known to carry pentastomids. Thus, a parasitological study is urgently required. Between 2016 and 2018, a total of 108 snakes were necropsied and stool samples examined. A single snake was infested with *Ophionyssus natricis*, and another individual with *Serpentirhabdias* sp. Only this latter snake presented gross lesions, characterized by granulomatous pneumonia. No Pentastomida were found. By contrast, almost the entire population (98.5%) was infested with larval helminths (three different nematode and two cestode species), characterized by granulomatous gastrointestinal serositis. This suggests the snake poses a 'dead end' host for local parasites. Based on these findings, snakes in Gran Canaria carry potential zoonotic mites, which along with *Serpentirhabdias* sp. could represent a threat to endemic lizards. The presence of metazoan parasites and their lesions are reported for the first time in the California kingsnake.

Introduction

Gran Canaria is an Atlantic island of the Canarian archipelago (27°57′31″N, 15°35′33″W), which belongs to Spanish Macaronesia. The island has a land area of just 1560 km^2 but reaches 1956 m at the highest point and contains a very diverse ecosystem for its relatively small size. Fifteen endemic species of reptile are found on the Canary Islands, but no snakes.

The introduction of foreign species poses one of the main threats to global biodiversity and ecosystem conservation and its effects are magnified on islands due to ecosystem isolation and high numbers of endemic species or subspecies (Carroll, 2007; Bezerra-Santos *et al.*, 2021). This effect has been observed on Gran Canaria where the introduced California kingsnake (*Lampropeltis californiae*) has resulted in a decrease in numbers of the endemic and endangered Gran Canaria giant lizard (*Gallotia stehlini*) in the areas where the snakes have been established the longest – a problem that seems to be getting worse (Gallo-Barneto *et al.*, 2016).

The California kingsnake is native from the southwestern USA to the northwestern part of Mexico (Fig. 1) and can be found in a wide range of habitats from forests, rocky areas, coastal, urban and suburban areas, deserts and lakes (Hubbs, 2009). The snake's natural diet is equally wide and includes lizards, geckoes, small turtles and birds, other snakes, skinks and rats among others. This species of snake is generally harmless to humans and has become popular as a pet (Hubbs, 2009). The invasion in Gran Canaria is presumed to be due to accidental escapes or through the release of pet snakes, since at least two of the four populations on the island were established by different groups of captive-bred individuals (Monzón-Argüello *et al.*, 2015). The general lack of natural predators, a diet based on at least three endemic reptiles; Gran Canaria giant lizard (*Gallotia stehlini*), Boettger's wall gecko (*Tarentola boettgeri boettgeri*) and Gran Canaria skink (*Chalcides sexlineatus*) and rats, together with its burrowing habits, have firmly established this species as a significant ecological problem (Monzón-Argüello *et al.*, 2015).

Invasive species pose a direct threat by preying on native fauna, but they can also harbour new parasites or other pathogens that may cause additional damage to the local environment (Taraschewski, 2006). Moreover, local parasites or pathogens can infect these exotic invaders and may be more harmful to their new (possibly naïve) hosts, than to their native counterparts (Kelehear and Jones, 2010).

Zoonotic parasites, such as *Raillietiella* sp. (Pentastomida) which can cause abdominal lesions in humans due to the migration of their larvae and nymphs, have been reported in *Lampropeltis getula* in the USA (Ali *et al.*, 1985; Tappe *et al.*, 2016; Mendoza-Roldán *et al.*, 2020). Their presence has already been demonstrated in *Gallotia* lizards from the islet of

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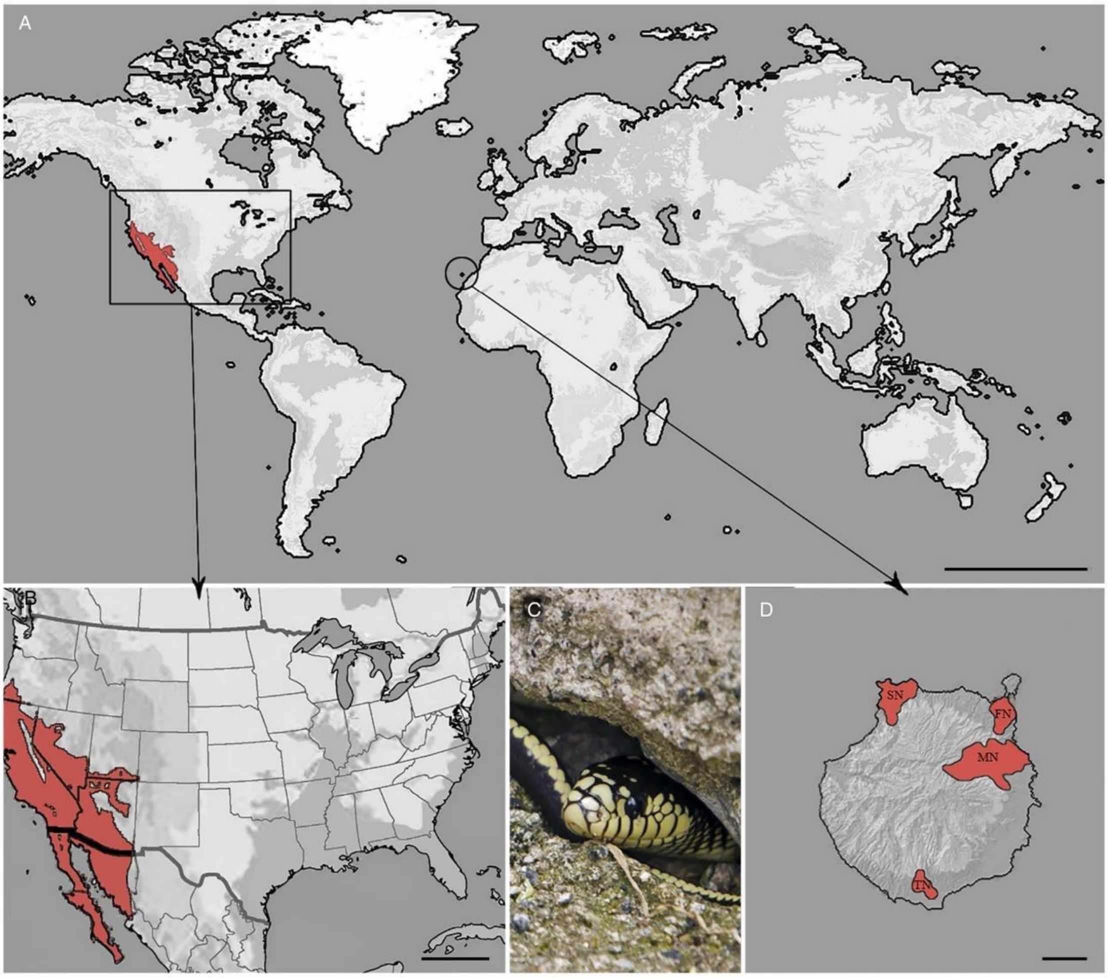

Fig. 1. (A) Distribution of California kingsnake in the world. Scale bar = 5000 km. (B) Native locality. Scale bar = 500 km. (C) An adult snake from Gran Canaria. (D) Populations of this invasive reptile in Gran Canaria, Canary Islands, Spain. Scale bar = 10 km. MN, main nucleus; SN, secondary nucleus; TN, tertiary nucleus; FN, fourth nucleus.

Alegranza (Abreu-Acosta *et al.*, 2006), in the eastern Canarian archipelago. If California kingsnakes are able to introduce this pentastomid genus into the island, they could also infect the Gran Canaria giant lizard, as well as potentially humans, and maintain the infection for many generations.

Despite its popularity as a pet, few parasitological studies have been carried out on the California kingsnake, even those kept in zoological collections. Two reports, totalling 11 individuals, describe only protozoan parasites (Van Peenen and Birdwell, 1968; Xiao *et al.*, 2004).

This is the first study that describes the lesions caused by helminth parasites in California kingsnakes on Gran Canaria and the trichinoscope (compression plates) is described as a useful tool for the detection of larvae. The possible biological hazard for local fauna, and potentially humans, due to the introduction of foreign parasites is discussed.

Material and methods

On Gran Canaria, the California kingsnake is located in three distinct nuclei (Fig. 1); the main nucleus (MN), characterized by a wetland area with abundant flora and fauna in the center-east of the island; the secondary nucleus (SN), a very dry and steep location with characteristic Tabaibal-Cardonal flora, in the northwest, and the tertiary nucleus (TN), with similar climatic conditions to the secondary, in the south. A fourth nucleus has recently been identified in the northeast, surrounding the capital city of Las Palmas de Gran Canaria.

Between 2016 and 2018, snakes from the main and secondary nucleus, captured under the extermination plan approved by the Canary Islands government, were necropsied at the Faculty of Veterinary Science of the University of Las Palmas de Gran Canaria following standard procedures (Farris *et al.*, 2013). Due to the few individuals captured, in both cases less than five, the tertiary and fourth nuclei were not included in this study. Biometrical parameters of weight, length, fat weight and sex were recorded. The infested tissues from the animals were fixed in 10% neutral-buffered formalin and routinely processed to paraffin blocks, serially sectioned at 5 μm and stained with hematoxylin and eosin.

Routine fecal examinations were performed following standard methods (Zajac and Conboy, 2012) and detailed notes made on the appearance and number of any gross pathological changes. Gross lesions were further assessed microscopically using compression plates (trichinoscopy). The results are given as prevalence, mean intensity and abundance (Bush *et al.*, 1997).

The parasites were extracted with the help of mounting needles and identified in temporary mounts with saline solution following

Table 1. Ecological indices for the larval parasites found in the main (MN) and secondary nucleus (SN)

	Group A *n* = 44 Prevalence (%)	Group B *n* = 64 Prevalence (%)	Mean intensity	Mean abundance
MN				
Helminths	55.2	97.0	81.1 ± 118.8	78.7 ± 117.7
Nematodes	[a]	69.7	62.7 ± 133.0	43.7 ± 114.0
Cestodes	[a]	81.5	42.7 ± 49.5	34.9 ± 47.7
SN				
Helminths	80.0	100.0	203.9 ± 294.9	203.9 ± 294.9
Nematodes	[a]	90.0	186.0 ± 306.5	167.4 ± 295.8
Cestodes	[a]	83.3	43.8 ± 33.2	36.5 ± 34.4

[a] Unable to calculate.

Table 2. Sample biometrical parameters

	Females	Males	ML (cm)	MW (g)	FW (g)	F%	Total
MN	33	26	88.3 ± 3.1	167.0 ± 15.9	14.1 ± 2.4	8.7 ± 1.9	59
SN	16	28	89.4 ± 3.7	163.9 ± 15.9	9.4 ± 1.2	5.7 ± 0.5	44
UN							5

ML, mean length; MW, mean weight; FW, mean fat weight; *F*%, mean fat percentage of the body weight; MN, main nucleus; SN, secondary nucleus; UN, undetermined sex and/or nuclei.

the keys cited in the bibliography for cestodes (Witenberg, 1932; Joyeux and Baer, 1936; Ryšavý, 1973; Jones, 1994), nematodes (Chitwood and Wehr, 1934; Moravec *et al.*, 1987; Anderson, 2000; Willmot and Chabaud, 2009; Kelehear and Jones, 2010) and Acari (Evans, 1955; Lindquist *et al.*, 2009; Moraza *et al.*, 2009).

Statistical analysis was performed with SPSS v. 24.0 (IBM SPSS Corp., Chicago, Illinois, USA). The parasite number variation was tested using the parametric Student *t*-test for each population, the correlations between both weight/length and parasite number were assessed by means of Pearson or Spearman correlation tests, after testing for normality. *P* values <0.05 were considered to be statistically significant.

Results

Biological sample data: ecological indices and correlations

In total, 108 snakes were examined, divided into two groups: Group A; the first 44 samples collected, in which coprological and postmortem examinations were performed using a stereoscopic microscope, and group B; the following 64 samples, in which the postmortem examinations were enhanced with the aid of a trichinoscope (Table 1). The biometrical parameters and sex by nuclei are represented in Table 2.

In group A snakes, assessed macroscopically, 59.1% had parasitic cysts, with 25.0% containing cysts in the stomach and 45.5% in the small intestine (Figs 2A and C). 80.0% of SN snakes harboured these cysts and 55.2% from the MN.

By contrast, group B, tested by trichinoscopy, revealed a prevalence of 98.4% with cysts – 100% of SN snakes and 96.9% of those from the MN. These cysts microscopically corresponded to cestode cysticercoids and nematode larvae. In summary, almost 30% more cysts were observed with trichinoscopy than by gross examination alone.

No significant differences in parasitic burden or prevalence were found between sex, colour and line patterns.

The prevalence of helminths was estimated using group B. No differences in cestode prevalence were observed between the MN and the SN (80.6 and 83.3% respectively). However, nematode prevalence was markedly different; 90.0% in the SN and 69.7% in the MN.

The two nuclei showed a significant difference in the average number of helminths per individual snake (Student *t*-test, $P = 0.008$), but there was no significant difference in numbers of cestodes between the two nuclei ($P = 0.307$; Mann–Whitney's *U* test).

In terms of intensity and abundance, the SN showed the highest mean nematode intensity and abundance, and the highest individual infestation (186 ± 306.5; 167.4 ± 296 and 492 larvae respectively). However, there were few differences between the mean intensity and abundance of tapeworms in the two nuclei. Highest tapeworm intensity was found in the MN.

The anatomic distribution of the helminths was not equal; nematodes were concentrated in the stomach, reducing in prevalence and abundance from stomach to large intestine (73.4% stomach, 67.2% small intestine and 42.2% large intestine). Similarly, cestodes were concentrated in the small intestine and reduced in numbers in the large intestine, with no records of cysticercoids in the stomach (81.0% small intestine, 51.6% large intestine and 0% stomach). This anatomic distribution was recorded in both populations studied. No correlations were found between the number of cestodes and the length of the snakes ($\rho = -0.144$, $P = 0.448$; Spearman ρ test for the SN and $\rho = -0.192$, $P = 0.285$; Spearman ρ test for the MN); however, the number of nematodes showed a positive correlation with the length of the snakes ($P = 0.022$ for the SN and $P = 0.008$ for the MN).

No significant correlations were found between the fat percentage of the body weight or fat weight with parasitic burden.

Coprological examination

Just one snake out of the 44 (group A) contained parasite eggs and larvae in its feces compatible with rhabditid nematodes

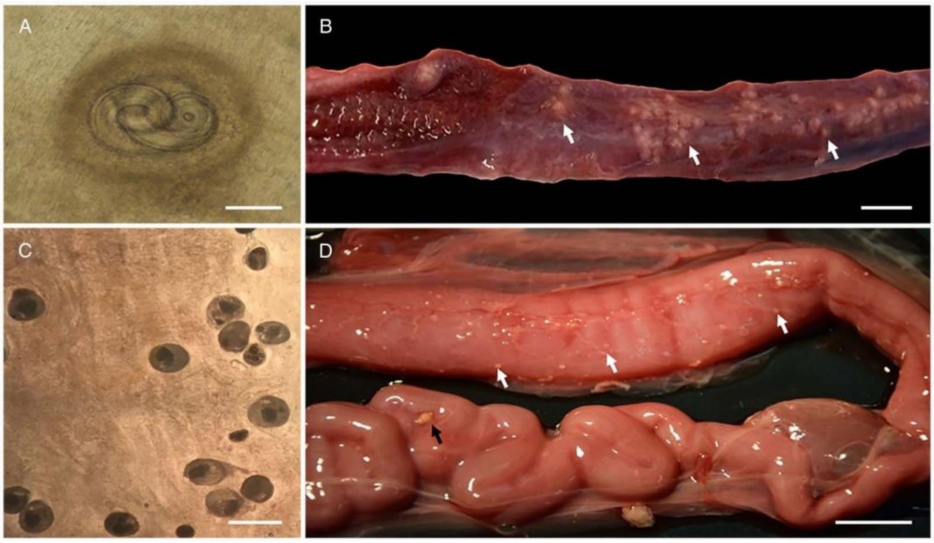

Fig. 2. (A) Larval nematode encysted in the gastric wall of a snake. Scale bar = 200 μm. (B) Gross appearance of a partly opened lung, showing multiple granulomas (white arrows). Scale bar = 10 mm. (C) Larval tapeworms encysted in the intestinal wall. Scale bar = 1 mm. (D) Gross image of granulomas on the gastric (top, white arrows) and intestinal (bottom, black arrow) serosa. Scale bar = 10 mm.

which, in addition to the observation of granulomatous lesions in the lung (Figs 2B, 3A and B), and no adult parasites in the intestine, confirms the diagnosis of rhabdiasid lungworm eggs and larvae, compatible with the genus *Serpentirhabdias*.

Adult parasites

Just two specimens out of the 108 snakes showed adult parasites, one specimen from each group (A and B). From group A, the snake passing parasite eggs in its feces was found infested with *Serpentirhabdias* lungworms. From group B, one snake harboured 19 Mesostigmatid skin mites of the family Macronyssidae, in various life stages, compatible with *Ophionyssus natricis* (Fig. 4F).

Identification of the larval helminths

The metacestodes (Figs 2C, 4A and C) found were characterized as a solid larval form, with 4 suckers and 4–5 rows of taenioid and rose thorn hooks which identifies them as two species of the genus *Diplopylidium*, *D. acanthotetra* (70.3% prevalence) and *D. nölleri* (21.9% prevalence). Several specimens of both species were deposited at the Parasites and Vectors collection of the Natural History Museum of London (accession numbers: NHMUK.2020.2.12.1-2 and NHMUK.2020.2.12.3).

Nematodes were characterized based on the divided oesophagus (muscular and glandular) and the three morphological types found correspond to the order Spirurida and, based on other morphological features, possibly to three superfamilies: Spiruroidea (Type 1), Acuarioidea (Type 2) and Physalopteroidea (Type 3).

The first and most common helminth (Fig. 4B) (45.3% prevalence) was characterized by two prominent papillae at the anterior end and thin lateral wings that begin posterior to the nerve ring, up to a few micrometres before the cloaca. These also possess a tuft of finger-shaped papillae at the posterior end. This description matches several larvae from genera of the same taxa: superfamily Spiruroidea, family Spirocercidae.

In the second type (Fig. 4D) (35.9% prevalence), there were two pseudo-labia at the anterior end, accompanied by four non-recurrent or anastomosed reticular cordons and two monocuspic cervical papillae. At the posterior end, no papillae or other ornaments were found. These characteristics match those described for larvae from the superfamily Acuarioidea, subfamily Acuarinae, which are the only ones in which larvae have cephalic cordons. Finally, the third (Fig. 4E) and smallest morphological type (4.7% prevalence) was characterized by two trilobed lips, a posterior end without papillae and thin lateral alae. These larvae likely belong to the superfamily Physalopteroidea.

Gross and microscopic findings

One snake presented with diffuse red discolouration of the lung with numerous, multifocal to coalescing white, round nodules, 0.5–2 mm diameter, affecting around 70% of the cranial lung parenchyma (Fig. 2B).

Microscopically, these lesions corresponded to multifocal mild verminous pneumonia composed of a mild infiltration of heterophils, macrophages and lymphocytes, as well as a small amount of cellular debris, fibrin and oedema surrounding adult and larval nematodes inside faveolar spaces. Mild pulmonary congestion as well as faveolar atelectasis were also observed (Figs 3B and A). In addition, occasional pleural granulomas with intralesional bacteria were observed (Fig. 3C).

The parasites were characterized by the presence of a body cavity and an external eosinophilic cuticle, surrounding an inner layer of degenerated platymyarian muscle fibres, a digestive tract and a reproductive structure compatible with ovary (Fig. 3A). Also present, predominantly within faveolar walls and the interstitium, were often highly cellular and basophilic larval forms. On sagittal sections, a single-cell digestive tract was observed – compatible with early larval stages (L1–L2).

In total, 98.4% of snakes presented with moderate to severe multifocal white nodular lesions from 0.7 to 5 mm in diameter on the serosal surface of the intestine and stomach, and coelomic wall (Fig. 2D). Microscopically, these lesions were present in various stages of maturity (Figs 3D and E) and were located mostly in the serosal and muscular layer of the intestine (Fig. 3F) and coelomic wall (Fig. 3E). The lesions were microscopically characterized as cystic granulomas, composed of an outer layer of fibroblasts, fibrocytes and collagen surrounded by several layers

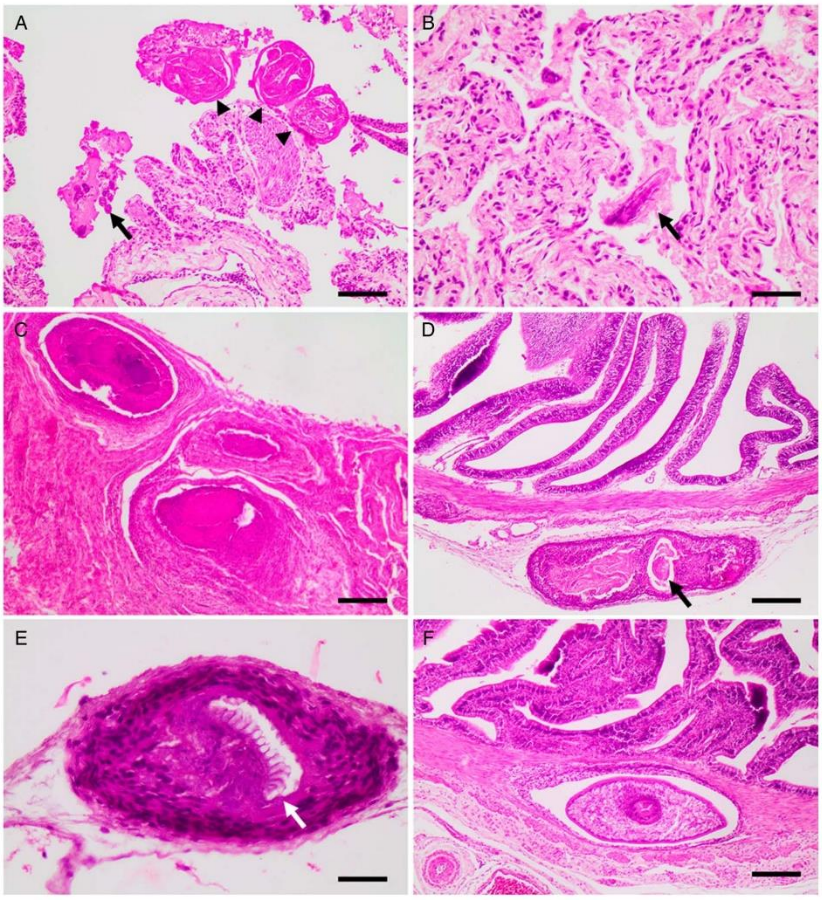

Fig. 3. Histological sections showing, (A) lung with adults (arrow heads) and larvae (arrow) of *Serpentirhabdias* sp. H&E. Scale bar = 100 μm. (B) Lung with atelectasis and mild suppurative inflammation. Note the longitudinal section of the head of a *Serpentirhabdias* larva (arrow). H&E. Scale bar = 40 μm. (C) Lung granulomas. H&E. Scale bar = 200 μm. (D): A chronic-active granuloma, with intralesional dead nematode, on the intestinal serosa (arrow). H&E. Scale bar = 200 μm. (E) A chronic granuloma, containing a dead tapeworm surrounded by a wall of fibrocytes. Note the preserved rostellum (arrow). H&E. Scale bar = 40 μm. (F) A cystic granuloma in the muscular layer of the intestine containing a cysticercoid. H&E. Scale bar = 200 μm.

of compacted macrophages and few lymphocytes and heterophils, surrounding a parasitic structure that floated in an unstained fluid. These parasites (Cestoda) were characterized by the presence of an outer thick eosinophilic tegumentary layer, lack of body cavity, and in its place a vacuolated space without digestive system, presence of armed scolex and suckers, lack of reproductive organs and presence of calcareous corpuscles.

Non-cystic granulomas were also found containing cysticercoids, with a well-preserved rostellum, surrounded by numerous macrophages, lymphocytes, fibroblasts and fibrocytes, or with more active inflammation including the presence of heterophils inside the granulomas (Figs 3D and E). Associated with these latter areas of inflammation were degenerated nematode larvae (Fig. 3D).

Discussion

Here the first record of two *Diplopylidium* species and three larval nematode morphological types in the California kingsnake are described. These findings are consistent with local parasites infecting an invasive species, which would act as a 'dead-end' paratenic host, since no predators are described for this snake in Gran Canaria.

Larval helminth cysts from reptiles, and snakes specifically, have been reported previously in the literature; however, just *Mesocestoides* tetrathyridia and *Macracanthorhynchus* cystacanths, have been described in a *Lampropeltis* sp. snake (Elkins and Nickol, 1983; Jacobson, 2007).

Only larvae from members of the superfamilies Spiruroidea, Acuarioidea and Physalopteroidea have been commonly described parasitizing reptiles (Anderson, 2000; Criscione and Font, 2001; Goldberg and Bursey, 2001; Santos *et al*., 2006). Specifically; *Physocephalus*, *Ascarops* and *Spirocerca lupi*, from Spirocercidae (Moravec *et al*., 1987; Goldberg and Bursey, 1988; Goldberg *et al*., 1994), Acuarinae *gen*. from Acuarioidea (Roca, 1985; Moravec *et al*., 1987) and *Physaloptera*, from Physalopteroidea (Widmer, 1970).

Regardless of the parasitic species, the burden and prevalence found in the Gran Canaria snake population (98.4%) is considerably higher than that reported for larval parasites from other reptiles in Spain, and indeed North America (usually less than 2%) (Widmer, 1967; Roca, 1985; Martín *et al*., 2003; Santos *et al*., 2006; Goldberg *et al*., 2013; Davis *et al*., 2016). Furthermore, there are no records of *Diplopylidium* species in North American fauna (Criscione and Font, 2001; Goldberg and Bursey, 2001; Yildirimhan *et al*., 2005; Goldberg *et al*., 2013; Davis *et al*., 2016; McAllister and Bursey, 2016). This difference could be due to the use of macroscopic examination during necropsy, instead of using compression plates (trichinoscopy) to see through the organ. In fact, the data obtained using the two

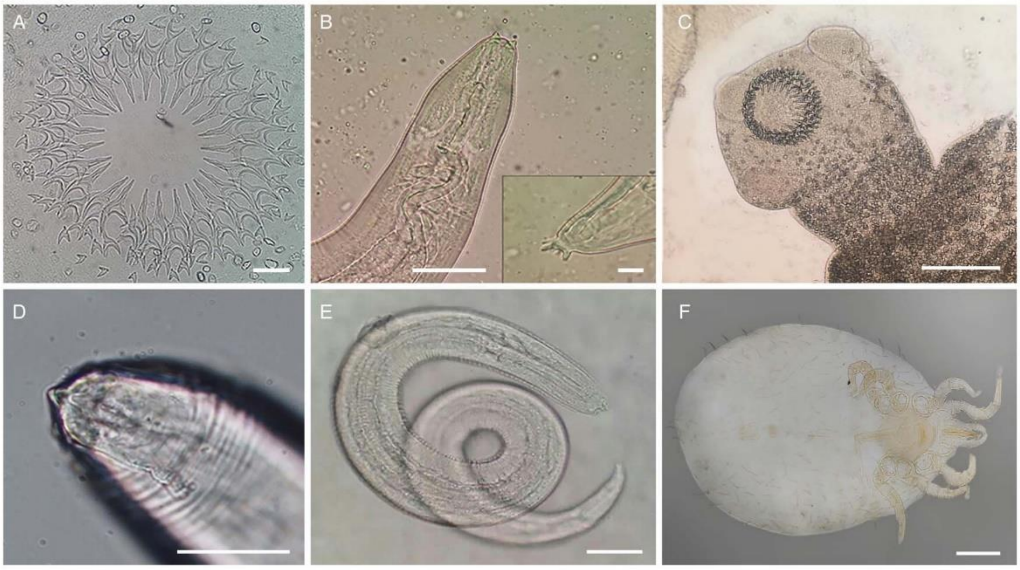

Fig. 4. (A) Rostellum in apical view, note the five rows of hooks. Scale bar = 50 μm of *Diplopylidium acanthotetra*. (B) Morphological type No. 1 of larval spirurids, possible Spirocercidae. Inset: posterior end. Note the two papillae on the anterior end, and the tuft of digitations on the tail. Scale bar (left) = 50 μm, scale bar (right) = 10 μm. (C) Cysticercoid of *Diplopylidium acanthotetra*. Scale bar = 200 μm. (D) Morphological type No. 2 of larval spirurids, possible Acuarinae. Note the two papillae and the straight reticular cordon. Scale bar = 50 μm. (E) Morphological type No. 3 of larval spirurids, possible Physalopteroidea. Scale bar = 50 μm. (F) Cleared specimen of *Ophionyssus naticis*. Scale bar = 200 μm.

techniques in this study differ by almost 30%, with the more sensitive utilization of trichinoscopy being developed through research on larval parasitic forms of *Trichinella*.

Due to the lack of studies in the California kingsnake, it is not possible to compare the epidemiological results here with the native American populations of these snakes. In the Canary Islands, a maximum prevalence of 21.7% for *D. nölleri* and 2.2% for *D. acanthotetra* (Roca *et al.*, 1999) is reported in the endemic gecko species described as natural hosts for the tapeworm species. Meanwhile in its invasive host the general prevalence was 25.8 and 76.7%, respectively. Moreover, the mean intensity registered for *Diplopylidium* species in endemic geckoes was also lower, reaching a maximum of 37.5 cysticercoids for *D. nölleri* from the Boettger's wall gecko (*T. boettgeri*) in Gran Canaria, *vs* 43.8 larvae in the snakes. In addition, the anatomic distribution of the cestodes was similar in the two hosts; in both species on the intestinal serosa, but in the case of geckoes, they were also found on the liver surface (Roca *et al.*, 1987). These findings could mean that *Diplopylidium* cysticercoids are being transferred from geckoes to the snakes. The only suitable definitive carnivore hosts on the island are cats and dogs, with only *Diplopylidium acanthotetra* described in cats from the island of Tenerife (Sánchez, 2013).

There is only one report of larval nematodes, which match the morphology described in this study as 'Type 1', parasitizing the endemic Boettger's lizard (*Gallotia caesaris*) on the island of El Hierro (Martín *et al.*, 2003). In that study, they reported a mean intensity of 2 larvae and a prevalence of 2.5%, which is significantly lower in comparison with the mean intensity of 186 larvae and 90% prevalence found in these kingsnakes. Apart from the report from *G. caesaris* from El Hierro, no other larval nematodes have been recorded in vertebrate hosts which can be eaten by the snakes in Gran Canaria, thus the previous paratenic host remains unknown.

Furthermore, it has been demonstrated that geckoes can harbour larval spirurids of the three morphological types described in this study (Criscione and Font, 2001; McAllister and Bursey, 2016), therefore despite the negative results found in Gran Canaria populations, Boettger's wall gecko would be a suitable previous paratenic host for these nematodes.

Ophionyssus natricis is a widely distributed mite that represents a potential zoonotic risk, predominantly in suburban areas, where the wildlife–human interface shrinks. There are no reported cases of human infestation from Gran Canaria, however, with concentrated sampling effort of habitations within the larger nuclei, its presence may be demonstrated. In addition to dermatitis in humans (Schultz, 1975), other reptiles can be infested with this mite (Norval *et al.*, 2020) which can also act as a vector for *Aeromonas* sp. (Wozniak and DeNardo, 2000), leading to fatal disease in certain cases. Hence, the possible spillover of these mites, as well as *Serpetirhabdias* sp., to local fauna, requires further research.

In addition to the ecosystem damage caused by predation of native fauna and competition with other native predators for food, these snakes can be considered 'dead-end' paratenic hosts. Most of the parasites found here will not be able to reach their respective definitive hosts, such as birds of prey, and thus are disrupting natural life cycles in the island. For example, once the population of geckoes decreases, the transmission of parasites, such as acuariid nematodes, will likely be affected, since these are transmitted to birds of prey by eating geckoes (as paratenic hosts) rather than consuming terrestrial isopods or other arthropods (intermediate hosts). This is a rather less obvious, but no less important, consequence of the presence of invasive snakes which will be contributing not only to the extinction of geckoes and other native reptiles, but also to their respective parasites of which several are endemic to the Canary Islands, e.g. *Thelandros filiformis*, *Alaeuris stehlini*, *Sarcocystis stehlini*, *Ophionyssus setosus*.

There was a positive correlation between nematode burden and the age of the snake, a correlation that did not exist for *Diplopylidium* sp. When an animal is exposed to a new parasite, the immune response is usually more severe than would occur in response to a parasite with which it had had co-evolved (Kelehear and Jones, 2010). No records of *Diplopylidium* have been found for North American fauna, unlike larval spirurids, therefore in its natural habitat, the California kingsnake would not be exposed to *Diplopylidium*. An increased immune response to new parasites could mean that the California kingsnake more efficiently controls tapeworms, rather than accumulating them during life as with nematodes, which would explain the positive correlation between longevity and parasite burden. Moreover, this could also explain how, if the two parasitic groups have the same donor host, such as the Boettger's wall gecko, that a correlation could exist for just one.

Rhabdiasid lungworms are considered common parasites of amphibians and reptiles (Anderson, 2000); however, their prevalence in North American snakes is very low. Only single cases of rhabdiasid-induced pneumonias, with limited gross and histological descriptions, are reported in snakes from various countries (Jacobson, 2007; Langford, 2010; Mihalca *et al.*, 2010; Goldberg *et al.*, 2013; Davis *et al.*, 2016).

Of the eight genera of Rhabdiasid nematodes which can infest reptiles, only one has been described in *Lampropeltis* spp. snakes, with two species: *Serpentirhabdias fuscovenosa* and *S. eustreptos* (Langford, 2010).

The low prevalence of *Serpentirhabdias* in kingsnakes in Gran Canaria could be explained by the relatively infrequent exposure of the snakes to their own feces in the environment, and their captive-bred origin. These first feral, presumed dewormed, animals had been released into an environment lacking existing snake parasites or natural paratenic hosts.

Parasitic pneumonias in snakes are reported with variable severity, from suppurative pneumonias with clear clinical signs and death (Jacobson, 2007), to those with only mild microscopic changes (Santos *et al.*, 2008).

Infective larvae of *Serpentirhabdias* sp. can inoculate soil bacteria, carried inside their intestines, within the snake lung, resulting in a significant secondary bacterial pneumonia with prominent gross lesions (Santos *et al.*, 2008). Various Gram-negative bacteria such as *Proteus* sp. and *Pseudomonas* sp. (Hilf *et al.*, 1990; Santos *et al.*, 2008) have been isolated from parasitized animals, likely acting as opportunistic agents, rather than the primary pathogen. Thus, the lung granulomas found in this study are possibly caused by inoculated soil bacteria, through indirect parasite damage.

Physaloptera sp. nematodes are described producing gastric lesions *via* larval penetration in a prairie rattlesnake (*Crotalus viridis)* in the USA (Widmer, 1970). In contrast, in this study, encysted larvae are described, with the small and large intestine representing a new anatomic location for these parasites. In addition, similar lesions to this report have been described for larval spirurids in several reptiles and amphibians (Goldberg and Bursey, 1988; McAllister *et al.*, 1993; Goldberg *et al.*, 1994).

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Paper 2:

Dispersion of adeleid oocysts by vertebrates in Gran Canaria, Spain: report and literature review.

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Dispersion of adeleid oocysts by vertebrates in Gran Canaria, Spain: report and literature review

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Abstract

Within the family Adeleidae, *Adelina* spp. belong to a group of arthropod pathogens. These parasites have been reported to have a wide geographic distribution, however, there are no reports of these protists in the Canary Islands, Spain. One of the peculiarities of the life cycle of *Adelina* spp. is the participation of a predator, because fecundation and sporulation occur inside the body cavity, and so necessitate destruction of the definitive host. The involvement therefore of a 'dispersion host', which eats the definitive host and spreads the oocysts through its faeces, is critical for the maintenance of certain *Adelina* spp. On the island of Gran Canaria, adeleid oocysts have been found in stool samples from four animals, three California kingsnakes (*Lampropeltis californiae*), and one feral cat. These animals were part of a larger coprological study of vertebrate parasites (117 snakes, 298 cats), where pseudoparasitic elements were also recorded. *L. californiae* and feral cats are invasive species which are widespread across the island and this novel finding of *Adelina* spp. oocysts in their faeces suggests that they could also serve as potential sentinel species for arthropod parasites.

Introduction

Adelina spp. (Apicomplexa: Adeleroina: Adeleidae) are parasitic protists of invertebrates, reported to have a worldwide distribution (Berto *et al.*, 2010). However, knowledge of the diversity of these protists is rather limited, particularly when compared to the diversity of their hosts. In the Canary Islands, an autonomous region of Spain located in the Macaronesian North Atlantic, there are no reports of *Adelina* spp. On the Iberian Peninsula, insect-related Adeleids have been observed as intra-abdominal oocysts in permanent mounts of sand flies (Morillas-Marquez *et al.*, 1983; Martinez-Ortega and Conesa-Gallego, 1987). These have only been identified to genus level which is understandable considering the large overlap in morphological parameters which exists between most of the described species (Purrini, 1984; Berto *et al.*, 2010).

The pathogenicity of these protozoa has not been studied extensively in natural invertebrate communities, however, their capacity to contribute to species competition, behavioural and colour changes, paralysis, darkening of internal organs and ultimately as a cause of death, have been demonstrated (Table 1). Thus, in addition to their likely natural role in population regulation, there may be a role for *Adelina* spp. as a means of biological pest control in farming (Yarwood, 1937; Park and Frank, 1950; Weisner, 1964; Purrini, 1984; El-Sufty and Boraei, 1989).

Adelina spp. are currently divided into two lineages; one group is found in the body cavity, while the second includes gut parasites. Classically, the genus *Adelina* (body cavity parasites) was erected from *Adelea* spp. (intestinal parasites), with differentiation of the two genera based on morphology of the sporocysts, which are spherical and discoidal, respectively (Yarwood, 1937). Based on these morphological features, several species from *Adelea* and *Klossia* were reclassified within the genus *Adelina*. However, with the exception of *Adelina dimidiata* and *A. schellacki,* which infect myriapods, all *Adelina* spp. are body cavity parasites (Purrini, 1984). Few molecular genetic studies have been undertaken in this genus, however comparing available sequences from NCBI (accession numbers in brackets), the difference of 4.3% between *A. dimidiata* (DQ096835.1) and *Adelina grylli* (body cavity) (DQ096836.2) is greater than other apicomplexans such as *Cystoisospora canis* (KT184368.1) compared with *Toxoplasma gondii* (2.2%, V03070.1;KX008033.1), *Neospora caninum* (1.9%, L24380.1) or *Besnoitia* spp. (*B. darlingi* (1.8%) MF872603.1; *B. besnoiti* (1.5%) XR_003828658.1). Further research is clearly needed to refine the current taxonomical status of these species and thus the intestinal infecting *Adelina* species are not considered further in this review.

The life cycle of *Adelina* spp. occurs inside the arthropod body cavity, with sporozoites piercing the gut to access the coelom (Merritt *et al.*, 1975). Asexual division takes place, forming two generations of merogonies (as described for *A. cryptocerci)* followed, after release of the

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Table 1. Recorded pathological effects of *Adelina* spp. on arthropod species around the world under laboratory or natural (Lab/Nat) conditions

Effect	Parasite	Host Order	Host family	*Host Spp*	Instar	Country	Lab/nat	Reference
Behavioural changes	*A. hypera*	Coleoptera	Curculionidae	*Hypera brunneipennis*	Larvae	Egypt	Lab	El-Sufty and Boraei (1989)
	A. tribolii	Coleoptera	Tenebrionidae	*Tribolium ferrugineum*	Larvae	Cambridge, UK	Lab	Bhatia (1937)
Colour changes	*A. hypera*	Coleoptera	Curculionidae	*Hypera brunneipennis*	Larvae	Egypt	Lab	El-Sufty and Boraei (1989)
	A. collembolae	Collembola	Neanuridae	*Neanura muscorum*	Adults	Germany	Nat	Purrini (1984)
Dark-brown spots in infected tissue	*A. hypera*	Coleoptera	Curculionidae	*Hypera brunneipennis*	Larvae	Egypt	Lab	El-Sufty and Boraei (1989)
	A. cryptocerci	Blattodea	Cryptocercidae	*Cryptocercus punctulatus*	Adults	Oregon, USA	Lab	Yarwood (1938)
	A. collembolae	Collembola	Neanuridae	*Neanura muscorum*	Adults	Germany	Nat	Purrini (1984)
Death	*Adelina* sp.	Coleoptera	Curculionidae	*Hypera brunneipennis*	Adults	Egypt	Nat	Merritt *et al.* (1975)
	Adelina sp.	Coleoptera	Curculionidae	*Hypera brunneipennis*	Larvae	Egypt	Nat	Merritt *et al.* (1975)
	Adelina sp.	Coleoptera	Curculionidae	*Hypera brunneipennis*	Larvae	Egypt	Nat	El-sufty and Boraei (1986)
	A. hypera	Coleoptera	Curculionidae	*Hypera brunneipennis*	Larvae	Egypt	Lab	El-sufty and Boraei (1989)
	A. hypera	Coleoptera	Curculionidae	*Hypera brunneipennis*	Cocoon	Egypt	Lab	El-sufty and Boraei (1989)
	A. cryptocerci	Blattodea	Cryptocercidae	*Cryptocercus punctulatus*	Adults	Oregon, USA	Lab	Yarwood (1937)
	A. tribolii	Coleoptera	Tenebrionidae	*Tribolium castaneum*	Larvae	Chicago, USA	Lab	Park and Frank (1950)
	A. tribolii	Coleoptera	Tenebrionidae	*Tribolium castaneum*	Pupae	Chicago, USA	Lab	Park and Frank (1950)
	A. tribolii	Coleoptera	Tenebrionidae	*Tribolium confusum*	Larvae	Chicago, USA	Lab	Park and Frank (1950)
	A. tribolii	Coleoptera	Tenebrionidae	*Tribolium confusum*	Pupae	Chicago, USA	Lab	Park and Frank (1950)
	A. tribolii	Coleoptera	Tenebrionidae	*Tribolium ferrugineum*	Larvae	Cambridge, UK	Lab	Bhatia (1937)
Population regulation	*A. tribolii*	Coleoptera	Tenebrionidae	*Tribolium castaneum*	–	Chicago, USA	Lab	Park and Frank (1950)

merozoites into fatty tissue, by sexual reproduction of gametoblasts (Yarwood, 1937). These macro and microgametoblasts fuse and develop into a zygote, which finally forms a sporont (Yarwood, 1937; Park and Frank, 1950; Ghosh *et al.,* 2000). Sporulation generally occurs within the fat bodies. As the infection spreads, the body tries to encapsulate the oocysts within tissue, to isolate them, and these appear as dark aggregates (Park and Frank, 1950; El-Sufty and Boraei, 1989). Finally, the adeleids begin to occupy the majority of the coelom and the rest of organs including muscles, resulting in death of the insect (Bhatia, 1937; Park and Frank, 1950; El-Sufty and Boraei, 1989). Other authors report secondary infections with gut bacteria as a cause of death in invertebrates, after penetration through the gut wall by the coccidia (Merritt *et al.,* 1975).

To infect other hosts, the oocysts must be released to the environment and then be ingested by other invertebrates. This can happen by cannibalism or through a 'dispersion host' (Sautet, 1930; Butaeva, 1996; De Quadros *et al.,* 2017). A dispersion host is typically a vertebrate predator which ingests an invertebrate whose tissues contain *Adelina* oocysts, and which are then released into its digestive tract and excreted. This phenomenon has been observed in several vertebrate species (reptiles, amphibians, birds and mammals), in which the parasite-infected invertebrates form part of their diet (Barnard *et al.,* 1974; Berto *et al.,* 2008; Lopes *et al.,* 2013; De Quadros *et al.,* 2017).

The Canary Islands are an archipelago composed by eight islands and five islets in Macaronesia. Despite their small size (7447 km^2), the Canaries are home to one of the largest number of endemic species in the temperate regions globally (Machado, 1998). Among the varied landscapes of the islands, which are considered 'hot-spots' of biodiversity, the laurel forests are particularly unique, found only in Macaronesia (Machado, 1998). Even considering their small size, there are between 2 and 5 isoclimatic zones, depending on the island, with four in the case of Gran Canaria: dry desert, dry steppe, temperate mild and temperate cold (Rodríguez-Ponce *et al.,* 1995).

On Gran Canaria, 5872 species of flora and fauna have been recorded to date, of which 22.7% are considered endemic. Arthropods comprise the largest and most diverse group with 3190 species recorded to date, of which 32.1% are endemic to the island (Arechavaleta *et al.,* 2010). Although arthropods constitute more than half the total species described on the island, there is a total dearth of knowledge of their coccidian parasites or their potential role in the regulation of arthropod populations within the islands. Moreover, considering the introduction of foreign parasitic species into the islands by exotic arthropods [612 introduced species and 66 invasive species. (Arechavaleta *et al.,* 2010)], an evaluation of current invertebrate parasites present on the island is much needed.

This study aims to contribute to baseline data for studies on invertebrate parasites in Macaronesia, their dissemination hosts as well as documenting the oocysts found.

Materials and methods

Between 2016 and 2019, faecal samples from various vertebrate animal species from Gran Canaria were analysed at the Laboratory of Parasitology, Faculty of Veterinary Sciences of the University of Las Palmas de Gran Canaria.

Faecal samples from cats were obtained from live animals during a larger study of feral cat colonies from across the island and donated from neutering release campaigns. For the remaining animals, the faeces were collected during *post-mortem* examination of fresh or frozen carcasses. The animals were obtained from the Tafira Wildlife Recovery Centre (naturally dead hedgehogs and birds) or Gestion y Planeamiento Territorial y Medioambiental (GesPlan) who conduct the eradication programme of invasive California kingsnakes (*Lampropeltis californiae*) in Gran Canaria. The samples from dogs were obtained during *post-mortem* examination of animals from the local animal shelter (Albergue insular de animales, Arucas) during practical classes in the Veterinary Faculty.

For species others than dogs and cats, all the collected faeces were used for concentration methods. For small amounts of sample, a minimum quantity of 0.5 mL of faeces were placed in each of three microcentrifuge tubes for processing. Samples with less than 0.5m L were discarded. For cats and dogs an average of 1.5 ***g*** of faeces were used for each concentration test. All faecal samples were tested for parasites using flotation in saturated sodium chloride solution (density 1.2 g mL^{-1}), zinc sulphate centrifugal flotation (density 1.18 g mL^{-1}) and formol-ether concentration method (7 parts of 10% formalin, 3 parts of pure diethyl-ether) (Willis, 1921; Faust *et al.,* 1938; Zajac and Conboy, 2012). Proper parasites and pseudoparasites were recorded.

The identification was carried by using the available references for pseudoparasitic elements in vertebrate faeces (Parker and Duszynski, 1986; Berto *et al.,* 2008; Lopes *et al.,* 2013; De Quadros *et al.,* 2017).

From each positive sample, oocysts were measured using a calibrated microscope (Leitz Laborlux S).

Results

In all, 476 faecal samples from 298 feral cats, 117 California kingsnakes, 10 Algerian hedgehogs (*Atelerix algirus caniculus*), 15 feral dogs and 36 birds from seven species were examined. Of these birds, many were species endemic to Macaronesia (M) or subspecies endemic to the Canary Islands (C) and included 10 *Turdus merula,* 9 *Falco tinnunculus canariensis* (C), 8 *Asio otus canariensis* (C), 3 *Passer hispaniolensis,* 3 *Serinus canaria* (M), 2 *Apus unicolor* (M) and 1 *Gallinula chloropus.*

Of the 476 samples, just four contained round to slightly ellipsoidal oocysts containing more than 4 (6–16) round sporocysts, consistent with the definition of the genus *Adelina.* These positive samples were from one cat, from the municipality of La Aldea de San Nicolás, in the west of the island; and three snakes from the municipality of Telde in the east giving a total *Adelina* spp. oocyst prevalence of 0.8% (4/476) across all samples, and 0.3% (1/298) and 2.6% (3/117) of feral cat and snake samples respectively. Measurements of oocysts and sporocysts in from each species are presented in Table 2 and compared with the other *Adelina* species described in the literature (Purrini, 1984).

Based on the size of the oocysts and sporocysts, the coccidia in the cat faeces resembled *Adelina picei* (two oocysts) (Fig. 1A), but the number of sporocysts found in these specimens was 6–8, while that described for *A. picei* is 8–18.

The coccidia from snake no. 1 (three oocysts) (Fig. 1B), were considered to be *Adelina tribolii*-like species, as the measurements and morphology (41 × 28–29 μm oocysts, slightly ellipsoidal 11 × 10–11 μm sporocysts, 8–9 sporocysts per oocyst) fell within the ranges of *A. tribolii* [26–50 × 22–36 μm oocysts, round sporocysts 10.4 μm and 2–24 sporocysts per oocyst (Purrini, 1984)]. In the faeces from snake no. 2 (two oocysts) (Fig. 1C), the coccidia most closely resembled *A. tribolii* based on the size of the oocysts and the number of sporocysts. Finally, the coccidia found in the faeces of snake no. 3 (two oocysts) (Fig. 1D) are possibly the same species as in snake no. 1 i.e. *A. tribolii*-like oocysts, but with slightly bigger sporocysts.

Discussion

In a diagnostic laboratory, pseudoparasitic elements, as well as pollen grains, fungal spores and yeasts, dust mite eggs and even

Table 2. Measurements of the stages of the parasite are given [meront (**M**), macrogametocyte (**Ma**), microgametocyte (**Mi**), and oocyst (**O**)], to summarize and facilitate the identification of future *Adelina* spp. in histological sections, fresh invertebrate tissues or as pseudoparasites in faeces

			Measurements in micrometres					NS	Author
Parasite	Host	Tissue	M	Ma	Mi	O	S		
A. acarinae	*Nothrus silvestris*	Body cavity	–	–	–	15–25	7–7.5	8–12	Purrini (1984)
A. castana	*Tribolium castaneum*	Body cavity	18–30 × 13–18	13–33 × 6.5–30	8.5–11.5 × 4–10	29.3 × 25.4	8.2	4–12	Ghosh *et al.* (2000)
A. collembolae	*Neanura muscorum*	Body cavity	–	–	–	40	7.5–8	24	Purrini (1984)
A. cryptocerci	*Cryptocercus punctulatus*	Different tissues	11 × 20	20 × 51	2.5–3	46–51 × 24–28	10–12	5–21	Yarwood (1937); Purrini (1984)
A. deronis	*Dero limosa*	Body cavity	25	19 × 17	7–9	17–21	9	8–16	Hauschka and Pennypacker (1942); Purrini (1984)
A. grylli	*Gryllus bimaculatus*	Body cavity	25.6 × 16.4	24.5 × 18.	3.5 × 2.45	32.5–36.3 × 24.7–30.1	9.9–13.3	4–22	Butaeva (1996)
A. melolonthae	*Melolontha melolontha*	Body cavity	18–22 × 11–14	30–50	10–11 × 6–7	30–35	11	6–14	Tuzet *et al.* (1965); Purrini (1984)
A. octospora	*Slavina appendiculata*	Body cavity	18–20	23 × 20	15 × 6	19–20	5–9	8	Hesse (1911); Purrini (1984)
A. palori	*Palorus ratzeburgii*	Body cavity	16.5–21.5 × 8–15	15–30 × 10–21	6–8	30.3 × 24.6	8	4–12	Ghosh *et al.* (2000)
A. picei	*Alphitobius picetus*	Body cavity	18–25 × 11.5–16.5	21–31 × 13–25	6.5–10	33.9 × 29.9	8.5	8–18	Ghosh *et al.* (2000)
A. sericesthis	*Sericesthis pruinose*	Body cavity	15–20 × 12–16	30–40	–	30–40	12–15	4–8	Weiser and Beard (1959); Purrini (1984)
A. tenebrionis	*Tenebrio molitor*	Body cavity	10–16	25	10	–	10–12	2–12	Sautet (1930); Purrini (1984)
A. transita	*Embia solieri*	Body cavity	30	30–40	8	30–40	10–11	6–20	Léger (1904); Purrini (1984)
A. tribolii	*Tribolium div.* sp.	Different tissues	15–30 × 6–20	21–49 × 16–33	8–15	26–50 × 22–36	10 × 4	2–24	Bhatia (1937) Purrini (1984)
A. zonula	*Blaps mortisaga*	Fat body	15–27 × 2–15	30–40	2–4 × 8–11	–	–	8	Morrof (1907); Purrini, (1984)
A. akidum	*Olocrates abbreviatus*	Body cavity	–	–	–	30–40	10	12–20	Léger (1900); Purrini (1984)
Adelina sp. '*picei*-like'	Cat faeces	–	–	–	–	32–33 × 28–30	8–10	6–8	This paper
Adelina sp. '*tribolii*-like'	Snake faeces 1 and 3	–	–	–	–	39–41 × 28–31	10–13	6–9	This paper
Adelina sp. '*tribolii*-like'	Snake faeces 2	–	–	–	–	52–53 × 34–35	10–11	14–16	This paper

Adelina spp. described, but thus far un-named, have not been considered. All the measurements are in micrometres. S, sporocyst; NS, number of sporocysts. In the author column the first one is the original description, authors in brackets are the source of the description represented in this table. If only an author in brackets is cited, represent also the original description.

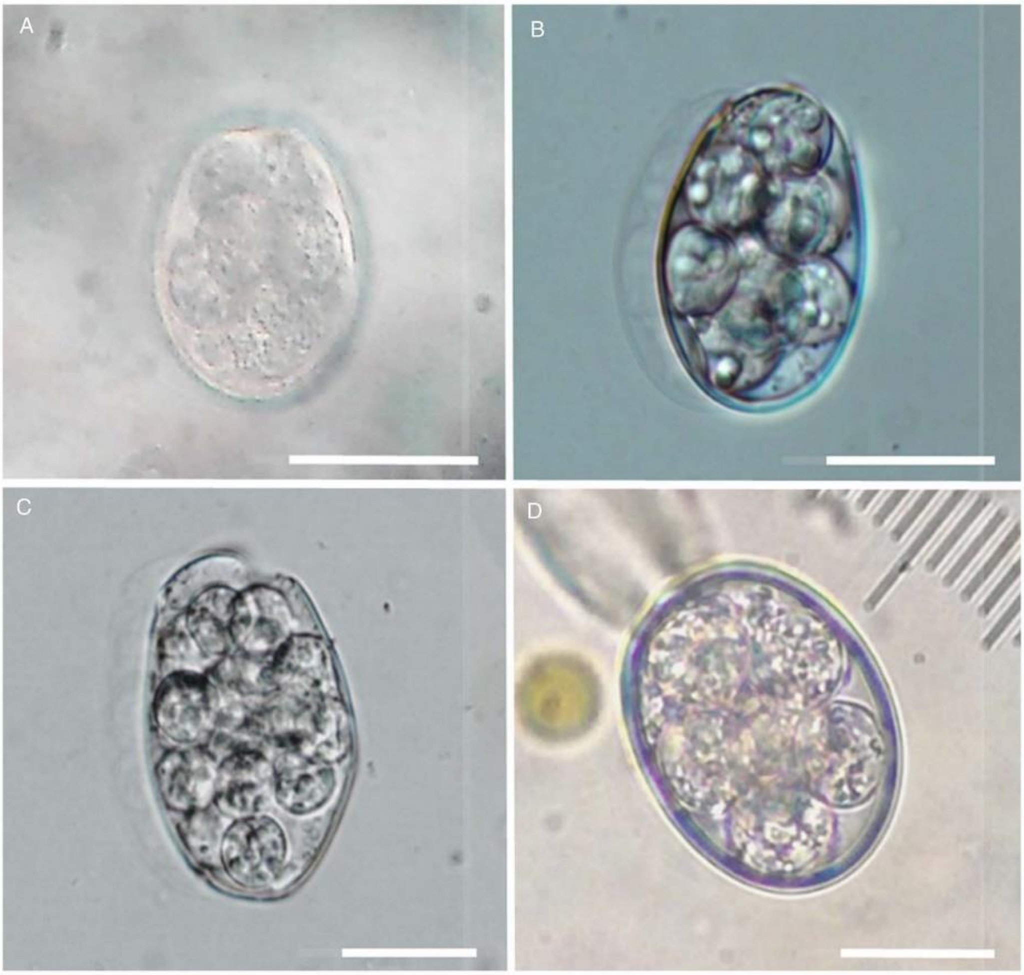

Fig. 1. Photomicrographs of sporulated *Adelina* spp. oocysts. (A) *A. picei* from a feral cat. (B) *A. tribolii* from snake 1. (C) *A. tribolii* from snake 2. (D) *A. tribolii* from snake 3. Scale bars = 20 μm.

fly larvae are usually present in faecal samples at the time of analysis. With experience, the technician can distinguish what is and what is not a parasitic element. However, in the case of carnivorous animals these pseudoparasitic elements could be parasites of their prey species. Frequently these prey parasites are disrupted and may appear 'dead', but in the case of *Adelina* the eggs survive inside the bowel of the predator (dispersion host) and are disseminated to the environment with the faeces, in the same way ingested plant seeds would also be dispersed.

The results of this study indicate the presence of at least two species of *Adelina* resembling *A. tribolii* and *A. picei* on the island of Gran Canaria. However, morphological measures of the oocysts are close to several reported species, but with potentially important differences in sporocyst numbers (Table 2). This fact may be important from the perspective of the identification of very similar species by molecular methods, considering the huge variation in *A. tribolii* sporocysts (from 2 to 24). This variation could be also explained by the process of sporulation, with two sporocysts being erroneously reported as mature oocysts, instead of 24, or the presence of several cryptic species. In addition, the lack of further ecological, morphological and molecular data from the actual definitive host, leave the speciation just presumptive at this stage.

California kingsnakes, unlike cats, are not known to eat invertebrates and thus the presence of adeleids in the faeces of a non-insectivorous snake could be explained through their regular prey on Gran Canaria: the Gran Canaria giant lizard (*Gallotia stehlini*), geckos (*Tarentola boettgeri*), skinks (*Chalcides sexlineatus*) and rodents (Monzón-Argüello *et al.*, 2015). These prey species usually consume arthropods and thus the oocysts may have originated from invertebrates within their gastrointestinal tract. In support of this theory is the finding, in the snake faeces, of other parasites from these prey reptile species such as eggshells of Pharyngodonidae oxiurids.

Despite all species in this study having a diet which includes insects, neither species of *Adelina* spp. was found. A possible explanation, given the low prevalence obtained from snakes and cats, could be the sample size of each species, as well as the scarcity of faeces in small animals. Furthermore, the accurate diet composition of the other species of the study could also influence the species of *Adelina* to be found e.g. swifts (*Apus* spp.) prey on tiny flying insects caught on the wing which may not contain *Adelina* spp.. Previous studies on wild invertebrates demonstrate a prevalence of *Adelina* spp. between 3 and 27% (Merritt *et al.*, 1975; El-Sufty and Boraei, 1986, 1989). What is not clear is if the low prevalence studies can be explained by selection failure of the sampled arthropods, due to death of infected immature stages. Considering the wide prevalence variation reported in other studies, it is not clear if the low figure of 0.8% in this study, is truly representative of the overall prevalence of *Adelina* in Gran Canaria. These two vertebrate species (cats and snakes) could amplify the number of oocysts in faeces by consuming more prey such as geckoes, serving as sentinel species for *Adelina* spp. surveys. Further studies are required to more accurately determine the prevalence of *Adelina* within definitive and other dispersion hosts.

Although data are scarce, Adeleid coccidia could be considered important ecosystem 'regulators', causing death of various arthropod species (Table 1). Under laboratory conditions, 20% fewer larval stages are reported *vs* non-infected insects, demonstrating how insect populations, can be influenced by these parasites (Park and Frank, 1950). Insects which are resistant to *Adelina* spp. have a significant selective advantage over those which are non-resistant (Park and Frank, 1950; Lange and Lord, 2012). Without the selective pressure of the parasite, the non-resistant insects dominate over the resistant ones.

The presence of *Adelina* spp. in stool samples from vertebrates is important from an ecological point of view, as digestion by vertebrates is required to release the oocysts from the invertebrate tissues, and disseminate within their faeces (Parker and Duszynski, 1986; De Quadros *et al.,* 2017). This has been widely studied in other parts of the world with Adeleorid coccidia demonstrated in vertebrate faeces as pseudoparasites (Parker and Duszynski, 1986; Berto *et al.,* 2008; Lopes *et al.,* 2013; De Quadros *et al.,* 2017). Indeed, a genus of coccidia (*Pythonella* spp.) was erroneously described as a reptile parasite when it is actually a pseudoparasite (Kawazoe and Gouvêa, 1999; Ghimire, 2010).

Dispersion hosts, on occasion, travel long distances or even, in the case of migratory birds, may move from one country or region to another, disseminating their parasites to their new habitat. This phenomenon has been widely demonstrated in ticks, with tick-borne diseases being carried from one country to another (Hasle, 2013). Furthermore, novel parasites introduced by these dispersion hosts or by exotic/invasive invertebrates may cause more significant disease in naïve invertebrate hosts than the natural infected host populations (Kelehear and Jones, 2010; Bacela-Spychalska *et al.,* 2012; Martín-Torrijos *et al.,* 2017). However, host specificity and thus the real impact of *Adelina* spp. in natural invertebrate populations, compared with laboratory populations, is not currently understood. Neither co-invasion nor host switch in natural insect populations infected with *Adelina* spp. has been reported in the literature, thus, further research is needed. Indeed, Gran Canaria, with its huge invertebrate diversity could be considered an ideal model island system to study this and other invertebrate parasites, starting with morphological and molecular surveys, and promotion of conservation programmes.

In general terms, coccidian parasites, including *Adelina* spp., are very host specific, affecting mostly animals from the same genus. *Adelina tribolii* has been described in three species of flour beetles (*Tribolium* spp.) (Table 1) (Park and Frank, 1950), a genus of beetle from the family Tenebrionidae. Based on this, *A. tribolii-like* records from Gran Canaria are most-likely parasites of a *Tribolium* sp., possibly the invasive species red flour beetle (*T. castaneum*) or confused flour beetle (*T. confusum*) which are the only known species recorded on the island. The other putative species recorded in this study, *Adelina picei* has been reported parasitizing *Alphitobius* sp., another tenebrionid beetle. Considering host specificity related to the genus of the host, for *Adelina picei* another two beetle species could be suitable hosts in Gran Canaria: the introduced lesser mealworm (*A. diaperinus*) and the black fungus beetle (*A. laevigatus).*

The definitive host species of the *Adelina* pseudoparasites remains unknown, however cats are known to consume Tenebrionid beetles often in feral life, unlike *L. californiae* (Medina and Nogales, 2009; Monzón-Argüello *et al.,* 2015; Gallo-Barneto *et al.,* 2016). Based on this data, *Adelina* could be present in Tenebrionids, of which several species are endemic and endangered (Arechavaleta *et al.,* 2010). Further sampling would be needed, in conjunction with molecular work, to address the accurate epidemiology of this parasite in Gran Canaria and other parts of the world.

Conclusions

Despite a low prevalence, these findings constitute the first baseline data for invertebrate pathology studies in the Canary Islands. Further epidemiological research on invertebrate parasites in these islands would be necessary to determine the invertebrate hosts, native or exotic, and the real epidemiological importance of insectivorous animals in the life cycle of *Adelina* spp. The further understanding of the role of this protozoan in invertebrate population dynamics is particularly important in an island setting where the vast majority of fauna is native/endemic and/or endangered. The Canaries, and other similar islands, could be utilized as model systems for arthropod parasites. Using morphological measures, the oocysts described here are close to several reported species, but with potentially important differences in sporocyst numbers. Further material should be studied to determine its accurate taxonomical status, considering the morphological variability of *A. tribolii.* With the appropriate molecular sampling of Adeleids within invertebrates, the vertebrate species studied here could be useful as sentinels for further research on *Adelina* spp. in the Canary Islands and further afield.

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Author contribution. Kevin M. Santana-Hernández and Eligia Rodríguez-Ponce conceived and designed the study. Kevin M. Santana-Hernández and Eligia Rodríguez-Ponce conducted data gathering. Kevin M. Santana-Hernández, Simon L. Priestnall, David Modrý and Eligia Rodríguez-Ponce wrote the article.

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Ethical standards. Not applicable.

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Paper 3:

Sarcocystis sp. infection (Apicomplexa: Sarcocystidae) in invasive California kingsnake *Lampropeltis californiae* (Serpentes: Colubridae) in Gran Canaria.

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Sarcocystis sp. infection (Apicomplexa: Sarcocystidae) in invasive California kingsnake *Lampropeltis californiae* (Serpentes: Colubridae) in Gran Canaria

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Abstract

Invasive species pose a threat not only to biodiversity because they displace or compete with native fauna, but also because of the pathogens they can host. The Canary Islands are an Atlantic biodiversity hotspot threatened by increasing numbers of invasive species, including the California kingsnake *Lampropeltis californiae*, which was recently introduced to Gran Canaria. Seventy-seven snakes were examined for gastrointestinal parasites in 2019–2020. Sporocysts of *Sarcocystis* sp. were detected in 10 of them; detection of gamogonia stages in histological sections of 3 snakes confirmed the snake as a definitive host. Partial ssrDNA was amplified using SarcoFext/SarcoRext primers; an additional sequence of *Sarcocystis* was obtained from the tail muscle of the endemic Gran Canaria giant lizard *Gallotia stehlini* for a comparison. Identical ssrDNA sequences of unknown *Sarcocystis* sp. were obtained from 5 different snakes. Phylogenetic analysis showed that *Sarcocystis* sp. isolated from invasive California kingsnakes is unrelated to *Sarcocystis* provisionally considered *S. stehlini* from the endemic lizard. The dixenous coccidia are rarely reported to invade new predator–prey systems. However, the present data suggest that previously unknown *Sarcocystis* sp. is circulating among invasive snakes and as yet unknown vertebrate intermediate hosts, with undetermined consequences for the Gran Canaria ecosystem.

Introduction

The Canary Islands are characterized by a great diversity of endemic reptiles, which constitute a large part of the terrestrial vertebrate fauna of the archipelago (Arechavaleta *et al.*, 2010). Like other volcanic islands, the Canary Islands were colonized by reptiles through a series of long-distance dispersal events, in this case from mainland Africa (Illera *et al.*, 2016). Three saurian genera, *Gallotia*, *Chalcides* and *Tarentola*, belonging to the families Lacertidae, Scincidae and Geckonidae, respectively, have diversified into 15 extant species *via* processes of adaptive radiation (Brown and Pestano, 1998; Carranza *et al.*, 2000; Cox *et al.*, 2010).

Invasive species threaten biodiversity not only through predation or competition with native fauna, but also through the pathogens they can transmit. Emerging infectious diseases are an important phenomenon underlying biological invasions, and oceanic islands are more vulnerable to biological invasions than continental ecosystems (Gurevitch and Padilla, 2004; Carroll, 2007). One invasive species in the Canary Islands stands out among the others and probably represents the greatest threat to the endemic vertebrates of the island of Gran Canaria: the California kingsnake, *Lampropeltis californiae* Blainville, 1835. The first observations of this North American colubrid were reported in southeastern Gran Canaria in 1998 (Pether and Mateo, 2007), but the eradication programme was not launched until 2007. Surveys in the following years revealed a large population that had expanded to the adjacent central and higher areas of the island, forming the so-called 'main nucleus' (NC1, Fig. 1). In 2010, the presence of a secondary nucleus (NC2) was confirmed in the northwest of the island (Gallo-Barneto *et al.*, 2016). Subsequent phenotypic and molecular studies suggested that the kingsnakes in these 2 nuclei originated from 2 different introductions (Monzón-Argüello *et al.*, 2015). Despite the eradication programme, the establishment of a small population was also confirmed in the south of the island (the third nucleus, NC3) and in the northeast (the fourth nucleus, NC4). In all cases, populations of *L. californiae* occur in habitats that are considered Special Conservation Area (BOC no. 60, 15 May 2000) and are inhabited by a variety of endemic species.

Dietary studies have confirmed the high proportion of endemic reptiles in the diet of snakes (69%), highlighting the Gran Canaria giant lizard *Gallotia stehlini* as the main component (43%), followed by the Gran Canaria skink *Chalcides sexlineatus* (23%) and the Boettger's

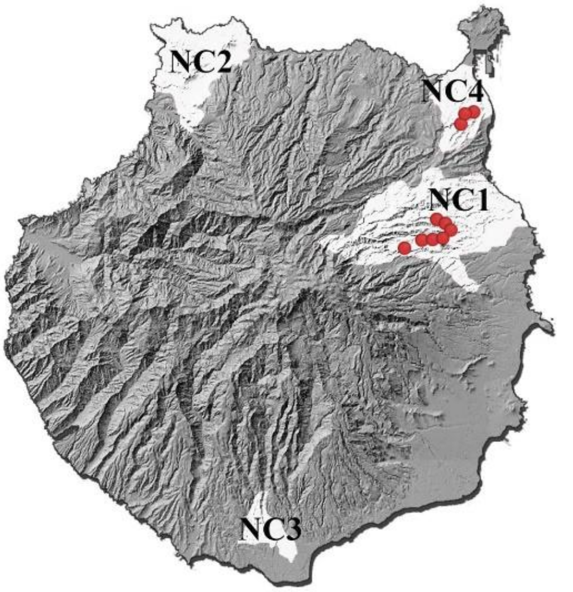

Fig. 1. A map of Gran Canaria island showing 4 populations of *Lampropeltis californiae* as white-shaded areas (NC1–NC4); the place of collection of 10 snakes positive for *Sarcocystis* sp. based on coproscopic examination marked by red dots. NC1: Main nucleus. NC2: Secondary nucleus. NC3: Third nucleus. NC4: Fourth nucleus.

wall gecko *Tarentola boettgeri* (3%) (Cabrera-Pérez *et al.*, 2012; Monzón-Argüello *et al.*, 2015). Small mammals (29.5%) and birds (1.5%) have also been detected in the digestive system of snakes. In addition to direct effects by predation on endemic fauna, possible co-invasive metazoan parasites infecting the snake have been detected, as well as ‘reverse’ infection with larval stages of parasites of feral cats and other vertebrates (Santana-Hernández *et al.*, 2021).

Well-described evolutionary processes and the diversity of reptiles make the Canary Islands an interesting model site that has stimulated several parasitological studies involving both protists and helminths (Roca *et al.*, 2012; Jorge *et al.*, 2018). Among protistan parasites with heteroxenous life cycles, 2 groups have recently gained attention – haemogregarines and *Sarcocystis* (e.g. Matuschka and Bannert, 1989; Bannert, 1992; Tomé *et al.*, 2018, 2019; Illera Cobo and Perera, 2020). The latter genus is characterized by a 2-host life cycle, that typically involves predators and their prey (in the case of reptiles, commonly colubrid snakes and lacertid or geckonid lizards). However, *Sarcocystis* species described in Canary Island lizards of the genus *Gallotia* are particularly adapted to transmission in an environment where there are no predators. In their life cycle (usually referred to as dihomoxenous), merogony and gamogony occur in the same host species (in this case *Gallotia* lizards), which serves alternately as the final and intermediate host. Infection is transmitted among lizards *via* sporocysts in feces and cannibalism (Matuschka and Bannert, 1987, 1989).

The introduction of an invasive snake predator into a Macaronesian ecosystem inhabited by endemic saurian reptiles provides an unintended ‘experimental’ system to evaluate the host specificity of *Sarcocystis* spp. in, and its potential interactions with, the invasive kingsnake *L. californiae*.

Material and methods

Reptiles collected, examination

In the period 2019–2020, samples of California kingsnakes were analysed. The snakes were captured by the staff of Gestión y planeamiento territorial y ambiental (GesPlan) manually and using box-traps in the framework of the eradication project (https://www.gesplan.es/content/orden-33620-que-modifica-la-n%C2%BA-12419-ejecucion-plan-post-life-lampropeltis-y-actuaciones-del). The locality data, colour pattern, sex, length and weight of each animal (Table 1) were collected. Captured snakes were euthanized with intracardiac premedication of mixed ketamine and medetomidine (5–10 mg kg^{-1} and 0.15–0.5 mg kg^{-1}), and a lethal dose (0.5–1 mL kg^{-1}) of pentobarbital by the veterinarians from the eradication programme.

Table 1. Distribution of snakes regarding nuclei, the municipalities which encompasses and positive/negative to *Sarcocystis* sp.

Nuclei (snakes examined)	Municipality	Positive (males/ females)	Total (males/ females)
1st (61)	Telde	6 (4/2)	52 (20/32)
	Valsequillo	1 (1/0)	8 (6/2)
	Santa Brígida	0	1 (1/0)
2nd (3)	Gáldar	0	2 (0/2)
	Agaete	0	1 (0/1)
3rd (5)	San Bartolomé	0	5 (0/5)
4th (8)	Las Palmas de Gran Canaria	3 (2/1)	8 (4/4)
Total	8 Municipalities	10 (7/3)	77(31/46)

The feces collected at the time of euthanasia was kept in sterile containers, transported in a refrigerator (3 °C) and examined within the following 12 h in the Parasitology Laboratory of the Faculty of Veterinary Sciences of the University of Las Palmas de Gran Canaria. The fecal samples were examined microscopically after centrifugal flotation with Sheather’s sugar solution (Zajac and Conboy, 2012), using a Nikon Eclipse Ni-U microscope with a Nikon Ds-Fi2 camera.

The small intestines of euthanized snakes were extracted and fixed in ethanol and 10% buffered formalin (Farris *et al.*, 2013). After coproscopic examination, the small intestine samples that were positive for *Sarcocystis* sp. (i.e. in which sporocysts/ocysts were detected by microscopy) were further processed. Specimens preserved in formalin were processed for standard histopathological examination and stained with haematoxylin and eosin (H&E). Sections were examined with Olympus BX53 light microscope and photographed with Olympus DP73 camera and Olympus Dimension CellSens imaging software. As comparative material, 2 Gran Canaria giant lizards *Gallotia stehlini* Schenkel, 1901 were received dead from the Wildlife Recovery Centre of Tafira, 1 from the municipality of San Mateo and 1 from Arucas. The tail muscles were microscopically examined as squash preparations for the presence of *Sarcocystis* tissue cysts and preserved in ethanol.

DNA isolation, PCR and sequencing

A total of 7 snake fecal samples, 3 snake gut tissue samples, and 2 lizard tail muscle tissue samples, all stored in ethanol, were used for DNA isolation. Genomic DNA was isolated using the GeneAll Exgene™ Stool DNA mini kit for fecal samples and the NucleoSpin® Tissue kit (Macherey-Nagel) for tissue samples according to the manufacturer’s instructions. The ssrRNA gene fragment was amplified using SarcoFext (5′-GGTGATTCATAGTAACCGAACG-3′)/SarcoRext (5′-GATTTCTCATAAGGTGCAGGAG-3′) primers (Moré *et al.*, 2013). To increase PCR sensitivity in isolates number 8 and 14, the following nested-PCR protocol was developed. A total volume of 25 μL PCR reaction mixture consisted of 12.5 μL PRCBIO Taq Mix Red Mastermix (PCR Biosystems Ltd.), 8.5 μL PCR H_2O, 1 μL of each primer and 2 μL extracted DNA. Amplification began with an initial denaturation at 95 °C for 1 min, followed by 40 cycles of 95 °C (15 s), 57 °C (15 s), and 72 °C (15 s), and ended with a final elongation at 72 °C for 5 min. The primers used in the first run were SarcoFext/SarcoRext (see above) and in the second run SarcoFint (5′-CGCAAATTACCCAATCCTGA-3′)/SarcoRint (5′-ATCGTCTTCGAGCCCCTAAC-3′) (Moré *et al.*, 2013). PCR products positive in gel electrophoresis (1% agarose gel) were purified using the Gel/PCR DNA Fragments Extraction Kit (Geneaid Biotech Ltd.) and sequenced by Macrogen Europe B.V.

Phylogenetic and statistical analyses

The sequences obtained were processed using Geneious Prime® 2020.2.5 software (Biomatters Ltd.). The following sequences from GenBank (accession numbers in parentheses) were selected for comparison in phylogenetic analysis: *Sarcocystis* sp. (KX453662), *S. lacertae* (AY015113), *S. gallotiae* (AY015112), *S. muris* (KC877996), *S. rodentifelis* (AY015111), *Frenkelia microti* (AF009244), *F. glareoli* (AF009245), *S. jamaicensis* (KY994649), *S. speeri* (KT207459), *S. neurona* (U07812), *S. ramphastosi* (EU263366), *S. falcatula* (MH626537), *S. lari* (MF946588), *S. rileyi* (KJ396583), *Sarcocystis* sp. (KX833709) and *Sarcocystis* sp. (KX453661). *Sarcocystis* sp. (U97524) and *S. atheridis* (AF120114) as outgroup. Phylogenetic analysis was performed using the maximum likelihood method of the IQ-TREE web server tool (Nguyen *et al.*, 2015) with ultrafast bootstrap (Minh *et al.*, 2013). The phylogenetic tree was visualized and processed using FigTree v1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/).

Basic statistical analysis was performed using SPSS v. 24.0 (IBM SPSS Corp., Chicago, Illinois, USA). The map was created using My map app by Google and GIMP 2.10 (GNU Image Manipulation program by Spencer Kimball, Peter Mattis and GIMP staff).

Table 2. Biometrical parameters of positive and negative snakes to *Sarcocystis* sp.

		Positive	Negative	Total
Males	Length	830.0 ± 214.0	967.6 ± 176.0	936.5 ± 190.6
	Weight	203.3 ± 121.8	289.7 ± 138.1	270 ± 137.6
	n	7	24	31
Females	Length	853.3 ± 134.3	877.1 ± 154.7	875.6 ± 152.3
	Weight	198.3 ± 134.3	242.6 ± 142.7	239.7 ± 136.7
	n	3	43	46
Total	Length	837.0 ± 186.2	909.5 ± 167.2	900.1 ± 170.2
	Weight	201.8 ± 108.4	259.5 ± 140.0	252.0 ± 137.0
	n	10	67	77

All the measurements for length and weight are in millimetres and grams.

Results

A total of 77 kingsnakes and 2 lizards were examined for the presence of *Sarcocystis* sp. during the study period. Snakes from 7

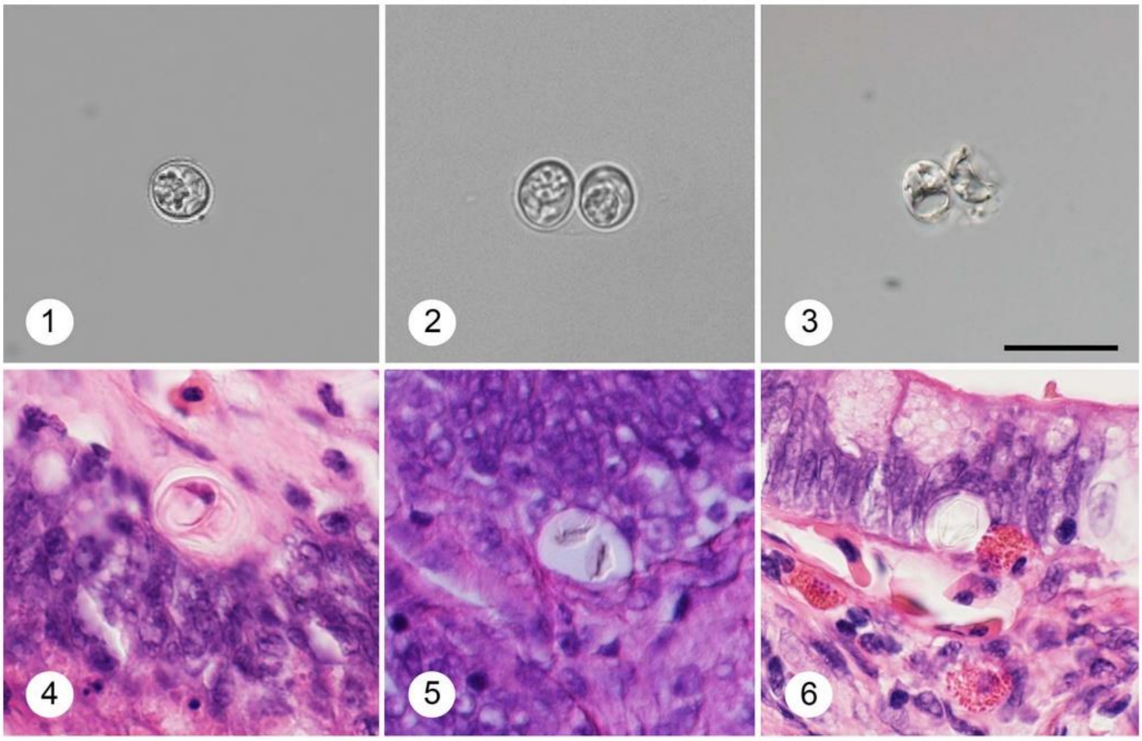

Fig. 2. Developmetal stages of *Sarcocystis* sp. in feces after flotation (1–3) and in intestinal tissue (4 and 5) stained with H&E. 1: Isolated sporocyst with apparent sporocyst residuum; 2: intact oocyst with apparent oocyst wall; 3: oocysts deformed by preservation; 4: *in situ* sporulated oocyst with apparent sporozoites; 5 and 6: cross-section through oocyst in parasitophorous vacuole showing the sporocyst wall. All figures in same scale, scale bar = 20 μm, figs 3 and 5 photographed with Nomarski differential contrast.

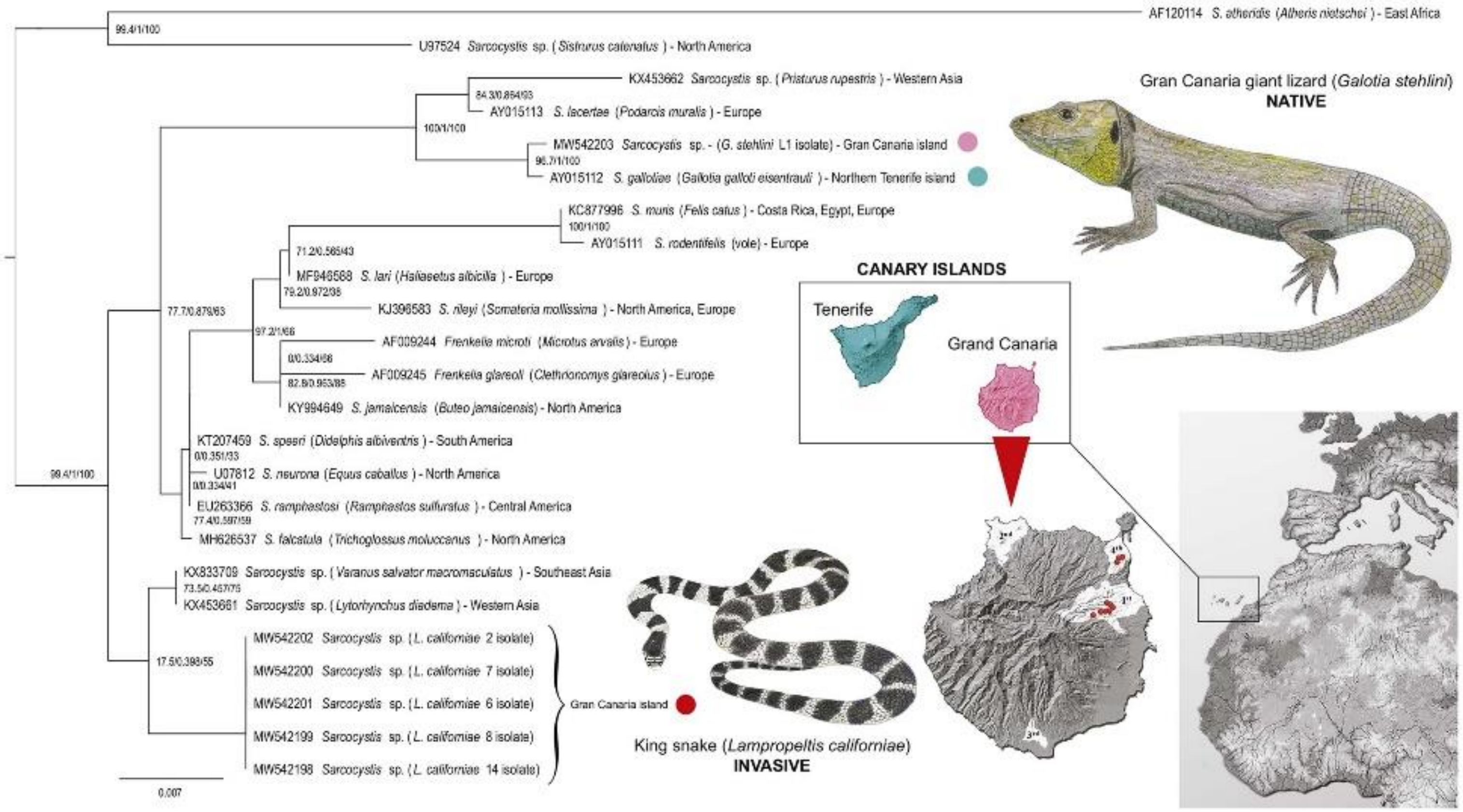

Fig. 3. Phylogenetic analysis of the partial ssrDNA sequences showing position of the 2 taxa discussed. The sequences used for comparison were chosen from a phylogenetic tree containing all GenBank accessible ssrDNA sequences of *Sarcocystis* spp. (data not shown) and the current tree consists of the sequences most related to those extracted from *L. californiae* and *G. stehlini*, rooted on *S. atheridis* (AF120114) and *Sarcocystis* sp. (U97524). Geographical origin was added to each selected sequence.

different localities comprising the 4 nuclei were examined (Fig. 1). Seventy-eight per cent of the snakes were from the main nucleus. Only individuals from the main nucleus (Telde and Valsequillo) and the fourth nucleus (Las Palmas de Gran Canaria) were positive for the presence of *Sarcocystis* sp. in feces (Table 1). The average biometric parameters of the snakes studied are summarized in Table 2. A subtle difference was observed between positive (shorter and lighter) and negative animals.

Coproscopy and histopathology

Oocysts and sporocysts of *Sarcocystis* sp. were detected by microscopy in the feces of 10 of the 77 snakes (Fig. 2). Liberated sporocysts (Fig. 2.1) were more common, although both types of exogenous stages were present in very low numbers. Sporocysts were broadly ellipsoidal, 11.5–12.5 × 9.7–10.3 ($n = 5$), with granular sporocyst residuum and 4 readily identifiable sporozoites.

Scattered developmental stages of gamogony of *Sarcocystis* sp. were observed in small intestinal sections of 3 California kingsnakes. The *in situ* sporulated oocysts were singly localized between enterocytes at the border of the lamina propria mucosae (Figs 2.4–2.6). No other developmental stages were seen.

Sequencing and phylogenetic analyses

Partial ssrRNA gene sequences of *Sarcocystis* sp. were obtained from 5 different California kingsnakes (2 from fecal sediment and 3 from small intestine tissue of snakes that were positive in histopathological sections) and from the tail muscle of a single Gran Canaria giant lizard. The sequences derived from the snakes were identical and 532–811 bp long (short sequences of 532 bp were from isolates processed by nested-PCR); the sequence from the tail muscle of the lizard was 785 bp long. The sequences obtained are deposited in GenBank under the following accession numbers: MW542198–MW542202 (from snakes) and MW542203 (from a lizard). The sequences obtained from the snakes and the lizard had 95.9% similarity. Phylogenetic analysis revealed that *Sarcocystis* sp. from the California kingsnakes in this study was most closely related to *Sarcocystis* sp. isolated from the crowned leaf-nosed snake (*Lytorhynchus diadema* – KX453661) and the common water monitor (*Varanus salvator macromaculatus* – KX833709), with pairwise similarity of 99.7% and 99.3%, respectively. *Sarcocystis* sp. isolated from the Gran Canaria giant lizard branched with *S. gallotiae* (AY015112) from *G. galloti* from Tenerife, with 99.7% similarity (Fig. 3).

Discussion

The finding of a *Sarcocystis* species in a population of invasive North American colubrid snakes recently introduced to Gran Canaria was unexpected, as the dixenous coccidia rarely invade new predator–prey systems. All the positive animals were found at the east nuclei of the island (main nucleus = 7/61 and fourth nucleus = 3/8). However, considering the huge difference of sample size between the nuclei no further statistical analyses were considered.

Three *Sarcocystis* spp. were previously described in endemic *Gallotia* lizards of Canary Islands, all of which have a dihomoxenous life cycle (Matuschka and Bannert, 1987, 1989; Bannert, 1992). In a previous phylogenetic study, *Sarcocystis gallotiae* was found to branch with Old World *Sarcocystis* species with a snake–lizard life cycle (Šlapeta *et al.*, 2001). Since *Gallotia* lizards evolved from North African ancestors (Cox *et al.*, 2010; Illera *et al.*, 2016), it is likely that the ancestral *Sarcocystis* had cycled between African lacertids and snakes. The DNA sequences of *Sarcocystis* that we obtained from invasive snakes and *Sarcocystis* from an endemic lizard *G. stehlini* differed significantly and branched into distant clades. On the contrary, the lizard-derived sequence was very close to *S. gallotiae* (>99 identity), which was previously isolated from *G. galloti* from Tenerife (Šlapeta *et al.*, 2001).

It is very likely that the *Sarcocystis* from the California kingsnakes described here is a snake-specific species that probably invaded the Macaronesian ecosystem along with its snake host. *Sarcocystis* species using reptiles as definitive hosts exhibit host specificity restricted to their host genus or family and are most likely unable to switch between hosts from different reptilian orders (Box and Smith, 1982; Lindsay *et al.*, 1992).

Small mammals (such as rodents) are the most common intermediate hosts for *Sarcocystis* species that have a snake as a definitive host. However, some *Sarcocystis* spp. have been described to cycle between snakes and saurian reptilian hosts (Volf *et al.*, 1999; Modrý *et al.*, 2000). The intermediate host of *Sarcocystis* sp. found in the kingsnakes of Gran Canaria is still unknown and further research should be directed towards the study of native (snakes, skins, geckos, passerine birds) or invasive (mice and rats) vertebrates, which inhabit in all nuclei.

The exact origin of the presumably invasive *Sarcocystis* species remains unknown, as does the origin of the introduced California kingsnakes themselves. Maintenance of *Sarcocystis* infection in captive-bred snakes is unlikely because they cannot sustain a 2 host life cycles. On the contrary, the presence of patent *Sarcocystis* spp. infection in captive-bred snakes strongly suggests that they are wild-caught (Moré *et al.*, 2014). In this sense, the presence of *Sarcocystis* sp. in invasive kingsnakes in Gran Canaria suggests that the population was established rather by individuals introduced from wild populations in the southwestern United States of northern Mexico than by captive-borne pet snakes.

Clarification of the life cycle of the snake sarcosporidian found in the Gran Canaria is necessary to answer questions related to possible impacts on the endangered endemic vertebrate fauna of Macaronesia.

Data. Data are available under reasonable requests.

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Author contributions. David Modrý conceived and designed the study. All authors conducted data gathering and wrote the article.

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Conflict of interest. The authors declare there are no conflicts of interest.

Ethical standards. Not applicable.

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Small islands as potential model ecosystems for parasitology: climatic influence on parasites of feral cats

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Abstract

The influence of climate on parasite distribution has been demonstrated in different regions worldwide. Despite its small size, Gran Canaria (Canary Islands, Spain) constitutes a 'biodiversity laboratory' due to the huge climatic differences between municipalities. Feral cats may represent a threat to biodiversity due to their predatory behaviour. In addition, they may be a source of pathogens zoonotic to humans. To study the climatic/seasonal influence and prevalence of feral cat parasites throughout the island, a total of 290 stool samples from 29 feral cat colonies were analysed following standard concentration protocols (sodium chloride, formol-ether and zinc sulphate). In total, 13 feline parasitic taxa were found, with the most common species being *Ancylostoma* spp., which, together with *Toxocara* spp., *Toxoplasma gondii* and *Giardia* spp., are considered a concern for human health. Nematodes were the most common parasites in all areas. Nematodes and protozoans were significantly more prevalent in temperate mild (75.0% and 30.0%) than in dry desert areas (29.3% and 18.7%). In contrast, cestodes were significantly more prevalent in dry desert than in temperate mild areas (26.0% and 13.3%). Only protozoans exhibited statistically significant seasonal patterns, mostly in the wet season. Data reported in this study endorse the usage of small and diverse islands such as Gran Canaria to study the climatic influence on parasitic communities in wild/feral animals. Cat colonies require better management to reduce their threat to endemic wildlife, domestic animals and public health, being invasive species that harbour zoonotic parasites.

Introduction

Seasonal and climatic variations have an important influence on vertebrate parasitic diseases worldwide (Elmaleck, 2015; Rondón *et al.*, 2017; Short *et al.*, 2017), including those affecting domestic carnivores like cats (Okoye *et al.*, 2013; Beugnet *et al.*, 2014). This climatic influence enables the transmission and maintenance of parasitic species in certain places or even restricts their spread to other regions in the same country (i.e. schistosomiasis in China), as clearly demonstrated in large continental areas, even between neighbouring countries (Martens, 1999; Zhou *et al.*, 2008). Marked climatic differences are not only present in large countries, but they can also be found in islands such as Gran Canaria, in Spanish Macaronesia.

Located off the north-western coast of Africa, Gran Canaria is described as a 'miniature continent' due to its 20–21°C temperature at sea level and less than 12°C in highland areas all year round, and a striking difference between the rainy municipalities in the centre-north and very dry ones in the south. Therefore, islands like Gran Canaria could serve as low-scale model ecosystems for parasite epidemiology among different communities of animal species. These different conditions between municipalities may also influence the distribution of vertebrate parasites present in different parts of the island, which include those found in feral cat communities, some of which have zoonotic potential.

Spillover and the emergence of zoonotic disease outbreaks potentially occur when the human–wildlife interface becomes narrower, facilitating the contamination of domestic and peridomestic areas (Mackenstedt *et al.*, 2015). In this sense, feral cats clearly act as reservoirs for zoonotic parasites, posing not only a hazard to humans by cohabiting with suburban colonies, but also infecting domestic animals (Morand *et al.*, 2014).

Feral cats were introduced to Gran Canaria during the Spanish colonization of the island in the 14th century (Medina & Nogales, 2009), resulting in the establishment of several colonies and their spread to all municipalities of the island. On this island, especially in suburban and rural areas, it is rather common to allow domestic cats to freely enter and leave houses, making them prone to infection from local feral cats through direct or indirect contact. Furthermore, indirect contact implies the consumption of prey with infective stages of parasites, which could later result in an indoor source of zoonotic parasite infection (Morand *et al.*, 2014).

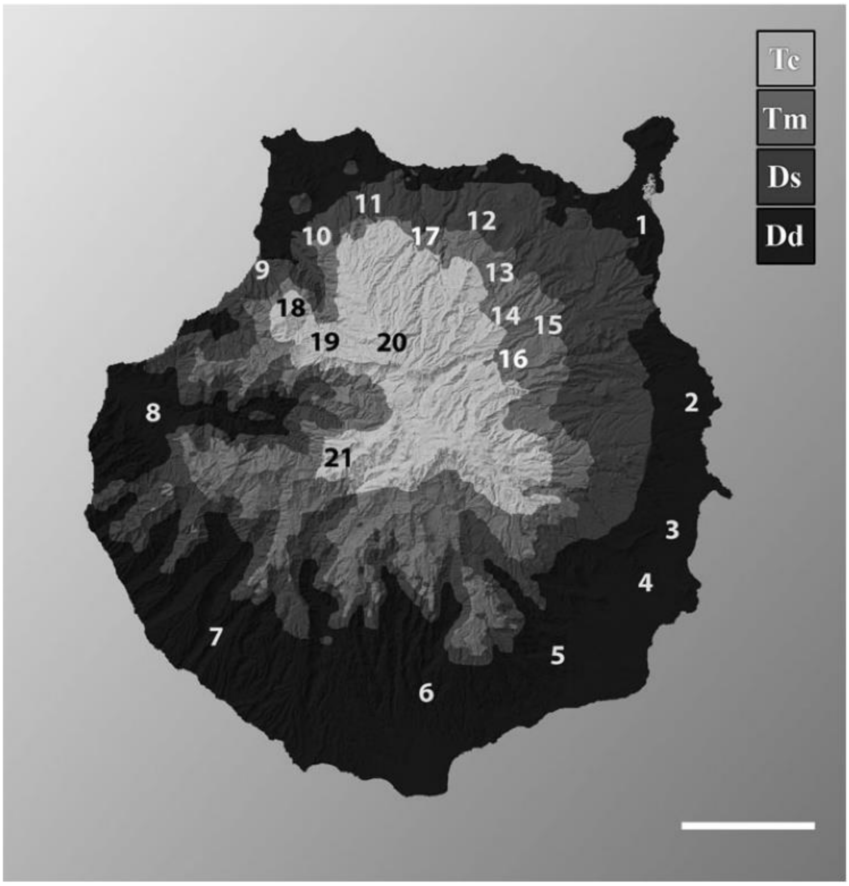

Fig. 1 Gran Canaria and its 21 municipalities. The greyscale represents the following isoclimatic zones: Dd, dry desert; Ds, dry steppe; Tm, temperate mild; Tc, temperate cold. *Municipalities tested twice each sampling row. 1: Las Palmas de Gran Canaria (capital). 2: Telde*. 3: Ingenio*. 4: Agüimes. 5: Santa Lucía*. 6: San Bartolomé de Tirajana*. 7: Mogán. 8: La Aldea de San Nicolás. 9: Agaete. 10: Gáldar. 11:Guía. 12: Firgas. 13: Arucas. 14: Teror. 15: Santa Brígida*. 16: Moya. 17: Valsequillo. 18: Artenara. 19: Valleseco. 20: San Mateo. 21: Tejeda. Scale bar = 10 km.

A neutering–releasing protocol is currently implemented to control these populations. The main objectives are to reduce the number of feral cats and their impact on endemic species, and prevent the public health risks (Rodríguez-Ponce *et al.*, 2016).

This study aims to deepen the epidemiology of feral cat parasites on Gran Canaria and investigate the influence of climatic conditions on their distribution, highlighting those with zoonotic potential. This research provides relevant information for use in cat colony control programmes.

Material and methods

Sampling and processing

Stool samples of randomly selected feral cats were analysed between January 2017 and May 2018. The cats lived in 29 colonies distributed throughout the island's 21 municipalities (fig. 1). The samples consisted of mixed faeces from at least three random cats, freshly deposited on soil. One sample was taken monthly from each municipality, except for Las Palmas de Gran Canaria, the island's capital, where four samples were taken, as this city has the largest number of inhabitants. In the municipalities with more than 30,000 inhabitants (Ingenio, Santa Lucía, San Bartolomé, Santa Brígida and Telde), two samples were taken.

Faeces were stored in dry sterile containers and sent to the Parasitology Laboratory of the Veterinary Faculty of Las Palmas de Gran Canaria University, where they were kept refrigerated until processing within 24–48 h. The European Scientific Counsel Companion Animal Parasites (ESCCAP) guidelines 2020 were considered to determine the techniques used, such as sodium chloride flotation (Carvalho *et al.*, 2012), formol-ether sedimentation (Ritchie, 1948) and zinc sulphate centrifugal flotation (Faust *et al.*, 1938). Larvae of lungworms were also tested using the Baerman–Wetzel method (Giannelli *et al.*, 2015).

Statistics and isoclimatic zones of Gran Canaria

All the statistics were processed using Microsoft Excel 2016 (Microsoft, Washington, US)®, applying the chi-square function, as well as prevalence ratios. Differences were considered significant at $P < 0.05$. A ratio was estimated to study the odds of finding a parasite in an isoclimatic zone depending on the season: the number of samples from an isoclimatic zone where a specific parasite was identified in the wet season was divided by the number of samples taken in the same isoclimatic zone during the dry season where that parasite was also identified.

The samples were grouped by isoclimatic zones and by precipitation: dry season (spring–summer) and wet/temperate season (autumn–winter). Gran Canaria has four different isoclimatic zones (Rodríguez-Ponce *et al.*, 1995), ranging from sea level to the central peak of the island describing concentric circles (fig. 1) as follows: dry desert zone (Dd), around sea level; dry steppe zone (Ds), higher places up to 200 m, with temperatures above 18°C all year round and very dry summers; temperate mild zone (Tm), 200 to 800 m in altitude with winter temperatures below 18°C and heavy rainfall in the wet season, followed by dry and hot summers; and temperate cold zone (Tc), from 800 m to the highest point of the island, midlands, with a similar climate throughout the year to the previous zone, but with a lower average temperature during the wet and dry seasons.

Results

Overall results

A total amount of 290 stool samples were analysed for parasites, distributed by municipalities, isoclimatic zones and season, as shown in tables 1–4.

Nematodes were the most prevalent parasites (52.4%). Within this group, *Ancylostoma* spp. appeared in a larger number of samples (28.6%), followed by bronchopulmonary nematodes (28.3%) and the roundworms *Toxocara cati* (11.7%) and *Toxascaris leonina* (2.1%). Other nematodes were present in lower percentages: *Physaloptera* spp. (2.1%) and *Trichuris* spp. (0.7%).

Protozoa were the second most isolated taxa (21.4%). *Giardia* spp. were found in the largest number of samples (12.4%), followed by *Cystoisospora felis* (9.3%), *Cystoisospora rivolta* (1.7%) and *Toxoplasma gondii* (0.7%).

The tapeworms (20.3%) were non-egg-cluster producers from the family Dipylidiidae (*Joyeuxiella* spp. and *Diplopylidium* spp.) (16.6%) followed by *Taenia* spp. (1.4%) and *Mesocestoides* spp. (0.3%).

Isoclimatic and seasonal statistics

Nematodes were identified more than any other parasite group in every isoclimatic zone ($P < 0.05$) (table 4), showing their lowest value in the Dd zone (29.3%). In terms of nematode families, *Ancylostoma* spp. and lungworms were more prevalent than roundworms in Tm and Ds zones ($P = 0.024$). The highest prevalence of all nematode taxa was recorded in the Tc zone. Protozoa were observed in greater numbers in the Tm zone, while cestodes in the Dd zone.

In terms of seasonal patterns, protozoans were significantly more frequent in the wet than in the dry season ($P = 0.003$). Furthermore, the prevalence ratio between wet and dry season was, in all cases, close to unity, except for *Giardia* spp., which was 3.1 times higher in the wet season.

Table 1. Number of samples (*n*) per municipality and their isoclimatic zones (IC).

IC	Municipality/no. of colonies	n
Tc	Artenara/1	10
	Tejeda/1	10
	Valleseco/1	10
	San Mateo/1	10
Tm	Arucas/1	10
	Moya/1	10
	Santa Brígida /2	20
	Teror/1	10
	Valsequillo/1	10
Ds	Agaete/1	10
	Firgas/1	10
	Gáldar/1	10
	Santa María de Guía/1	10
Dd	Agüimes/1	10
	Ingenio/2	20
	Mogán/1	10
	San Bartolomé/2	20
	Santa Lucía/2	20
	Telde/2	20
	Las Palmas de Gran Canaria/4	40
	La Aldea/1	10
Total	21 Municipalities/29 colonies	290

Dd, dry desert; Ds, dry steppe; Tc, temperate cold; Tm, temperate mild.

Discussion

Zoonotic risk and disease reservoir

Few zoonotic species have been isolated in this work such as *Ancylostoma* spp., *T. cati* and *Giardia* spp., with a low prevalence of *T. gondii*. However, the ecology of *T. gondii* on Gran Canaria is a clear example of how much feral cat colonies can affect not only humans but also livestock and wildlife.

Previous studies on Gran Canaria alarmingly revealed more than 60% seropositivity to *T. gondii* in humans and livestock (Rodríguez-Ponce *et al.*, 1995), affecting almost 80% of humans aged over 60 years (Rodríguez-Ponce, 1994). The low detection of oocysts in faeces may also be influenced by the erratic pattern of excretion of this parasite, usually reaching a prevalence of 1% in cats shedding oocysts at any given time (Dubey, 2010; Elmore *et al.*, 2010).

Larva migrans is a relatively important zoonotic disease in certain countries of the world, even regarded as a hazard to travellers (Norris, 1971; Jelinek *et al.*, 1994; Sow *et al.*, 2017). Furthermore, a seroepidemiological study on the visceral *larva migrans* producer, *Toxocara* spp., revealed a prevalence of less than 6% in humans (Jiménez *et al.*, 1997), including a paediatric case of ocular toxocariosis (Cejas *et al.*, 2016). These diseases seem to be more related to children, as they can be more exposed at playgrounds, which are frequented by either cats or dogs, contaminated with larvated eggs of *Toxocara* spp. (Wright *et al.*, 2016) or third-stage larvae of *Ancylostoma* (Jelinek *et al.*, 1994). In addition to direct infection through the environment, toxocariasis can be regarded as a food-borne pathogen since contaminated vegetables are a common source of infection (Healy *et al.*, 2022). Hence, the high prevalences of *Ancylostoma* spp. and *T. cati* reported in this study represent a clear public health problem that must be addressed, particularly considering the probable indoor shedding by contaminated domestic cats.

While genotype F or *Giardia cati* (Feng & Xiao, 2011) (non-zoonotic) is the most common in cats (55.8%) (Ramírez-Ocampo *et al.*, 2017), almost four out of ten isolations (Ramírez-Ocampo *et al.*, 2017) belong to zoonotic species: *Giardia duodenalis sensu stricto* or genotype A (Feng & Xiao, 2011). Every year, more than 150 native cases of giardiosis are documented on the Canary Islands, mainly on the two capital islands – that is, 101 cases in Gran Canaria in 2018 (Canary Islands Health Service, 2018). Thus, the 12% reported in this study should be treated as potential zoonoses. Furthermore, kittens may also harbour other zoonotic species, *Giardia enterica* or genotype B, which in the Canary Islands is only documented

Table 2. Epidemiological data of the protozoans ordered by isoclimate and season studied.

Season	IZ	Protozoa	*Giardia* spp.	Coccidia	*C. felis*	*C. rivolta*	*T. gondii*	n
O	Tc	17.5	5.0	12.5	12.5	0.00	0.0	40
	Tm	30.0	21.7	15.0	8.3	3.3	3.3	60
	Ds	25.0	15.0	10.0	10.0	0.0	0.0	40
	Dd	18.7	10.0	10.7	8.7	2.0	0.0	150
	O	21.4	12.4	11.7	9.3	1.7	0.7	290
W	Tc	25	7.1	25.1	17.9	3.6	3.6	28
	Tm	35.7	18.6	11.9	9.5	2.4	0.0	42
	Ds	19.2	19.2	0.0	0.0	0.0	0.0	26
	Dd	22.5	11.2	14.2	11.2	3.1	0.0	98
	O	25.3	15.5	13.4	10.3	2.6	0.5	194
D	Tc	0.0	0.0	8.3	0.0	0.0	8.3	12
	Tm	16.7	5.6	5.6	5.6	0.0	0.0	18
	Ds	35.7	7.1	28.6	28.6	0.0	0.0	14
	Dd	11.5	7.7	3.9	3.9	0.0	0.0	52
	O	14.6	6.3	8.3	7.3	0.0	1.0	96

Tc, temperate cold; Tm, temperate mild; Ds, dry desert; Ds, dry steppe; W, wet; D, dry; O, overall; IZ, isoclimatic zone; *n*, total number of samples analysed.

Table 3. Epidemiological data of the cestodes ordered by isoclimate and season studied.

Season	IZ	Cestoda	*Mesocestoides* spp.	Dipylidiidae	*Taenia* spp.	n
O	Tc	12.5	0.0	15.0	0.0	40
	Tm	13.3	0.0	11.7	1.7	60
	Ds	17.5	2.5	12.5	0.0	40
	Dd	26.0	0.0	20.0	2.0	150
	O	20.3	0.3	16.6	1.4	290
W	Tc	10.7	0.0	35.7	0.0	28
	Tm	11.9	0.0	4.8	0.0	42
	Ds	26.9	3.8	11.5	3.8	26
	Dd	31.6	0.0	14.3	3.1	98
	O	23.7	0.5	14.9	2.1	194
D	Tc	16.7	0.0	25.0	0.0	12
	Tm	16.7	0.0	5.6	0.0	18
	Ds	0.0	0.0	21.4	0.0	14
	Dd	15.4	0.0	23.1	0.0	52
	O	13.5	0.0	19.8	0.0	96

Tc, temperate cold; Tm, temperate mild; Ds, dry desert; Ds, dry steppe; W, wet; D, dry; O, overall; IZ, isoclimatic zone; *n*, total number of samples analysed.

in rodents from La Palma (Western Canary Islands) (Fernández-Álvarez *et al.*, 2014). Since no other known potential wildlife sources of *Giardia duodenalis* have been identified, further sampling for molecular studies should be considered to evaluate the real risk of this flagellate from feral cats.

Climatic influence

These data suggest that local climatic conditions seem to be more important than seasons for most parasites, except for protists. In the same line, warmer and more humid areas like the Tm are more ideal for parasites to reproduce. However, linked to the transmission of zoonotic parasites in suburban areas is the adaptation of intermediate hosts to these environments, which is influenced not only by the direct effect of urban development but also climatic conditions (Mackenstedt *et al.*, 2015). The best example highlighted in this study are cestodes.

The tapeworms reported here are non-egg cluster producers from the family Dipylidiidae, which comprises two genera with similar ecology: *Joyeuxiella* spp. and *Diplopylidium* spp. No

Table 4. Epidemiological data of the nematodes ordered by isoclimate and season studied.

Season	IZ	Nematoda	*Ancylostoma* spp.	*T. cati*	*T. leonina*	*Physaloptera* spp.	*Trichuris* spp.	Lungworms	n
O	Tc	87.5	42.5	35.0	0.15	0.05	0.05	52.5	40
	Tm	75.0	53.3	18.3	0.0	0.05	0.0	51.7	60
	Ds	70.0	52.5	7.5	0.0	0.0	0.0	50.0	40
	Dd	29.3	8.7	4.0	0.0	0.0	0.0	6.7	150
	O	52.4	28.6	11.7	2.1	2.1	0.7	28.3	290
W	Tc	89.3	46.4	71.4	7.1	14.3	7.1	50	28
	Tm	73.8	50	2.4	0.0	2.4	0.0	42.9	42
	Ds	76.9	57.7	19.2	0.0	0.0	0.0	50	26
	Dd	34.7	8.2	0.0	0.0	0.0	0.0	10.2	98
	O	56.7	29.4	13.4	1.0	2.6	1.0	28.4	194
D	Tc	83.3	33.3	41.7	33.3	0.0	0.0	58.3	12
	Tm	77.8	61.1	0.0	0.0	5.6	0.0	72.2	18
	Ds	57.1	42.9	14.3	0.0	0.0	0.0	50	14
	Dd	19.2	9.6	1.9	0.0	0.0	0.0	0.0	52
	O	43.8	27.1	7.3	4.2	1.0	0.0	28.1	96

Tc, temperate cold; Tm, temperate mild; Ds, dry desert; Ds, dry steppe; W, wet; D, dry; O, overall; IZ, isoclimatic zone; *n*, total number of samples analysed.

information is available on the actual intermediate host of these cestodes, but their cysticercoids have been extensively reported in several poikilothermic animals (Witenberg, 1932; Roca, 1985). In Gran Canaria, the endemic Boettger's wall gecko (*Tarentola boettgeri*) is the only reptile included in the diet of cats, reported to be infected with either cestode (Roca *et al.*, 1987, 1999; Santana-Hernández & Rodríguez-Ponce, 2019). The population density of this gecko decreases with altitude (from sea level up to 750 m), with higher densities in the Dd areas (Mateo, 2002) – a fact which could account for the higher prevalence of cestodes in cats from Dd zones on the island. In addition, by preying on geckoes, two species of *Diplopylidium* are being transmitted to another important invasive species on Gran Canaria, the California Kingsnake (*Lampropeltis californiae)* (Santana-Hernández *et al.*, 2021). Hence, the life cycle of *Diplopylidium* spp. highlights the urgent need to control invasive species such as feral cat colonies and their co-invasive parasites to preserve Gran Canaria's fragile biodiversity.

However, added to the benefits of paratenic/invertebrate host usage, environmental resistance is a key feature for the survival of parasites. *Ancylostoma*, for example, is clearly sensitive to desiccation and sunlight, which explains its higher prevalence in Tc–Ds areas in contrast to Dd areas, where the action of the paratenic host may have a crucial role in their survival. Similarly, the use of land molluscs by lungworms is evidently limited to areas with higher densities of these animals, such as Ds and higher areas, with superior humidity to Dd areas. Thus, it suggests more benevolent climatic conditions for egg and larvae to survive.

Despite these patterns, mean temperature and precipitation in a specific location seemed to have a stronger influence on parasite prevalence than seasonal fluctuations in this study. The only recorded seasonal fluctuation was the prevalence of *C. felis* and *Giardia* spp. during the wet season. This high humidity could suggest more benevolent conditions for protists to survive. Similar patterns have been described by other authors, but no further conclusions have been formulated (Barutzki & Schaper, 2011).

In conclusion, islands similar to Gran Canaria could be used as model ecosystems to evaluate the influence of climate on parasite communities in wildlife and with further sampling, the potential consequences of climate change on parasitic biodiversity.

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Conflicts of interest. None.

Ethical standards. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional guides on the care and use of laboratory animals.

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Section III:
Conclusions

Parasitological findings in the invasive California kingsnake (*Lampropeltis californiae*) in Gran Canaria, Spain.

1. Low prevalence of adult snake-parasites, instead, almost the whole sample (98.4%) was infected with larval helminths, acting as 'dead-end' paratenic hosts.

2. Most of the parasites found here will not be able to reach their respective definitive hosts, such as birds of prey, and thus are disrupting natural life cycles in the island.

3. These invasive snakes will be contributing not only to the extinction of geckoes and other native reptiles, but also to their respective parasites of which several are endemic to the Canary Islands, e.g. *Thelandros filiformis, Alaeuris stehlini, Sarcocystis stehlini, Ophionyssus setosus*.

4. First record of two *Diplopylidium* species and three larval nematode types in the California kingsnake

5. Regardless of the parasitic species, the burden and prevalence found in the Gran Canaria snake population (98.4%) is considerably higher than that reported for larval parasites from other reptiles in Spain, and indeed North America (usually less than 2%).

6. First record of *Diplopylidium* species in a North American snake.

7. Potentially increased immune response against cestodes compared to nematodes due to a lack of co-evolution.

8. *Ophionyssus natricis* is a widely distributed mite that represents a potential zoonotic risk in Gran Canaria.

9. The possible spillover of these mites, as well as *Serpetirhabdias* sp., to local fauna, requires further research.

Dispersion of adeleid oocysts by vertebrates in Gran Canaria, Spain: report and literature review.

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1. Despite a low prevalence, these findings constitute the first baseline data for invertebrate pathology studies in the Canary Islands.

2. Further epidemiological research on invertebrate parasites in these islands would be necessary to determine the invertebrate hosts, native or exotic, and the real epidemiological importance of insectivorous animals in the life cycle of *Adelina* spp.

3. The further understanding of the role of this protozoan in invertebrate population dynamics is particularly important in an island setting where most of the fauna is native/endemic and/or endangered.

4. The Canaries, and other similar islands, could be utilized as model systems for arthropod parasites.

5. Using morphological measures, the oocysts described here are close to several reported species, but with potentially important differences in sporocyst numbers.

6. Further material should be studied to determine its accurate taxonomical status, considering the morphological variability of *A. tribolii*.

7. With the appropriate molecular sampling of Adeleids within invertebrates, the vertebrate species studied here could be useful as sentinels for further research on *Adelina* spp. in the Canary Islands and further afield.

Sarcocystis sp. infection (Apicomplexa: Sarcocystidae) in invasive California kingsnake *Lampropeltis californiae* (Serpentes: Colubridae) in Gran Canaria.

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1. All the positive animals were found at the east nuclei of the island (main nucleus = 7/61 and fourth nucleus = 3/8).

2. The DNA sequences of *Sarcocystis* that were obtained from invasive snakes and *Sarcocystis* from an endemic lizard *G. stehlini* differed significantly and branched into distant clades.

3. On the contrary, the lizard-derived sequence was very close to *S. gallotiae* (>99 identity), which was previously isolated from *G. galloti* from Tenerife.

4. It is very likely that the *Sarcocystis* from the California kingsnakes described here is a snake-specific species that probably invaded the Macaronesian ecosystem along with its snake host.

5. The intermediate host of *Sarcocystis* sp. found in the kingsnakes of Gran Canaria is still unknown and further research should be directed towards the study of native (skinks, geckos, passerine birds…) or invasive (mice and rats) vertebrates, which inhabit in all nuclei.

6. The presence of *Sarcocystis* sp. in invasive kingsnakes in Gran Canaria suggests that the population was established rather by individuals introduced from wild populations in the southwestern United States of northern Mexico than by captive-borne pet snakes.

7. Clarification of the life cycle of the snake sarcosporidian found in the Gran Canaria is necessary to answer questions related to possible impacts on the endangered endemic vertebrate fauna of Macaronesia.

Small islands as potential model ecosystems for parasitology: climatic influence on parasites of feral cats.

1. Few zoonotic species have been isolated in this work such as *Ancylostoma* spp., *T. cati Giardia* spp. and *T. gondii*.

2. The ecology of *T. gondii* on Gran Canaria is a clear example of how much feral cat colonies can affect not only humans but also livestock and wildlife by having a low prevalence in cats, and over 60% in livestock and humans.

3. The high prevalence of *Ancylostoma* spp. and *T. cati* reported in this study represent a clear public health problem that must be addressed, particularly considering the probable indoor shedding by contaminated domestic cats.

4. These data suggest that local climatic conditions seem to be more important than seasons for most parasites, except for protists (*C. felis* and *Giardia* spp.).

5. Linked to the transmission of zoonotic parasites in suburban areas is the adaptation of intermediate hosts to these environments, which is influenced not only by the direct effect of urban development but also climatic conditions. Such, likely, the ecology of the transmission of *Diplopylidium* spp. The population density of this gecko decreases with altitude, with higher densities in the Dd areas – matching with higher prevalence of this parasite in cats from Dd zones.

6. The life cycle of *Diplopylidium* spp. highlights the urgent need to control invasive species such as feral cat colonies and their co-invasive parasites to preserve Gran Canaria's fragile biodiversity not only by predation, but by disseminating co-invasive parasitic species.

7. In conclusion, islands similar to Gran Canaria could be used as model ecosystems to evaluate the influence of climate on parasite communities in wildlife and with further sampling, the potential consequences of climate change on parasitic biodiversity.

Section IV:
Ongoing work and final remarks

Issue I: Parasites of snakes.

Issue II: Larval nematodes in the snake's prey and other hosts.

Issue III: Larval nematodes in intermediate hosts.

Issue IV: Studies on *Diplopylidium* spp. and other cestodes of cats.

Issue V: The fifth paper, a "microparasite".

During the studies with stool samples of California kingsnakes, two additional parasites were detected: *Caryospora* sp., and *Eimeria* sp. (1 out of 121 samples each). These were presented at the 14th EWDA/69th WDA conference:

Coprological approach to parasites of the California kingsnake (*Lampropeltis californiae*); an invasive species in Gran Canaria, Spain.

Kevin M. Santana-Hernández[a], Simon L. Priestnal[b], Eligia Rodríguez-Ponce[a]

a Department of Animal Pathology, Faculty of Veterinary Sciences, University of Las Palmas de Gran Canaria, Arucas, Spain.

b Department of Pathobiology and Population Sciences, The Royal Veterinary College, Hatfield, United Kingdom

Introduction

The worldwide demand for novel, more exotic species of companion animal has also led to growth in the illegal release of invasive species into non-native ecosystems.

Whilst the animals themselves can cause significant imbalances to biodiversity, the pathogens they can carry to their new habitats are less well documented.

The California kingsnake (*Lampropeltis californiae*), native to south-western North America, has emerged as a significant ecological problem on the island of Gran Canaria, Spain, and its control has proved challenging. The snake preys on endemic reptile species and current data supports the threat of extinction for some of these animals.

Material and methods

Between 2016 and 2020, to assess the possible introduction of foreign parasites which could pose an additional risk to local biodiversity, faecal samples from this snake species were analysed at the Laboratory of Parasitology, Faculty of Veterinary Sciences, University of Las Palmas de Gran Canaria. Faeces extracted from dead and frozen animals (A) were tested using NaCl flotation and formol-ether sedimentation, and freshly collected material from live snakes (B) with sugar centrifugal flotation.

Results and discussion

In total, 118 samples were analysed, with true parasites identified in only three snakes (2.5%): *Serpentirhabdias* sp., *Eimeria lampropeltis* and *Caryospora* sp., one in each of three samples (0.8% each). Parasites from the snakes prey species were also present, including disrupted eggs of oxiurids (5.1%), tapeworms (2.5%) and *Adelina tribolii* (3.4%). *Sarcocystis* sp. were also found in eight animals (6.8%), but due the poor state of preservation, it was not possible to determine if they were proper parasites, or those from prey.

This is the first report of *Adelina tribolii* (coccidia from beetles) in the Canary Islands. No known zoonotic or parasites important to ecosystem conservation were found in the faeces, however this needs to be further monitored through ongoing *post-mortem* and coprological examinations.

Coprological approach to parasites of the California kingsnake (Lampropeltis californiae); an invasive species in Gran Canaria, Spain

***Kevin M. Santana-Hernández*[a]**, *Simon L. Priestnall*[b], *Eligia Rodríguez-Ponce*[a]

[a] Department of Animal Pathology, Faculty of Veterinary Sciences, University of Las Palmas de Gran Canaria, Arucas, Spain.
[b] Department of Pathobiology and Population Sciences, The Royal Veterinary College, Hatfield, United Kingdom

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Introduction

The worldwide demand for novel, more exotic species of companion animal has also led to growth in the illegal release of invasive species into non-native ecosystems. Whilst the animals themselves can cause significant imbalances to biodiversity, the pathogens they can carry to their new habitats are less well documented. The California kingsnake (*Lampropeltis californiae*), native to south-western North America, has emerged as a significant ecological problem on the island of Gran Canaria, Spain, and its control has proved challenging. The snake preys on endemic reptile species and current data supports the threat of extinction for some of these animals.

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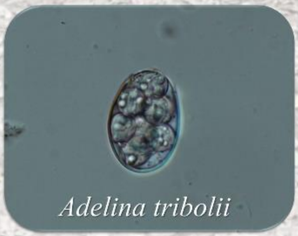
Caryospora sp.


Eimeria lampropeltis


Sarcocystis sp.


Serpentirhabdias sp.


Adelina tribolii


Photograph courtesy of Mr. de Blas

Background picture courtesy of Raúl E. Bruno

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Issue II: Larval nematodes in the snake’s prey and other hosts

For further knowledge on which of the snake’s prey could be transmitting these larval parasites (mainly Spirurida from rodents), carcasses of miscellaneous vertebrates have been inspected from 2014 to the current date of this thesis.

Eighteen boettger’s wall gecko (*Tarentola boettgeri*), 9 Gran Canaria giant lizards (*Gallotia stehlini*), 7 grancanarian skinks (*Chalcides sexlineatus*), 22 brown rats (*Rattus norvegicus*), 4 shrews (*Crocidura russula*) and 4 house mice (*Mus musculus*) have been examined.

The Boettger’s wall gecko and grancanarian skink are the vertebrate species, known to be included in the diet of the snake on the island of Gran Canaria, found to be infected with larval Spirurida. In addition to larval parasites from rodents, larval acuarioidea were found in geckoes and shrews (*Crocidura russula*).

These suspected rodent parasites (likely *Mastophorus muris* and *Streptopharagus greenbergi*) seem to have a rather broad spectrum of possible paratenic hosts since they have been found in feral ferrets (*Mustela putorius*), houbara bustards (*Chlamydotis undulata*), Eurasian stone curlew (*Buhrinus oedicnemus*), Eurasian hobby (*Falco Subbuteo*), barbary falcon (*Falco peregrinus pelegrinoides*) and common chaffinch (*Fringilla coelebs*) from several islands.

Different morphological types of larval nematodes in geckoes from Gran Canaria. **A**: Probable Spirocercidae **B**: Acuaridae.

Issue III: Larval nematodes in intermediate hosts

Few species of soil invertebrates have been inspected for larval spirurids; however, the most extensive study was performed using an invasive myriapod, *Omatoidulus moreletii*. This was presented as a poster at the 9th Conference of the Baltic-Scandinavian Society of Parasitology.

Parasites in a changing world: Transmission among three invasive species of *Mastophorus muris* in Gran Canaria, Spain.

Kevin M. Santana-Hernández[1]; Pilar Foronda[2]; Simon L. Priestnall[3]; Natalia Martín-Carrillo[2]; Jose Pestano-Brito[4]; Eva Betancor[4]; Eligia Rodríguez-Ponce[1]

1 Department of Pathology, Faculty of Veterinary Science, Universidad de Las Palmas de Gran Canaria (ULPGC), Las Palmas, Spain

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Invasive species represent one of the most important hazards for biodiversity loss, especially on isolated and unique ecosystems, such as islands. This threat is not solely through competition for resources or predation, but invasive species can act as 'Trojan horses' for important pathogens and parasites transmissible to wildlife, domestic animals and humans. Understandably, most reports focus on interactions between invasive and native species or humans, however interactions amongst invasive species, for example *Mastophorus muris*, a common nematode of rodents, are seldom studied.

Twenty-two brown rats (*Rattus norvegicus*), 108 California Kingsnakes (*Lampropeltis californiae*), 15 geckoes (*Tarentola boettgeri*) and 87 millipedes (*Ommatoiulus moreletii*) collected in Gran Canaria, were examined for parasites. Millipedes were digested with pepsin in pools of 2g. Where parasites were identified from these pools, DNA was extracted and a fragment of the ITS1 region amplified by PCR and sequenced.

Larval nematodes consistent with *M. muris* were found in 3/6 pools of millipedes, 5/15 geckoes and 81/108 snakes. Adult *M. muris* were found in 3/22 rats. ITS1 gene sequences from millipedes revealed 100% homology with *M. muris* (accession number MK829005).

These findings suggest a model of transmission using three invasive and one endemic species. Empiric data describes M. muris as a generalist parasite in terms of intermediate hosts, thus, rats not only pose a threat to vertebrates (including humans) by harbouring dangerous pathogens, but also to invertebrate conservation.

This is the first description of larval spirurids in Spanish geckoes and *O. moreletii* as an intermediate host of *M. muris*.

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This is the first description of larval spirurids in Spanish geckoes and *O. moreletii* as an intermediate host of *M. muris.*

Issue IV: Studies on *Diplopylidium* spp. and other cestodes of cats

Post-mortem examinations were performed on cat carcasses from different dates (2008, 2015-2019), searching specifically for tapeworms.

These dissections showed that tapeworm species in feral cats were more prevalent (62 out of the 78 cats) than expected from faecal samples in the fourth paper of this thesis.

The most common parasite was *Joyeuxiella* (39/78) with material from 22 cats identifiable as *J. pasqualei*. The genus *Diplopylidium* was the second most prevalent parasite (23/78) registering 9 positive cats for *D. acanthotetra* and 8 for *D. nölleri*. The most common co-existing parasites were species of *Joyeuxiella* and *Diplopylidium,* biologically linked by infecting geckoes. Therefore, part of these data on *Joyeuxiella* from Issues II and IV were presented as a poster the 21st SOCEPA conference.

Definitive and paratenic hosts for *Joyeuxiella pasqualei* in Gran Canaria: a new host record for Spain.

Santana-Hernández, K.M.& Rodríguez-Ponce, E.

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Joyeuxiella pasqualei is a cyclophillidean tapeworm, common in cats from diverse parts of the world, using as paratenic hosts several vertebrate species such as geckoes and shrews. The intermediate hosts nowadays remains unknown, but due to experimental studies, would be an invertebrate, which can be eaten by the geckoes or other small vertebrates.

In the case of Canary Islands, several epidemiological works has been published about cats and geckoes, reporting mainly *Dipylidium caninum*. The similarities between these two species often cause misidentifications of these tapeworms. Usually they loss several rows of hooks, and frequently the submitted samples have no gravid proglottids, being these two features crucial to differentiate between the two genera.

To approach in the epidemiology of *Joyeuxiella pasqualei* in Gran Canaria island, corpses from 61 feral cats, 15 dogs, 15 geckoes (*Tarentola boettgeri*), 7 Gran Canaria giant lizards (*Gallotia stehlini*), 3 skinks (*Chalcides sexlineatus*), 22 brown rats (*Rattus norvegicus*), and 4 shrews (*Crocidura russula osorio*) submitted for their post-mortem and parasitological exam were dissecte d at the Faculty of Veterinary Sciences, University of las Palmas de Gran Canaria.

Joyeuxiella pasqualei was present in two out of the eight species analysed: cats, with the presence of adults in 80.3%, but also in geckoes, where the 40.0% presented cysticercoids.

These discoveries on geckoes, and the previous mention of *Joyeuxiella* sp. in shrews, mice and cats as cysticercoids and adults in other islands, suggests that the natural host would be the feral cats, having a night-related life cycle using these three nocturnal vertebrates as paratenic hosts.

This is the first report of the definitive host of *Joyeuxiella pasqualei* in Gran Canaria as well as a new paratenic host record for this tapeworm in Spain.

Definitive and paratenic hosts for *Joyeuxiella pasqualei* in Gran Canaria: a new host record for Spain

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Fig. 1: Part of the *Joyeuxilla* life cycle elucidated in this work

To approach in the epidemiology of *Joyeuxiella pasqualei* in Gran Canaria island, corpses from 61 feral cats, 15 dogs, 15 geckoes (*Tarentola boettgeri*), 7 Gran Canaria giant lizards (*Gallotia stehlini*), 3 skinks (*Chalcides sexlineatus*), 22 brown rats (*Rattus norvegicus*), and 4 shrews (*Crocidura russula osorio*) submitted for their post-mortem and parasitological exam were dissecte d at the Faculty of Veterinary Sciences, University of las Palmas de Gran Canaria.

Fig. 2: Definitive and paratenic hosts described in the Canary islands.

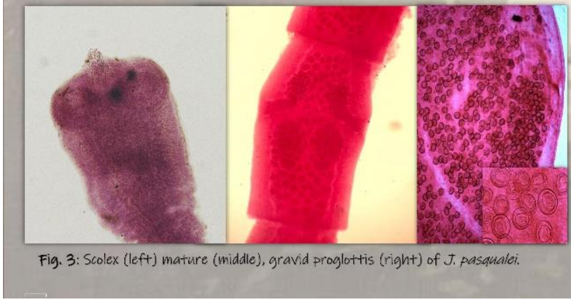

Fig. 3: Scolex (left) mature (middle), gravid proglottis (right) of *J. pasqualei*.

RESULTS

Joyeuxiella pasqualei was present in two out of the eight species analysed: cats, with the presence of adults in 80.3%, but also in geckoes, where the 40.0% presented cysticercoids. These discoveries on geckoes, and the previous mention of *Joyeuxiella* sp. in shrews, mice and cats as cysticercoids and adults in other islands, suggests that the natural host would be the feral cats, having a night-related life cycle using these three nocturnal vertebrates as paratenic hosts.

CONCLUSIONS

This is the first report of the definitive host of *Joyeuxiella pasqualei* in Gran Canaria as well as a new paratenic host record for this tapeworm in Spain.

Issue V: The fifth paper, a "microparasite"

It was decided during the sampling process to test for other microparasitic pathogens that could be carried by invasive reptiles, such as *Salmonella enterica.* This paper is presented as a final note since it finds itself outside of the main *corpus* of pure taxonomy and invasion ecology.

Salmonella was the chosen bacteria to start this part of the thesis; however, there are a few more options that can be explored in future research, as well as samples from other hosts.

animals

MDPI

Article

One Health Approach: Invasive California Kingsnake (*Lampropeltis californiae*) as an Important Source of Antimicrobial Drug-Resistant *Salmonella* Clones on Gran Canaria Island

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Simple Summary: The aim of this study was to investigate the invasive species *Lampropeltis californiae* (California kingsnake) as a reservoir of *Salmonella* and its ability to spread different clones of the bacterium with zoonotic potential into the environment, as well as study its antimicrobial resistance patterns in Gran Canaria (Spain). The main results showed that a high diversity of *Salmonella* subsp. *salamae* strains circulate in Gran Canaria with a high prevalence of resistance shown for antimicrobials of public health importance, as summarised in the European Decision 2013/652/EU.

Abstract: The increase in the reptile population has led to a rise in the number of zoonotic infections due to close contact with reptiles, with reptile-associated salmonellosis being particularly relevant. California kingsnake invasion not only threatens the endemic reptile population of the island of Gran Canaria (Spain) but also poses serious public health problems by spreading zoonotic pathogens and their antimicrobial resistance (AMR) to the environment. Thus, the aim of this study was to assess the occurrence, genetic diversity, and AMR among *Salmonella* spp. strains isolated from California kingsnakes in Gran Canaria Island (Spain). Of 73 invasive individuals captured, 20.5% carried *Salmonella* spp., belonging to different subspecies and serovars, with subsp. *salamae* as the most abundant. Pulsed-field electrophoresis showed high genetic diversity among subsp. *salamae* isolates, and among these, 73.3% showed resistance to at least one of the antimicrobials tested. In conclusion, the present study revealed the importance of wild invasive California kingsnakes as reservoirs of drug-resistant *Salmonella* spp. that could pose a direct threat to livestock and humans. Identification of drug-resistant *Salmonella* strains in wildlife provides valuable information on potential routes of transmission that involve risks to public and animal health.

Keywords: *Salmonella*; multi-drug resistance; PFGE; *Lampropeltis californiae*

1. Introduction

The Canary Islands are considered a hotspot of Atlantic biodiversity due to their strategic geographical location, volcanic origin, and close contact with Africa and Europe [1]. This archipelago has a great diversity of endemic reptiles that form a large part of its terrestrial fauna. However, the endemic reptile species of the Canary Islands are threatened by a growing number of invasive species [2,3].

In recent years, the population of exotic and native reptiles as pets has increased considerably, with a total population of more than 11 million in European households [4]. The population of free-living exotic reptiles has also increased due to their accidental release or escape into the wild [5], becoming a major threat with a huge ecological impact and favouring the spread of pathogens in the environment [6]. Different studies have described how reptiles act as natural reservoirs of *Salmonella* spp. with a prevalence of up to 90% and are able to carry a wide variety of serovars asymptomatically [7–10]. The increase in the reptile population has led to a rise in the number of zoonotic infections due to close contact with reptiles, with reptile-associated salmonellosis (RAS) being particularly relevant in at-risk populations such as children, the elderly or immunocompromised adults [11–13]. In addition, some authors have described the ability of *Salmonella* strains isolated from domestic reptiles to develop antimicrobial resistance (AMR) [14], thus posing a serious threat to free-living reptiles because of their role in the dissemination of AMR in the environment [15]. *Salmonella* is a very complex gender with a most complex taxonomy than other bacteria [16]. *S.* enterica has long been subdivided by differential antibody reactions into serovars [17]. The use of specific antibodies that could identify distinct cell-surface antigens within lipopolysaccharide and flagella has led to the distinction of over 2500 serovars that differ in their antigenic formulas [18]. In addition, *Salmonella* is also subdivided taxonomically into *S. enterica*, which contains multiple subspecies, and a separate species, *S. bongori* [18].

In this context, the invasion of *Lampropeltis californiae* (California kingsnake) that has occurred in recent years in Gran Canaria (Canary Islands, Spain) is of particular relevance.

The California kingsnake was first detected free-living in Gran Canaria in 1998, and its population has been rising since then, mainly due to the accidental or intentional release of this invasive snake species into the island ecosystem [3,19]. The increase of the California kingsnake has caused a decrease in the population of different reptiles endemic to Gran Canaria because their diet on the island is mainly based on the endemic reptile species: the Gran Canaria gigant lizard (*Gallotia stehlini*), the Gran Canaria skink (*Chalcides sexlineatus*), and Boettger's wall gecko (*Tarantula boettgeri*) [20]. In fact, in the areas where the California kingsnake lives, the gigant lizard is locally extinct, the skink has reduced its population by almost 83%, and the Boettger´s wall gecko population has been reduced by half [3], causing a serious ecological impact in Gran Canaria [20]. In addition, this California kingsnake invasion also poses serious public health problems by spreading zoonotic pathogens and their AMR to the environment, where they can be transmitted to humans and other animals (wild or domestic), contributing to the growing problem of AMR [21].

The World Health Organisation (WHO) has declared AMR and its ability to transmit between different animal species and humans through the environment as one of the ten most significant threats to public health, encompassing this problem under the "One Health" perspective [22], a concept that refers to a global strategy that seeks to increase interdisciplinary collaboration in the health care of people, animals and the environment to develop and implement programs, policies and laws to improve public, animal and environmental health [23].

Pathogens like *Salmonella* spp. can easily acquire resistance genes through contact with commensal bacteria [24], so the high prevalence of RAS combined with this ability of bacteria to acquire AMR leads to salmonellosis caused by AMR- *Salmonella* strains [25]. Therefore, *Salmonella* spp. could entail therapeutic consequences for humans in close contact with reptiles [26].

With this perspective, the aim of this study is to assess the genetic diversity and the AMR patterns among *Salmonella* spp. strains isolated from California kingsnakes in Gran Canaria Island (Spain).

2. Materials and Methods

The animals that are part of the study originate from a program for eradicating invasive species by the Government of the Canary Islands (Spanish Royal Degree 216/2019 and Order 336/20). All the procedures used in this study were performed in accordance with Directive 2010/63/EU EEC for animal experiments.

2.1. Sample Collection

From June to October 2019, a total of 73 individuals of California kingsnake were captured by the staff of *Gestión y Planeamiento territorial y ambiental* (GesPlan) manually and using box-traps in the framework of the eradication project (https://www.gesplan.es/content/orden-33620-que-modifica-la-n%C2%BA-12419-ejecucion-plan-post-life-lampropeltis-y-actuaciones-del, accessed on 1 March 2023). Individuals were captured at four different nuclei in the island of Gran Canaria (1. Main nucleus. 2. Secondary nucleus. 3. Third nucleus. 4. Fourth nucleus, represented in Figure 1). Prior to euthanasia, they were sexed, measured, and weighed. In addition, for *Salmonella* detection, cloacal samples were taken from asymptomatic individuals using sterile cotton swabs immediately after animals were euthanased (Cary–Blair sterile transport swabs, DELTALAB, Barcelona, Spain). The swab was inserted approximately 1 cm into the cloaca to obtain the sample and then kept in Cary–Blair transport medium. All collected samples were transported refrigerated at ≤4 °C to the microbiology laboratory at the Faculty of Veterinary Sciences of the University of Las Palmas de Gran Canaria for microbial analyses within 24 h of collection.

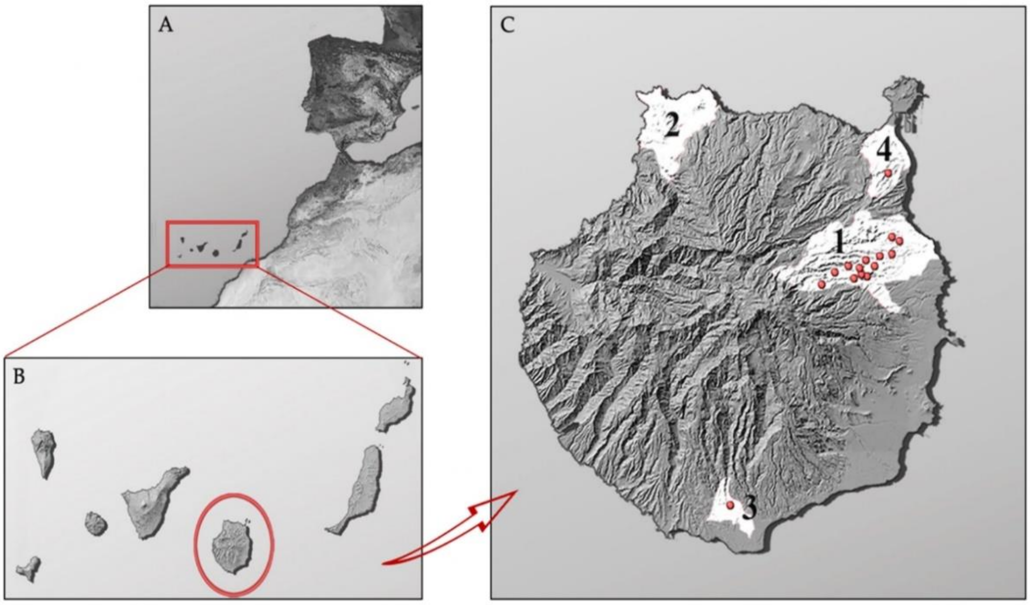


Figure 1. Distribution of California kingsnakes sampled in the island of Gran Canaria. (**A**). Location of the Canary Islands in the Atlantic Ocean; (**B**). Location of the island of Gran Canaria in the Canary Archipelago; (**C**). Representation of the locations where the samples were taken (Nucleus 1, Nucleus 2, Nucleus 3, Nucleus 4). Note: distribution of positive snakes in this study is represented by red dots.

2.2. Salmonella Isolation

For *Salmonella* detection, strains were isolated and identified using conventional culture methods as follows: The cloacal swabs were pre-enriched for 24 h in Buffered Peptone water (1:10 vol:vol, Becton, Dickinson and Company, Le Pont de Claix, France (BDC).

The pre-enriched samples (100 µL) were transferred onto Rappaport Vassiliadis semisolid agar (MRSV, BDC) at 42 °C for 24 h. The culture obtained onto MRSV was transferred to two different selective agar plates, Xylose Lysine Deoxycholate agar (BDC) and Hektoen Enteric agar (BDC), which were incubated at 37 °C for 24 h. Suspicious colonies that produced sulfhydric acid were selected for biochemical tests (Kliger Iron (BDC), Citrate agar (BDC), Motility–indole (BDC), Phenylalanine agar (BDC), and Voges Proskauer (BDC)). Considering variabilities in the fermentation of lactose by common serovars of subspecies *arizonae* and *diarizonae*, fermenting and non-fermenting colonies were selected. Then, the Analytical Profile Index (API) 20E (BioMérieux, Madrid, Spain) was performed to carry out the confirmation of *Salmonella* spp., following the manufacturer's indications. Then, to determine the subspecies of *Salmonella* isolates, an analysis was performed following the method proposed by Popoff and Le Minor based on the determination of biochemical characteristics and susceptibility to phage O1 [18]. Finally, confirmed *Salmonella* spp. strains were serotyped in accordance with Kauffman–White–Le Minor technique [18] at the National Reference Laboratory for Animal Health (Algete, Madrid, Spain).

2.3. Molecular Typing of Salmonella Isolates

Genotyping of *Salmonella* spp. isolates was performed by pulsed-field gel electrophoresis (PGFE) according to the PulseNet standardised protocol (www.pulsenetinternational.org/protocols/pfge/, accessed on 15 March 2023). The genomic DNA of the isolates was digested with Xbal and BlnI restriction enzymes (Roche Applied Science, Indianapolis, IN). We analysed the resulting PFGE band patterns using Fingerprinting II v3.0 software (Bio-Rad, Hercules, CA, USA). Similarity matrices were calculated using the Dice coefficient with a band position tolerance of 1.5%, and cluster analysis was performed by the unweighted-pair group method with arithmetic mean (UPGMA). A cut-off of 90% was used for the determination of the different profiles (PFGE type or pulsotype).

2.4. Antimicrobial Susceptibility Testing

Salmonella spp. strains were inoculated onto Müller–Hinton agar (BDC) to form a bacterial lawn; then, antibiotic discs were put on the plates, which were incubated at 37 °C for 24 h. Antimicrobial agents were selected following those set out in Decision 2013/652/EU [27], including two quinolones: ciprofloxacin (CIP; 5 µg) and nalidixic acid (NAL; 30 µg); one aminoglycoside: gentamicin (GEN, 10 µg); one potentiated sulfonamide: trimethoprim-sulfamethoxazole (TRS; 25 µg); one phenicol: chloramphenicol (CHL; 30 µg); one pyrimidine: trimethoprim (TRI; 5 µg); three ß-lactams: ampicillin (AMP; 10 µg), cefotaxime (CTA; 30 µg), ceftazidime (CTZ; 30 µg); one macrolide: azithromycin (AZI; 15 µg); one polymyxin: colistin (COL; 10 µg); and one glycylcycline: tigecycline (TIG; 15 µg). After the 24 h incubation period at 37 °C, the inhibition zone around each disc was measured. These zones were interpreted as susceptible (S) or resistant (R) according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) indications [28] (http://www.eucast.org/clinical_breakpoints/, accessed on 6 February 2023) for Enterobacteriaceae, and when not possible, Clinical and Laboratory Standards Institute (CLSI) indications were used (https://clsi.org/media/2663/m100ed29_sample.pdf, accessed on 6 February 2023) [29]. Multidrug resistance (MDR) was defined as acquired resistance to at least one agent in three or more antimicrobial classes [30].

2.5. Statistical Analysis

A Generalised Linear Model (GLM), which assumed a binomial distribution for *Salmonella* spp. shedding was fitted to the data to determine whether there was an association with the categorical variables (sex, body length, and weight) or not. A $p \leq 0.05$ was considered to indicate a statistically significant difference. Data are presented as least squares means ± standard error of the least squares means for the body length and weight. In addition, a GLM was performed to assess the serovars isolated in this study. Finally, a GLM was performed to study the relationship between *Salmonella* spp. and their AMR.

Analyses were carried out using a commercially available software application (SPSS 24.0 software package; SPSS Inc., Chicago, IL, USA, 2002).

3. Results

During this study, a total of 73 California kingsnakes were captured at four different nuclei in the Gran Canaria Island: Nucleus 1 (n = 57), Nucleus 2 (n = 4), Nucleus 3 (n = 4) and Nucleus 4 (n = 8). From all animals sampled, 50.7% (37/73) were females and 49.3% (36/73) were males. The mean body length and weight of the total animals were 922.5 ± 18.4 cm and 264.7 ± 15.0 g, respectively.

3.1. *Salmonella spp. Identification and Serotyping*

From all animals sampled, 20.5% (15/73) tested positive for *Salmonella* spp. (Table 1). Due to the difference in the number of samples taken between the different nuclei, it was not possible to establish statistical significance among the different nuclei. In addition, statistically significant differences were observed between the presence of *Salmonella* and the sex of the sampled animals, with the presence of *Salmonella* being more prevalent in females than in males ($p < 0.05$). However, no statistically significant differences were observed between the presence of *Salmonella* and the measure and weight of the positive animals ($p > 0.05$).

Table 1. Relationship between positive animals and their location, sex, body length, and weight.

		n	**% *Salmonella* spp.**	***p*-Value**
Location (nuclei)	1	57	86.6	
	2	4	0	
	3	4	6.7	
	4	8	6.7	
Sex	Female	37	73.3 ^{a}	$p = 0.042$
	Male	36	26.7 ^{b}	
Body Length	≤900 cm	33	40.0	$p = 0.647$
	>900 cm	40	60.0	
Weight	≤250 g	39	46.7	$p = 0.732$
	251–500 g	28	40.0	
	>500 g	6	13.3	

n: total number of animals sampled. $^{a, b}$: different superscripts in each column mean significant differences with a p-value < 0.05. %: percentage of *Salmonella* positive animals.

All *Salmonella* spp. isolates were classified as *Salmonella enterica* (n = 15). The subspecies isolated were, in decreasing order, *S. salamae* (66.6%, 10/15), *S. enterica* (20.0%, 3/15), *S. diarizonae* (6.7%, 1/15), and *S. houtenae* (6.7%, 1/15). Seven different serovars of *S. enterica* were identified (Table 2).

Table 2. *Salmonella enterica* serovars isolated from California kingsnake.

Subspecies	**Serovar**	**n**	**Nuclei**	**Prevalence (%)**
salamae	42:z:e,n.x.z15	8	1	53.3 ^{a}
	41:d:z6	2	1	13.2 ^{b}
enterica	Cerro	1	1	6.7 ^{b}
	Kentucky	1	3	6.7 ^{b}
	Midway	1	1	6.7 ^{b}
diarizonae	60:-:-	1	4	6.7 ^{b}

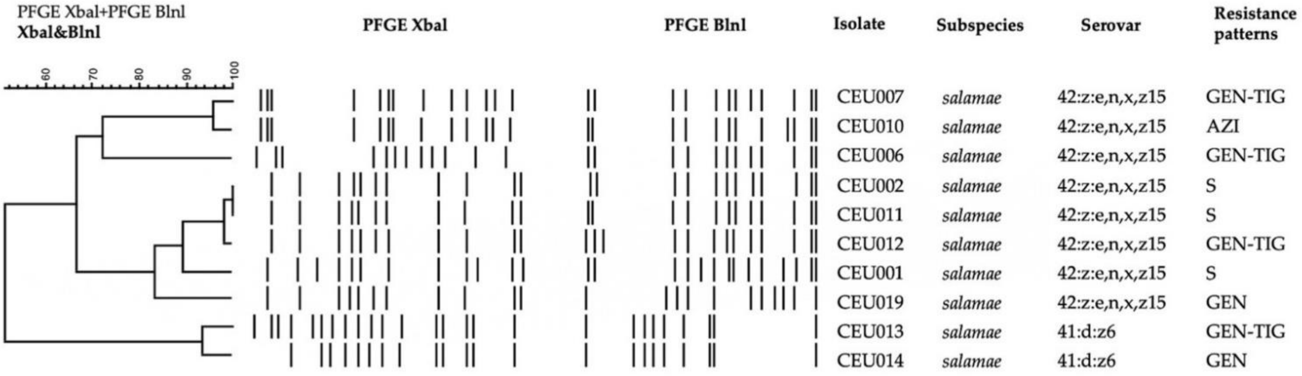
Table 2. *Cont.*

Subspecies	Serovar	n	Nuclei	Prevalence (%)
houtenae	43:z4,z23:-	1	1	6.7 ^{b}

n: Number of *Salmonella* strains per serovar. %: Percentage of positive samples. Nuclei: 1. Main nucleus, 3. Third nucleus, 4. Fourth nucleus. ^{a,b} Different superscripts in each column mean significant differences with a *p*-value < 0.05.

3.2. *Salmonella* Molecular Typing

PFGE analysis revealed six different pulsotypes within the ten subsp. *salamae* isolates, five of them belonging to serovar 42:z:e,n.x.z15 and the remaining one corresponding to isolates of serovar 41:d:z_6 (Figure 2). Isolates of ser. 42:z:e,n.x.z15 were grouped in two main clusters at 83.11% and 71.92% similarity, respectively. The single isolates of ser. Cerro and ser. Kentucky were not typable with the enzyme BlnI.



Isolate	Subspecies	Serovar	Resistance patterns
CEU007	*salamae*	42:z:e,n,x,z15	GEN-TIG
CEU010	*salamae*	42:z:e,n,x,z15	AZI
CEU006	*salamae*	42:z:e,n,x,z15	GEN-TIG
CEU002	*salamae*	42:z:e,n,x,z15	S
CEU011	*salamae*	42:z:e,n,x,z15	S
CEU012	*salamae*	42:z:e,n,x,z15	GEN-TIG
CEU001	*salamae*	42:z:e,n,x,z15	S
CEU019	*salamae*	42:z:e,n,x,z15	GEN
CEU013	*salamae*	41:d:z6	GEN-TIG
CEU014	*salamae*	41:d:z6	GEN

Figure 2. PFGE dendrogram of Xbal and Blnl profiles of *Salmonella enterica* subsp. *salamae*. isolates from California kingsankes in Gran Canaria and their resistance patterns. GEN: gentamicin, TIG: tigecycline, AZI: azithromycin. S: sensible.

3.3. Antimicrobial Susceptibility

From all strains isolated, 73.3% (11/15) were resistant to at least one of the twelve antimicrobials tested. The highest frequency of AMR was found to GEN (60%, 9/15) and TIG (40%, 6/15), followed by AZI (13.3%, 2/15), and AMP and CTZ with only one strain resistant (6.7%, 1/15) ($p < 0.001$). No resistance was found against CHL, CIP, COL, CTA, NAL, TRS, and TRI (Figure 2). Furthermore, the *S. enterica* ser. Cerro isolate was resistant to three different antimicrobials.

Overall, four different resistance patterns were found (Table 3, Figure 2). GEN alone (36.4%, 4/11) and GEN-TIG (36.4%, 4/11) were the most frequent patterns observed. Isolates with either of these AMR patterns also showed different PFGE profiles (Figure 2). AZI alone and the combination of GEN-AZI-TIG and AMP-CTZ-TIG were only observed once (9.1%, 1/11).

Table 3. Antimicrobial resistance according to the antibiotic and the *Salmonella* species and serovars isolated.

Species	Serovar	n	CIP	AMP	NAL	GEN	CHL	TRS	TRI	COL	CTA	AZI	CTZ	TIG
S. enterica subsp. *salamae*	42:z:e,n.x.z15	8	0	0	0	4	0	0	0	0	0	1	0	3
	41:d:z6	2	0	0	0	2	0	0	0	0	0	0	0	1
S. enterica subsp. *enterica*	Cerro	1	0	0	0	1	0	0	0	0	0	1	0	1
	Kentucky	1	0	0	0	1	0	0	0	0	0	0	0	0
	Midway	1	0	0	0	1	0	0	0	0	0	0	0	0

Table 3. *Cont.*

Species	Serovar	n	CIP	AMP	NAL	GEN	CHL	TRS	TRI	COL	CTA	AZI	CTZ	TIG
S. enterica subsp. *diarizonae*	60:-:-	1	0	1	0	0	0	0	0	0	0	0	1	1
S. enterica subsp. *houtenae*	43:z4,z23:-	1	0	0	0	0	0	0	0	0	0	0	0	0

n: number of *Salmonella* strains. CIP: ciprofloxacin, AMP: ampicillin, NAL: nalidixic acid, GEN: gentamicin, CHL: chloramphenicol, TRS: trimethoprim-sulphamethoxazole, TRI: trimethoprim, COL: colistin, CTA: cefotaxime, AZI: azithromycin, CTZ: ceftazidime, TIG: tigecycline.

4. Discussion

The present study demonstrates that 20.5% of invasive California kingsnakes from the island of Gran Canaria (Canary Islands, Spain) carry *Salmonella* spp. Genotyping analysis showed high diversity among isolates of subsp. *salamae*. From these isolates, 73.3% presented resistance to at least one of the antimicrobials tested, included in Decision 2013/652/EU. To our knowledge, this is the first study in the literature assessing the prevalence, genetic relatedness, and AMR of this zoonotic pathogen in the California kingsnake on Gran Canaria Island.

Reptiles have been considered carriers of *Salmonella* spp. worldwide, and their serological variety and antimicrobial resistance have been studied [10,29,31,32]. Therefore, they may pose a danger as a source of dissemination of the bacterium in the environment, as well as an important cause of animal and human infection, especially in at-risk populations [11,29,33]. Different studies worldwide have shown a wide variety of *Salmonella* spp. prevalence in domestic and wild snakes (being less prevalent in the latter), based on the geographic area, reptile species, time of sampling (as shedding is intermittent), and methodology used [31]. This vast difference among studies highlights the poor knowledge about *Salmonella* epidemiology in wild reptiles [31]. *Salmonella* spp. is an enterobacterium that is highly associated with stress. Hence, situations that increase stress in these animals increase the shedding of the bacteria into the environment [11]. Therefore, as has been done in other animals (such as livestock), it seems mandatory to unify methodology in terms of sampling and analysis of samples to be able to compare results between different regions and thus obtain more information from all the research carried out. In line with previous studies, our results showed that California kingsnake carries different subspecies and serovars of *Salmonella* [31] and, as in other studies, our results showed that sex directly influences the shedding of *Salmonella* spp. in reptiles, as the prevalence has been found in our study to be higher in California kingsnake females than in males [34,35]. However, as also seen in other studies, our results showed that size and weight have no influence on *Salmonella* shedding in reptiles [36].

Similarly to other reports, in this study, *Salmonella enterica* was the most common species isolated from reptiles [10,36,37]. The four subspecies we isolated (*S. enterica enterica* [I] (3/15), *S. enterica salamae* [II] (10/15), *S. enterica diarizonae* [IIIb] (1/15) and *S. enterica houtenae* [IV] (1/15)) have also been widely reported in different reptile studies, including snakes, turtles, and lizards [6,10,14,29,32]. In addition, *Salmonella enterica enterica* has been reported in the Gran Canaria Island as the main species isolated from feral cats, although the identified subspecies do not coincide with those found in the California kingsnake [34].

In some studies, snakes have been found to have the greatest diversity of *Salmonella* subspecies [32]. Of all *Salmonella enterica* subspecies, *enterica* has been considered the most prevalent subspecies in reptiles [38,39]. However, we have isolated this subspecies with a low prevalence. Instead, subsp. *salamae* was the most prevalent subspecies isolated in this study (53.3%). One of the hypotheses that could explain this fact is that the Canary Islands are a limited geographical area, which has allowed subsp. *salamae* to colonise these populations. Subsp. *salamae* has previously been isolated from other animals, such as carnivores [40], wild boars [41], and poultry [42]. However, this subspecies has not frequently been associated with human infections [43–45]. Finally, subsp. *diarizonae* and subsp. *houtenae* are two species especially related to snakes [46], and these subspecies have also been occasionally found in other animal species [41,47,48]. subsp. *diarizonae* and subsp. *houtenae* have also been described in human cases as a result of the close contact between humans and their exotic pets [49–52].

Salmonella serovars, with major epidemiological importance in human medicine and domestic animals, occur less frequently in cold-blooded animals [31]. To the authors' knowledge, this is the first report of the isolation of *Salmonella* ser. 42:z:e,n.x.z15 in the world, as well as the first report of *Salmonella* ser. Cerro, Midway, Kentucky, 41:d:z6, 60:-:-, and 43:z4,z23:- from California kingsnakes. Among them, ser. Kentucky has been considered a public health threat [52], as it has been identified as causing human pathologies, such as urinary tract infections or gastroenteritis [53,54]. This serovar has been isolated from a wide range of animals, ranging from pet reptiles (snakes, turtles, or lizards) [55] to poultry, dairy, cattle, or food [56], being an important source of human infections, directly through pets [55] or indirectly through food consumption [56]. Regarding *S.* ser. Cerro, it is a serovar infrequent in humans and widely adapted and associated with cattle pathologies [57,58], but it has also been described in some snake species [59,60] and other reptiles such as lizards [61] and turtles [11]. In turn, *S.* ser. Midway [32,62] and 43:z4,z23:- [9,52,59,61] have been widely reported in different snake species, as they are serovars closely related to snakes. To the authors' knowledge, the serovar 41:d:z6 has only been reported once, isolated from a green lizard, and it was designed as *S.* Hennepi [63], but no more references have been found.

The high genetic diversity of subsp. *salamae* isolates, which were all recovered from snakes from the same nuclei, suggests different sources of infection. Nevertheless, a broader sampling and a greater number of isolates to compare would be needed to confirm this. In addition, further studies, including whole genome sequencing (WGS), could be interesting for improving the information related to the epidemiology of *Salmonella* in reptiles [64].

Studies on AMR in *Salmonella* spp. isolates from wild snakes are scarce [15,65], but some studies suggest similar patterns of drug resistance to strains isolated from captive or pet snakes [29,52,66]. In line with previous studies, resistance against GEN was the most common AMR observed [15,65,67], followed by TIG and AZI [68]. This study has also shown resistance against AMP and CTZ [29,52]. Bacterial resistance to these antimicrobials has been widely documented due to its extensive use in veterinary medicine [65,67,68]. Hence, the close contact of these snakes with humans and livestock could explain the high frequency of GEN-resistant strains in this study and, in addition, to TIG and AZI. These results, along with those of other authors, suggest that the problem of AMR is not limited to its initial niches, potentially livestock, but that wildlife could also play an important role in the spread of these strains in households [69].

In our study, no resistance against CIP, NAL, CHL, TRS, TRI, COL, and CTA was found in *Salmonella* isolates, as seen in other studies [32,68]. However, there is a huge difference in AMR patterns of *Salmonella* spp. strains depending on the geographical location [31,32,52], ranging from 14% in Taiwan [70] to 90% in Italy [68]. These results demonstrate that external factors other than the animal species could influence the development of AMR patterns.

5. Conclusions

The present study revealed the importance of wild invasive California kingsnakes as reservoirs of *Salmonella* spp., which could pose a direct threat to livestock and humans. Identification of drug-resistant *Salmonella* subsp. and serovars in wildlife provides valuable information on potential routes of transmission that involve risks to public and animal health.

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H., S.L.P., S.V., C.M., A.M.-F., M.C.-C., T.A., T.G.B. and P.A.L.-G.; data curation, C.M. and A.M.-F.; writing—original draft preparation, C.M. and A.M.-F.; writing—review and editing, K.M.S.-H., E.R.-P., I.R.M., B.A.-H., S.L.P., S.V., C.M., A.M.-F., M.C.-C., T.A., T.G.B. and P.A.L.-G.; visualization, K.M.S.-H., E.R.-P., I.R.M., B.A.-H., S.L.P., S.V., C.M., A.M.-F., M.C.-C., T.A., T.G.B. and P.A.L.-G.; supervision, K.M.S.-H., E.R.-P., I.R.M., B.A.-H., S.L.P., S.V., C.M., A.M.-F., M.C.-C., T.A., T.G.B. and P.A.L.-G.; project administration, K.M.S.-H., E.R.-P., I.R.M., B.A.-H., S.L.P., S.V., C.M., A.M.-F., M.C.-C., T.A., T.G.B. and P.A.L.-G.; funding acquisition, K.M.S.-H., E.R.-P., I.R.M., B.A.-H., S.L.P., S.V., C.M., A.M.-F., M.C.-C., T.A., T.G.B. and P.A.L.-G. All authors have read and agreed to the published version of the manuscript.

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Section V: Appendices

Appendix 1: Endemic parasites in the Canary Islands.

Appendix 2: Non-endemic parasites in the Canary Islands.

Appendix 3: Accidental or non-obligate parasites in the Canary Islands.

Appendix 4: Type material of *Lampropeltis californiae* hold by Museums**.**

Appendix 5: Parasitic species in snakes of the genus *Lampropeltis*.

Appendix 1:

Endemic parasites in the Canary Islands

Species of endemic parasites, according to BIOTA**. FLH:** Free living, haematophagous invertebrate**. C:** Ectoparasite. **N:** Endoparasite. *Island endemism **H**: El Hierro**; P**: La Palma**; Go**: La Gomera; **Tn**: Tenerife; **GC**: Gran Canaria; **F**: Fuerteventura; **L**: Lanzarote.

Class	Order	Family	Species	Category	Host	-	H	P	Go	Tn	GC	F	L
Insecta	**Diptera**	**Psychodidae**	*Phlebotomus fortunatarum*	FLH	Generalist	-	1	0	1	1	1	0	0
		Simuliidae	*Simulium guimari*	FLH	Generalist	-	0	1	1	1	1	0	0
			Simulium paraloutetense	FLH	Generalist	-	0	0	1	0	1	0	0
			Simulium velutinum	FLH	Generalist	-	0	1	1	1	0	0	0
		Tabanidae	*Atylotus canarius*	FLH	Generalist	-	0	0	0	1	0	1	1
	Siphonaptera	**Leptopsyllidae**	*Leptopsylla algira atlantidis**	C	Mammalia	Rodentia	0	0	0	0	0	0	1
		Pulicidae	*Xenopsylla guancha*	C	Mammalia	Rodentia	0	0	0	0	0	1	1
Arachnida	**Mesostigmata**	**Laelapidae**	*Echinonyssus molinae**	C	Mammalia	Insectivora	0	0	0	0	1	0	0
		Macronyssidae	*Ophionyssus dolatelacensis**	C	Squamata	Lacertida	0	0	0	0	0	0	1
			*Ophionyssus galloticolus**	C	Squamata	Lacertida	0	0	0	1	0	0	0
			*Ophionyssus setosus**	C	Squamata	Lacertida	0	0	0	0	1	0	0
			Steatonyssus balcellsi	C	Mammalia	Chiroptera	0	1	1	1	0	0	0
			Steatonyssus teidae	C	Mammalia	Chiroptera	0	1	0	1	0	0	0
	Trombidiformes	**Pterigosomatidae**	*Geckobia canariensis*	C	Squamata	Gekkota	1	1	1	1	0	0	0
			Geckobia tinerfensis	C	Squamata	Gekkota	1	1	1	1	0	0	0
Maxillopoda	**Cephalobaenida**	**Cephalobaenidae**	*Raillietiella morenoi**	N	Squamata	Lacertida	0	0	0	0	0	0	1
Chromadorea	**Rhabditida**	**Pharyngodonidae**	*Alaeuris numida canariensis**	N	Squamata	Lacertida	0	0	0	0	1	0	0
			*Alaeuris stehlini**	N	Squamata	Lacertida	0	0	0	0	1	0	0
			*Pharyngodon hierrensis**	N	Squamata	Lacertida	1	0	0	0	0	0	0
			*Pharyngodon hispanicus**	N	Squamata	Lacertida	0	0	0	1	0	0	0
			Skrjabinodon canariensis	N	Squamata	Lacertida	0	0	1	1	1	0	0
			Spauligodon atlanticus	N	Squamata	Lacertida	1	1	1	1	1	1	1
			Thelandros filiformis	N	Squamata	Lacertida	1	1	1	1	1	0	0
			Thelandros galloti Astasio-	N	Squamata	Lacertida	1	1	1	1	0	0	0
			Thelandros tinerfensis	N	Squamata	Lacertida	1	1	1	1	0	0	0
Trematoda	**Plagiorchiida**	**Dicrocoeliidae**	*Pseudoparadistomum yaizaense**	N	Squamata	Lacertida	0	0	0	0	0	0	1

Appendix 2:

Non-endemic parasites in the Canary Islands

M/T: Habitat; **M**: Marine; **T**:Terrestrial; **Dub**: Dubious taxon or report; **Ct**: Category **C**: Ectoparasite; **CM**: Cavitary myiasis; **N**: Endoparasite; **FLH**: Free-living, haematophagous invertebrate; **Hg**: Host group; **Hsg**: Host subgroup; **H**: El Hierro; **P**: La Palma; **Go**: La Gomera; **Tn**: Tenerife; **GC**: Gran Canaria; **F**: Fuerteventura; **L**: Lanzarote; $^{\mathrm{Tx1}}$: Cited as *Hyalomma detritum*. $^{\mathrm{Tx2}}$: Cited also as *Ixodes pari*.

In the case of the phylum Nematoda, all chromadoreans in this list belong to the order Rhabditida. Hence, the taxonomic name given bellow in the "Order" column is the suborder instead.

Phylum	Clases	Order	Family	Species	M/T	Ct	Hg	Hsg	H	P	Go	Tn	GC	F	L
Arthropoda	**Insecta**	**Diptera**	**Ceratopogonidae**	*Culicoides obsoletus*	T	FLH	Generalist	Generalist	0	1	0	1	0	0	0
				Forcipomyia fuliginosa	T	FLH	Generalist	Generalist	0	1	0	0	0	0	0
				Forcipomyia nitens	T	FLH	Generalist	Generalist	0	1	0	0	0	0	0
			Culicidae	*Anopheles sergentii*	T	FLH	Generalist	Generalist	0	0	0	1	1	0	0
				Anopheles multicolor	T	FLH	Generalist	Generalist	0	0	1	1	0	1	0
				Anopheles cinereus	T	FLH	Generalist	Generalist	0	1	1	1	1	1	0
				Culex hortensis	T	FLH	Generalist	Generalist	0	0	0	0	1	0	0
				Culex arbieeni	T	FLH	Generalist	Generalist	0	1	1	1	1	0	0
				Culex laticinctus	T	FLH	Generalist	Generalist	1	1	1	1	1	0	0
				Culex theileri	T	FLH	Generalist	Generalist	0	1	1	1	1	0	1
				Culex pipiens	T	FLH	Generalist	Generalist	1	1	1	1	1	1	1
				Culiseta longiareolata	T	FLH	Generalist	Generalist	1	1	1	1	1	1	1
				Ochlerotatus caspius	T	FLH	Generalist	Generalist	0	0	0	0	1	0	0
				Ochlerotatus eatoni	T	FLH	Generalist	Generalist	0	1	0	1	0	0	0
			Hippoboscidae	*Crataerina acutipennis*	T	C	Aves	Apodiformes	0	0	0	1	1	1	0
				Hippobosca equina	T	C	Mammalia	Artyodactyla	0	1	0	1	1	0	0
				Icosta minor	T	C	Aves	Passeriformes	0	0	0	1	0	0	0
				Icosta pilosa	T	C	Aves	Otidiformes	0	0	0	0	0	1	0
				Melophagus ovinus	T	C	Mammalia	Artyodactyla	0	0	0	1	0	0	0
				Olfersia fumipennis	T	C	Aves	Generalist	0	0	0	0	0	0	0
				Ornithomyia avicularia	T	C	Aves	Generalist	0	0	0	1	0	0	0
				Ornithomyia chloropus	T	C	Aves	Generalist	0	1	0	1	0	0	0
				Ornithophila metallica	T	C	Aves	Generalist	0	0	0	1	0	0	1
				Pseudolynchia canariensis	T	C	Aves	Generalist	0	1	0	1	1	1	0
			Muscidae	*Haematobia titillans*	T	FLH	Generalist	-	0	0	0	0	0	0	0
				Stomoxys calcitrans	T	FLH	Generalist	-	1	1	1	1	1	1	1
			Oestridae	*Oestrus ovis*	T	CM	Generalist	-	1	0	0	1	1	1	0
			Psychodidae	*Phlebotomus ariasi*	T	FLH	Generalist	-	0	0	0	0	0	1	0
				Phlebotomus perniciosus	T	FLH	Generalist	-	0	0	0	1	0	0	0
				Phlebotomus sergenti	T	FLH	Generalist	-	0	0	0	1	0	0	0
			Simuliidae	*Simulium intermedium*	T	FLH	Generalist	-	0	1	1	1	1	0	0
				Simulium pseudequinum	T	FLH	Generalist	-	0	1	1	1	1	0	0

Phylum	Clases	Order	Family	Species	M/T	Ct	Hg	Hsg	H	P	Go	Tn	GC	F	L
Arthropoda	**Insecta**	**Diptera**	**Simuliidae**	*Simulium ruficorne*	T	FLH	Generalist	-	0	1	1	1	1	1	0
			Tabanidae	*Tabanus cordiger*	T	FLH	Generalist	-	0	0	0	1	0	1	0
		Phthiraptera	**Haematopinidae**	*Haematopinus suis*	T	C	Mammalia	Artyodactyla	0	0	0	1	0	0	0
				Neohaematopinus pectinifer	T	C	Mammalia	Rodentia	0	0	0	0	0	1	0
			Haplopleuridae	*Polyplax spinulosa*	T	C	Mammalia	Rodentia	1	0	0	1	0	0	0
			Linognathidae	*Linognathus setosus*	T	C	Mammalia	Carnivora	0	0	0	1	0	0	0
			Menoponidae	*Austromenopon echinatum*	T	C	Aves	Procellariiformes	0	0	0	1	1	0	1
				Dennyus hirundinis	T	C	Aves	Apodiformes	0	0	0	0	0	0	0
				Longimenopon infans	T	C	Aves	Procellariiformes	0	0	0	0	0	0	0
			Pediculidae	*Pediculus humanus*	T	C	Mammalia	Primates	0	0	0	0	0	0	0
			Philopteridae	*Campanulotes bidentatus*	T	C	Aves	Columbiformes	0	0	0	1	0	0	0
				Coloceras quadraticus	T	C	Aves	Columbiformes	0	0	0	0	0	0	0
				Columbicola columbae	T	C	Aves	Columbiformes	0	0	0	1	0	0	0
				Cuclotogaster barbara	T	C	Aves	Galliformes	0	0	0	1	0	0	0
				Degeeriella fulva	T	C	Aves	Accipitriformes	0	0	0	1	0	0	0
				Halipeurus abnormis	T	C	Aves	Procellariiformes	0	0	0	1	1	0	1
				Saemundssonia peusi	T	C	Aves	Procellariiformes	0	0	0	1	1	0	1
				Strigiphilus goniodicerus	T	C	Aves	Strigiformes	0	0	0	0	0	0	0
			Phthiridae	*Phthirus pubis*	T	C	Mammalia	Primates	0	0	0	0	0	0	0
		Siphonaptera	**Ceratophyllidae**	*Dasypsyllus gallinulae*	T	C	Aves	Generalist	0	0	1	1	0	0	0
				Nosopsyllus barbarus	T	C	Mammalia	Rodentia	1	1	1	0	1	1	0
			Ctenophthalmidae	*Stenoponia tripectinata*	T	C	Mammalia	Rodentia	1	1	0	1	1	0	0
			Ischnopsyllidae	*Ischnopsyllus octactenus*	T	C	Mammalia	Chiroptera	0	0	1	0	0	0	0
				Ischnopsyllus intermedius	T	C	Mammalia	Chiroptera	0	1	1	0	0	0	0
			Leptopsyllidae	*Leptopsylla algira*	T	C	Mammalia	Insectivora	0	0	0	0	0	0	1
				Leptopsylla segnis	T	C	Mammalia	Rodentia	1	1	1	1	1	1	0
			Pulicidae	*Ctenocephalides canis*	T	C	Mammalia	Generalist	0	0	0	1	0	0	0
				Ctenocephalides felis	T	C	Mammalia	Generalist	0	0	0	1	1	0	0
				Echidnophaga gallinacea	T	C	Generalist	Generalist	0	0	0	1	1	1	0
				Echidnophaga murina	T	C	Mammalia	Rodentia	1	0	0	0	1	1	0
				Pulex irritans	T	C	Mammalia	Generalist	0	0	1	1	0	0	0
				Xenopsylla cheopis	T	C	Mammalia	Rodentia	1	0	0	1	1	1	0

Phylum	Clases	Order	Family	*Species*	M/T	Ct	Hg	Hsg	H	P	Go	Tn	GC	F	L
Arthropoda	**Insecta**	**Siphonaptera**	**Pulicidae**	*Xenopsylla gratiosa*	T	C	Aves	Procellariiformes	1	0	0	1	1	0	1
				Xenopsylla brasiliensis	T	C	Mammalia	Rodentia	0	1	1	1	1	0	1
	Arachnida	**Ixodida**	**Argasidae**	*Argas persicus*	T	C	Aves	Generalist	0	0	0	1	0	0	0
				Argas vespertilionis	T	C	Mammalia	Chiroptera	0	1	1	1	0	0	0
			Ixodidae	*Haemaphysalis punctata*	T	C	Generalist	Generalist	0	0	0	1	0	0	0
				Haemaphysalis sulcata	T	C	Mammalia,	Generalist	0	0	0	1	0	0	0
				Hyalomma dromedarii	T	C	Generalist	Generalist	0	0	0	0	0	0	0
				Hyalomma impressum	T	C	Mammals	Generalist	0	0	0	0	0	0	0
				Hyalomma truncatum	T	C	Mammals	Generalist	0	0	0	0	0	0	0
				Hyalomma scupense [Tx1]	T	C	Mammals	Artyodactyla	0	1	0	1	0	0	0
				Hyalomma marginatum	T	C	Generalist	Generalist	0	0	0	1	0	1	1
				Hyalomma lusitanicum	T	C	Mammals	Generalist	1	0	0	1	1	1	0
				Ixodes frontalis[Tx2]	T	C	Aves	Generalist	0	0	0	1	0	0	0
				Ixodes ventalloi	T	C	Mammalia	Generalist	0	0	0	1	0	0	0
				Rhipicephalus turanicus	T	C	Generalist	Generalist	0	0	0	0	0	1	1
				Rhipicephalus pusillus	T	C	Mammalia	Lagomorpha	0	1	0	0	1	1	1
				Rhipicephalus sanguineus	T	C	Mammalia	Carnivora	0	1	0	1	1	1	1
		Mesostigmata	**Laelapidae**	*Eulaelaps stabularis*	T	C	Mammalia	Rodentia	0	0	0	1	0	0	0
				Laelaps echidninus	T	C	Mammalia	Rodentia	0	0	0	1	0	0	0
				Laelaps nuttalli	T	C	Mammalia	Rodentia	0	0	0	1	0	0	0
			Macronyssidae	*Ornithonyssus bacoti*	T	C	Mammalia	Rodentia	0	1	0	1	0	0	0
				Parasteatonyssus hoogstraali	T	C	Mammalia	Chiroptera	0	0	0	1	0	0	0
			Spinturnicidae	*Spinturnix plecotinus*	T	C	Mammalia	Chiroptera	0	1	0	0	0	0	0
		Sarcoptiformes	**Sarcoptidae**	*Sarcoptes scabiei*	T	C	Mammalia	Generalist	0	0	0	0	0	0	0
		Trombidiformes	**Myobiidae**	*Myobia musculi*	T	C	Mammalia	Rodentia	0	1	0	0	0	0	0
				Radfordia ensifera	T	C	Mammalia	Rodentia	0	0	0	1	0	0	0
	Hexanauplia	**Siphonostomatoida**	**Caligidae**	*Alebion crassus*	M	C	Chondrichthyes	Carcharhiniformes	0	0	0	0	0	0	0
			Eudactylinidae	*Eudactylina acuta*	M	C	Chondrichthyes	Squatiniformes	0	1	0	0	0	0	0
			Lernaeopodidae	*Pseudocharopinus pillaii*	M	C	Chondrichthyes	Myliobatiformes	0	0	0	0	0	0	0
			Pandaridae	*Pandarus cranchii*	M	C	Chondrichthyes	Carcharhiniformes	0	0	0	0	0	0	0
	Malacostraca	**Isopoda**	**Aegidae**	*Aegapheles deshaysiana*	M	C	Chondrichthyes	Squatiniformes	0	0	0	1	1	1	1
			Cymothoidae	*Anilocra capensis*	M	C	Osteichthyes	Generalist	0	0	0	0	1	0	0

Phylum	Clases	Order	Family	Species	M/T	Ct	Hg	Hsg	H	P	Go	Tn	GC	F	L
Arthropoda	**Malacostraca**	**Isopoda**	**Cymothoidae**	*Ceratothoa capri*	M	C	Osteichthyes	-	0	0	0	0	0	0	0
				Ceratothoa oestroides	M	C	Osteichthyes	Gadiformes	0	0	0	1	1	0	0
				Ceratothoa parallela	M	C	Osteichthyes	Perciformes	0	0	0	1	1	0	0
				Ceratothoa steindachneri	M	C	Osteichthyes	Perciformes	0	1	0	0	1	0	0
				Livoneca sulcata	M	C	Osteichthyes	Perciformes	0	0	0	1	1	1	1
				Nerocila bivittata	M	C	Osteichthyes	Tetraodontiformes	0	0	0	1	0	0	0
				Nerocila armata	M	C	Osteichthyes	-	0	0	0	1	1	0	0
			Gnathiidae	*Paragnathia formica*^{Dub}	M	C	Osteichthyes	-	0	0	0	0	0	1	0
	Thecostraca	**Pollicipedomorpha**	**Pollicipedidae**	*Anelasma squalicola*	M	C	Chondrichthyes	Squaliformes	0	0	0	1	1	0	0
Nematoda	**Chromadorea**	**Spirurina**	**Anisakidae**	*Anisakis simplex*	M	N	Mammalia	Odontoceti	0	0	0	0	0	0	0
			Ascarididae	*Ascaridia columbae*	T	N	Aves	Columbiformes	0	0	0	1	0	0	0
				Ascaridia galli	T	N	Aves	Galliformes	0	0	0	1	0	0	0
				Ascaris lumbricoides	T	N	Mammalia	Primates	1	1	1	1	0	0	0
				Lyruterina nigropunctata	T	N	Aves	Galliformes	0	0	0	1	0	0	0
				Toxascaris leonina	T	N	Mammalia	Carnivora	0	0	0	1	0	0	0
				Toxocara cati	T	N	Mammalia	Carnivora	0	0	0	0	0	0	0
				Toxocara canis	T	N	Mammalia	Carnivora	1	1	1	1	1	1	1
			Cosmocercidae	*Cosmocerca ornata*	T	N	Amphibia	Anura	0	0	0	1	0	0	0
			Heterakidae	*Heterakis gallinarum*	T	N	Aves	Galliformes	0	0	0	1	0	0	0
			Heteroxynematidae	*Aspiculuris tetraptera*	T	N	Mammalia	Rodentia	0	0	0	0	0	0	0
				Dermatoxys getula	T	N	Mammalia	Rodentia	0	0	0	0	0	1	0
			Oxyuridae	*Enterobius vermicularis*	T	N	Mammalia	Primates	1	1	1	1	1	1	1
				Passalurus ambiguus	T	N	Mammalia	Lagomorpha	0	0	0	1	0	0	1
				Skrjabinema ovis	T	N	Mammalia	Artyodactyla	0	0	0	1	0	0	0
				Syphacia muris	T	N	Mammalia	Rodentia	1	0	0	0	0	0	0
				Syphacia pallaryi	T	N	Mammalia	Rodentia	0	0	0	0	0	1	0
			Pharyngodonidae	*Alaeuris numida*	T	N	Squamata	Lacertida	1	0	0	0	1	0	0
				Parapharyngodon astasioae^{Dub}	T	N	Squamata	Lacertida	0	0	0	0	0	0	0
				Parapharyngodon corderoi^{Dub}	T	N	Squamata	Lacertida	0	0	0	0	0	0	0
				Parapharyngodon lamasi^{Dub}	T	N	Squamata	Lacertida	0	0	0	0	0	0	0
				Parapharyngodon tirma^{Dub}	T	N	Squamata	Lacertida	0	0	0	0	0	0	0
				Parapharyngodon bulbosus	T	N	Squamata	Lacertida	0	0	0	0	1	0	0

Phylum	Clases	Order	Family	Species	M/T	Ct	Hg	Hsg	H	P	Go	Tn	GC	F	L
Nematoda	**Chromadorea**	**Spirurina**	**Pharyngodonidae**	*Parapharyngodon lilfordii*	T	N	Squamata	Lacertida	0	1	1	0	0	0	1
				Parapharyngodon echinatus	T	N	Squamata	Generalist	0	1	1	1	1	1	1
				Parapharyngodon micipsae	T	N	Squamata	Generalist	1	1	1	1	1	1	1
				Pseudolaeuris galloti^{Dub}	T	N	Squamata	Lacertida	0	0	0	0	0	0	0
				Pseudolaeuris zapateroi^{Dub}	T	N	Squamata	Lacertida	0	0	0	0	0	0	0
				Skrjabinodon medinae	T	N	Squamata	Lacertida	0	1	0	1	0	0	1
				Spauligodon tarentolae	T	N	Squamata	Gekkota	0	1	1	0	0	0	0
				Tachygonetria martinezi^{Dub}	T	N	Squamata	Lacertida	0	0	0	0	0	0	0
				Tachygonetria palmarum^{Dub}	T	N	Squamata	Lacertida	0	0	0	0	0	0	0
				Tachygonetria conica	T	N	Squamata	Lacertida	0	0	0	0	1	0	0
				Tachygonetria numidica	T	N	Squamata	Lacertida	0	0	0	0	1	0	0
				Tachygonetria dentata	T	N	Squamata	Lacertida	1	0	0	0	1	0	0
				Tachygonetria palearcticus	T	N	Squamata	Lacertida	1	0	0	0	1	0	0
				Thelandros zoiloi^{Dub}	T	N	Squamata	Lacertida	0	0	0	0	0	0	0
			Seuratidae	*Skrjabinelazia hoffmanni*	T	N	Squamata	Generalist	0	0	0	0	0	1	0
				Skrjabinelazia pyrenaica	T	N	Squamata	Generalist	1	0	1	0	0	0	0
			Onchocercidae	*Dirofilaria repens*	T	N	Mammalia	Carnivora	0	0	0	0	0	0	0
				Dirofilaria immitis	T	N	Mammalia	Carnivora	0	0	0	1	1	0	0
			Spirocercidae	*Mastophorus muris*	T	N	Mammalia	Rodentia	1	0	0	0	0	0	0
				Protospirura muricola	T	N	Mammalia	Rodentia	0	0	0	0	0	1	0
				Streptopharagus greenbergi	T	N	Mammalia	Rodentia	1	0	0	0	0	0	0
			Tetrameridae	*Crassicauda grampicola*	M	N	Mammalia	Odontoceti	0	0	0	0	0	0	0
		Rhabditina	**Ancylostomatidae**	*Ancylostoma caninum*	T	N	Mammalia	Carnivora	0	0	0	1	0	0	0
				Bunostomum trigonocephalum	T	N	Mammalia	Artyodactyla	0	0	0	1	0	0	0
			Angiostrongylidae	*Aelurostrongylus abstrusus*	T	N	Mammalia	Carnivora	0	0	0	1	0	0	0
			Dictyocaulidae	*Dictyocaulus filaria*	T	N	Mammalia	Artyodactyla	0	0	0	0	0	0	0
			Metastrongylidae	*Angiostrongylus cantonensis*	T	N	Mammalia	Rodentia	0	0	0	1	0	0	0
			Molineidae	*Nematodirella dromedarii*^{Dub}	T	N	Mammalia	Artyodactyla	0	0	0	0	0	0	0
				Oswaldocruzia filiformis	T	N	Amphibia	Anura	0	0	0	1	1	0	0
			Pseudaliidae	*Stenurus minor*	M	N	Mammalia	Odontoceti	0	0	0	0	0	0	0
			Strongylidae	*Strongylus galdosianus*^{Dub}	T	N	Amphibia	Anura	0	0	0	0	0	0	0
			Trichostrongylidae	*Cooperia curticei*	T	N	Mammalia	Artyodactyla	0	0	0	0	0	0	0

Phylum	Clases	Order	Family	Species	M/T	Ct	Hg	Hsg	H	P	Go	Tn	GC	F	L
Nematoda	**Chromadorea**	**Rhabditina**	**Trichostrongylidae**	*Trichostrongylus retortaeformis*	T	N	Mammalia	Artyodactyla	0	0	0	1	0	0	0
		Tylenchina	**Strongyloididae**	*Strongyloides papillosus*	T	N	Mammalia	Artyodactyla	0	0	0	0	0	0	0
	Dorylaimia	**Trichinellida**	**Capillariidae**	*Aonchotheca annulosa*	T	N	Mammalia	-	0	0	0	0	0	0	0
				Aonchotheca caudinflata	T	N	Aves	Galliformes	0	0	0	1	0	0	0
				Baruscapillaria obsignata	T	N	Aves	Galliformes	0	0	0	1	0	0	0
				Eucoleus annulatus	T	N	Aves	Galliformes	0	0	0	1	0	0	0
			Trichuridae	*Trichuris muris*	T	N	Mammalia	Rodentia	1	0	0	0	0	0	0
				Trichuris vulpis	T	N	Mammalia	Carnivora	0	0	0	1	0	0	0
				Trichuris trichiura	T	N	Mammalia	Primates	0	1	1	1	0	0	0
Platyhelminth	**Cestoda**	**Cyclophyllidea**	**Anoplocephalidae**	*Andrya cuniculi*	T	N	Mammalia	Lagomorpha	0	0	0	1	0	0	0
				Killigrewia delafondi	T	N	Aves	Columbiformes	0	0	0	0	0	0	0
				Mosgovoyia ctenoides	T	N	Mammalia	Lagomorpha	0	0	0	1	0	0	1
				Oochoristica agamae	T	N	Squamata	Generalist	0	0	0	0	1	0	0
				Oochoristica tuberculate	T	N	Squamata	Generalist	0	0	0	1	1	0	0
			Davaineidae	*Ophryocotyle proteus*	T	N	Aves	Charadriiformes	0	0	0	0	0	0	0
				Raillietina micracantha	T	N	Aves	Columbiformes	0	0	0	1	0	0	0
			Dilepididae	*Biuterina passerine*	T	N	Aves	Passeriformes	0	0	0	0	0	0	0
				Choanotaenia ibanezi	T	N	Aves	Charadriiformes	0	0	0	0	0	0	0
				Choanotaenia infundibulum	T	N	Aves	Galliformes	0	0	0	1	0	0	0
				Dilepis undula	T	N	Aves	Passeriformes	0	0	0	0	0	0	0
				Fuhrmannolepis decacantha	T	N	Aves	Charadriiformes	0	0	0	0	0	0	0
				Neogryporhynchus	T	N	Aves	Pelecaniformes	0	0	0	0	0	0	0
				Paricterotaenia porosa	T	N	Aves	Charadriiformes	0	0	0	0	0	0	0
				Pseudangularia brachycolpos	T	N	Aves	Apodiformes	0	0	0	0	0	0	0
				Sobolevitaenia moldavica	T	N	Aves	Passeriformes	0	0	0	0	0	0	0
				Sobolevitaenia similis	T	N	Aves	Passeriformes	0	0	0	0	0	0	0
				Spiniglans constricta	T	N	Aves	Passeriformes	0	0	0	0	0	0	0
			Dipylidiidae	*Diplopylidium noelleri*	T	N	Mammalia	Carnivora	0	1	1	1	1	0	0
				Diplopylidium acanthotetra	T	N	Mammalia	Carnivora	0	1	0	1	1	0	1
				Dipylidium caninum	T	N	Mammalia	Carnivora	0	0	0	1	0	0	0
			Hymenolepididae	*Echinocotyle glareolae*	T	N	Aves	Charadriiformes	0	0	0	0	0	0	0
				Echinocotyle multiglandularis	T	N	Aves	Charadriiformes	0	0	0	0	0	0	0

Phylum	Clases	Order	Family	Species	M/T	Ct	Hg	Hsg	H	P	Go	Tn	GC	F	L
Platyhelminth	**Cestoda**	**Cyclophyllidea**	**Hymenolepididae**	*Echinocotyle paradoxa*	T	N	Aves	Charadriiformes	0	0	0	0	0	0	0
				Hymenolepis diminuta	T	N	Mammalia	Rodentia	1	0	0	0	0	0	0
				Rodentolepis nana	T	N	Mammalia	Rodentia	0	0	1	1	1	1	1
				Wardium calumnacantha	T	N	Aves	Charadriiformes	0	0	0	0	0	0	0
				Wardium paraclavicirrus	T	N	Aves	Charadriiformes	0	0	0	0	0	0	0
			Mesocestoididae	*Mesocestoides lineatus*	T	N	Mammalia	Carnivora	0	0	0	0	0	0	0
			Nematotaeniidae	*Nematotaenia tarentolae*	T	N	Squamata	Generalist	0	0	0	1	1	1	0
			Paruterinidae	*Notopentorchis iduncula*	T	N	Aves	Apodiformes	0	0	0	0	0	0	0
			Taeniidae	*Taenia hydatigena*	T	N	Mammalia	Carnivora	0	0	0	0	0	0	0
				Taenia saginata	T	N	Mammalia	Carnivora	0	0	0	0	0	0	0
				Taenia taeniaeformis	T	N	Mammalia	Carnivora	1	0	0	0	0	0	0
				Taenia pisiformis	T	N	Mammalia	Carnivora	0	0	0	1	0	0	0
				Taenia solium	T	N	Mammalia	Primates	0	0	0	1	0	0	0
		Tetrabothriidea	**Tetrabothriidae**	*Tetrabothrius erostris*	M	N	Aves	Charadriiformes	0	0	0	0	0	0	0
		Tetraphyllidea	**Phyllobothriidae**	*Monorygma grimaldii*	M	N	Chondrichthyes	-	0	0	0	0	0	0	0
				Phyllobothrium delphini	M	N	Chondrichthyes	-	0	0	0	0	0	0	0
	Monogenea	**Capsalidea**	**Capsalidae**	*Capsala martinieri*	M	C	Osteichthyes	Tetraodontiformes	0	0	0	0	0	0	1
				Neobenedenia melleni	M	C	Osteichthyes	-	0	0	0	1	0	0	0
		Polyopisthocotylea	**Axinidae**	*Axine belones*	M	C	Osteichthyes	-	0	0	0	0	0	0	0
				Heteraxine louiseuzeti	M	C	Osteichthyes	-	0	0	0	0	0	0	0
			Diclidophoridae	*Choricotyle chrysophryi*	M	C	Osteichthyes	-	0	0	0	0	0	0	0
				Cyclocotyla bellones	M	C	Osteichthyes	-	0	0	0	0	0	0	0
				Gempylitrema longipedunculatum	M	C	Osteichthyes	-	0	0	0	0	0	0	0
				Kuhnia scombri	M	C	Osteichthyes	-	0	0	0	0	0	0	0
				Mazocraeoides georgei	M	C	Osteichthyes	-	0	0	0	0	0	0	0
			Discocotylidae	*Winkenthughesia bramae*	M	C	Osteichthyes	-	0	0	0	0	0	0	0
			Gastrocotylidae	*Gastrocotyle trachuri*	M	C	Osteichthyes	-	0	0	0	0	0	0	0
				Gotocotyla acanthura	M	C	Osteichthyes	-	0	0	0	0	0	0	0
				Pseudaxine trachuri	M	C	Osteichthyes	-	0	0	0	0	0	0	0
			Hexabothriidae	*Hexabothrium mustelid*	M	C	Chondrichthyes	-	0	0	0	0	0	0	0
			Hexostomatidae	*Hexostoma thynni*	M	C	Osteichthyes	-	0	0	0	0	0	0	0
			Microcotylidae	*Atriaster heterodus*	M	C	Osteichthyes	-	0	0	0	0	0	0	0

Phylum	Clases	Order	Family	Species	M/T	Ct	Hg	Hsg	H	P	Go	Tn	GC	F	L
Platyhelminth	**Monogenea**	**Polyopisthocotylea**	**Microcotylidae**	*Atriaster maillardi*	M	C	Osteichthyes	-	0	0	0	0	0	0	0
				Atrispinum salpae	M	C	Osteichthyes	-	0	0	0	0	0	0	0
				Atrispinum seminalis	M	C	Osteichthyes	-	0	0	0	0	0	0	0
				Bivagina alcedinis	M	C	Osteichthyes	-	0	0	0	0	0	0	0
				Bychowskicotyla mormyrid	M	C	Osteichthyes	-	0	0	0	0	0	0	0
				Intracotyle hannibali	M	C	Osteichthyes	-	0	0	0	0	0	0	0
				Microcotyle erythrini	M	C	Osteichthyes	-	0	0	0	0	0	0	0
				Microcotyle pomatomid	M	C	Osteichthyes	-	0	0	0	0	0	0	0
				Polylabris tubicirrus	M	C	Osteichthyes	-	0	0	0	0	0	0	0
				Pseudoaspinatrium gallieni	M	C	Osteichthyes	-	0	0	0	0	0	0	0
				Rhinecotyle crepitacula	M	C	Osteichthyes	-	0	0	0	0	0	0	0
			Pyragraphoridae	*Pyragraphorus hollisae*	M	C	Osteichthyes	-	0	0	0	0	0	0	0
	Trematoda	**Diplostomida**	**Brachylaimidae**	*Brachylaima fuscata*	T	N	Aves	Passeriformes	0	0	0	0	0	0	0
				Postharmostomum gallinum	T	N	Aves	Passeriformes	0	0	0	0	0	0	0
			Cyclocoelidae	*Cyclocoelum mutabile*	T	N	Aves	Charadriiformes	0	0	0	0	0	0	0
			Leucochloridiidae	*Leucochloridium perturbatum*	T	N	Aves	Passeriformes	0	0	0	0	0	0	0
				Urogonimus macrostomus	T	N	Aves	Passeriformes	0	0	0	0	0	0	0
				Urogonimus turdi	T	N	Aves	Passeriformes	0	0	0	0	0	0	0
			Schistosomatidae	*Ornithobilharzia canaliculata*	T	N	Aves	Charadriiformes	0	0	0	0	0	0	0
			Strigeidae	*Apharyngostrigea cornu*	T	N	Aves	Pelecaniformes	0	0	0	0	0	0	0
				Apharyngostrigea ramai	T	N	Aves	Pelecaniformes	0	0	0	0	0	0	0
				Cardiocephaloides hillii	T	N	Aves	Charadriiformes	0	0	0	0	0	0	0
				Cardiocephaloides longocollis	T	N	Aves	Charadriiformes	0	0	0	0	0	0	0
		Plagiorchiida	**Brachycladiidae**	*Brachycladium atlanticum*	M	N	Mammalia	Odontoceti	0	0	0	0	0	0	0
			Echinostomatidae	*Aporchis massiliensis*	T	N	Aves	Charadriiformes	0	0	0	0	0	0	0
				Echinostoma chloropodis	T	N	Aves	Gruiformes	0	0	0	0	0	0	0
			Eucotylidae	*Tamerlania zarudnyi*	T	N	Aves	Passeriformes	0	0	0	0	0	0	0
			Fellodistomidae	*Steringophorus blackeri*	M	C	Osteichthyes	-	0	0	0	0	0	1	0
			Hemiuridae	*Paradinurus manteri*	M	N	Osteichthyes	-	0	0	0	0	0	1	0
			Heterophyidae	*Pholeter gastrophilus*	M	N	Mammalia	Odontoceti	0	0	0	0	0	0	0
			Microphallidae	*Diacetabulum curvicolom*	T	N	Aves	Charadriiformes	0	0	0	0	0	0	0
				Maritrema opisthometra	T	N	Aves	Charadriiformes	0	0	0	0	0	0	0

Phylum	Clases	Order	Family	Species	M/T	Ct	Hg	Hsg	H	P	Go	Tn	GC	F	L
Platyhelminth	**Trematoda**	**Plagiorchiida**	**Microphallidae**	*Megalophallus pentadactylus*	T	N	Aves	Charadriiformes	0	0	0	0	0	0	0
				Sphairiotrema prudhoei	T	N	Aves	Charadriiformes	0	0	0	0	0	0	0
			Notocotylidae	*Notocotylus attenuates*	T	N	Aves	Gruiformes	0	0	0	0	0	0	0
				Notocotylus gibbus	T	N	Aves	Gruiformes	0	0	0	0	0	0	0
Annelida	**Clitellata**	**Arhynchobdellida**	**Hirudinidae**	*Limnatis nilotica*	T	C	Generalist	Generalist	0	0	0	1	1	0	0
		Rhynchobdellida	**Glossiphoniidae**	*Helobdella europaea*	T	C	Generalist	Generalist	0	0	0	1	0	0	0
				Helobdella stagnalis	T	C	Generalist	Generalist	0	0	0	1	0	0	0
			Piscicolidae	*Branchellion torpedinis*	M	C	Generalist	Generalist	0	0	0	0	1	0	0
				Stibarobdella macrothela	M	C	Generalist	Generalist	0	0	0	1	1	1	1
				Trachelobdella lubrica	M	C	Generalist	Generalist	0	0	1	1	0	1	0

Appendix 3:

Accidental or non-obligate parasites in the Canary Islands

T: Terrestrial; **M**: Marine; **E**: Endemic; **NE**: Non-endemic; **Ct**: Category; **My**: Oportunistic myiasis; **Ep**: Epibiota; **Ec**: Oportunistic ectoparasite; **G**: Generalist; **Tes**: Testudines; **Mam**: Mammalia; **Dub**: Dubious presence or report; **H**: El Hierro; **P**: La Palma; **Go**: La Gomera; **Tn**: Tenerife; **GC**: Gran Canaria; **F**: Fuerteventura; **L**: Lanzarote.

Phyllum	Class	Order	Family	Species	Habitat	E/NE	Ct.	Host	H	P	Go	Tn	GC	F	L
Arthropoda	**Insecta**	**Diptera**	**Calliphoridae**	*Calliphora splendens*	T	E	My	G	1	1	1	1	0	0	0
				Calliphora vicina	T	NE	My	G	1	1	1	1	1	1	1
				Calliphora vomitoria	T	NE	My	G	1	1	1	1	1	0	0
				Chrysomya albiceps	T	NE	My	G	1	1	1	1	1	1	0
				Chrysomya chloropyga	T	NE	My	G	0	0	0	1	0	1	1
				Chrysomya megacephala	T	NE	My	G	0	1	0	1	1	1	1
				Lucilia sericata	T	NE	My	G	1	1	1	1	1	1	1
			Muscidae	*Musca biseta*	T	NE	My	G	0	0	0	0	0	0	0
				Musca domestica	T	NE	My	G	1	1	1	1	1	1	1
				Musca osiris	T	NE	My	G	1	1	0	1	1	1	0
				Musca sorbens	T	NE	My	G	0	1	1	1	1	1	1
				Musca tempestiva	T	NE	My	G	0	0	0	1	1	0	0
				Musca vitripennis	T	NE	My	G	1	1	0	1	1	1	0
				Muscina levida	T	NE	My	G	0	1	1	1	1	0	0
				Muscina prolapsa	T	NE	My	G	1	1	1	1	1	0	0
				Muscina stabulans	T	NE	My	G	1	1	1	1	1	1	1
			Sarcophagidae	*Blaesoxipha lapidosa*	T	NE	My	G	0	1	0	0	0	0	0
				Blaesoxipha rufipes	T	NE	My	G	0	0	0	0	1	0	0
				Parasarcophaga exuberans	T	NE	My	G	0	0	0	1	0	0	0
				Ravinia pernix	T	NE	My	G	1	1	0	1	1	0	0
				Sarcophaga metopina	T	E	My	G	0	1	0	0	0	0	0
				Sarcophaga santospintosi	T	E	My	G	0	0	0	0	0	1	1
				Sarcophaga tricolor	T	E	My	G	0	1	1	1	1	0	0
				Sarcophaga africa	T	NE	My	G	1	1	1	1	1	0	0
				Sarcophaga argyrostoma	T	NE	My	G	0	0	1	1	1	0	0
				Sarcophaga crassipalpis	T	NE	My	G	0	1	1	1	0	0	0
				Sarcophaga deviedmani	T	NE	My	G	0	0	0	0	0	1	1

Phyllum	Class	Order	Family	Species	Habitat	E/NE	Ct.	Host	H	P	Go	Tn	GC	F	L
				Sarcophaga dux	T	NE	My	G	0	0	0	0	0	0	0
				Sarcophaga ferox	T	NE	My	G	0	1	0	1	1	0	0
				Sarcophaga jacobsoni	T	NE	My	G	0	0	0	1	0	0	0
				Sarcophaga maculata	T	NE	My	G	0	0	0	0	0	0	0
				Sarcophaga melanura	T	NE	My	G	0	0	0	0	0	0	0
				Sarcophaga sexpunctata	T	NE	My	G	0	0	0	0	0	0	0
				Sarcophaga soror	T	NE	My	G	0	0	0	0	0	0	0
				Sarcophaga tibialis	T	NE	My	G	0	1	1	1	0	0	0
				Sarcophaga uncicurva^{Dub}	T	NE	My	G	0	0	0	0	0	0	0
				Sarcophila latifrons	T	NE	My	G	0	1	1	1	1	0	0
				Wohlfahrtia bella	T	NE	My	G	0	1	0	1	1	0	0
				Wohlfahrtia indigens	T	NE	My	G	0	0	0	1	1	1	1
				Wohlfahrtia trina	T	NE	My	G	0	0	0	1	0	0	0
			Syrphidae	*Eristalis tenax*	T	NE	My	G	1	1	1	1	1	1	1
	Thecostraca	**Balanomorpha**	**Chelonibiidae**	*Chelonibia testudinaria*	M	NE	Ep	Tes	1	0	0	0	1	0	0
			Coronulidae	*Xenobalanus globicipitis*	M	NE	Ep	Mam	0	0	0	0	1	0	1
		Scalpellomorpha	**Lepadidae**	*Lepas anatifera*	M	NE	Ep	Tes	0	1	1	1	1	0	1
				Lepas hillii	M	NE	Ep	Tes	0	0	0	0	1	0	0
Nematoda	**Chromadorea**	**Rhabditida**	**Peloderidae**	*Pelodera teres*	T	NE	Ec	Mam	0	1	0	1	0	0	0

Appendix 4:

Type material of *Lampropeltis californiae* hold by Museums

Type material of *Lampropeltis californiae* hold by Museums

Museum specimens extracted and corrected from **www.reptile-base.org**:

ANSP: Academy of Natural Sciences of Philadelphia, US.

CAS: California Academy of Sciences, US.

MNHN: Muséum national d'histoire naturelle, France.

MVZ: Museum of Vertebrate Zoology, University of California, Berkely, US.

USNM: Smithsonian institution, United States National Museum.

The holotype for *Coluber (Ophis) californiae* is supposed to be unknown. However, there exists a registered specimen collected by Paul-Émile Botta (1820's) (MNHN-RA-0.732) in the National museum of natural history in Paris classified as "type" material, which could have been used for the definition of the species in 1835.

Holotypes of synonyms

MVZ 50814 – *Lampropeltis getulus nigritus* - 30.6 miles (by road) south of Hermosillo, Sonora, Mexico

CAS 800 (Lost in the earthquake and fire of 1906) - *Lampropeltis nitida* – San Jose del Cabo, Baja California, Mexico. Neotype: USNM 64585 - Miraflores, Baja California Sur, Mexico, North America

USNM 1698 - *Lampropeltis getulus boylii* - El Dorado County, Calif.

USNM 11788 - *Ophibolus getulus eiseni* - Fresno, California, United States, North America

USNM 61318 - *Lampropeltis getulus yumensis* —Twenty-seven miles west of Indian Oasis, PimaCounty, Ariz.

A decent amount of non-type specimens (456) of *Lampropeltis californiae* is held by CAS.

Appendix 5:

Parasitic species in snakes of the genus *Lampropeltis*

I/P: Intermediary or paratenic host. **ND**: Not determined.

Kingdom	**Phylum**	**Common tags**	**Parasite**	**Current host name**	**Host cited as**	**Location**	**Presumed location**	**Host type**	**Locality**	**Reference**
Protozoa	**Amebozoa**	**Amebozoa**	***Entamoeba invadens***	*L. getula*	*L. getulus*	-	Intestine	Definitive	-	Ratcliffe and Geiman 1938
				L. triangulum	*L. doliata*	Intestine	Intestine	Definitive	Michigan, USA	Barrow and Stockton 1960
			Entamoeba serpentis	*L. holbrooki*	*L. getulus holbrooki*	Liver and large intestine	Intestine	Definitive	Montreal Canada	Fantham and Porter 1953-1954
	Metamonada	**Metamonada**	***Giardia* sp.**	*L. holbrooki*	*L. g. holbrooki*	Small intestine	Intestine	Definitive	Montreal, Canada	Fantham and Porter 1953-1954
			***Trichomonas* sp.**	*L. holbrooki*	*L. g. holbrooki*	Rectum and cloaca	Intestine	Definitive	Montreal, Canada	Fantham and Porter 1953-1954
			Eutrichomastix serpentis	*L. holbrooki*	*L. g. holbrooki*	Rectum and cloaca	Intestine	Definitive	Montreal, Canada	Fantham and Porter, 1953-1954
Chromista	**Miozoa**	**Apicomplexa**	***Criptosporidium lampropeltis***	*L. calligaster*	*L. c. calligaster*	-	Stomach	Definitive	Illinois, USA	Anderson *et al.* 1968
			Criptosporidium serpentis	*L. californiae*	*L. getula californiae*	Stomach	Stomach	Definitive	St. Louis zoo, USA	Xiao *et al.* 2004
				L. getula	*L. getula*	Stomach	Stomach	Definitive	Thailand	Yimming *et al.* 2016
			Caryospora guatemalensis	*L. triangulum*	*L. triangulum*	-	Intestine	Definitive	Guatemala	Seville et al. 2005
			Caryospora duszynskii	*L. holbrooki*	*L. holbrooki*	-	Intestine	Definitive	Arkansas, USA	McAllister *et al.* 2011
				L. triangulum	*L. triangulum syspila*	-	Intestine	Definitive	Arkansas, USA	McAllister *et al.* 2011
				L. calligaster	*L. c. calligaster*	-	Intestine	Definitive	-	McAllister *et al.* 1995

Kingdom	Phylum	Common tags	Parasite	Current host name	Host cited as	Location	Presumed location	Host type	Locality	Reference
			Caryospora lampropeltis	*L. calligaster*	*L. c. calligaster*	-	Intestine	Definitive	Illinois, USA	Anderson *et al.* 1968
				L. calligaster	*L. c. calligaster*	-	Intestine	Definitive	Texas, USA	McAllister *et al.* 2017
				L. splendida	*L. getula splendida*	-	Intestine	Definitive	Texas, USA	McAllister *et al.* 1995
				L. triangulum	*L. triangulum syspila*	-	Intestine	Definitive	Arkansas, USA	McAllister *et al.* 1995
			Eimeria sp	*L. californiae*	*L. g. californiae*	-	Gallblader	Definitive	California, USA	Van Peenen and Birdwell 1968
			Eimeria lampropeltis	*L. calligaster*	*L. c. calligaster*	-	Gallblader	Definitive	Illinois, USA	Anderson *et al.* 1968
			Eimeria zamenis	*L. triangulum*	*L. triangulum triangulum*	Gallblader	Gallblader	Definitive	Iowa, USA	Wacha and Christiansen 1974
				L. calligaster	*L c. calligaster*	Gallblader	Gallblader	Definitive	Iowa Illinois	Anderson *et al.* 1968
				L. holbrooki	*L g holbrooki*	Gallblader	Gallblader	Definitive	Brasil	Anderson *et al.* 1968
			***Sarcocystis* sp.**	*L. calligaster*	*L. calligaster calligaster*	-	Intestine	Definitive	Arkansas and Oklahoma, USA	McAllister *et al.* 1995
				L. californiae	*L. g. californiae*	-	Intestine	Definitive	California, USA	Van Peenen and Birdwell 1968

Kingdom	**Phylum**	**Common tags**	**Parasite**	**Current host name**	**Host cited as**	**Location**	**Presumed location**	**Host type**	**Locality**	**Reference**
			Sarcocystis lampropeltii	*L. holbrooki*	*L. g. holbrooki*	Intestine	Intestine	Definitive	Arkansas, USA	Lindsay *et al*. 1992 Duszynski and Upton 2009
			Haemogregarinidae gen.	*L. californiae*	*L. g. californiae*	Blood	Blood	I/P	California, USA	Van Peenen and Birdwell 1968
			***Haemogregarina* sp.**	*L. floridana*	*L. g. floridanus*	*Blood*	*Blood*	I/P	USA	Hull and Camin 1960
				L. getula	*L. g. getulus*	Blood	Blood	I/P	USA	Hull and Camin 1960
				L. holbrooki	*L. g. holbrooki*	Blood	Blood	I/P	USA	Hull and Camin 1960
				L. holbrooki	*L. g. holbrooki*	Blood	Blood	I/P	Montreal, Canada	Fantham and Porter 1953-1954
			Hepatozoon eurytopis	*L. floridana*	*L. getula floridana*	Blood	Blood	I/P	Florida, USA	Telford 2010
			Hepatozoon karyolysi	*L. floridana*	*L. g. floridana*	Blood	Blood	I/P	Florida, USA	Telford 2010
			Hepatozoon rexi	*L. floridana*	*L. g. floridana*	Blood	Blood	I/P	Florida, USA	Telford 2010
Animalia	**Platyhelminthes**	**Trematoda**	***Lechriorchis primus***	*L. holbrooki*	*L. g. holbrooki*	Lung	Lung	Definitive	Montreal, Canada	Fantham and Porter 1953-1954
			Lechriorchis validus	*L. holbrooki*	*L. g. holbrooki*	Mouth & lung	Lung	Definitive	Texas, USA	Hardwood 1932
			Ochetosoma georgianum	*L. floridana*	*L. g. floridana*	-	Mouth	Definitive	Florida, USA	Parker 1941

Kingdom	Phylum	Common tags	Parasite	Current host name	Host cited as	Location	Presumed location	Host type	Locality	Reference
			Ochetosoma ellipticus	*L. holbrooki*	*L. g. holbrooki*	-	Mouth	Definitive	Tenessee, USA	Parker 1941
				L. floridana	*L. g. floridana*	-	Mouth	Definitive	Florida, USA	Parker 1941
				L. holbrooki	*L. g. holbrooki*	-	Mouth	Definitive	Louisiana, USA	Rabalais 1969
				L. getula	*L. getula*	Mouth	Mouth	Definitive	-	Ernst and Ernst 2006
			Renifer aniarum	*L. holbrooki*	*L. holbrooki*	-	Mouth	Definitive	-	McAllister and Bursey 2008
			Styphlodora horrida	*L. polyzona*	*L. triangulum polyzona*	Ureters, kidneys and cloaca	Urinary	Definitive	Mexico	Thatcher 1963
		Cestoda	***Ophiotaenia filarioides***	*L. holbrooki*	*L. g. holbrooki*	Small intestine	Intestine	Definitive	Montreal, Canada	Fantham and Porter 1953-1954
			***Mesocestoides sp.* (Tetrathyridia)**	*L. getula*	*L. getula*	Coelomic cavity	Coelom	I/P	-	Goldberg and Bursey 2004
				L. pyromelana	*L. pyromelana*	Pancreas	Coelom	I/P	-	Jacobson 2007
			***Spirometra mansonoides* (Plerocercoid)**	*L. holbrooki*	*L. g. holbrooki*	Subcutis	Subcutaneus	I/P	Louisiana, USA	Corkum 1966
	Acanthocephala	**Acanthocephala**	***Macracanthorhynchus ingens* (Cystacanth)**	*L. holbrooki*	*L. g. holbrooki*	Coelomic cavity	Coelom	I/P	Louisiana, USA	Elkins and Nickol 1983
	Nematoda	**Nematoda**	***Kalicephalus agkistrodontis***	*L. holbrooki*	*L. g. holbrooki*	Stomach	Stomach	Definitive	Texas, USA	Hardwood 1932
			Kalicephalus coronellae	*L. triangulum*	*L. triangulum*	Stomach	Stomach	Definitive	USA	Orlepp 1923

Kingdom	**Phylum**	**Common tags**	**Parasite**	**Current host name**	**Host cited as**	**Location**	**Presumed location**	**Host type**	**Locality**	**Reference**
			Kalicephalus inermis	*L. holbrooki*	*L. g. holbrooki*	Stomach	Stomach	Definitive	-	Baker 1987
				L. nigra	*L. getula niger*	Stomach	Stomach	Definitive	-	Baker 1987
				L. triangulum	*L. triangulum*	Stomach	Stomach	Definitive	-	Baker 1987
			Kalicephalus parvus	*L. getula*	*L. getula*	Stomach	Stomach	Definitive	London zoo, UK	McAllister et al 2008
			Kalicephalus parvus	*L. holbrooki*	*L. g. holbrooki*	Stomach	Stomach	Definitive	Montreal, Canada	Fantham and Porter 1953-1954
			Kalicephalus rectiphilus	*L. getula*	*L. getula*	Stomach	Stomach	Definitive	London zoo, UK	Schad 1955
			Macdonaldius oschei	-	*Lampropeltis sp.*	Mesenteric arteries	Arteries	Definitive	-	Telford 1965
			Ophidascaris labitopapilosa	*L. getula*	*L. getula*	Stomach	Stomach	Definitive	Florida, USA	Ash and Beaver 1963
			Physaloptera abjecta	*L. calligaster*	*L. c. calligaster*	Stomach	Stomach	Definitive	-	McAllister *et al.* 2008
				L. holbrooki	*L. g. holbrooki*	Stomach	Stomach	Definitive	Montreal, Canada	Fantham and Porter 1953-1954
				L. nigra	*L. getula nigra*	Stomach	Stomach	Definitive	-	Morgan 1941
			***Physaloptera* sp. (free larvae)**	*L. pyromelana*	*L. pyromelana*	Stomach	Stomach	ND	Arizona, USA	Goldberg *et al.* 2007
			Polydelphis anoura	*L. triangulum*	*L. doliata triangulum*	Stomach	Stomach	Definitive	-	Kutzer and Grünberd 1965
			Serpentirhabdias fuscovenosa	*L. triangulum*	*L. triangulum*	Lung	Lung	Definitive	-	Railliet 1899

Kingdom	Phylum	Common tags	Parasite	Current host name	Host cited as	Location	Presumed location	Host type	Locality	Reference
			Serpentirhabdias eustreptos	-	*Lampropeltis sp*	Lung	Lung	Definitive	-	Langford 2010
				L. getula	*L. getula*	Lung	Lung	Definitive	-	Langford 2010
			Strongyloides sp.	*L. holbrooki*	*L. g. hoolbroki*	Small Intestine	Intestine	Definitive	-	Holt 1978
	Arthropoda	**Acari**	***Entophionyssus fragilis***	*L. getula*	*L. getula getula*	Lung	Lung	Definitive	Texas, USA	Keegan 1946
			Entophionyssus glasmacheri	*L. holbrooki*	*L. g. holbrooki*	Lung	Lung	Definitive	-	Fain 1961
			Entophionyssus heterodontos	*L. calligaster*	*L. calligaster*	Lung	Lung	Definitive	-	Keegan 1943a
			Hyponeocula imitator	*L. getula*	*L. getula*	Skin	Skin	I/P	Baja California, Mexico	Tanigoshi and Loomis 1974
			Leiognathus triangulus	*L. calligaster*	*L. calligaster*	Skin	Skin	Definitive	Maryland, USA	Fonseca 1948
			Ophionyssus natricis	*L. triangulum*	*L. triangulum*	Skin	Skin	Definitive	Mexico	Aguirre-Medina 2005
			Ophionyssus serpentinum	-	*Lampropeltis sp.*	Skin	Skin	Definitive	-	Schroeder 1934
		Pentastomida	***Raillietiella bicaudata***	*L. getula*	*L. getula*	Lung	Lung	Definitive	-	Ali *et al*. 1985
			Kiricephalus coarctatus	*L. getula*	*L. getula*	Lung	Lung	Definitive	-	Sambon 1922
			K. coarctatus **(Nymphs)**	*L. floridana*	*L. g. floridana*	-	Coelom	I/P	-	Keegan 1943b

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