Checklist and state of knowledge of helminths in wild birds from Chile: an update

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\textbf{ABSTRACT.} Helminths are an important component of biodiversity with over 24,000 species parasitising wild birds globally, with this figure on the rise given the growing interest in wildlife parasitology. The present study aimed to establish an updated baseline of the helminthological surveys on wild birds from Chile. Available publications were reviewed to build a parasite-host association checklist and also to discuss the state of knowledge regarding these parasites. A total of 92 publications were found between the years 1892 and 2019. Regarding helminth parasites, 174 taxa belonging to 3 phyla and 37 families were recorded, 114 taxa were identified at species level, with the rest remaining incompletely described. A lso, 4 taxa corresponded to new genera and 16 to new at species for science. The most reported parasites were platyhelminthes (53.9\%) followed by nematodes (36.2\%) and acanthocephalans (9.2\%). Sixty-five avian species from 19 orders have been recorded as hosts, with most of them having been studied only once (64.6\%). Out of these, the order Charadriiformes had the highest number of publications (n=23). In the case of the avian species present in the country, 14.2\% of native, 40\% of endemic and 22.2\% of exotic species have been recorded hosting helminths. Regarding heteroxenous parasites, only 2 species have had their life cycles elucidated. Among the methodologies used for parasitic identification, 48.9\% of the studies used morphological tools, 5.4\% used molecular tools and 4.3\% used both tools. For that reason, there are evident gaps in the data concerning the hosts sampled, methodologies and issues related to the biology of parasites such as life cycles, among others. In this sense, the need for specialists and cooperative research becomes indispensable to improve our understanding of helminths.

**Key words:** wildlife, parasitology, helminths, Chile.

\textbf{INTRODUCTION}

Helminths are defined as metazoan parasites including phyla Platyhelminthes (class Cestoda and Trematoda), Nematoda and Acanthocephala, all of them having been reported parasitising wild birds around the world (Wobeser 2008, Roberts \textit{et al} 2013). A round 24,000 helminth species have been estimated as infecting birds, although this figure could underestimate the real number of parasitic species for this host group (Carlson \textit{et al} 2019). Thus, these organisms should also be considered as an important component of the biodiversity from any territory (Poulin and Morand 2004).

Helminth parasites can be found not only in the digestive tract but potentially in all tissues, organs, and cavities, depending on the preference for every taxon in every host (Wobeser 2008). The complexity of life cycles varies between different species, with direct or monoxenous life cycles (1 host) and indirect or heteroxenous life cycles (2 or more hosts) (Roberts \textit{et al} 2013).

Parasites from wild birds have been widely studied in Europe and North America providing a vast knowledge of taxonomy, disease, and ecology (Wobeser 2008). In South America, the helminth fauna of wild birds have been mostly studied in countries such as Brazil and Argentina with several species and life cycles elucidated thanks to the constant surveys of several groups of local and international parasitologists interested in those hosts (e.g. Lopes \textit{et al} 2017\textsuperscript{a,b}, Drago and Lunaschi 2015, Hernández-Orts \textit{et al} 2019). Notwithstanding the above, the knowledge of helminth parasites in wild birds from Chile is fragmentary, with several avian species without records of its helminth fauna, which could be related to the reduced number of parasitologists performing active research in the country (Hinojosa-Sáez and González-Acuña 2005). The last review on helminth parasites in wild birds from Chile was published by Hinojosa-Sáez and González-Acuña (2005) who reported a total of 49 taxa from all phyla of metazoan parasites parasitising 30 avian species.

Based on a literature review, this study aimed known the number of helminth species parasitising wild birds in Chile and provide a concise description and discussion of the state of knowledge related to parasites, hosts, and methodology used by authors, among others.

\textbf{MATERIAL AND METHODS}

The present study included literature on helminth fauna of wild birds from Chile and it considered both native and introduced species. A time period between the years 1800 and December 2019 was established. Articles and books were searched through the databases of NCBI,
RESULTS

PUBLICATIONS

A total of 92 publications (88 scientific articles and 4 books) reporting helminths in wild birds from Chile have been published between the years 1896 and December 2019. During that period, and considering the 3 centuries (1800-1899; 1900-1999; 2000-2019), the number of publications per period was 1, 44 and 47, respectively. The highest number of publications was registered between 2011 and 2019, with 26 publications (figure 1).

HELMINTH TAXA

One hundred and seventy-four taxa of helminths organised in 3 phyla, 6 classes, 9 orders, 37 families and 95 genera have been found parasitising wild birds in Chile. Out of these taxa, 114 were classified at species level with 4 of them constituting new genera, while 16 are new species. A nother 47 helminths were identified at genus level only, 9 at family level, 3 at class and 1 at phylum level. Furthermore, 23 out 47 genera have only been recorded up to this taxonomic level in the country without a specific identification. Other two taxa correspond to accidental parasites of birds. *Anisakis* sp. and *Pseudoterranova* sp. A checklist of parasite-host associations is shown in supplementary table S1.

When analysing taxa for every phylum, 36.2% (63/174) belong to Nematoda, 54.6% (95/174) to Platyhelminthes (52 taxa belonging to class Cestoda and 43 taxa to class Trematoda) and 9.2% (16/174) to Acanthocephala. Also, 63% (58/92) of publications recorded platyhelminthes, 57.6% (53/92) nematodes and 18.5% (17/92) acanthocephalans. Only 33.7% (31/92) recorded mixed infections between different phyla. About the type of mixed infections, 91.3% (84/92) of the publications recorded natural mixed infections, 14.1% (13/92) experimental mixed infections and 4.4% (4/92) had no details on the type of infection.

HOSTS

A total of 65 species from 19 orders and 32 families of birds were recorded as being parasitised by helminths. Also, 2 birds were not identified at species level, i.e. *Larus* sp. and *Anas* sp. A total of 63 bird species were catalogued as native, 4 of them being also considered as endemic species, while another 2 were catalogued as exotic species. Consequently, and considering the number of avian species present in Chile, 14.2% (63/443) of native species, 40% (4/10) of endemic species and 22.2% (2/9) of exotic species have been recorded harbouring helminths to date. With regard to the conservation status of hosts, 57

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1 Available at: www.australjvs.cl/ajvs
species were classified as Least concern (LC), 3 as Near threatened (NT) and 5 as Vulnerable (VU).

The avian order with the highest number of publications was Charadriiformes (n=23) followed by Sphenisciformes (n=14), Passeriformes (n=11) and Suliformes (n=10). In the case of the number of species of hosts studied, the order Passeriformes denoted the highest number of publications (13 out of 81 species). Regarding the number of publications for every avian species, kelp gull (*Larus dominicanus*) recorded the highest number (n=17) followed by Brown-hooded gull (*Chroicocephalus maculipennis*) (n=10) and the Neotropic cormorant (*Phalacrocorax brasilianus*) (n=8). Despite the above-mentioned information, 64.6% (42/65) of avian hosts have been studied only once.

When considering the origin of birds, 79.4% (73/92) of publications were based on wild birds, 7.6% (7/92) came from wildlife rehabilitation centres, 4.4% (4/92) from zoos and 9.8% (9/92) of publications did not detail the source.

The analysis of publications covering the subject of infection sites showed that 60.9% (56/92) of them recorded parasites from the digestive system, 2.2% (2/92) from the respiratory system and 6.5% (6/92) from other organs/sites (e.g. articulations, blood, bursa of Fabricius, coelomic cavity, pancreas and tendons), where as 34.8% (32/92) did not detail the organ/tissue.

**LIFE CYCLES**

Seventy-four out 114 species have indirect life cycles, 20 have direct life cycles and 20 have unknown life cycles. Two out of 74 heteroxenous species have had their life cycles elucidated in Chile: *Profilicollis altmani* and *Profilicollis antarcticus*. Additionally, there are other 3 heteroxenous helminth species with incomplete knowledge of their life cycles: *Dibothriocephalus dendriticus*, *Stephanoprora uruguayense* and *Tylodelphys cf. destructor*. Considering the 47 parasitic taxa identified only to genus level, 43 of them would have indirect and 4 direct life cycles.

**PARASITES BY REGION**

Avian hosts from 12 out of 16 regions of Chile have been recorded as parasitised by helminths. The most studied regions were Los Ríos region (19.6% of publications, 18/92), Ñuble and Biobío regions (16.3%, 15/92 each one), and Los Lagos region (10.9%, 10/92). In contrast, wild birds from Tarapacá (I), Atacama (III), Libertador General Bernardo O'Higgins (VI) and Aysén del General Carlos Ibáñez del Campo (XI) regions have not been surveyed for helminths yet. Furthermore, in 15.2% (14/92) of publications, the authors did not record the locality of collection of birds.

**ADDITIONAL INFORMATION**

Although all the publications were based on taxonomy, some of them considered additional focuses, e.g. 6.5% (6/92) considered pathology and 2.2% (2/92) had an ecological overview. These views were always combined with taxonomy (8.7%, 8/92).
METHODOLOGIES

The results on the methodology used showed that 48.9% (45/92) of publications used only morphological tools, while 5.4% (5/92) of surveys used molecular tools with 4 out 5 studies using it together with morphological description. Also, one study was based on the sole use of molecular tools and another used Giemsa stain. Other techniques used with the morphological descriptions of isolated helminths were histopathological analysis with hematoxylin-eosin stain (6.5%, 6/92) and coproparasitological techniques (4.4%, 4/92). In contrast, 44.6% (41/92) of studies did not detail the technique used to identify the parasites. All surveys, except for 1 publication which used only Giemsa stain for blood smear examination, were based on necropsy of birds.

HELMINTHOLOGICAL COLLECTIONS

Only 45.7% (42/92) of the studies deposited parasites in a helminthological collection, 21.7% (20/92) of publications did so in a national collection, 19.6% (18/92) placed them in an international collection, and 4.4% (4/92) placed them in both collections. Additionally, only one study out 5, which used molecular tools, detailed the sequences of the parasites deposited in GenBank.

DISCUSSION

Since the review by Hinojosa-Sáez and González-Acuña (2005) that was published 14 years ago, a substantial increase in the helminthological knowledge of birds has been recorded until 2019; 49 taxa in the former review versus 174 taxa in this work. This prominent increase could be the consequence of a higher number of publications, i.e. 42 additional scientific articles since 2005, plus another 21 studies that were not considered in the former review (see Lönnberg 1896, Fuhrmann 1908, 1908b; Baylis 1928, 1932, Duthoit 1931, Baudet 1937, Porter 1937, Tagle 1942, 1966, Rausch and M organ 1947, Schuurmans-Stekhoven 1950, Dubois and Rausch 1960, Yamaguti 1963, Szidat 1969, Forrester et al 1978, Torres et al 1981a, 1982b, Bartlett and Greiner 1986, M cDonald 1988, Wong and Anderson 1991).

The first study reporting helminths in a Chilean wild bird was made by Lönnberg (1896) and the last by Oyarzún-Ruiz et al (2019). On a time scale, the number of publications from the year 2000 to present is higher than that registered during the past century (47 vs 43), which is noteworthy because in 19 years there have been more articles published than in one century. This could be related to a major interest of researchers in this group of hosts.

The increase in the number of species is remarkable as well, from 34 in the former review to 115 species up to 2019. Also, 6 new species were described after the former review was published in the year 2005. In contrast, although there are 16 new species for science described to date, 2 have questionable taxonomic validity, i.e. Contracaecum macronectidis (Schuurmans-Stekhoven 1950) and Taenia diaphoracantha (Fuhrmann 1908b). The first species is the only record of a helminth species for Southern giant-petrel (Macronectes giganteus) from Chile, however, the description was brief and based on one immature worm only, therefore, additional surveys are needed to collect and describe mature worms which are necessary for an accurate identification (see Anderson et al 2009). For the second species, isolated from a Southern rockhopper penguin (Eudyptes chrysocome), it must be noted that the genus Taenia only parasitises mammals as definitive hosts (Khalil et al 1994). Also, the genera Tetrabothrius and Parorchites are the only tapeworms recorded infecting penguins (Brandão et al 2014). The description for T. diaphoracantha provided by Fuhrmann (1908b) detailed several hooks which could be related to genus Parorchites, which is also the only penguin tapeworm with a rostellum (Khalil et al 1994). However, this genus has not been recorded in this penguin, E. chrysocome. On the other hand, the figure of an adult Streptocara sp. from Adelie penguin (Pygoscelis adeliae) by F redes et al (2008) resembles specimens of Stegophorus spp. with some features that are typical for this genus (e.g. well-developed helmet-like structure with several denticles, thin and long vestibule, and deirids at vestibule-esophagus union) which are absent in Streptocara (see Yamaguti 1961, Anderson et al 2009). For this reason, a revision of the material is needed to establish the identity of these worms.

Furthermore, 23 genera have been recorded only once in Chile, highlighting the need of reviewing the available material and carry out additional surveys to classify these parasites and to determine if they correspond to new species or previously described taxa in other countries, thus amplifying its geographic range to our country.

Anisakis sp. and Pseudoterranova sp. are parasites whose definitive hosts are marine mammals such as cetaceans and pinnipeds meanwhile fishes act as intermediate hosts (Anderson 2000). These nematodes occasionally are found in the stomachs or intestines of wild birds (George-Nascimento and Carvajal 1980, Torres et al 2005) which get parasitised through predation of fishes. Only larval stages are found in birds because these nematodes mature exclusively in the former mammals, therefore, birds are considered to be accidental hosts (Anderson 2000).

When considering the total native avifauna from Chile (443 species) (Martínez and González 2017, MAA 2018), it is found that only a few species have been studied for helminthological fauna (14.2%). Even exotic species lack attention (22.2%), e.g. the house sparrow (Passer domesticus) and the ring-necked pheasant (Phasianus colchicus) have no published articles regarding endoparasitic fauna. Some possible explanations could be the few groups of parasitologists working on helminths in wildlife in the country. Besides, several avian hosts have restricted distributions in rugged terrain, such as different birds...
that are only present in the highlands (e.g., puna hawk, Geranoaetus poecilochrous) or in Patagonia (e.g., lesser rhea, Rhea pennata pennata) (Martínez and González 2017). Furthermore, 10 avian species are considered endemic in Chile (Martínez and González 2017) and 4 out of them have been studied for helminths. Those studies on endemic species have identified helminth species that seem to be restricted to Chile, with no further records of the country, e.g., Navonia pterodroma from pink-footed shearwater (Ardena creatopus) and Juan Fernández petrel (Pterodroma externa) (Díaz et al 2007), and Aploparaksistinamou from Chilean tinamou (Nothoprocta perdicaria) (Olsen 1970, Rubilar et al 1996), therefore, they might be considered as endemic parasites. In this way, helminthological studies in the rest of the endemic species could provide similar results, however, the threatened state of conservation of these host populations and their recondite habitats (Martínez and González 2017, MMA 2018) limit the efforts of sampling.

In the case of the avian orders and species, the order Charadriiformes and the kelp gull concentrated most of the published studies, a result similar to that suggested by Hinojosa-Sáez and González-A cuña (2005). In fact, most publications covering this avian order correspond to those on kelp gull (17 out of 23). This could be associated to the wide geographical distribution and population size of this bird in the country, a species with no conservation issues and, consequently, allowed to be captured according to the Hunting Law of the State (SAG 2018).

There are other cases in which certain birds have been surveyed but no helminths have been isolated, such as the Chilean pigeon (Patagioenas araucana) and the invasive monk parakeet (Myiopsitta monachus) (Arriagada et al 2010, Briceño et al 2017). This absence could be the consequence of factors such as the age of birds, necropsy procedure focused only on gastrointestinal parasites or small sample size. Thus, additional surveys including a complete parasitic necropsy are encouraged to determine their helminth fauna in the country. Also, there is an erroneous report by Führmann (1908a) who indicated helminths in a great black-hawk (Buteogallus urubitinga) coming from Chile. However, this species is not distributed in the country but it is found in Central America and Northern Argentina (Ferguson-Lees and Christie 2005).

With regard to infection site in hosts, only three helminth species were recorded as extra-intestinal parasites, i.e., Cyathostoma (C.) phenisci infecting the respiratory system (Baudet 1937, Oyarzún-Ruíz et al 2015) and Pelecitus circularis and Pelecitus fulicaeaevae in the joints of birds (Bartlett and Greiner 1986, González-A cuña et al 2017). The reduced record for extra-intestinal parasites could be related to the non-sampling of such organs at the moment of necropsy. For example, Prosthogonimus ovatus, a fluke from the bursa of Fabricius in the Neotropic cormorant, and the avian schistosomes in the blood vessels of kelp gull have been recorded in Argentina (Drago and Lunachi 2015, Brant et al 2017) but not in Chile. At first, it could be considered that these helminths are not present in the country. However, all studies related to these two birds in Chile have focused only on digestive parasites. In consequence, their presence in the national territory cannot be discarded. Thus, future surveys should consider all organs and tissues as possible habitats of parasites, providing data to increase the number of host-parasite associations and helminth biodiversity for Chile.

As for the species with indirect life cycles, only 2 have known life cycles in the country: Profilicollis altmani with marine birds (Franklin’s gull Leucophaeus pipixcan, grey gull Leucophaeus modestus, silvery grole Podiceps occipitalis, whimbrel Numenius phaeopus, brown-hooded gull and kelp gull) as definitive hosts (Riquelme et al 2006, González-A cuña et al 2017, Rodríguez et al 2017b) and sandy-shore molecrab Emerita analoga as intermediate host (Erez et al 2010, Rodríguez et al 2017b); Profilicollis antarcticus with marine birds (Imperial shag Leucocarbo atriceps and kelp gull) as definitive hosts (Torres et al 1991b, 1992, Rodríguez et al 2017). On the other hand, there are examples of incomplete known life cycles, e.g., Stephanoprora uruguayense whose definitive hosts in Chile are piscivorous birds (major grebe Podiceps major, Peruvian pelican Pelecanus thagus, brown-hooded gull and kelp gull), and second intermediate hosts are galaxiid fishes (Torres et al 1982, 1983b, 1991b, 1992, 1993, Viozzi et al 2008). However, the first intermediate host remains unknown. In Argentina, this life cycle was elucidated by Ostrowski de Núñez (2007), indicating the aquatic snail Heleobia parchappei as the first intermediate host. This genus of snail is also present in Chile (Valdovinos 1999), therefore, surveying these mollusks could elucidate the life cycle in the country. A another example is Dibothriocephalus dendriticus whose definitive hosts are gulls (brown-hooded gull and kelp gull) and teleostean fishes (galaxiids, perchs and salmonids) as second intermediate hosts (Muñoz and Olmos 2008), however, the first intermediate host has not been reported. Torres et al (2007) recorded the copepod crustacean Mesocyclops longiseta as the first intermediate host for another broad tapeworm, Dibothriocephalus latus, in Southern Chile. Thus, a relative to these crustaceans, probably, could act as a suitable host as well.

In some cases, there is incomplete identification of larval or adult stages belonging to the same genus, e.g., Wardium spp. and Confluaria spp. The former has been recorded as larvae in the brine shrimp (Artemia persimilis) and adult tapeworm in auroral thrushes (Turdus falcklandii). Nevertheless, in both cases there is no species identification (Llanos-Soto et al 2019, Redón et al 2019), therefore, a relationship between both remains uncertain. Furthermore, shorebirds act as definitive hosts as well (Käll et al 1994). For the second example, this tapeworm has been isolated in the same brine shrimp as Confluaria podicipina, which use grebes as definitive hosts (Redón et al 2019). However, in the silvery grebe from Chile, this
group has been identified only to genus level (González-Acuña et al. 2017). Besides, there are records of larval stages of helminths which use birds as definitive hosts (DH), however, these remain unknown in Chile, e.g. third larval stages of Contraecacum multipapillatum (herons and pelicans as DH) in the flathead grey mullet (Mugil cephalus) (Fernández 1987, Anderson 2000), cysticanchans of Profiticollis chasmagnathi (aquatic birds as DH) in estuarine crabs (Rodriguez et al. 2017), Hernández-Orts et al. 2019) and cysticercoids of Fimbriarioides sp. (waterfowl and shorebirds as DH) in brine shrimps (Khalil et al. 2019, Redón et al. 2019). Hereafter, additional surveys on wild hosts incorporating a precise identification of worms through the use of morphological and molecular tools, and also experimental infections, could elucidate the hosts and features of these unknown life cycles in the country (e.g. Ostrowski de Núñez 2007, Rodriguez et al. 2017).

There is an evident lack of knowledge for newly described species in avian hosts regarding their life cycles in the country with only the avian hosts known to date and no data on the intermediate hosts (heteroxenous life cycles). However, there is a particular case with the possible larval stage isolated reported by Redón et al. (2019) who found the larval stage of Flamingolepis in brine shrimp. Hence, this crustacean could act as an intermediate host for Flamingolepis chileno, the only species recorded for this genus in Chile (see supplementary table S1).

Although there were other areas of science considered in conjunction with helminthological research, these were just a few; only 2 studies were focused on ecology and other 6 had a pathological focus. In consequence, and considering the importance for understanding the effects of parasitism on wild bird populations (Wobeser 2008), there is a poor understanding of the diverse interactions between helminth fauna and wild birds in Chile. This situation highlights the obvious need for specialists and cooperative work on these and other fields of research, a situation mentioned previously by Hinojosa-Sáez and González-Acuña (2005).

The present review showed that most studies were based on necropsy of birds except for 1 publication which used blood smears for detecting microfilariae (see Forrest et al. 1977). Thus, the limits imposed by accessing carcasses in the non-studied birds could be bypassed through their capture with mist nets or sampling captive birds in rehabilitation centres (Lutz et al. 2017), without the need to euthanize specimens. In those cases, the collection of faeces would allow the description of digestive, urinary and respiratory helminths through coprological techniques such as simple flotation or modified Baermann technique. However, the limitations of these techniques could be related to the specific identification of parasitic structures, i.e. species, for example (Smith et al. 2007). Also, the screening of blood smear should be considered to look for microfilariae, a group of nematodes parasitising various orders of birds around the globe (Bartlett 2008) but with minimal attention here in Chile.

A nother taxonomy-related issue recently discussed is the deposition of organisms in accessible collections such as museums, which is also applicable to parasites (Krell 2016). A not negligible percentage of surveys did not specify if isolated helminths were placed in a helminthological collection (53.3%), including some cases of new species such as Variolepis fernandensis (Nybelin 1929), Notocotylus tachyeretis (Duthoit 1931) and C. (C.) phenisci (Baudet 1937). Moreover, there was a case where material belonged to a private collection (e.g., Tetrabothrius (Neotetrabothrius) eudyptidis (Lönngberg 1896)). The deposition in accessible collections is important particularly for new species, species with conflictive identification as stated in the present review with the helminths T. diaphoracantha and C. macronectidis, and parasites not completely identified as in the case of the 23 taxa recorded only to genus level, for example. These collections would allow researchers to review material and solve those taxonomic conflicts. In Chile, the only available collection allowing the deposition of helminths is the museum of Zoology at the Universidad de Concepción, Concepción (see https://www.naturalesudec.cl/zoologi-a-museo/). For these reasons, we suggest that researchers interested in parasitology should deposit holotypes and paratypes of parasites in this public national collection.

Since parasites are an important component of the biodiversity, although generally neglected by non-parasitologists, research programs should consider parasites as an overall goal in regions considered as hotspots of biodiversity (Poulin and Morand 2004, Mairaix and Georgiev 2018).

As a final recommendation, and considering that there is a conspicuous lack of information mostly related to the methodology, forthcoming researches should fill these voids of data to allow future comprehensive analysis and comparisons related to different species of helminths.

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