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Morphometrical and molecular evidence suggests cryptic diversity among hookworms (Nematoda: *Uncinaria*) that parasitize pinnipeds from the south-eastern Pacific coasts

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Abstract

Hookworms of the genus *Uncinaria* parasitize pinniped pups in various locations worldwide. Four species have been described, two of which parasitize pinniped pups in the southern hemisphere: *Uncinaria hamiltoni* parasitizes *Otaria flavescens* and *Arctocephalus australis* from the South American coast, and *Uncinaria sanguinis* parasitizes *Neophoca cinerea* from the Australian coast. However, their geographical ranges and host specificity are unknown. *Uncinaria* spp. are morphologically similar, but molecular analyses have allowed the recognition of new species in the genus *Uncinaria*. We used nuclear genetic markers (internal transcribed spacer (ITS) and large subunit (LSU) rDNA) and a mitochondrial genetic marker (cytochrome *c* oxidase subunit I (COI)) to evaluate the phylogenetic relationships of *Uncinaria* spp. parasitizing *A. australis* and *O. flavescens* from South American coasts (Atlantic and Pacific coasts). We compared our sequences with published *Uncinaria* sequences. A Generalized Mixed Yule Coalescent (GMYC) analysis was also used to delimit species, and principal component analysis was used to compare morphometry among *Uncinaria* specimens. Parasites were sampled from *A. australis* from Peru (12°S), southern Chile (42°S), and the Uruguayan coast, and from *O. flavescens* from northern Chile (24°S) and the Uruguayan coast. Morphometric differences were observed between *Uncinaria* specimens from both South American coasts and between *Uncinaria* specimens from *A. australis* in Peru and southern Chile. Phylogenetic and GMYC analyses suggest that south-eastern Pacific otariid species harbour *U. hamiltoni* and an undescribed putative species *of Uncinaria*. However, more samples from *A. australis* and *O. flavescens* are necessary to understand the phylogenetic patterns of *Uncinaria* spp. across the South Pacific.

Introduction

Hookworms of the genus *Uncinaria* Frölich, 1789 (Nematoda: Ancylostomatidae) have been widely reported to parasitize pinniped pups from various geographical regions worldwide, and they are sometimes associated with pup mortality (Castinel *et al.*, 2006; Nadler *et al.*, 2013). Despite their high potential pathogenicity, little is known about their diversity and impact on wildlife populations (Seguel and Gottdenker, 2017). Currently, four species of *Uncinaria* are recognized in pinniped pups: *Uncinaria lucasi* Stiles, 1901, which is found in northern fur seals (*Callorhinus ursinus*), *Uncinaria hamiltoni* Baylis, 1933, which is found in South American sea lions (*Otaria flavescens*), *Uncinaria sanguinis* Marcus *et al.*, 2014, which is found in Australian sea lions (*Neophoca cineria*), and *Uncinaria lyonsi* Kuzmina & Kuzmin, 2015, which is found in Californian sea lions (*Zalophus californianus*).

Taxa that cannot readily be distinguished morphologically, but for which there is evidence that they are different evolutionary units, are commonly named cryptic species (Struck *et al.*, 2018). In the last decade, the number of publications referring to cryptic species has increased dramatically due to the increased use of molecular markers (e.g. Sepúlveda and González, 2014; Halnet *et al.*, 2015). Morphological (mainly morphometric) differences have often been reported between *Uncinaria* specimens collected from different pinniped species, but it is unclear whether these are interspecific differences or whether they correspond to

intraspecific morphometric variations associated either with host geography and/or host–species identity (George-Nascimento *et al.*, 1992; Nadler *et al.*, 2000, 2013; Castinel *et al.*, 2006; Ramos *et al.*, 2013).

A comprehensive phylogenetic molecular analysis of *Uncinaria* spp. from pinnipeds demonstrated seven independent evolutionary lineages (Nadler *et al.*, 2013). Five of these species were associated with specific hosts: one species (currently named *U. lyonsi)* parasitizes the Californian sea lion (*Z. californianus*), and the remaining undescribed species parasitize the New Zealand sea lion (*Phocarctos hookeri*), Australian fur seal (*Arctocephallus pusillus doriferus*), southern elephant seal (*Mirounga leonina*), and Mediterranean monk seal (*Monachus monachus*). Additionally, Nadler *et al.* (2013) demonstrated that *U. lucasi* parasitizes *C. ursinus* and *Eumetopias jubatus*, whereas *U. hamiltoni* parasitizes the South American fur seal (*Arctocephallus australis*) and South American sea lion (*O. flavescens*). However, *Uncinaria* specimens from *A. australis* and *O. flavescens* from the south-eastern Pacific (SEP) coast have not been examined previously, so the identity of *Uncinaria* parasitizing pinniped populations from SEP remains unknown, in addition to the geographical range of *U. hamiltoni* along the South American coast.

The South American pinnipeds (*A. australis* and *O. flavescens*) are distributed from the Peruvian coasts (SEP) to the Brazilian coasts (South Atlantic). However, *A. australis* is absent along 2300 km of the Chilean coastline (SEP), from the Antofagasta coast (23°S) to Chiloe Island (43°S) (Pavés, 2008; Túnez *et al.*, 2013). Moreover, based on genetic and morphological differences, it has been postulated that *A. australis* colonies from the Peruvian, southern Chilean, and Atlantic coasts are subspecies (Oliveira *et al.*, 2008; Berta and Churchill, 2012). It is therefore possible that these SEP otariids harbour a species of *Uncinaria* that is distinct from *U. hamiltoni*.

In this study, we present morphometric and molecular analyses of *Uncinaria* populations based on partial sequences of nuclear ribosomal DNA (internal transcribed spacer (ITS) rDNA and the D2/D3 region of the large subunit (LSU) rDNA) and mitochondrial DNA (cytochrome *c* oxidase subunit I (COI)). In addition, using developed theoretical models that combine species phylogenies and gene genealogies via ancestral coalescent processes (Pons *et al.*, 2006; Monaghan *et al.*, 2009; Powell *et al.*, 2011), ITS rDNA sequence data were used in a species delimitation analysis.

Materials and methods

Sampling areas and collection of hookworms

Adult specimens of *Uncinaria* were collected from five *A. australis* pups in Ica, Peru (12°S; n = 30), six *A. australis* pups in southern Chile (43°S, n = 30), and two *O. flavescens* pups in northern Chile (20°S; n = 30). *Otaria flavescens* pups sampled in central Chile (36°S) were also found to be parasitized with *Uncinaria* specimens. Additionally, ten parasites were collected from two *A. australis* pups and ten parasites were collected from two *O. flavescens* pups from the Uruguayan coast (fig. 1). Sampling in the Punta San Juan reserve, Peru, was authorized by SERNANP-RNSIIPG (resolution no. 09-2013-SERNANP-RNSIIPG). Sampling in Uruguay was authorized by DINARA (no. 584/2006). Sampling in southern Chile was performed using the framework of the "Guafo Island Conservation Program", and SERNAPESCA authorized sampling in northern and central Chile. One female specimen, preserved in ethanol, was deposited in Natural History Museum of Chile, and it was labelled as MNHNCL NEM-15015.

Morphometric study

The *Uncinaria* specimens were preserved in 70% ethanol, cleared, and temporarily mounted on slides with lactophenol. Each *Uncinaria* specimen was observed and measured under light microscopy and body measurements were performed (table 1). Principal component analysis (PCA) was performed based on parasite body measurements relative to total body length (BL). The measurements for both *Uncinaria* sexes were as follows: body width/BL, oesophagus length/BL, buccal capsule length/BL, buccal capsule width/BL, and tail length/BL. For females, the distance from the vulva to the posterior end/BL was also measured, and for males, the spicule length/BL was measured.

Molecular analyses

For sequencing, 19 adult *Uncinaria* from *A. australis* and *O. flavescens* were isolated individually and placed into a 1.5 ml microcentrifuge tube. Details of the numbers of specimens sequenced by host species and host geography as well as the GenBank accession numbers are shown in table 2. DNA extraction was performed by adding 500 µl of 5% Chelex and 2.5 ml of proteinase K (20 mg/ml) to each tube. The samples were incubated at 60°C for 4 h and boiled for 8 minutes. Two regions of nuclear rDNA and one region of mitochondrial DNA were amplified individually by polymerase chain reaction (PCR). The rDNA involved the ITS and 5.8S subunit regions (ITS-1, 5.8S, and ITS-2) and the D2/D3 region of the LSU (large subunit ribosomal DNA (lsrDNA)). The primers and cycling conditions followed the protocol described by Nadler *et al.* (2013). The mitochondrial DNA involved the COI region, which was amplified using the following specially designed primers: UCoxF (5′-TTCCTTTAATGTTGGGTGCT-3′) and UCoxR (5′-GTAGCAGCCGTAAAATAAGC-3′). The amplifications were performed using the following parameters: initial denaturation at 95°C for 2 minutes; 35 cycles of 30 s at 95°C, 40 s at 56°C, 1 minute at 72°C; and a final extension for 10 minutes at 72°C. All amplifications, including positive and negative controls, were checked using 1.5% agarose gels with Tris/acetic acid buffer, with an appropriate molecular-weight ladder. The gels were visualized in an ultraviolet (UV) transilluminator. The PCR products were sequenced by Macrogen, Inc. (Seoul, South Korea; http://www.macrogen.com). Complementary sequences were assembled and edited using ProSeq v2.9 (Filatov, 2002). The fragments were aligned using the ClustalW algorithm with BioEdit software (Hall, 1999).

For the phylogenetic analysis, the ITS and LSU datasets were analysed using Bayesian inference (BI), maximum-likelihood (ML), and maximum-parsimony (MP) methods. The MP method was performed using PAUP* 4.0b10 (Swofford, 2001). The ML method was performed using raxmlGUI version 1.5 (Silvestro and Michalak, 2012), with the GTR + G model being used for ITS and the GTR + I and GTR + G models being used for LSU. The BI method was performed using MrBayes software (Huelsenbeck *et al.*, 2001). The MP analyses were performed using a heuristic search strategy with random-addition sequences. For the BI analyses, the HKY + G and HKY + I models were used for ITS, and the HKY + G model was used for LSU.

The node supports were evaluated statistically by bootstrapping involving 1000 resamples for ML and MP, respectively (Efron, 1982). JModeltest 2 (Darriba *et al.*, 2012) was used to

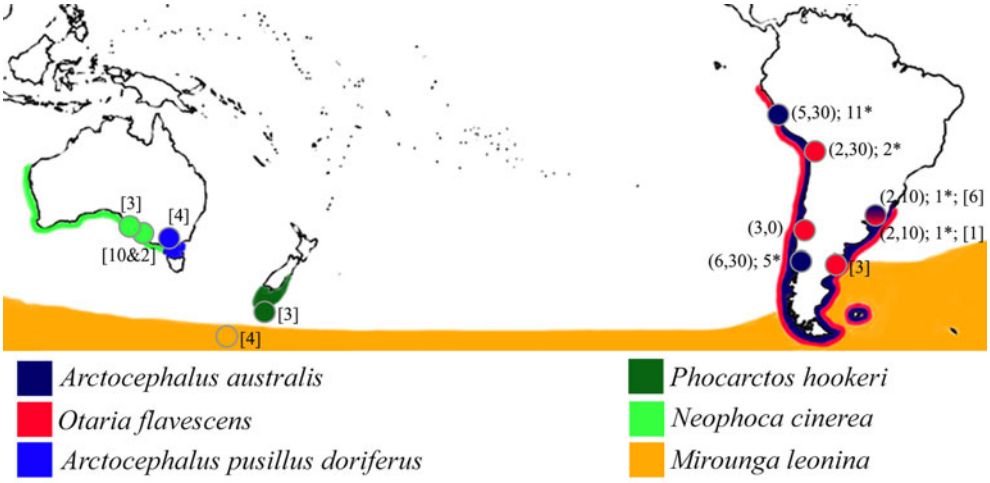

Fig. 1. Map showing host distributions and sampled localities. The number of examined hosts per locality and the number of parasites used for morphometric analyses are indicated in parentheses, and the numbers of parasites used for DNA analyses are indicated in square brackets. * indicates sequences obtained from GenBank.

Table 1. Mean body measurements (mm) of *Uncinaria* specimens collected from *Arctocephalus australis* and *Otaria flavescens* in different geographical areas. Aa,P = *A. australis* from Peru; Aa,G = *A. australis* from Guafo Island, southern Chile; Aa,U = *A. australis* from Uruguay; Of,Iq = *O. flavescens* from Iquique (northen Chile, 20°S); Of,U = *O. flavescens* from Uruguay.

Body measurements	Aa,P	Aa,G	Aa,U	Of,Iq	Of,U
Females					
Body length	20.176	18.147	17.620	19.624	11.107
Body width	0.503	0.505	0.567	0.608	0.398
Buccal capsule length	0.258	0.349	0.274	0.318	0.268
Buccal capsule width	0.209	0.263	0.237	0.281	0.231
Oesophagus length	1.274	1.377	1.317	1.419	1.151
Vulva-posterior extreme	8.712	7.744	6.651	7.528	4.300
Tail length	0.147	0.202	0.203	0.269	0.176
Males					
Body length	11.251	9.863	11.069	11.570	8.834
Body width	0.450	0.376	0.435	0.514	0.357
Buccal capsule length	0.215	0.277	0.280	0.265	0.222
Buccal capsule width	0.156	0.194	0.216	0.235	0.182
Oesophagus length	1.055	1.118	1.065	1.225	1.090
Spicule length	1.026	1.025	0.873	0.934	0.948
Tail length	–	0.542	–	0.579	0.000

compare the fit of nucleotide substitution models using the Akaike criterion. For BI inference, posterior probabilities were estimated over 10,000,000 generations via three runs with Markov Chain Monte Carlo (MCMC), with every 1000th tree saved. The first 1000 generations (10% burn-in) were discarded, as suggested by Nylander *et al.* (2004), and the consensus trees were built with 1000 trees. The sequences were contrasted with *Uncinaria* spp. sequences (Nadler *et al.*, 2013; Ramos *et al.*, 2013; Haynes *et al.*, 2014; Catalano *et al.*, 2015). *Ancylostoma caninum* (GenBank accession no.: AM039739.1) (Chilton *et al.*, 2006), *A. caninum* (JQ812694) (Lucio-Forster *et al.*, 2012), and *U. stenocephala* (AJ407939.1) (Hu *et al.*, 2002) were used as outgroup species for the LSU and ITS genes. Individual parasites were classified into groups according to host species and host geography, and the distances were computed based on the mean number of mutations (substitutions). The divergence between groups was determined using the TN93 + G model and the number of mutations, in base pairs (bp), which was performed using Mega version 7 software (Kumar *et al.*, 2016). In the ITS gene matrix, there were gaps and the sequences for *U. rauschi* and *U. yukonensis* were shorter; therefore, these spaces were filled with the "-" symbol representing missing. The LSU gene matrix did not have gaps.

Species delimitation analyses

We used the ITS sequences and the Generalized Mixed Yule Coalescent (GMYC) coalescent-based approach to test the

Table 2. GenBank accession numbers for sequences of ITS, LSU and COI genes, respectively, corresponding to parasite individuals collected for this study. Individuals 1–11: *Uncinaria* specimens from *A. australis* from Peru; 12–16: *Uncinaria* specimens from *A. australis* from Guafo, southern Chile; 17–18: *Uncinaria* specimens from *O. flavescens* from northern Chile; 19: *Uncinaria* specimens from *O. flavescens* from Uruguay.

No.	Individual	ITS	LSU	COI
1	Uncinaria_sp._A.aus_P_K2	KY465444		KY465461
2	Uncinaria_sp._A.aus_P_K3	KY465445		KY465462
3	Uncinaria_sp._A.aus_P_Un1	KY465446		KY465468
4	Uncinaria_sp._A.aus_P_Un2	KY465447		KY465469
5	Uncinaria_sp._A.aus_P_Un3	KY465448		
6	Uncinaria_sp._A.aus_P_Un4	KY465449		
7	Uncinaria_sp._A.aus_P_Un5	KY465450		KY465470
8	Uncinaria_sp._A.aus_P_Un6	KY465451		KY465471
9	Uncinaria_sp._A.aus_P_Un7	KY465452		KY465472
10	Uncinaria_sp._A.aus_P_K4	KY465453	KY465457	KY465467
11	Uncinaria_sp._A.aus_P_K5	KY465454	KY465458	
12	Uncinaria_sp._A.aus_Ch_GI_1U	KX276196	KX276202	KY465459
13	Uncinaria_sp._A.aus_Ch_GI_2U	KX276197	KX276203	KY465460
14	Uncinaria_sp._A.aus_Ch_GI_3U	KX276198	KX276204	KY465464
15	Uncinaria_sp._A.aus_Ch_GI_4U	KX276199	KX276205	KY465465
16	Uncinaria_sp._A.aus_Ch_GI_7U	KX276200	KX276206	KY465466
17	Uncinaria_sp._O.fla_Ch_An_3UA	KY465455	KY465456	KY465463
18	U._hamiltoni_O.fla_Ch_Iq_10		KX276208	
19	U._hamiltoni_A.aus_U_AAUY	KX276201	KX276207	

alternative hypotheses of species delimitation outlined by Pons *et al.* (2006). Based on this, we used three Bayesian methods for reconstructing ultrametric trees: a strict-clock analysis with a coalescent prior, and two relaxed lognormal clock analyses, one using a coalescent prior and one using a Yule prior with the program BEAST 2 (Bouckaert *et al.*, 2014). The DNA substitution model was chosen using jModelTest. We performed several initial runs considering a range of ITS mutation rates from 0.10 to 3.0% per Ma as the lower and higher thresholds. A Bayes factor analysis (Li and Drummond, 2012) indicated that the uncorrelated lognormal relaxed-clock model using a mutation rate equal to a 1% per Ma divergence rate received decisive support in comparison with the uncorrelated exponential or strict-clock models. All other parameters were set to default. Two independent MCMC chains were run for 50 million generations and sampled every 1000 generations, resulting in 50,000 trees for each run. The first 10,000 trees were then discarded from each run, and the independent log and tree files were combined using LogCombiner version 2.4.7 (Bouckaert *et al.*, 2014). The maximum clade credibility tree found using TreeAnnotator version 2.4.7 (Bouckaert *et al.*, 2014) with all options set to default was used as input data for the GMYC model. The GMYC model was optimized using the script available within the SPLITS package (http://r-forge.r-project.org/projects/splits/) for R.

Results

Morphometry

The morphometry of *Uncinaria* females infesting *A. australis* from Peru differed significantly from that of the *Uncinaria* females infesting *O. flavescens* and *A. australis* from the Uruguayan and Chilean coasts (table 1; fig. 2a). The most important relative measurements for distinguishing between *Uncinaria* females were buccal capsule length/BL, buccal capsule width/BL, and oesophagus length/BL (factor loads > 0.90). The morphometric differences between *Uncinaria* males of different hosts and locations were less clear than the differences between *Uncinaria* females. Nevertheless, *Uncinaria* males infesting *A. australis* from Peru differed from *Uncinaria* males infesting *A. australis* from southern Chile and *A. australis* from the Uruguayan coast, and from *Uncinaria* males infesting *O. flavescens* from northern Chile (fig. 2b). Only the length and width of the buccal capsule/BL were important for distinguishing among males from different hosts and locations (factor load > 0.89).

Phylogenetic analyses

The total lengths of the ITS, LSU and COI sequences were 782, 511 and 688 bp, respectively. The ITS analyses showed high resolution in the middle and basal nodes, and the LSU analyses showed high resolution in the middle and terminal branches. For both genes, the terminal branches did not exhibit good resolution due to polytomies. The phylogenetic relationships, based on different analyses (BI, ML and MP), showed low congruence, but the bootstrap values for IB, ML and MP were high. Four clades of *Uncinaria* were observed based on the ITS gene (fig. 3a).

The first ancestral clade was composed of *U. stenocephala*, *U. rauschi* and *U. yukonensis*. The second clade was composed

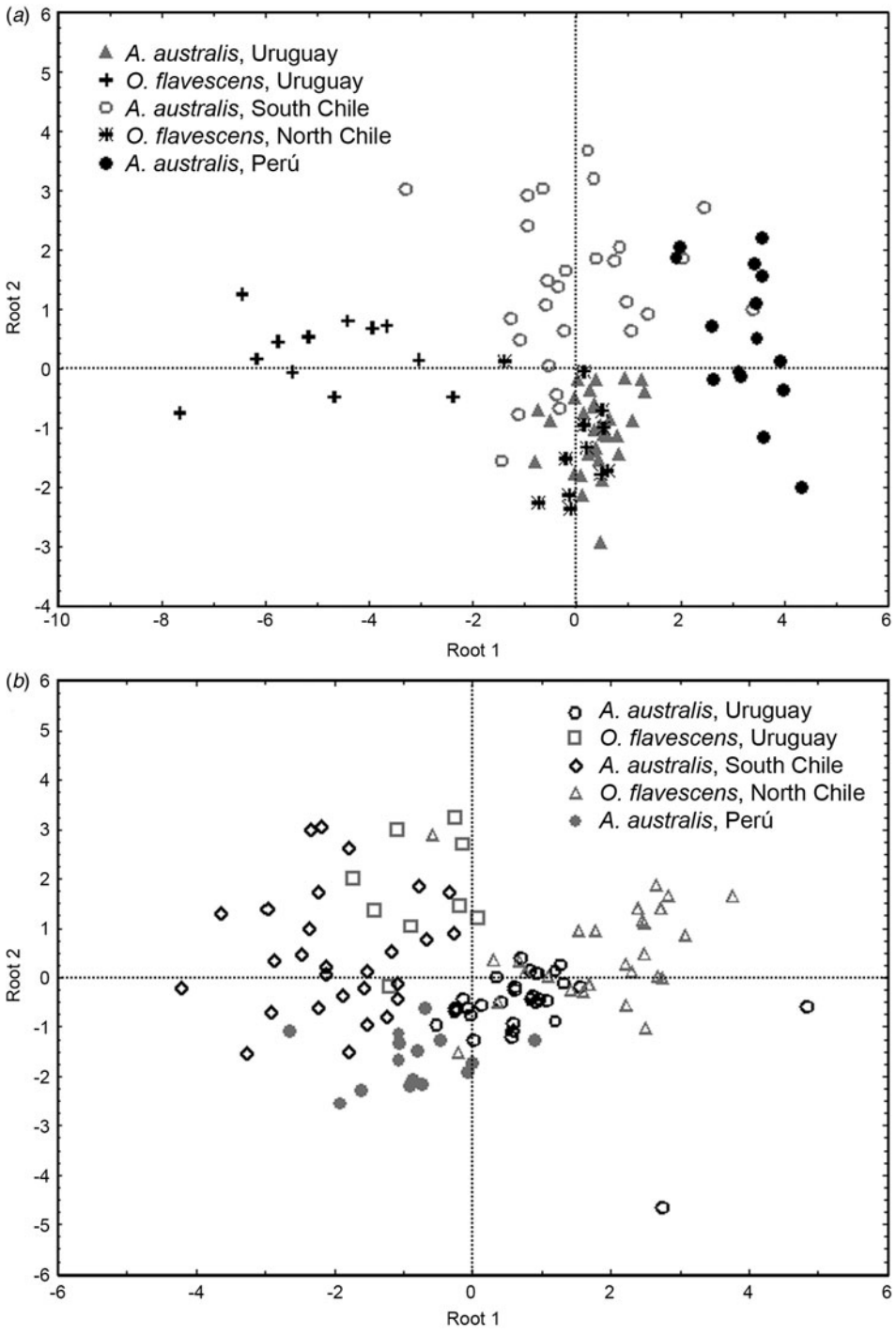

Fig. 2. Results of principal component analyses using morphometric measurements of (a) female and (b) male *Uncinaria* specimens collected from *A. australis* and *O. flavescens* from the coasts of Uruguay, northern Chile (20–24°S), southern Chile (42°S) and Peru (11°S).

of *Uncinaria* spp. from Phocidae hosts (*M. leonina* and *M. monachus*). The third clade was composed of *U. lucasi* and *U. lyonsi*, which parasitize Otariidae hosts from the northern hemisphere. The fourth clade was composed of *Uncinaria* spp. parasitizing pinnipeds from South America (*U. hamiltoni* and *Uncinaria* sp. from Peru and Chile), Australia *(U. sanguinis* and *Uncinaria* sp. from *A. pusillus doriferus*), and New Zealand (*Uncinaria* sp. from *P. hockeri*), which formed a polytomy. In turn, the LSU gene analysis showed that *U. hamiltoni* was the most recent species obtained from Otariidae hosts along the South American coasts.

Regarding the MP analysis of the ITS gene, 170 characters were included, obtaining six equally parsimonious trees with a length of 198 steps, with high homology indices (CI = 0.94; HI = 0.06; RI = 0.98; RCI = 0.92). Regarding the MP analysis of the LSU gene, 14 equally parsimonious trees were obtained with a length of 22 steps. Twenty characters were included, and the homology indices were also high (CI = 0.91; HI = 0.09; RI = 0.99; RCI = 0.90). The strict consensus tree obtained using both genes (not shown) was similar to the ML topology (fig. 3b). Phylogenetic analyses, based on COI sequences, are not shown because COI sequences are available for *U. sanguinis* only.

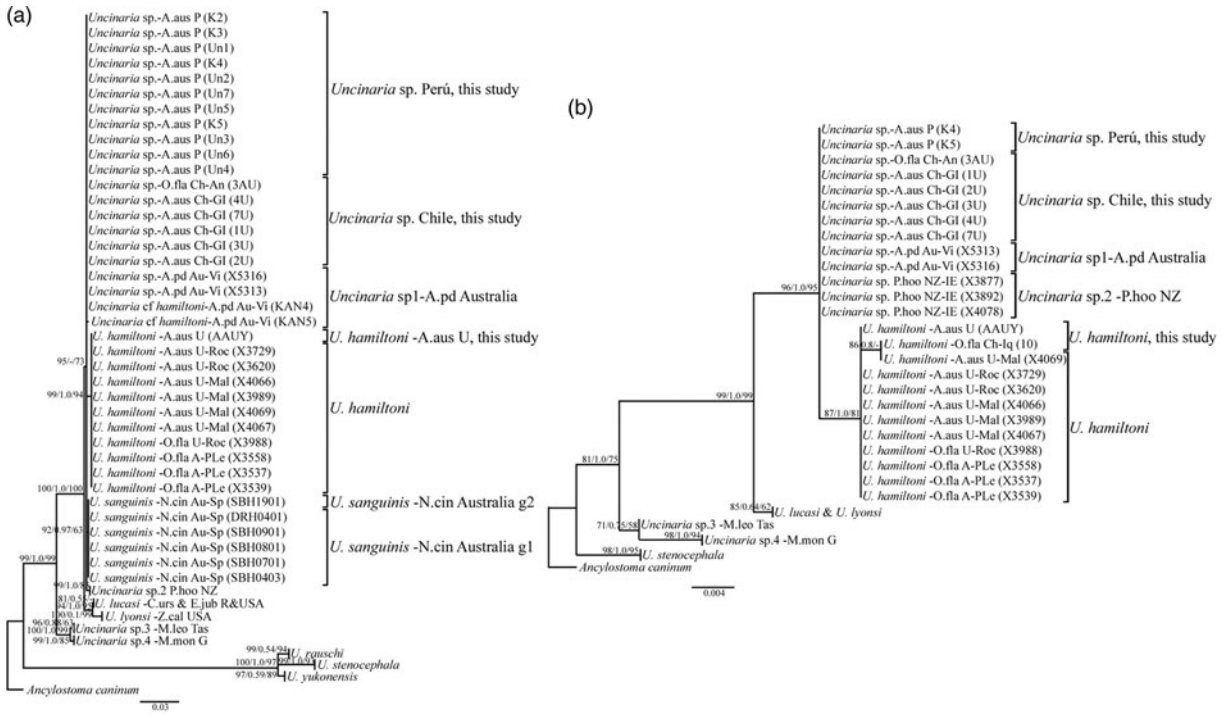

Fig. 3. Phylogenetic tree of specimens of *Uncinaria* spp. obtained from the hosts *Arctocephalus australis* (A. aus) and *Otaria flavescens* (O. fla) from Guafo Island-Chile (Ch-IG), Antofagasta-Chile (Ch-An), Iquique-Chile (Ch) and Peru (P), based on maximum likelihood (ML) analyses of the (a) ITS and (b) LSU genes. Numbers along branches indicate the percentages of support values resulting from the different analyses in the order ML/IB/MP for ITS gene and LSU gene. Values lower than 50% are indicated by dashes or are not indicated. The abbreviations for localities are Maldonado-Uruguay (U-Mal), Rocha-Uruguay (U-Roc), Punta Leon-Argentina (A-PLe), Victoria-Australia (Au-Vi), Australia (Au), New Zealand (NZ), Russia (R), Alaska-United States of America (USA-AK), and United States of America (USA). Hosts: *Arctocephalus pusillus doriferus* (A.pd), *Neophoca cinerea* (N. cin), *Eumetopias jubatus* (E. jub), *Mirounga leonina* (M. leo), *Monachus monachus* (M.mon), *Zalophus californianus* (Z. cal), *Phocarctos hookeri* (P. hoo), and *Callorhinus ursinus* (C. urs).

Species delimitation analyses

The total GMYC analysis, including the outgroup, was represented by eight ML entities (CI = 1–9) (fig. 4). Delimited GMYC clusters were largely also congruent with the species clades defined by the tree-based methods. Based on the supported monophyly of these lineages, combined with the pattern and number of genetic clusters seen in the GMYC analyses, we suggest that there are three putative species of *Uncinaria* along southern South American coasts: *U. hamiltoni*, *Uncinaria* sp.1 and *Uncinaria* sp. 2 (fig. 4).

Discussion

Understanding the specificity and diversity of *Uncinaria* spp. in pinnipeds is important for understanding hookworm diseases in pinniped pups. *Uncinaria lucasi* parasitizing *C. ursinus* in the northern hemisphere and *U. hamiltoni* parasitizing *O. flavescens* in the southern hemisphere were described in 1933 (Baylis, 1933). However, the geographical distribution and host range of *U. hamiltoni* has been controversial for many years (Baylis, 1933; Dailey and Hill, 1970; George-Nascimento *et al.*, 1992; Nadler *et al.*, 2000). Nadler *et al.* (2013) provided strong molecular evidence to definitively separate a third *Uncinaria* species from the two previously described species (*U. lucasi* and *U. hamiltoni*), recognizing, in turn, five undescribed *Uncinaria* species parasitizing pinnipeds. Since then, two new species have been formally described: *U. sanguinis* from the Australian sea lion (*N. cinerea*) (Marcus *et al.*, 2014) and *U. lyonsi* from the California sea lion (*Z. californianus*) (Kuzmina and Kuzmin, 2015). Additionally, Nadler *et al.* (2013) identified *U. hamiltoni* as a parasite infesting both *O. flavescens* and *A. australis* from the Atlantic coasts. The breeding ranges of these host species overlap along the southern coasts of South America. Therefore, *U. hamiltoni* could be present in South American fur seals (*A. australis*) and sea lions (*O. flavescens*) from the Pacific and Atlantic coasts. Our study, however, showed that *A. australis* populations from the SEP coast harbour an undescribed taxon of *Uncinaria*, although *U. hamiltoni* was also detected in an *O. flavescens* pup from the SEP coast (northern Chile). Currently, it is proposed that *A. australis* from the Pacific coast and *A. australis* from the Atlantic coast should be considered different evolutionary units (Oliveira *et al.*, 2008). Additionally, *A. australis* from the Peruvian coasts has been recognized recently as a distinct subspecies (Berta and Churchill, 2012; Oliveira and Brownell, 2014). Thus, it is possible that host habitat separations occurred during the Pleistocene when the southern tip of South America was fragmented by glacial tectonic processes (Túnez *et al.*, 2013). This may have led to host–parasite isolation and consequent genetic differentiation of the *Uncinaria* parasite.

The phylogenetic analyses of *Uncinaria* indicated a polytomy involving parasites from otariid hosts from the South Pacific coast (the Australian, New Zealand, Peruvian, and Chilean coasts), which concurs with the recent historical and ecological connections between these host species. Molecular analyses support the theory of a monophyletic group involving the southern hemisphere otariids (i.e. *Otaria*, *Neophoca*, *Phocarctos* and *Arctocephalus*), but the genus *Arctocephalus* has not been found

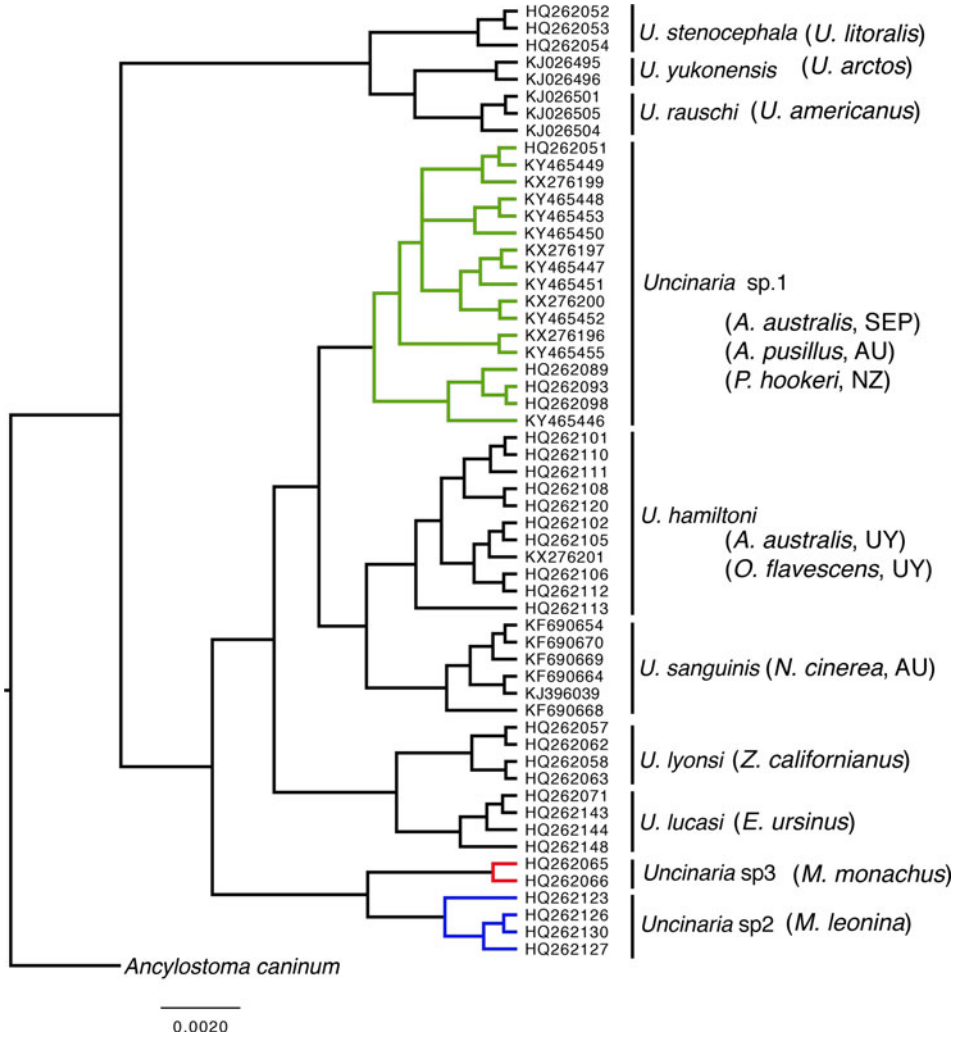

Fig. 4. Generalized Mixed Yule Coalescent species delimitation tree of *Uncinaria*, using ITS sequences. GMYC clusters were delimited using the single threshold model. Hosts are shown in parentheses.

to be monophyletic (Berta and Churchill, 2012). In turn, *A. pusillus* and *O. flavescens* are considered to be the closest species, belonging to the same phylogenetic clade (Nyacatura and Bininda-Emonds, 2012). Otariidae represent a group of recent radiation, and there was a distinct lag time between their origin and initial diversification (20.4 versus 8.1 Ma ago, respectively). Therefore, the phylogenetic relationships among otariids from the southern hemisphere remain an unresolved issue (Berta and Churchill, 2012; Nyacatura and Bininda-Emonds, 2012). Regardless of the *Arctocephalus* phylogeny, *A. australis* and *O. flavescens* are distributed along the SEP coasts, whereas *A. pusillus doriferus* is distributed along the south-western Pacific coasts (Australia). Therefore, it is possible that a recent speciation event that led to these host species could be reflected in the close phylogenetic relationships (and the observed polytomy) of the *Uncinaria* specimens parasitizing them.

The genetic markers used in this study, despite their different resolutions, indicated *Uncinaria* spp. phylogenetic relationships similar to the phylogeny proposed by Nadler *et al.* (2013). In both studies, one clade was composed of hookworms from terrestrial hosts that are ancestral to *Uncinaria* spp. of marine hosts (according to ITS gene analyses). Another clade included *Uncinaria* spp. from Phocidae hosts that formed an ancestral clade (according to ITS gene analyses); this pattern concurs with phylogenetic relationships among host species because the Phocidae family is older than the Otariidae family (Berta and Churchill, 2012). The clade involving *U. lucasi* and *U. lyonsi* from northern otariids (*C. ursinus* and *Z. californianus*) evolved in parallel to the clade from the southern hemisphere (Nadler *et al.*, 2013) and the clade involving *U. hamiltoni* from otariids from the South Atlantic coast. This latter clade could not be resolved with the genes used in this study (ITS and LSU); however, *U. hamiltoni* was observed to be a recent species. In addition, according to GMYC analysis (involving the ITS gene), *Uncinaria* sp. 1 is present in three host species (*A. pusillus*, *P. hookeri* and *A. australis*) from the South Pacific, suggesting a low host specificity.

Uncinaria specimens parasitizing otariids from the South Pacific could be considered cryptic species due to their similar morphology (Ramos *et al.*, 2013; Kuzmina and Kusmin, 2015), which makes it difficult to distinguish species only based on morphological characteristics. However, the morphometric variations between *Uncinaria* specimens parasitizing *A. australis* (Peru) and *U. hamiltoni* parasitizing *O. flavescens* (Uruguay) are concordant with interspecific parasite variations. On the other hand, the morphometric differences between *Uncinaria* sp. 1 parasitizing *A. australis* from Peru and *A. australis* from southern Chile (fig. 2), could be due to the geographical separation of the host populations along the SEP coast (Túnez *et al.*, 2013; Pavés *et al.*, 2016), which in

turn could be concordant with the existence of two lineages of *Uncinaria* sp. 1 along SEP, as suggested by a preliminary analysis (not included here) based on our COI sequences. This isolation of host populations, with *A. australis* being absent along *c.* 2300 km of the Chilean coastline, is also supported by the temporal differences in breeding phenology (and pre-mating isolation mechanisms) between *A. australis* populations from the Peruvian coast and from the south of Chile (Pavés *et al.*, 2016).

In summary, the phylogenetic and GMYC analyses suggest that the host *A. australis* from the SEP coast harbours an undescribed taxon of *Uncinaria* and that *U. hamiltoni* is distributed along both South American coasts. On the other hand, it is interesting to note that this putative species (*Uncinaria* sp. 1) could be a generalist parasite of pinnipeds, because it is present in *A. australis*, and *O. flavescens* from the SEP coast, in the fur sea lion species *A. pusillus doriferus* from Australia, and possibly in *P. hookeri* from New Zealand, thereby exhibiting an extensive distributional range across the South Pacific. *Uncinaria* sp. 2 and *Uncinaria* sp. 3 seem to be specialist parasites of *M. leonina* and *M. monachus*, respectively (fig. 4). Nevertheless, a more extensive study, including more samples from different colonies of both otariid species, will be necessary to clarify the genetic patterns of *Uncinaria* spp. distributed across the South Pacific.

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