The sympatric occurrence of two genetically divergent lineages of sucking louse, *Polyplax arvicanthis* (Phthiraptera: Anoplura), on the four-striped mouse genus, *Rhabdomys* (Rodentia: Muridae)

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SUMMARY

Within southern Africa, the widely distributed four-striped mouse genus (*Rhabdomys*) is parasitized by, amongst others, the specific ectoparasitic sucking louse, *Polyplax arvicanthis*. Given the presence of significant geographically structured genetic divergence in *Rhabdomys*, and the propensity of parasites to harbour cryptic diversity, the molecular systematics of *P. arvicanthis* was investigated. Representatives of *P. arvicanthis* were sampled from *Rhabdomys* at 16 localities throughout southern Africa. Parsimony and Bayesian gene trees were constructed for the mitochondrial COI, 12S rRNA, 16S rRNA and nuclear CAD genes. Our findings support the existence of 2 genetic groups within *P. arvicanthis* separated by at least 25% COI sequence divergence, which is comparable to that observed among recognized *Polyplax* species. We therefore propose that these 2 genetic lineages probably represent distinct species and that the apparent absence of clear morphological differences may point to cryptic speciation. The 2 taxa have sympatric distributions throughout most of the sampled host range and also occasionally occur sympatrically on the same host individual. The co-occurrence of these genetically distinct lineages probably resulted from parasite duplication via host-associated allopatric divergence and subsequent reciprocal range expansions of the 2 parasite taxa throughout southern Africa.

Key words: *Polyplax*, *Rhabdomys*, Anoplura, Rodentia, parasite duplication, sympatry, allopatric speciation, molecular phylogeny.

INTRODUCTION

Sucking lice (Anoplura) are obligate, permanent ectoparasites of eutherian mammals and are believed to have shared an intimate biological relationship with their hosts through evolutionary time, as evidenced by the high incidence of host specificity and monoxeny (Kim, 2006). The probable origin of Anoplura dates back to the mid-Cretaceous period and the diversity within the group is frequently correlated to the divergence of placental mammals (Hopkins, 1949; Ledger, 1980; Smith *et al*. 2011). It is believed that global parasite biodiversity is currently greatly underestimated (Bensch *et al*. 2000; Poulin and Morand, 2004; Locke *et al*. 2010). This is also exemplified by the Anoplura since the number of recognized species worldwide ($n = 532$) has quadrupled within the last century (Durden and Musser, 1994) and it has been speculated that the true number of species is probably between 1000–1500 (Kim *et al*. 1990).

The genus *Polyplax* (Phthiraptera: Anoplura) contains 78 known species, which occur predominantly on members of the rodent family Muridae (Durden and Musser, 1994). *Polyplax arvicanthis* (Bedford, 1919) has been documented exclusively on the four-striped mouse genus, *Rhabdomys* (Ledger, 1980) which is widely distributed throughout the southern African subregion (Skinner and Chimimba, 2005). The specificity of *P. arvicanthis* is reflected in the nomenclature (*Rhabdomys* was originally assigned to the genus *Arvicanthis*; Meester *et al*. 1986), and where this association has been thoroughly studied, *P. arvicanthis* has been recorded to have high prevalence (60%) and abundance (Matthee *et al*. 2007, 2010).

Within *Rhabdomys* (Thomas, 1916), a single species (*R. pumilio*) was originally recognized, until molecular evidence (supported by ecological divergence) led to the recognition of 2 species; the arid-adapted *R. pumilio* (Sparrman, 1784) and mesic-adapted *R. dilectus* (De Winton, 1897; Rambau *et al*. 2003; Musser and Carleton, 2005). Subsequently,

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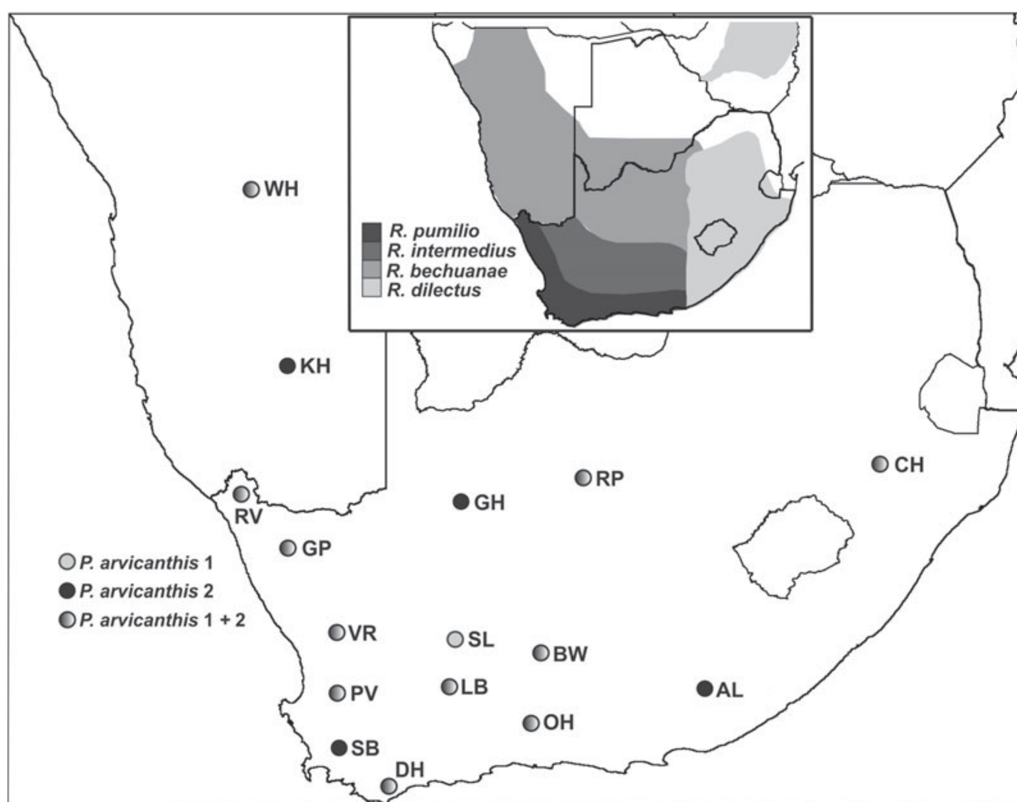

Fig. 1. Localities from which *Polyplax arvicanthis* were sampled, indicating areas of sympatric and allopatric occurrence of the 2 clades (*P. arvicanthis* 1 and 2), with locality codes as in Table 1. The inset represents the distribution of the different host species following Rambau *et al*. (2003) and du Toit *et al*. (2012).

Castiglia *et al*. (2011) and du Toit *et al*. (2012) conducted more in-depth analyses and indicated that both *R. dilectus* and *R. pumilio* contain multiple genetic lineages. Pertinent to the focus of the current study, the arid-adapted *R. pumilio* consists of 3 geographically structured genetic lineages representing distinct species (du Toit *et al*. 2012). When this diversity is incorporated into *Rhabdomys* taxonomy, it can be argued that at least 4 species exist within southern Africa. The names *R. dilectus, R. pumilio, R. intermedius* and *R. bechuanae* have been suggested based on the distributions of previously described subspecies and are further used herein (Fig. 1; Rambau *et al*. 2003; du Toit *et al*. 2012).

Cryptic species (morphologically similar, but genetically distinct; Andrews *et al*. 1998) have been documented within a wide range of parasitic groups (see de León and Nadler, 2010; Nadler and de León, 2011; Perkins *et al*. 2011). Parasites are especially prone to harbour cryptic diversity since their reduced bodily features, small size, and morphological stasis often make finding variable morphological characters problematic (Perkins *et al*. 2011), particularly among closely related species (Nadler and de León, 2011). Hence, the importance of molecular tools for cataloguing diversity within parasites, preferably using multiple genes, is being increasingly recognized (McManus and Bowles, 1996; Nadler, 1990, 2002; Nadler and de León, 2011; Perkins *et al*. 2011). Since the current description of *P. arvicanthis* is based solely on morphological characteristics (Johnson, 1960; Ledger, 1980) and given that this parasite has a large geographical range spanning 4 host species within southern Africa (du Toit *et al*. 2012), it is probable that undetected evolutionary divergence may exist within what is currently recognized as a single species.

To test the hypothesis that hidden genetic diversity, potentially associated with *Rhabdomys* divergences (Rambau *et al*. 2003; du Toit *et al*. 2012), may be present within *P. arvicanthis* we embarked on an investigation using multiple molecular markers and included several *P. arvicanthis* specimens sampled throughout southern Africa.

MATERIALS AND METHODS

Taxon and gene sampling

Polyplax arvicanthis specimens were collected from 16 localities across the distribution of the four *Rhabdomys* species (Table 1; Fig. 1). Live traps (Sherman-type) baited with a mixture of peanut butter and oats were used to capture host individuals (Permit numbers: Northern Cape, 0904/07; Western Cape, AAA004-00034-0035; Namibia, 1198/2007; Eastern Cape, CRO37/11CR and CRO38/11CR; and SANPARKS, 2007-08-08SMAT). Mice were

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Table 1. Geo-referenced localities and hosts from which *Polyplax arvicanthis* were collected

(The total number of hosts captured, number of host with lice, total number of lice collected, and the subsample of lice (specified in Table A1) used in subsequent analyses are indicated per sampled locality.)

Locality	Code	Geographical coordinates	Host species	Total hosts	Hosts with lice	Total lice	Subsampled lice
Windhoek	WH	22°31′S, 17°25′E	*R. bechuanae*	20	17	20	2
Keetmanshoop	KH	26°21′S, 18°29′E	*R. bechuanae*	21	12	20	1
Richtersveld	RV	28°12′S, 17°06′E	*R. pumilio*	31	27	27	2
Springbok	GP	29°42′S, 18°02′E	*R. pumilio*	30	28	28	2
Groblershoop	GH	28°37′S, 21°42′E	*R. bechuanae*	14	8	18	1
Sutherland	SL	32°24′S, 20′54′E	*R. intermedius*	13	6	17	1
Rooipoort	RP	28°39′S, 24°08′E	*R. bechuanae*	15	11	28	2
Vanrhynsdorp	VR	31°44′S, 18°46′E	*R. pumilio*	30	23	23	2
Porterville	PV	32°59′S, 19°01′E	*R. pumilio*	30	18	18	2
Stellenbosch	SB	33°55′S, 18°49′E	*R. pumilio*	31	12	15	1
De Hoop	DH	34°29′S, 20°24′E	*R. pumilio*	19	7	14	2
Oudtshoorn	OH	33°36′S, 22°08′E	*R. pumilio*	31	29	29	2
Beaufort West	BW	32°13′S, 22°48′E	*R. intermedius*	33	15	20	2
Laingsburg	LB	33°10′S, 20°55′E	*R. intermedius*	23	7	10	2
Chelmsford	CH	28°00′S, 29°54′E	*R. dilectus*	7	6	8	2
Alice	AL	32°47′S, 26°50′E	*R. dilectus*	6	2	4	1

euthanized with 0·2–0·4 ml sodium pentobarbitone (200 mg kg^{-1}) and placed in individual plastic bags to prevent the loss of ectoparasites post-mortem (Stellenbosch University ethics clearance: 2006B01007). Host specimens were frozen in the field at −20 °C and subsequently thawed in the laboratory, where all lice were removed with forceps under a stereoscopic microscope. For the DNA analyses, *P. arvicanthis* specimens were selected from as many different host individuals as possible per sampled locality (determined by parasite prevalence and abundance; Table 1), and placed in a 100% EtOH solution. The remainder of the lice collected from *Rhabdomys* at each site were preserved in 70% EtOH for morphological confirmation (identifications provided by L. A. Durden, Department of Biology, Georgia Southern University, USA). All specimens were identified as belonging to a single species, *P. arvicanthis,* based on gross morphological examination.

Mitochondrial COI (Cytochrome Oxidase I) sequence data were generated for 299 *P. arvicanthis* specimens from the 16 sampled localities (Table 1) and these sequences were collapsed to haplotypes (Collapse 1.2; Posada, 2004). Phylogenetic reconstructions (outlined below) were performed on these haplotypes, which indicated the presence of 2 highly divergent genetic clades (see Results section). Based on the need to include multiple markers for accurate phylogenetic inference (Nadler, 2002), the COI gene tree was supplemented with data derived from the mitochondrial 12S and 16S rRNA, and nuclear CAD (carbamoyl-phosphate synthetase 2, aspartate transcarbamylase, and dihydroorotase) genes. The latter was performed for a representative subsample of the COI haplotypes, specifically selected to optimally cover the genetic and geographical variation present within *P. arvicanthis* based on the results of the phylogenetic analyses (see below). One haplotype from each COI clade present at each of the 16 sampled localities was included (in most instances 2 haplotypes per locality, Tables 1, A1, A2; Fig. 1).

Molecular techniques

Total genomic DNA was extracted from whole individual louse specimens with a commercial kit (Qiagen, DNeasy® Blood and Tissue). PCR and sequencing of all gene fragments were performed following standard polymerase chain reaction (PCR) protocols in a GeneAmp® PCR system 2700 thermal cycler (Applied Biosystems). General PCR cycling conditions for the 12S rRNA, 16S rRNA and CAD genes included an initial denaturation of 3 min at 94 °C followed by 30–40 cycles of 30 s denaturation at 94 °C, 45–60 s annealing at the primer-specific temperature (Table 2), and 45–60 s extension at 72 °C, followed by a final extension period of 5 min at 72 °C. For the COI gene, the primers LCOIP6625 and HCOIPrev were used (Table 2) and the PCR conditions were as follows: an initial denaturation of 1 min at 95 °C followed by 10 cycles of 1 min denaturation at 95 °C, 1 min annealing at 45 °C, and 1 min extension at 72 °C after which 30 cycles of 1 min denaturation at 93 °C, 1 min annealing at either 58 °C or 59 °C (depending on amplification success), and 1 min extension at 72 °C was performed followed by a final extension of 5 min at 72 °C. Aliquots of PCR products (5 μL) were separated by electrophoresis on a 1% agarose gel for visual inspection, after which purification was performed on the remaining

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Table 2. Primers used for PCR amplification of the various gene fragments

Target gene	Primer name	Forward/ Reverse	Sequence (5′–3′)	Annealing temperature (°C)	Reference
COI	LCOIP6625	F	CCCGATCCTTTTGGTTTTTTGGGCATCC	45–58/59 °C	Adapted from L6625; Hafner *et al*. (1994)
	COIPRev	R	CCYCCTACNGTAAAYAGRAARATRAARC		Designed in this study
12S	12SAL	F	AAACTGGGATTAGATACCCCACTAT	50 °C	Palumbi *et al*. (1991)
	12SBH	R	AGAGTGACGGGGGGTGTGT		Palumbi *et al*. (1991)
16S	16SF	F	TTAATTCAACATCGAGGTCGCAA	58 °C	Shao *et al*. (2009)
	Lx16SR	R	GACTGTGCTAAGGTAGCATAAT		Shao *et al*. (2009)
CAD	CADPfor	F	ACGACAACTGCATTACCGTTTGCA	55 °C	Adapted from ApCADfor1; Danforth *et al*. (2006)
	CADPrev	R	CCACCGGGGAATTTTGACAAC		Adapted from Ap835rev1; Danforth *et al*. (2006)

PCR product with a commercial kit (Macherey-Nagel, NucleoFast 96 PCR Kit). In some instances fragments were excised and purified using a commercial gel purification kit (Promega, Wizard® SV Gel Clean-Up System). All cycle-sequencing reactions were performed using BigDye Chemistry and products were analysed on an automated sequencer (ABI 3730 XL DNA Analyzer, Applied Biosystems).

Data analysis

Sequences were edited in BioEdit Sequence Alignment editor 7.0.5 (Hall, 2005) and aligned with multiple alignment mode in Clustal X2 (Larkin *et al*. 2007). The ends of sequences were trimmed to avoid the inclusion of missing data, with the final alignment lengths as indicated in Table 3. Within the COI alignment, a 3 bp or 6 bp insert was present within *P. arvicanthis* specimens when compared with outgroup taxa. No double reads were present in the original chromatograms and to further ensure that these sequences represented the functional copy of the protein coding COI gene, all sequences were translated into protein sequences using the online tool EMBOSStranseq (www.ebi.ac.uk/Tools/st/emboss_transeq).

Phylogenetic reconstructions

A single specimen of *Haematopinus phacochoeri* (sampled from the warthog, *Phacochoerus africanus*) was used as a distant outgroup in all analyses (GenBank Accession nos JX 218028–218030; Table 4). Several additional species of Anoplura, for which sequence data were available on GenBank, were used as reference taxa in the various phylogenetic analyses (Table 4). Parsimony and Bayesian reconstructions were performed on all the COI haplotypes. For the subsampled datasets, parsimony and Bayesian trees were constructed for each gene fragment individually (COI, 12S, 16S and CAD) followed by combined analyses of the mtDNA fragments as well as a combined mtDNA and nDNA dataset. The COI insertions were coded as present/absent data and included in the analyses. Sequence ambiguities resulting from heterozygous positions of the nuclear CAD gene were treated as polymorphisms. To further investigate the divergence among the 2 *P. arvicanthis* clades with respect to other recognized *Polyplax* species, a reduced COI Bayesian topology was constructed using data derived from GenBank (Tables 4 and A3).

Unweighted parsimony analyses were conducted in PAUP* v 4.0b10 (Swofford, 2000). In each analysis, the heuristic search option with random taxon addition (10 replicates) and tree bisection and reconnection (TBR) branch swapping was implemented and a maximum of 100 equally parsimonious

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Table 3. The number of ingroup samples (*N*), amplified and final alignment length, polymorphic sites (P), and parsimony informative sites (PI) for each gene fragment

Gene fragment	*N*	Amplified length	Alignment length	P	PI
COI	27	300	270*	147	115
12S	26	420	406	185	141
16S	27	350	336	133	92
CAD	27	370	349	80	30

* Excluding the two 3 bp indels coded as presence/absence.

Table 4. GenBank Accession numbers for outgroup taxa used in the different gene analyses

Taxon	Gene fragment	Accession no.
Fahrenholzia pinnata	COI	EF152557.1
	CAD	FJ267404.1
Hoplopleura ferrisi	COI	HM171428.1
Pedicinus badii	CAD	FJ267414.1
Pediculus humanus capitis	12S	AY139881.1
	16S	AY139928.1
	CAD	FJ267404.1
Polyplax serrata	COI	EU162172.1
Haematopinus phacochoeri	COI	JX218028
	12S	JX218029
	16S	JX218030

trees were saved during each step. Nodal support was assessed with 1000 bootstrap replicates (Felsenstein, 1985). Best-fit models of sequence evolution for all gene fragments were determined under the AICc (Akaike, 1973; Burnham and Anderson, 2002, 2004) in jModelTest v0.1.1 (Guindon and Gascuel, 2003; Posada, 2008) and implemented in Bayesian tree reconstructions in MrBayes v3.2 (Ronquist *et al.* 2012). The effect of codon partitioning on the marginal likelihoods for the protein coding COI and CAD genes was evaluated with Bayes factors (Kass and Raftery, 1995), as calculated in Tracer v1.5 (Newton and Raftery, 1994; Suchard *et al.* 2001; Rambaut and Drummond, 2007). For all analyses, only the general structure of the model was defined and the default priors were used to estimate parameters. In each analysis, 2 parallel Markov Chain Monte Carlo (MCMC) simulations, consisting of 5 chains each, were run for 2–5 million generations depending on when it was estimated that stationarity had been reached. Trees and parameters were sampled every 100 generations, and 25% of the total number of generations sampled were discarded as burn-in after convergence and ESS values were assessed in Tracer v1.5 (Rambaut and Drummond, 2007). Posterior probabilities for nodal support were obtained by using the sumt command in MrBayes. COI GTR-corrected sequence distances were calculated in PAUP* v4.0b10 (Swofford, 2000) to compare sequence divergence estimates among species/lineages.

RESULTS

The 299 *P. arvicanthis* specimens revealed 94 COI haplotypes (Table A1; GenBank Accession nos JX629372–JX629438, JX198372–JX198398). A total of 27 COI haplotypes were selected (see above, Table A1) and sequences were generated for the 16S rRNA and CAD gene fragments (GenBank Accession nos JX198319–JX198345, JX198399–JX198425; Tables 3 and A2). Despite numerous attempts, 12S rRNA data for 1 haplotype (LB_1) could not be obtained, resulting in a total of 26 haplotypes for this dataset (GenBank Accession nos JX198346–JX198371; Tables 3 and A2).

Bayes factors indicated that partitioning by codon position with a separate model assigned to each partition was preferred over an unpartitioned scheme with a single model, for both the protein coding COI and CAD genes (Table A4). Thus, the JC model (nst = 1, rates = equal) was assigned to the first and second codon positions of COI and all 3 codon positions of CAD. The HKY (nst = 2, rates = equal), GTR + I + G (nst = 6; rates = invgamma), and GTR + G (nst = 6; rates = gamma) models were specified for the third codon position of COI, the 12S rRNA, and 16S rRNA genes respectively.

Phylogenetic analyses of the 94 COI haplotypes revealed the presence of 2 well-supported genetic clades (Table 5 and Fig. B1) differentiated by an average of 25% (±0·02) sequence divergence, which is comparable to values observed among other recognized species of *Polyplax* (Table A4). Considerable variation is also present within the 2 *P. arvicanthis* clades, especially within *P. arvicanthis* 2 (Fig. 2) which has an average intra-clade divergence of 16% (±7·34) compared with 11% (±7·91) within *P. arvicanthis* 1. For the representative subset of 26/27 haplotypes, the monophyly of *P. arvicanthis* was strongly supported in nearly all individual and combined analyses (parsimony and Bayesian; Table 5; Fig. 2) of the various genes. The combined analyses (parsimony and Bayesian) of the 3 mtDNA fragments (Fig. 2) provided the highest posterior probability support for the existence of 2 clades within *P. arvicanthis* (1 and 2) and the same pattern emerged when all the data were combined (Table 5; also see Light and Reed (2009) and Light *et al.* (2010) showing greater support from combined analyses). Support for the 2 clades varied among the individual parsimony and Bayesian gene trees (Table 5), and in instances where the nodes were not obtained they reflect polytomies (unresolved). All of the nodal uncertainty found in the individual gene analyses surrounded the monophyly of *P. arvicanthis* 2. Importantly, however, individuals belonging to the

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Table 5. Bootstrap and posterior probability support values for the monophyly of *Polyplax arvicanthis* and the 2 clades therein resulting from the various single and combined gene analyses

Clade	Gene fragment						
	COI (full)	COI (subset)	12S	16S	mtDNA	CAD	mtDNA + nDNA
Polyplax arvicanthis	78/1	82/0·99	100/0·8	98/0·99	85/1	100/1	96/1
P. arvicanthis 1	72/0·99	82/0·99	59/0·57	82/0·98	93/0·99	78/0·90	98/1
P. arvicanthis 2	76/0·99	82/0·98	89/0·89	nf/nf	95/0·96	nf/nf	97/0·91

* nf, Not found.

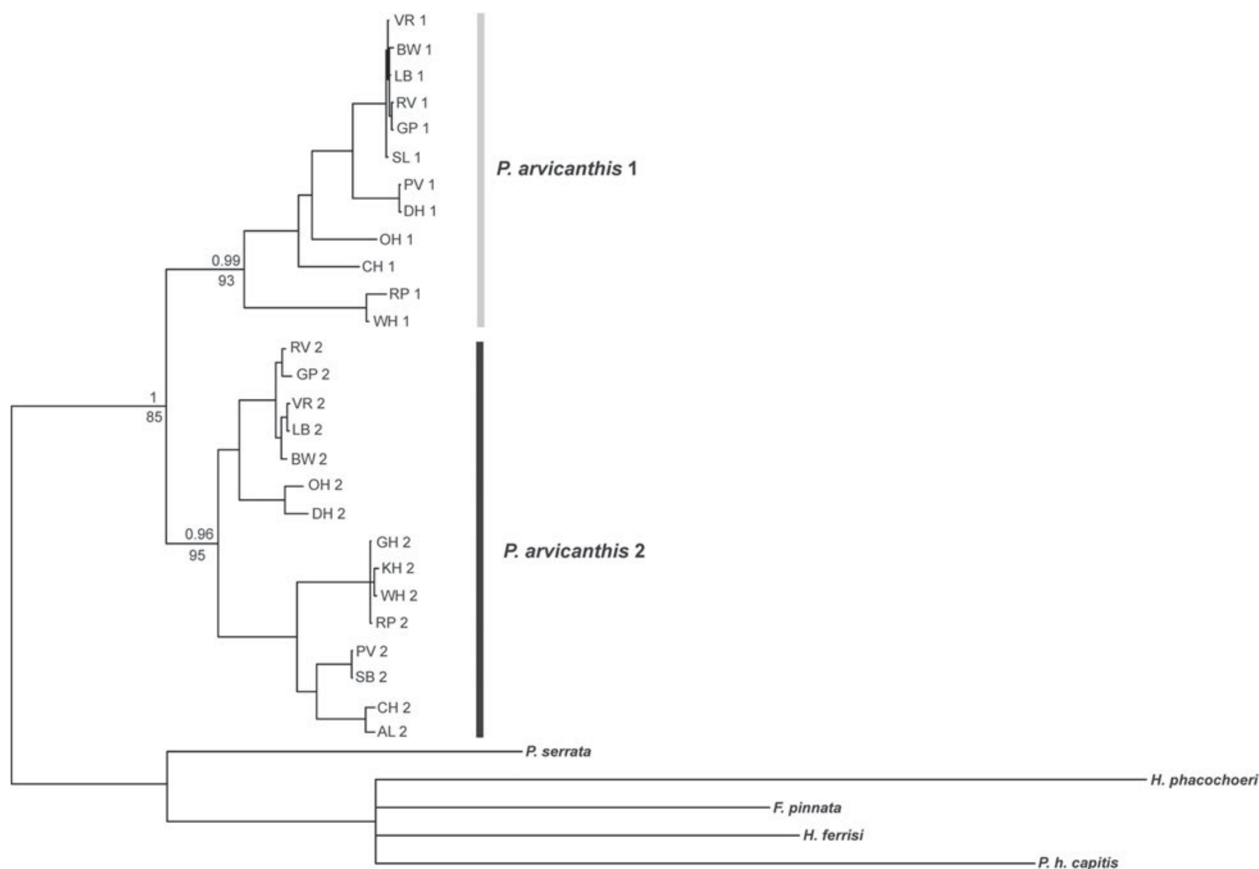

Fig. 2. Consensus parsimony and Bayesian topology of the combined mtDNA dataset (posterior probabilities above and bootstraps below nodes), indicating the 2 clades within *Polyplax arvicanthis*.

2 *Polyplax* clades are consistently differentiated by the presence of the 6 bp (*P. arvicanthis* 1) or 3 bp (*P. arvicanthis* 2) insert within COI when compared with other *Polyplax* species, and this more conservative substitution (Matthee *et al.* 2001) provides further support for the monophyly of each of the 2 lineages.

Analysis of the phylogenetic relationships within *Polyplax* (using COI data obtained from GenBank; Fig. 3), place the 2 *P. arvicanthis* lineages as sister taxa within a clade containing *P. spinulosa, P. borealis* and *P. serrata*. Branch lengths separating the *P. arvicanthis* lineages are comparable to those separating the other recognized species. The topology also supports the previously reported non-monophyly of the family *Polyplacidae* (Light *et al.* 2010). It is, however, important to realize that the relationships within the genus as portrayed herein is based on a fraction of the total number of known *Polyplax* species and additional data (species and molecular markers) are needed to obtain more robust support for these preliminary phylogenetic findings.

DISCUSSION

The existence of 2 genetically distinct, reciprocally monophyletic clades within *P. arvicanthis* is strongly supported in nearly all our analyses. The COI sequence divergence between these clades, also illustrated by branch lengths, is comparable to that found between other *Polyplax* species. Although the exact taxonomic status of these clades is not yet clear

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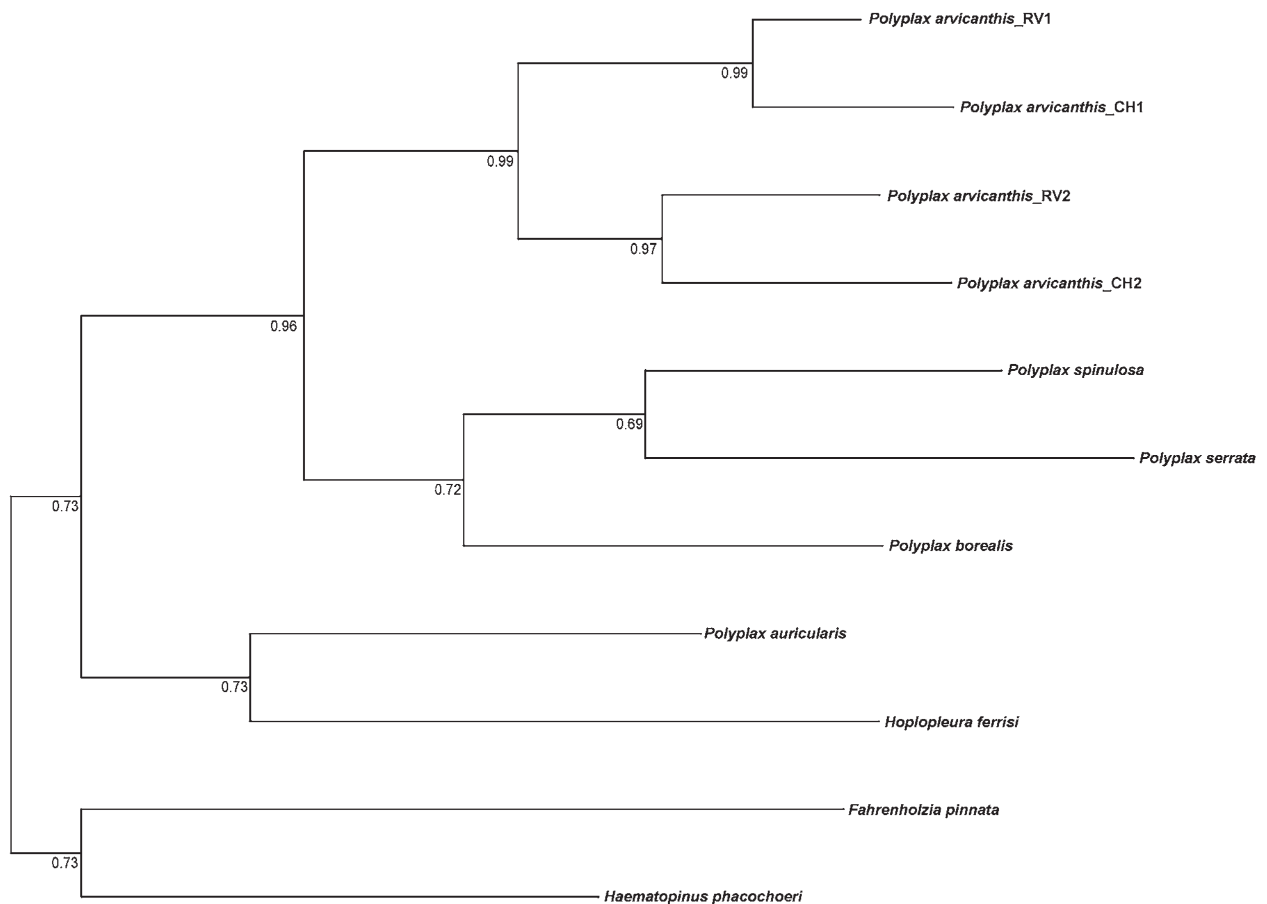

Fig. 3. Bayesian COI topology indicating the phylogenetic position of the 2 clades within *Polyplax arvicanthis* with respect to other recognized *Polyplax* species (GenBank Accession numbers listed in Tables 4 and A4).

(and data from more *Polyplax* species are needed to determine whether they represent sister-taxa), this questions the notion that *P. arvicanthis* represents a single species (Bedford, 1919; Ledger, 1980).

The apparent absence of morphological differentiation among the 2 genetically divergent *P. arvicanthis* lineages suggests that they may represent cryptic species. The discovery of cryptic species through the use of molecular tools has been documented widely in several parasitic groups (de León and Nadler, 2010) and this may be attributed to the reduced morphological features associated with the parasitic mode of life (Nadler and de León, 2011; Perkins *et al*. 2011). It should, however, be noted that further morphological investigation, especially using features that have not previously been used in alpha taxonomy, may reveal differences among the *P. arvicanthis* lineages (see Yoshizawa and Johnson, 2006; Schlick-Steiner *et al*. 2007) which will provide further evidence for the existence of 2 species on *Rhabdomys*. Until such investigations have been conducted, the 2 genetic lineages identified in our study should therefore remain provisionally cryptic (de León and Nadler, 2010).

Parasite genetic divergence does not mirror that observed within the host since, for both *P. arvicanthis* lineages, the lice collected from *R. dilectus, R. pumilio, R. intermedius* and *R. bechuanae* do not form monophyletic entities (du Toit *et al*. 2012). In addition, the 2 parasite lineages have sympatric distributions throughout most of the sampled range of all host lineages. Our findings are not unique in the sense that closely related lineages of sucking lice occurring sympatrically on a single host species has been documented previously (Reed *et al*. 2004; Štefka and Hypša, 2008). It has been postulated that the sympatric occurrence of closely related parasitic groups may arise through colonization (switching from another host) or parasite duplication (shared common ancestor on particular host; Page, 2003). A sister relationship between the 2 lineages would thus favour parasite duplication over colonization due to host-switching. In the present analysis the 2 divergent *P. arvicanthis* lineages represent sister-taxa (0·99 posterior probability support) which, in combination with the presence of unique insertions within both *P. arvicanthis* lineages (when compared with other available *Polyplax* species), supports the idea of a common ancestor on *Rhabdomys* followed by duplication. Unfortunately, the paucity of sequence data for African *Polyplax* precludes a firm statement regarding the sister-taxon status of these lineages and we cannot rule out the possibility that the insertions may also be present in other African

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Polyplax. However, *P. arvicanthis* is specific to *Rhabdomys* as it has not been documented on any other co-occurring rodent taxa within southern Africa (Ledger, 1980; Durden and Musser, 1994), and is also morphologically distinct from the *Polyplax* species parasitizing these co-occurring hosts (Ledger, 1980). Given the specificity to *Rhabdomys* and the apparent lack of morphological differences among the 2 *P. arvicanthis* lineages, a recent common ancestor on *Rhabdomys* followed by a parasite duplication event is a more parsimonious evolutionary scenario than morphological convergence of distantly related louse lineages following colonization of one of the lineages from another host (i.e. host switching).

Parasite duplication may occur via sympatric or allopatric speciation (Johnson and Clayton, 2004). Multiple instances of sympatric speciation would be needed to explain the broad sympatric occurrence of the 2 louse taxa in this study. Thus, parasite duplication through allopatric speciation caused by temporary fragmentation of host populations (Page, 2003), followed by mutual secondary colonizations of hosts, appears to be the most parsimonious explanation for the pattern we observed. *Rhabdomys* probably experienced multiple expansion-contraction cycles during glacial–interglacial phases (Zachos, 2001) and allopatric divergence of the parasite would be enhanced by the short generation times (Huyse *et al*. 2005; Whiteman and Parker, 2005) and faster evolutionary rates (Hafner *et al*. 1994; Paterson and Banks, 2001; Nieberding *et al*. 2004) generally observed in parasites. It is thus plausible that the 2 lineages within *P. arvicanthis* diverged in allopatry while isolated on host lineages within different refugia (du Toit *et al*. 2012). If this holds, the current pattern resulted from mutual host colonizations of the 2 parasite taxa across the region. This dispersal would have been facilitated through the secondary contact observed among hosts (du Toit *et al*. 2012). Importantly, however, host sympatry does not necessarily equate to parasite sympatry (McCoy, 2003), especially for *P. arvicanthis* which requires bodily contact to move between hosts (Hopkins, 1949; Ledger, 1980; Marshall, 1981). From the parasite's perspective, host syntopy (occupation of same macro-habitat; Rivas, 1964) will therefore be more important than host sympatry in terms of dispersal opportunity between hosts. For *P. arvicanthis*, host syntopy probably does occur within the host contact zone and the co-occurrence of both cryptic lineages on the same host individuals implies that parasite syntopy also occurs in certain areas.

The present study clearly lends support to the suggestions of Kim *et al*. (1990), in that much of Anoplura diversity is yet to be discovered. Within *P. arvicanthis,* detailed morphological comparsions are needed to fully resolve the taxonomic status of the 2 genetic lineages and given the extensive variation observed within each of the 2 *Polyplax* assemblages, more fine-scale comparative phylogeographical analyses are required to unravel the complex patterns observed.

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APPENDIX A

Table A1. COI haplotypes identified from the 299 *Polyplax arvicanthis* specimens sampled from 16 localities (codes as in Table 1)

(GenBank Accession numbers and haplotypes selected for the representative subset (sequenced for additional markers) are also indicated. Subscripts indicate *P. arvicanthis* clade 1 or 2.)

Haplotype	Accession No.	Frequency	Subset	Individuals
H01_1	JX 198378	52	GP_1	RV1_1, 3_1, 4_1, 6_1, 7_1, 10_1, 11_1, 12_1, 13_1, 18_1, 20_1, 21_1, 23_1, 24_1, 30_1; GP3_1, 4_1, 5_1, 9_1, 10_1, 12_1, 13_1, 14_1, 16_1, 17_1, 20_1, 22_1, 24_1, 27_1; VR1_1, 2_1, 4_1, 5_1, 6_1, 8_1, 9_1, 17_1, 21_1, 23_1, 24_1, 27_1; BW10·2_1; LB2_1; SL3·6_1, 4·1_1, 4·2_1, 4·3_1, 4·4_1, 4·5_1, 4·6_1, 6·1_1, 8·2_1;
H02_1	JX 198372	1	RV_1	RV14_1
H03_1	JX 198377	1	RP_1	RP12·2_1
H04_1	JX 629372	1		GP1_1
H05_1	JX 629373	4		GP2_1, 6_1, 8_1, 28_1
H06_1	JX 629374	1		GP7_1
H07_1	JX 629375	1		GP23_1
H08_1	JX 198380	12	PV_1	PV4_1, 5_1, 9_1, 10_1, 14_1, 17_1, 20_1, 23_1, 29_1, 30_1; DH3·2_1, 15·1_1
H09_1	JX 629376	1		PV18_1
H10_1	JX 629377	1		PV26_1
H11_1	JX 198382	20	OH_1	OH3_1, 5_1, 6_1, 9_1, 10_1, 12_1, 13_1, 15_1, 16_1, 17_1, 18_1, 19_1, 21_1, 22_1, 25_1, 26_1, 27_1, 30_1, 31_1, 32_1
H12_1	JX 629378	1		OH4_1
H13_1	JX 198384	1	VR_1	VR7_1
H14_1	JX 629379	1		VR19_1
H15_1	JX 629380	2		VR22_1 , 26_1
H16_1	JX 629381	1		BW1_1
H17_1	JX 629382	1		BW8_1
H18_1	JX 198387	5	BW_1	BW9_1, 10·1_1, 10·4_1, 18_1, 19_1
H19_1	JX 629383	1		BW17·1_1
H20_1	JX 629384	1		BW21_1
H21_1	JX 629385	1		BW28·1_1
H22_1	JX 629386	1		BW28·2_1
H23_1	JX 629387	1		BW32_1
H24_1	JX 198389	1	LB_1	LB1·3_1
H25_1	JX 629388	1		WH3·4_1
H26_1	JX 629389	12		WH4_1, 5_1, 6_1, 8_1, 9_1, 10_1, 12_1, 13_1, 16_1, 17_1, 18_1, 21_1
H27_1	JX 629390	3		WH7_1, 19_1, 23_1
H28_1	JX 198391	1	WH_1	WH11_1
H29_1	JX 198393	2	SL_1	SL3·1_1, 3·2_1
H30_1	JX 629391	2		SL3·3_1, 7·3_1
H31_1	JX 629392	1		SL8·1_1
H32_1	JX 629393	1		SL8·3_1
H33_1	JX 629394	4		DH2_1, 5·1_1, 5·2_1, 5·3_1
H34_1	JX 198394	1	DH_1	DH3·1_1
H35_1	JX 629395	1		DH3·5_1
H36_1	JX 629396	1		DH5·5_1
H37_1	JX 629397	1		DH5·6_1
H40_1	JX 629398	1		CH6·1_1
H41_1	JX 198397	1	CH_1	CH7_1
H42_1	JX 629399	2		CH14·1_1, 14·2_1
H01_2	JX 629400	3		RV2_2, 19_2, 28_2
H02_2	JX 629401	5		RV8_2, 9_2, 15_2, 16_2, 17_2
H03_2	JX 198373	1	RV_2	RV25_2
H04_2	JX 629402	1		RV26_2
H05_2	JX 629403	1		RV29_2
H06_2	JX 629404	1		GH1·2_2
H07_2	JX 198376	39	RP_2	GH2·1_2, 2·2_2, 2·3_2, 4·2_2, 4·3_2, 4·4_2, 4·6_2, 5·1_2, 5·2_2, 8·1_2, 8·2_2, 9·1_2, 14·1_2, 14·3_2, 14·4_2; RP1·1_2, 1·2_2, 3·2_2, 3·1_2, 4·1_2, 4·2_2, 5·1_2, 5·2_2, 6·1_2, 7·1_2, 7·2_2, 9·1_2, 9·2_2, 9·3_2, 11·1_2,12·1_2, 12·3_2, 12·5_2, 12·7_2, 12·8_2,

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Table A1. (*Cont.*)

Haplotype	Accession No.	Frequency	Subset	Individuals
				12·9_2, 12·10_2, 14·1_2, 15·1_2
H08_2	JX 198374	1	GH_2	GH4·5_2
H09_2	JX 629405	1		GH10_2
H10_2	JX 198375	4	KH_2	KH1_2, 3_2, 6_2, 14·2_2
H11_2	JX 629406	15		KH4·1_2, 4·2_2, 4·3_2, 7_2, 11_2, 15_2, 16_2, 18·1_2, 18·2_2, 20·1_2, 20·3_2, 21·1_2, 21·2_2, 21·3_2; WH14_2
H12_2	JX 629407	2		KH14·1_2, 20·2_2
H13_2	JX 629408	3		RP11·3_2, 12·4_2, 12·6_2
H14_2	JX 198379	1	GP_2	GP11_2
H15_2	JX 629409	1		GP15_2
H16_2	JX 629410	1		GP18_2
H17_2	JX 629411	3		GP19_2, 21_2, 30_2
H18_2	JX 629412	1		GP26_2
H19_2	JX 629413	1		PV1_2
H20_2	JX 198381	16	PV_2	PV2_2, 7_2, 21_2, 24_2, 27_2; SB2·2_2, 2·4_2, 4·1_2, 4·2_2, 5_2, 8_2, 14_2, 18_2, 22_2, 24_2, 26_2
H21_2	JX 629414	3		OH1_2, 2_2, 20_2
H22_2	JX 629415	1		OH8_2
H23_2	JX 198386	2	OH_2	OH11_2, 33_2
H24_2	JX 629416	1		OH14_2
H25_2	JX 629417	1		OH24_2
H26_2	JX 198385	4	VR_2	VR3_2, 11_2, 16_2, 29_2
H27_2	JX 629418	1		VR12_2
H28_2	JX 629419	1		VR15_2
H29_2	JX 629420	1		VR18_2
H30_2	JX 629421	1		SB2·1_2
H31_2	JX 629422	1		SB12_2
H32_2	JX 198386	1	SB_2	SB25_2
H33_2	JX 629423	1		SB30_2
H34_2	JX 629424	3		BW7_2, 10·5_2, 10·6_2
H35_2	JX 198388	1	BW_2	BW20_2
H36_2	JX 629425	1		BW22_2
H37_2	JX 629426	1		BW26_2
H38_2	JX 629427	1		BW27_2
H39_2	JX 629428	1		LB9·1_2
H40_2	JX 198390	2	LB_2	LB9·3_2 , 14·1_2
H41_2	JX 629429	1		LB10_2
H42_2	JX 629430	1		LB12·1_2
H43_2	JX 629431	1		LB12·2_2
H44_2	JX 629432	1		LB12·4_2
H45_2	JX 629433	1		LB17_2
H46_2	JX 198392	2	WH_2	WH3·1_2, 3·3_2
H47_2	JX 629434	2		SL6·2_2, 13·2_2
H48_2	JX 198395	2	DH_2	DH4·1_2, 8_2
H49_2	JX 629435	2		DH14·2_2, 14·3_2
H66_2	JX 629436	1		CH2_2
H67_2	JX 198396	3	CH_2	CH6·2_2, 12_2, 8_2
H68_2	JX 629437	2		AL 4·2, 5·1
H69_2	JX 629438	1		AL5·2
H70_2	JX 198398	1	AL_2	AL4·1

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Table A2. GenBank Accession numbers for the gene sequences generated for the subset of specimens, with locality codes as in Table 1 and subscripts indicating *P. arvicanthis* clade 1 or 2

Specimen	16S	12S	CAD
RV_1	JX198319	JX198346	JX198399
RV_2	JX198320	JX198347	JX198400
GH_2	JX198321	JX198348	JX198401
KH_2	JX198322	JX198349	JX198402
RP_2	JX198323	JX198350	JX198403
RP_1	JX198324	JX198351	JX198404
GP_1	JX198325	JX198352	JX198405
GP_2	JX198326	JX198353	JX198406
PV_1	JX198327	JX198354	JX198407
PV_2	JX198328	JX198355	JX198408
OH_1	JX198329	JX198356	JX198409
OH_2	JX198330	JX198357	JX198410
VR_1	JX198331	JX198358	JX198411
VR_2	JX198332	JX198359	JX198412
SB_2	JX198333	JX198360	JX198413
BW_1	JX198334	JX198361	JX198414
BW_2	JX198335	JX198362	JX198415
LB_1	JX198336	JX198363	JX198416
LB_2	JX198337	no data	JX198417
WH_1	JX198338	JX198364	JX198418
WH_2	JX198339	JX198365	JX198419
SL_1	JX198340	JX198366	JX198420
DH_1	JX198341	JX198367	JX198421
DH_2	JX198342	JX198368	JX198422
CH_2	JX198343	JX198369	JX198423
CH_1	JX198344	JX198370	JX198424
AL_2	JX198345	JX198371	JX198425

Table A3. GTR-corrected COI sequence distances among recognized *Polyplax* species, with GenBank Accession numbers

	P. spinulosa	*P. auricularis*	*P. borealis*	*P. serrata*
Accession no.	HQ542196·1	DQ324549·1	DQ324548·1	EU162264·1
P. spinulosa	–			
P. auricularis	0·28	–		
P. borealis	0·23	0·28	–	
P. serrata	0·27	0·29	0·32	–

Table A4. Log_{10} Bayes Factors for alternative partitioning schemes (unpartitioned and partitioned by codon) of the protein coding COI and CAD genes

(Standard errors were estimated with 1000 bootstrap replicates.)

Gene	lnP	s.e.	Partitioned	Unpartitioned
COI				
Partitioned	−2442·79	±0·132	–	38·206
Unpartitioned	−2530·76	±0·126	−38·206	–
CAD				
Partitioned	−898·67	±0·201	–	35·869
Unpartitioned	−981·262	±0·296	−35·869	–

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APPENDIX B

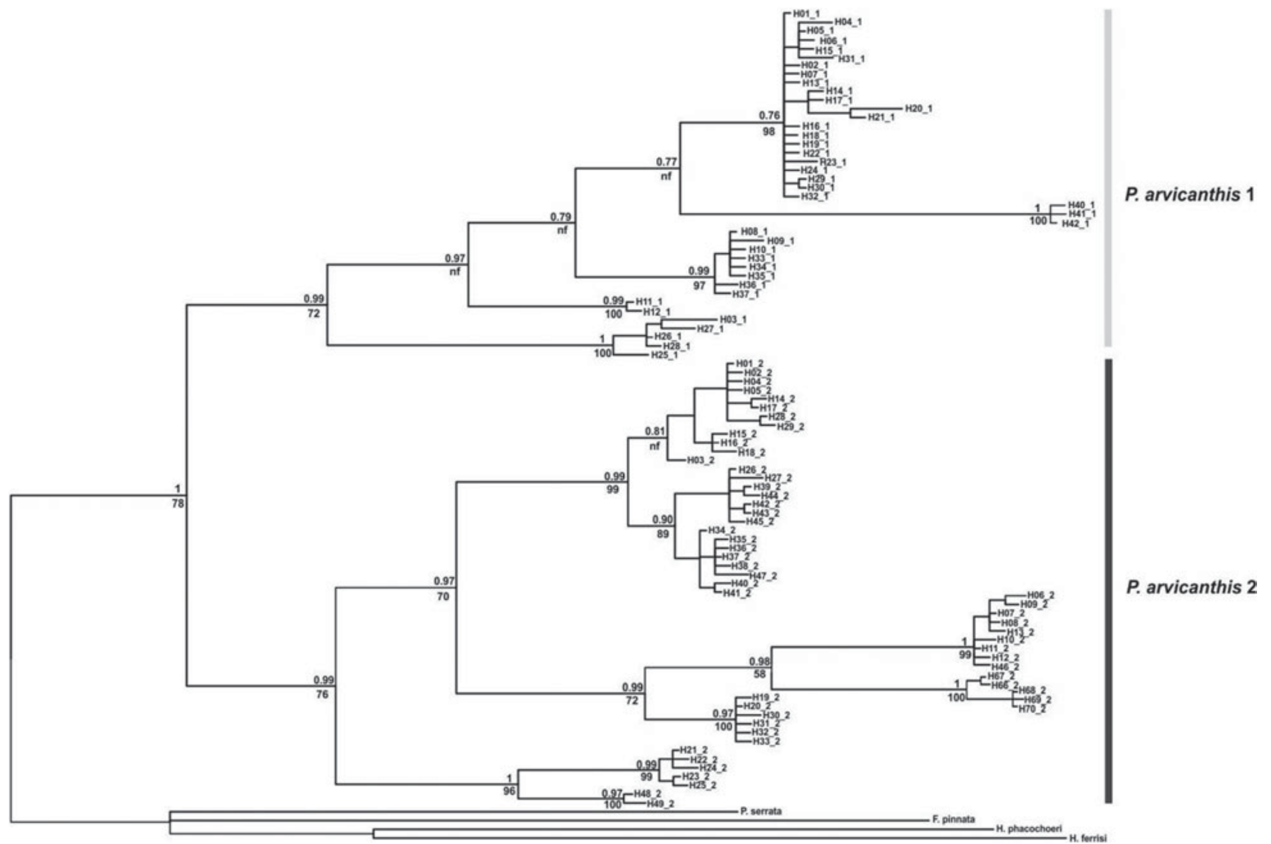

Fig. B1. Consensus Bayesian and Parsimony COI topology indicating the relationships among the 94 haplotypes within *P. arvicanthis* (codes as in Table 1). Posterior probabilities and bootstrap support values are indicated above and below nodes, respectively.

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