Evidence of cryptic speciation in mesostigmatid mites from South Africa

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SUMMARY

Laelaps giganteus and *Laelaps muricola* (Mesostigmata; Laelapidae) are widespread and locally abundant host generalists on small mammals in southern Africa. The large host range and complex life history of these ectoparasites may allude to possible intraspecific cryptic diversity in these taxa. To assess genetic and morphological diversity in *L. giganteus* and *L. muricola*, we sampled 228 rodents at eight localities in South Africa. This sample included nine previously recorded host species and on these, *L. muricola* was only recorded from *Mastomys natalensis* and *Micaelamys namaquensis* while *L. giganteus* was found on *Rhabdomys dilectus* and *Lemniscomys rosalia*. Phylogenetic analyses of partial mtDNA cytochrome oxidase subunit I (COI) and nuclear ITS1 data strongly supported the recognition of *L. giganteus* and *L. muricola*, a scenario partly supported by the Tropomyosin intron. Strong support for evolutionary distinct lineages within *L. giganteus* is found: *L. giganteus* lineage 1 is confined to *R. dilectus* and *L. giganteus* lineage 2 is confined to *L. rosalia*. These host specific monophyletic lineages were also separated by 9·84% mtDNA sequence divergence and 3·44% nuclear DNA sequence divergence. Since quantitative morphometric analyses were not congruent with these findings, these two lineages more than likely represent cryptic species.

Key words: *Laelaps*, COI, ITS1, Tropomyosin, host range, cryptic species.

INTRODUCTION

Recent molecular studies highlighted the need to re-address parasite taxonomy previously based on traditional morphological approaches (Williams *et al*. 2006; Smith *et al*. 2007; Ståhls and Savolainen, 2008; Perkins *et al*. 2011; du Toit *et al*. 2013*a*, *b*). This holds true especially for small-bodied invertebrates which are often characterized by a slow rate of change in morphological features (Clayton *et al*. 2003; Huyse *et al*. 2005; Whiteman and Parker, 2005), and in the case of ectoparasites, speciation may also be subjected to evolutionary processes related to their host (Roy *et al*. 2008, 2010; Perkins *et al*. 2011; du Toit *et al*. 2013*a*, *b*).

Since a comprehensive understanding of cryptic diversity is needed to better understand taxonomy and the processes generating biodiversity, most modern taxonomic studies have a total evidence approach incorporating both morphology and DNA sequencing (Bickford *et al*. 2007; Morelli and Spicer, 2007; Detwiler *et al*. 2010; Shäffer *et al*. 2010; Skoracka and Dabert, 2010; Apanaskevich *et al*. 2011; Knee *et al*. 2012). Apart from providing new insights into the systematics of the taxa concerned, a thorough phylogenetic analysis can also be used to explore the mechanisms involved in parasite speciation. For example, well-resolved congruent phylogenies of a particular parasite and host system can enable co-evolutionary scenarios to be described between the complementary parasite and host lineages (Page, 1996; Morelli and Spicer, 2007; du Toit *et al*. 2013*a*, *b*).

The evolutionary history of ectoparasite taxa is complex. They can be structured spatially between microhabitats within individual hosts, between individuals or populations of the same host species, and between different host species (de Meeûs, 2000; Clayton *et al*. 2003; Noureddine *et al*. 2011). Differences in several factors, such as life cycle, mode of transmission, interspecific competition and host specificity could result in more pronounced genetic structure in a given parasite species or population (Blouin *et al*. 1995; Nadler, 1995; Criscione and Blouin, 2004, 2005; Barrett *et al*. 2008; Cangi *et al*. 2013) and different parasite races may even be

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formed as a result of disruptive selection in the host (Maynard Smith, 1966; Bush, 1994). However, without sufficient data, the contemporary taxonomy and evolutionary predictions for ectoparasites remain highly speculative.

In the African context, molecular investigations focusing on the taxonomy and co-evolution of mammalian ectoparasites are virtually non-existent (but see Cangi *et al*. 2013; du Toit *et al*. 2013*a*, *b*). Pertinent to the focus of the present study, regional investigations on mites of the order Mesostigmata seem to be limited to species surveys only (Hirst, 1925; Zumpt, 1961; Matthee *et al*. 2007, 2010; Matthee and Ueckermann, 2008, 2009; Viljoen *et al*. 2011). Since mite studies conducted elsewhere show pronounced genetic disparity when compared with morphological traits (Morelli and Spicer, 2007; Knee *et al*. 2012), it seems reasonable to hypothesize that the current diversity of parasitic mites in southern Africa is also underestimated (also see de León and Nadler, 2010; Nadler and de León, 2011).

To gain more insights into the evolution and taxonomy of mesostigmatid mites we performed a morphological and molecular investigation on two mite species, *Laelaps giganteus* (Berlese, 1918) and *Laelaps muricola* (Träghardh, 1910). The mites belong to the subfamily Laelapinae (Mesostigmata: Laelapidae) and can be differentiated from other species in the genus by a unique opisthogenital shield with four pairs of setae on adult females (Hirst, 1925). Both *Laelaps* species are geographically widespread in sub-Saharan Africa and are reported from multiple rodent species (Hirst, 1925; Zumpt, 1961). In addition, Zumpt (1961) hypothesized about the possibility of multiple sub-species within *L. giganteus*.

In this study the evolutionary history and taxonomic status of two recognized southern African Mesotigmatid mites, *L. giganteus* and *L. muricola*, was investigated using a combination of partial mitochondrial cytochrome oxidase subunit I (COI), the nuclear internal transcribed spacer 1 (ITS1) and the intron Tropomyosin (TropoM). Selected morphological characters were also included. The relative importance of host range as a contributing factor towards lineage diversification was tested by sampling multiple previously described hosts of these parasites at different localities.

MATERIALS AND METHODS

Samples collected

Hirst (1925) and Zumpt (1961) lists the South African hosts of *L. giganteus* and *L. muricola* as being *Rhabdomys dilectus, Rhabdomys pumilio, Lemniscomys rosalia, Mastomys natalensis, Dasymys incomtus* (type host for *L. giganteus*), *Aethomys chrysophilus, Micaelamys namaquensis, Saccostomys campestris, Otomys irroratus, Parotomys littledalei, Rattus rattus* and *Tatera afra* while several other hosts are recorded for the rest of sub-Saharan Africa. To achieve maximum host overlap at sampling sites, efforts focused on the eastern side of South Africa (see Skinner and Chimimba, 2005 for host distributions). Attempts were made to sample as many host species possible (Table 1) at eight different collection sites (Fig. 1). The mite *Androlaelaps marshalii* (Berlese, 1911) collected in this study from *Tatera brantsii* was used as an outgroup for some of the phylogenetic analyses. In instances where *A. marshalii* failed to amplify, the GenBank sequence of the more distantly related *Dermanyssus apodis* (FM897373.1) was also used as an alternative outgroup.

Rodents were trapped using Sherman-type live traps that were set in trap lines (each trap 10 m apart). Trapping was done for a minimum period of 4 days (dependent on the trapping success). Adult hosts were selected for parasite screening and placed in a plastic bag and euthanized using an intra-peritoneal injection with sodium pentobarbitone (200 mg kg^{-1}) (ethical approval for euthanasia method was granted by Stellenbosch University: SU-ACUM11-00004). Ectoparasites were obtained by brushing the pelage of the host using standard procedures (Ignoffo, 1958; Burger *et al*. 2012). To prevent cross contamination between conspecific samples, brushes were cleaned with 100% ethanol after each use. Cross contamination among host species was avoided by using separate clean brushes for each host species at each site. Only female mites were selected for inclusion in the genetic analyses as there is a significant female bias on rodent hosts (1 male : 128 females per host; Matthee *et al*. 2007) and females also possess more distinct morphological characters for identification (Matthee and Ueckermann, 2009). Mites were preserved in 100% ethanol for molecular and morphological analysis.

DNA extraction and sequencing

Total genomic DNA was extracted using a Macherey-Nagel kit (GmbH & Co.) following the protocol of the manufacturer. Whole animals were placed in the extraction buffer without grinding and digested at 56 °C for a minimum of 3 h. Individual extraction reactions were mixed every 30 min using a vortex. Following extraction, the remaining exoskeletons of all mites were stored individually and used for the morphological component of the study (see below). Extracted DNA was stored at −20 °C and later thawed for PCR use.

Universal primers LCO1490 and HCO2198 of COI were used to amplify 708 base pairs (bp) of the gene (Folmer *et al*. 1994). Also, two nuclear genes were included in this study namely ITS1, for which 700 bp were amplified using the primers described in Roy *et al*. (2008) and TropoM for which 570 bp were amplified as described in Roy *et al*. (2010).

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Table 1. Collection localities, host species, total number of individuals per host species and number of host individuals that harboured either *L. giganteus* or *L. muricola*

Province	Locality	Code	Host species	Total hosts	*L. giganteus*	*L. muricola*
Eastern Cape	Alice	AL	*Micealamys namaquensis*	15	–	14
			Otomys irroratus	8	–	–
			Rattus rattus	2	–	–
			Rhabdomys dilectus	6	–	–
	Hogsback	HB	*Micealamys namaquensis*	4	–	8
			Mus musculus	3	–	–
			Otomys irroratus	2	–	–
			Rhabdomys dilectus	10	2	–
KwaZulu Natal	Vryheid	VH	*Lemniscomys rosalia*	1	1	–
			Mastomys natalensis	5	–	–
			Micealamys namaquensis	3	–	–
			Rhabdomys dilectus	3	–	–
	Inkunzi	IN	*Aethomys chrosophilus*	4	–	–
			Mastomys natalensis	9	–	–
			Otomys irroratus	2	–	–
			Rattus rattus	3	–	–
			Rhabdomys dilectus	9	2	–
Gauteng	Rietvlei	RV	*Mastomys coucha*	18	–	2
			Rhabdomys dilectus	24	10	–
	Kaalplaas	KP	*Mastomys coucha*	18	–	7
			Rhabdomys dilectus	30	18	–
			Steatomys pratensis	10	–	–
North West	Zeerust	ZE	*Lemniscomys rosalia*	2	–	–
			Mastomys coucha	2	–	1
	Mooinooi	MN	*Lemniscomys rosalia*	14	7	–
			Mastomys coucha	21	–	12

Fig. 1. Collection localities in South Africa from where *Laelaps giganteus* (filled circles) and *L. muricola* (open circles) were recorded.

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Table 2. The morphological characters measured for three *Laelaps* lineages in micrometres following the nomenclature of Evans and Till (1979)[a]

Morphological characters	Animal number Nr. 1	Nr. 2	Nr. 3	Nr. 4	Nr. 5	Nr. 6	Nr. 7	Nr. 8	Nr. 9	Nr. 10	Average	S.D.	Size range
Laelaps giganteus from *Rhabdomys dilectus*													
Length of dorsal shield	1325	1294	1324	1300	1406	1235	1228	1248	1191	1271	1282	61·59	1191–1406
Width of dorsal shield	970	1002	984	948	906	901	837	839	872	937	920	58·41	837–1002
Spine on Trochnater I	39	38	40	34	42	35	47	43	42	47	41	4·42	34–47
Spine on Coxa I	60	65	59	58	63	60	65	74	65	68	64	4·85	58–74
Anterior spine on Coxa II	74	69	60	68	61	61	0	69	61	70	59	21·40	60–74
Posterior spine on Coxa II	72	74	64	61	64	66	74	63	71	76	69	5·46	61–76
Anterior spine on Coxa III	73	62	50	68	57	71	62	60	71	70	64	7·44	50–73
Posterior spine on Coxa III	63	70	66	67	65	65	63	64	65	65	65	2·06	63–70
Spine Coxa IV	52	58	55	56	50	53	53	56	51	57	54	2·69	50–58
Distance Sternal setae 1–3	236	238	234	241	226	236	237	232	237	235	235	4·02	226–238
Distance Sternal setae 2–2	248	270	268	251	251	245	247	237	251	243	251	10·39	243–270
Para-anal setae	166	195	182	173	164	176	175	196	180	172	178	10·79	164–196
Post anal seta	256	282	268	256	240	249	244	250	247	250	254	12·43	240–282
Width of genital shield, across 2nd pair of setae	310	298	290	286	298	281	278	292	289	294	292	9·22	278–310

	Animal number Nr. 1	Nr. 2	Nr. 3	Nr. 4	Nr. 5	Nr. 6	Nr. 7	Nr. 8	Nr. 9	Average	S.D.	Size range
Laelaps giganteus from *Lemniscomys rosalia*												
Length of dorsal shield	1286	1211	1257	1131	1104	1189	1214	1264	1241	1211	61·02	1104–1286
Width of dorsal shield	944	842	895	824	879	915	817	947	850	879	49·35	824–947
Spine on Trochnater I	43	38	39	42	37	31	41	39	36	38	3·61	31–43
Spine on Coxa I	68	63	67	66	59	71	76	75	69	68	5·40	59–76
Anterior spine on Coxa II	70	73	71	72	70	65	70	63	70	69	3·24	63–73
Posterior spine on Coxa II	82	78	72	69	63	67	71	78	64	72	6·62	63–82
Anterior spine on Coxa III	65	65	54	62	61	71	70	68	62	64	5·24	54–71
Posterior spine on Coxa III	68	77	65	61	58	67	59	67	63	65	5·77	59–77
Spine Coxa IV	52	57	56	40	41	56	51	44	36	48	7·96	36–57
Distance Sternal setae 1–3	239	238	245	239	232	249	231	238	236	239	5·68	231–239
Distance Sternal setae 2–2	233	228	232	235	218	239	237	226	233	231	6·40	218–239
Para-anal setae	166	175	162	200	184	150	136	168	179	169	18·81	136–200
Post anal seta	247	258	248	251	0	289	0	221	0	168	127·35	221–289
Width of genital shield, across 2nd pair of setae	277	276	286	285	278	303	316	0	320	260	98·97	276–320

	Animal number												
	Nr. 1	Nr. 2	Nr. 3	Nr. 4	Nr. 5	Nr. 6	Nr. 7	Nr. 8	Nr. 9	Nr. 10	Average	S.D.	Size range
Laelaps muricola from *Mastomys coucha* and *Micealamys namaquensis*													
Length of dorsal shield	1461	1440	1528	1470	1451	1434	1526	1432	1334	1387	1446	58·1	1334–1528
Width of dorsal shield	980	1004	1028	1009	1021	977	982	923	909	917	975	44·0	909–1028
Spine on Trochnater I	–	–	–	–	–	–	–	–	–	–	–	–	–
Spine on Coxa I	60	52	53	37	53	44	51	55	48	54	51	6·4	37–60
Anterior spine on Coxa II	–	–	–	–	–	–	–	–	–	–	–	–	–
Posterior spine on Coxa II	66	65	57	65	62	66	60	70	68	53	63	5·2	53–70
Anterior spine on Coxa III	–	–	–	–	–	–	–	–	–	–	–	–	–
Posterior spine on Coxa III	49	46	0	50	56	51	48	52	51	49	45	16·1	46–56
Spine Coxa IV	28	26	22	23	21	26	27	26	29	23	25	2·7	21–29
Distance Sternal setae 1–3	265	272	275	262	278	269	291	269	262	259	270	9·5	259–291
Distance Sternal setae 2–2	336	327	348	339	341	323	334	339	323	324	333	8·7	323–341
Para-anal setae	129	144	139	147	127	129	138	134	146	118	135	9·4	118–147
Post anal seta	191	157	172	0	0	186	205	182	181	174	145	77·3	172–205
Width of genital shield, across 2nd pair of setae	420	425	446	445	432	427	420	419	428	396	426	14·2	396–446

[a] Zeros in the table indicate that the particular appendage broke during DNA extraction and could not be measured.

All PCR reactions were optimized and carried out using 25 μL reaction volumes with a GeneAmp® PCR system 2700 thermal cycler (Applied Biosystems). COI regions were amplified via a 'cold start' reaction consisting of a denaturation cycle of 1 min at 95 °C followed by a 10-cycle loop of 1 min at 95, 45 and 72 °C, respectively. A 30-cycle loop was then followed using the exact same conditions apart from increasing the 45 °C annealing temperature to 59 °C. All reactions were ended off by a final 5 min extension period at 72 °C. PCR conditions for ITS1 and TropoM followed Roy *et al*. (2010) with annealing temperatures of 49 and 54 °C, respectively. After amplifications, 5 μL of the PCR products were visualized on a 1% agarose gel. The remainder of the PCR product was purified with a NucleoFast 96 PCR kit (Macherey-Nagel). Cleaned products were then cycle sequenced using BigDye Chemistry and analysed with an ABI 3730 XL DNA Analyzer (Applied Biosystems, Inc.).

Sequence processing and alignment

Sequences were authenticated using the BLASTN tool on GenBank (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and the mtDNA sequences were also translated to amino acids with the online tool EMBOSS/Transec (www.ebi.ac.uk/Tools/emboss/transeq/index.html). All usable sequences were manually aligned and edited using BioEdit Sequence Alignment Editor v. 7.0.9 (Hall, 2005). To avoid the inclusion of missing data and ambiguities, small end sections of the sequences were truncated.

Phylogenetic analyses

Phylogenetic relationships among individuals sequenced were firstly inferred using maximum parsimony (MP) in PAUP* v4.0b10 (Swofford, 2002). A heuristic search was run following the tree bisection-reconnection branch exchange method (TBR) with all characters assigned equal weights and unordered. Stability of the nodes on the MP tree was assessed with bootstrapping using 1000 re-sampling pseudo-replicates and the TBR method. Bootstrap values above 75% were considered well supported while bootstrap values below 75% were considered poorly supported (Felsenstein, 1985). Using the program JModeltest v. 2.1.2 (Guindon and Gascuel, 2003; Posada, 2008) and the Akaike information criteria (AIC), the best-fit model of evolution was determined for each gene fragment (Akaike, 1973; Nylander, 2004). Using the latter as a guide for prior input, Bayesian analysis were performed in MrBayes v. 3.2 (Ronquist and Huelsenbeck, 2003), including five parallel Monte Carlo Markov chains. A total of five million generations were used while the chains were sampled every

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100th generation. The generated samples were summarized with the sump command in MrBayes to determine statistical stationarity and based on these results 25% were discarded as burn-in. The PSRF (potential scale reduction factor) value was also used to assess whether the data were adequately sampled (Rambaut and Drummond, 2007). The sumt command in MrBayes was used to obtain statistical support values for the nodes on the trees. Trees were then visualized with the program FigTree v. 1.2.2 (http://tree.bio.ed.ac.uk/software/gtree/). Nodes with posterior probabilities (P) < 0·95 were considered not significantly supported. After individual analyses, a concatenated dataset was created and analysed using Parsimony (as described above) and Bayesian analyses in a partitioned fashion for each gene fragment (COI, ITS1 and TropoM). The latter Bayesian analysis was run for 22 million generations (until the S.D. of split frequencies were below 0·01) including 58 representative specimens for which all three gene complements were available.

In order to also incorporate population level processes, the individual genes were also analysed in SplitsTree v. 4.5 (Huson and Bryant, 2006). For each gene, uncorrected P distances were used to draw a neighbour-net network (Bryant and Moulton, 2004), using equal angle splits to present the relationships (Dress and Huson, 2004).

Morphological analysis

All specimens sequenced were mounted in Heinze-PVA medium following the protocol stipulated in Matthee and Ueckermann (2009). Following the key of Evans and Till (1979), 14 morphologically diagnostic characters were recorded (Table 2) and measured in micrometres using a Zeiss Axioscope Research microscope (Zeiss). *Laelaps giganteus* has a genital plate that is distinctly separate from the anal shield and is narrower relative to other species within the genus (especially *L. muricola*). The venter is inundated with short stout setae. Trochanter I has one spine and five small setae. Coxa I has one spine and one spine-like seta while coxae II–III each has two spines and coxa IV one spine (Hirst, 1925). In contrast, *L. muricola* has a smooth and fairly wide heart-shaped genital plate extending greatly behind the hind legs. Hairs on the venter are numerous, thick and longer than those of *L. giganteus* (Hirst, 1925). Trochanter I has six small setae, coxae II–III each with one spine and one acute and stout seta and coxa IV with one small spine. Morphological measurements were analysed with a ZEN Imaging Software system (Zeiss). To test for a significant size difference between specimens, 9–10 individuals from each genetic lineage were measured for all characters and the mean was calculated for each. To exclude the effects of missing measurements on the principal component analysis, the data for each measured character group was mean centred. This was done by determining the mean for each character group and deducting those values from each data point in the character group and dividing the subsequent value by the S.D. of the particular variable. The mean centred data were then normalized in the open source software program GNU Octave (www.gnu.org/software/octave/) in order to assign the same weight to each character. The first component (PC1) gave information on how samples differed from each other while the second component (PC2) showed how variables relate to each other.

RESULTS

Host and parasite demographics

By sampling eight localities, 228 specimens were collected from 10 different potential host species (Table 1). Of the 10 different Muridae host species that were collected only four carried the mites of interest (Table 1). The four-striped grass mouse, *R. dilectus* was the most common host collected at all sampling sites (with the exclusion of Zeerust). Based on morphology *L. giganteus* was only recorded from *R. dilectus* and the single-striped grass mouse, *L. rosalia* (Table 1). *Laelaps muricola* were collected at six localities from two host species, namely the Southern multimammate mouse, *M. coucha*, and the Namaqua rock mouse, *M. namaquensis* (Table 1). The remaining hosts were predominantly infested with mites belonging to the genus *Androlaelaps*. These findings do not deviate significantly from that reported by Matthee *et al*. (2007).

Gene sequence characteristics

Attempts were made to sequence 84 *Laelaps* specimens for the COI locus, ITS1 and TropoM regions. We were successful in obtaining sequences for all specimens for COI and ITS1, but despite numerous attempts only managed to get 58 sequences for the TropoM gene (GenBank accession numbers: COI: KF805772–KF805856; ITS1: KF805857–KF805940; TropoM: KF505941–KF805998). Sampling data corresponded to the two morphologically recognized species *L. giganteus* ($N = 40$) and *L. muricola* ($N = 44$), respectively (Table 1). JModeltest selected the GTR + G model as the best-fit model of substitution for all three gene fragments. A total of 644 bp were analysed for the COI region and excluding the outgroup, this resulted in 522 (81·05%) invariant and 105 (16·30%) parsimony informative characters. The ITS1 region presented 468 base pairs, of which 382 (81·62%) were invariant and 47 (10·04%) parsimony informative while TropoM produced 464 useable base pairs, of which 384 (82·75%) were invariant and 44 (9·48%) parsimony informative characters.

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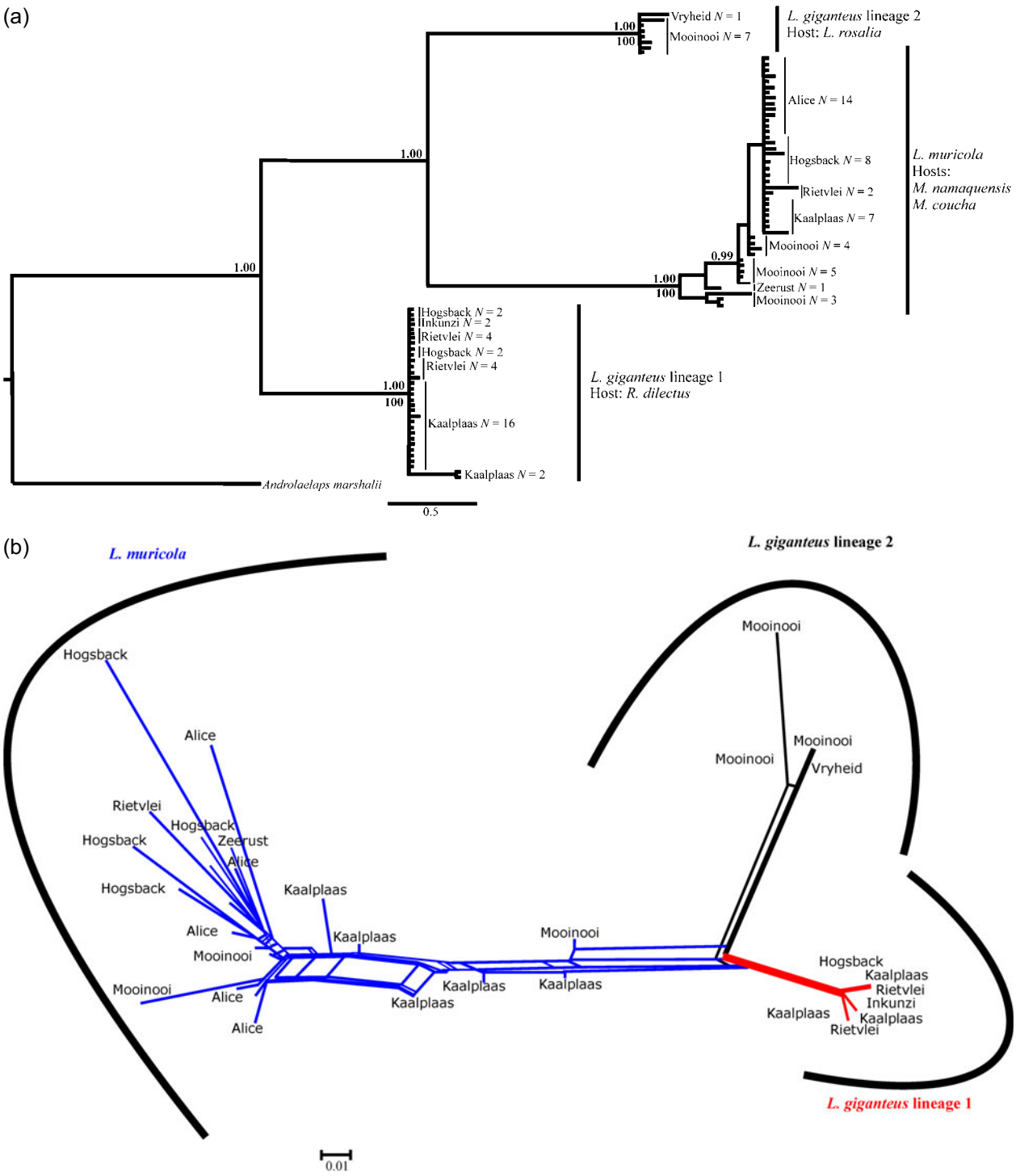

Fig. 2. See the following page for legend.

Pair-wise divergence and phylogenetic reconstructions

Bayesian and Parsimony analyses of the COI data revealed the existence of at least three monophyletic lineages (Fig. 2a). The three lineages support the distinction between the morphologically recognized *L. muricola* and *L. giganteus* and furthermore provide strong evidence for the existence of at least two genetic lineages within *L. giganteus* (Fig. 2a). The three genetic lineages are separated by between 9·84–10·51% mtDNA sequence divergence and 3·55–7·72% nuclear DNA divergence (Table 3). The distinctions of the three genetic clades are supported by intra-lineage sequence divergences that, apart from TropoM were markedly lower than inter-lineage sequence divergences (Table 3).

Parsimony and Bayesian analyses of the ITS1 data consistently support the recognition of the two recognized species, but analyses based on TropoM were unresolved (data not shown). This result is best illustrated by the Neighbour-net network analyses of the faster-evolving ITS1 data (Fig. 2b) when

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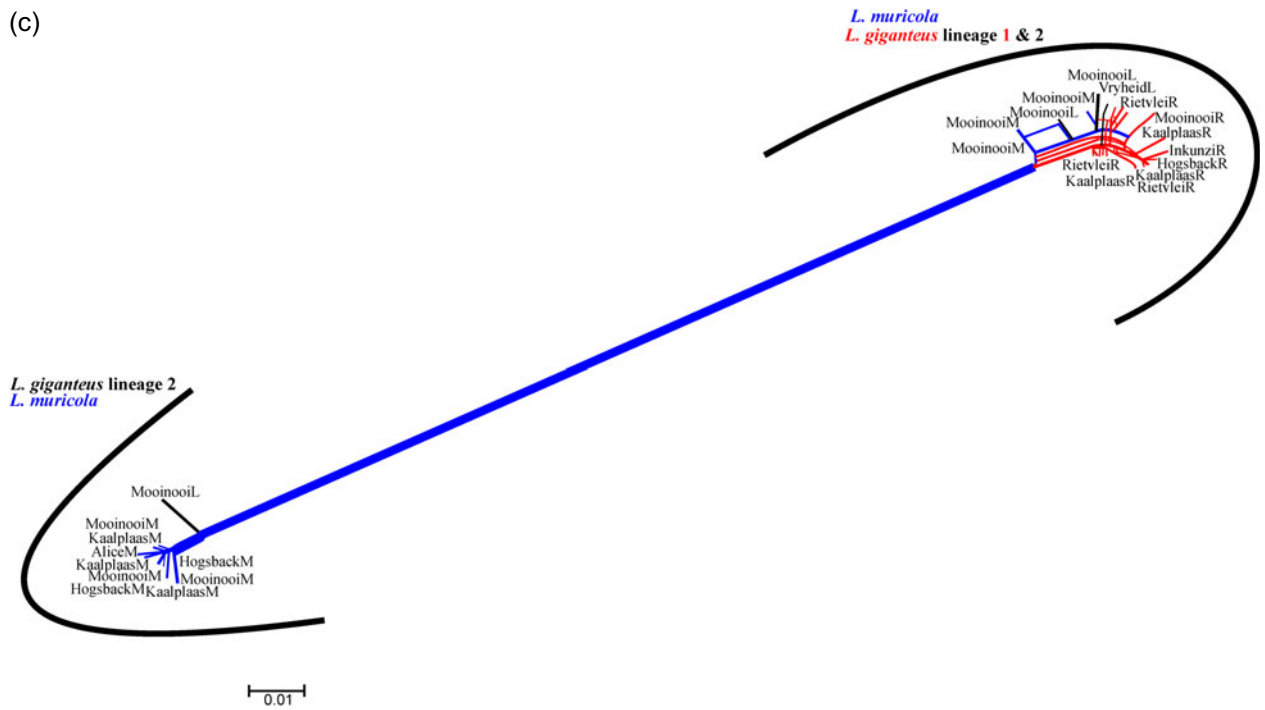

Fig. 2. (a) Bayesian phylogeny indicating the three major clades retrieved from the COI dataset. The two cryptic lineages within *L. giganteus* are indicated as *L. giganteus* lineage 1 and *L. giganteus* lineage 2, with *L. muricola* grouped sister to *L. giganteus* lineage 2. (b) *Laelaps* Neighbour-Net phylogenetic network of the ITS1 dataset indicating the three major groupings recovered in the COI phylogeny (labelled as such). Ambiguous signal and conflicts are indicated by multiple connections. (c) *Laelaps* Neighbour-Net phylogenetic network of the TropoM dataset indicating the three major groupings recovered in the COI phylogeny (labelled as such). Ambiguous signal and conflicts are indicated by multiple connections.

compared with the mixed signals obtained for TropoM (Fig. 2c). Combining the data in a single matrix provided robust support for the two recognized species but due to the TropoM data failed to support the strict monophyly of the two *L. giganteus* lineages (combined analyses not shown).

Morphological analysis

Morphological measurements of $N = 29$ mites originating respectively from each of the three clades (Fig. 2a and b) showed strong morphological differentiation between *L. giganteus* and *L. muricola* but show a large overlap in range sizes for all morphological characters that were measured for the two *L. giganteus* lineages (Table 2; Fig. 3a). A test of explained variance showed that more than 95% of the data was described by the first and second principal components. Comparing only the samples from the two genetic lineages in *L. giganteus* showed that no single component described the data; adding support to the finding that these two lineages are possibly cryptic species (Fig. 3b).

DISCUSSION

The current study provides: (i) novel genetic data to support the currently recognized *L. giganteus* and *L. muricola* as distinct species; (ii) new insights into host range of *L. giganteus* and *L. muricola* in South Africa; and (iii) the first published genetic evidence for cryptic speciation in a mesostigmatid mite occurring in southern Africa. In concert, these findings allow for new insights into the taxonomy and evolution of *L. giganteus* and *L. muricola*. Broadly this study also contributes towards the global need for more investigations examining parasite biodiversity (de León and Nadler, 2010).

The marked genetic differentiation between the two recognized species based on mtDNA and nuclear DNA data confirm the original morphological distinction between the two *Laelaps* species (Hirst, 1925). With the exception of some evidence (paraphyletic clustering for the TropoM dataset), phylogenetic analyses of all remaining datasets (together with the morphological measurements) support this taxonomic division. Contrary to published findings (Hirst, 1925; Zumpt, 1961), these two species also seem to be ecologically differentiated based on host preferences. *Laelaps giganteus* was absent on eight of the 10 possible host species but instead was only recorded on Arvicanthini rodents, while *L. muricola* seems to be more of a rodent generalist but was never found on *Rhabdomys*. This absence of *L. muricola* on *Rhabdomys* is seemingly not seasonally influenced (see Matthee *et al*. 2007) and the host specificity

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Table 3. Pair-wise genetic divergence values within and between the described *Laelaps* lineages

	COI (%±S.D.)	ITS1 (%±S.D.)	TropoM (%±S.D.)
Sequence divergence between *L. muricola* + *L. giganteus*	10·51±0·43	5·18±1.67	7·72±5·13
Sequence divergence between *L. giganteus* lineage 1 and 2	9·84±0·18	3·55±0·71	3·45±0·39
Sequence diversity within *L. giganteus* lineage 1	0·16±0·35	0·84±1.49	0·25±0·15
Sequence diversity within *L. giganteus* lineage 2	0·38±0·35	0·42±0·69	1·91±1·05
Sequence diversity within *L. muricola*	0·79±0·72	1·56±1·22	5·90±5·20

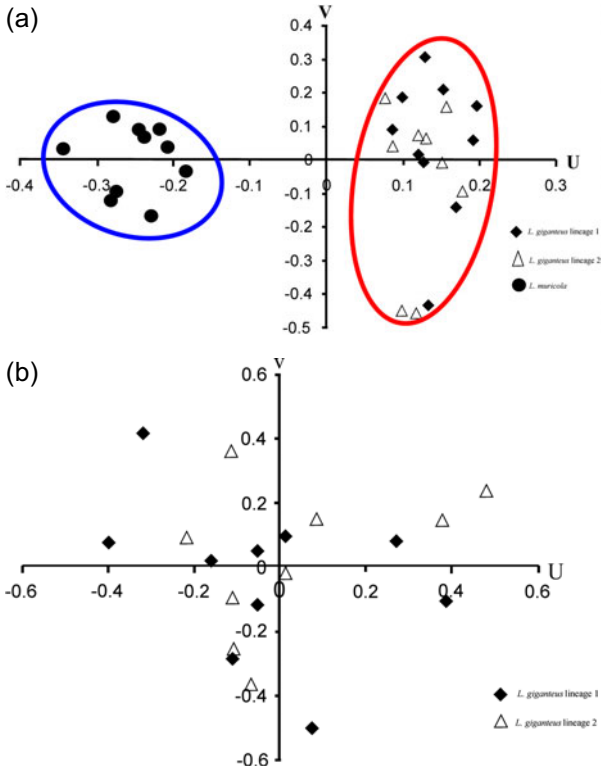

Fig. 3. (a) Principal component analysis of morphological characteristics recorded for the three *Laelaps* lineages. *L. giganteus* lineage 1 ($N = 10$) (diamond shapes) and *L. giganteus* lineage 2 ($N = 9$) (open triangles) and *L. muricola* ($N = 10$) (filled circles). (b) Principal component analysis of morphological characteristics recorded for two *L. giganteus* lineages. *L. giganteus* lineage1 (diamond shapes) and *L. giganteus* lineage 2 (open triangles).

is furthermore also in agreement with previous diversity studies based on much larger sample sizes (Matthee *et al*. 2010; Froeschke *et al*. 2013).

The most prominent finding of this study was the discovery of genetically differentiated clades within *L. giganteus* that are morphologically similar. These two lineages form well-supported monophyletic clades when two (COI and ITS1) of the three gene trees are considered and these lineages are separated by a mtDNA sequence divergence value of 9·84% (10·51% separate *L. giganteus* and *L. muricola*; it is also comparable to species level distinctions in other mesostigmatid mites; Roy *et al*. 2008, 2010; Knee *et al*. 2012). Several factors can be put forward as to why the third genetic dataset (TropoM) did not recover the same monophyletic conclusions. Among these, the possible retention of ancestral polymorphisms cannot be discarded as a potential explanation and it is also possible that hybridization between individuals of the two species, and/or individuals belonging to the two *L. giganteus* lineages, could have resulted in some allele sharing at some loci (Ballard and Whitlock, 2004; Felsenstein, 2004; Maddison and Knowles, 2006; Degnan and Rosenberg, 2009; du Toit *et al*. 2013*b*). It is prudent that more genetic data are needed before a firm conclusion can be reached to explain the conflict in the TropoM data.

A concrete taxonomic revision is not possible at this stage since the samples reviewed in this study cover only a small area of the overall distribution of the formerly described *L. giganteus* (Hirst, 1925; Zumpt, 1961). A second confounding difficulty with a revised taxonomy is that the type specimen for *L. giganteus* was described from *Dasymys incomptus* which was collected in Pulima, Ghana, West Africa (Zumpt, 1961). Unfortunately, despite several attempts to sample *D. incomptus* locally, no host specimens could be retrieved in the present study. If the genetic pattern obtained for *Laelaps* can be seen as indicative for the entire species, then it is quite likely that *L. giganteus* sampled on *D. incomptus* may in fact also represent a distinct cryptic lineage, and this lineage will then have priority in name. What makes a proper taxonomic assessment also problematic is the fact that only the range of *R. dilectus* was sampled in the present study (which spans the mesic eastern side of South Africa; Skinner and Chimimba, 2005; Mucina and Rutherford, 2006). Previous studies indicate that *L. giganteus* is commonly found on *R. pumilio* also (occurring along the xeric western regions of South Africa; Matthee *et al*. 2007) but at least four distinct *Rhabdomys* species exist in the region (du Toit *et al*. 2012). Several sibling species have also been described in *D. incomptus* based on chromosomal rearrangements (Volobouev *et al*. 2000). Given the narrow host range observed for *L. giganteus* in the present study, it is quite plausible that *L. giganteus* (*senso stricto*) may harbour significantly more cryptic diversity than is currently recognized. In turn the pattern presented in this study is also consistent with global trends suggesting that mites previously described as one species often harbour multiple cryptic lineages tightly linked to

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a primary host (Morelli and Spicer, 2007; Roy *et al*. 2008; Shäffer *et al*. 2010; Skoracka and Dabert, 2010; Martin *et al*. 2010; Knee *et al*. 2012).

In the present study we find strong evidence to suggest that host evolution played some role in the evolution of these ectoparasites since both *L. giganteus* lineages seem to follow a lifestyle reminiscent of a host-specific parasite (the two clades also showed strong host exclusivity independent of geography). This holds despite the fact that 10 different host species of this parasite were collected in partial sympatry. Unfortunately, our taxonomic sampling of hosts is not sufficient to make strong co-evolutionary conclusions but it is interesting to note that the average mtDNA and nuclear DNA sequence distances suggest a closer relationship between the two *L. giganteus* lineages. Along these lines, the hosts of the two *L. giganteus* lineages (*R. dilectus* and *L. rosalia*) detected in this study are also phylogenetically closely related in the 'Arvicanthini' group of Muroid rodents (Watts and Baverstock, 1995; Ducroz *et al*. 2001; Steppan *et al*. 2005). Some molecular evidence also suggests that the type host of *L. giganteus, D. incomptus*, is basal to the Arvicanthini rodents (Ducroz *et al*. 2001). Contrasting to the pattern observed in *L. giganteus, L. muricola* was recorded on *M. coucha* (grass/plain dwelling rodent) and *M. namaquensis* (preferring rocky habitats). In support of the more generalist lifestyle, no inter-specific genetic structure was recorded in this species despite the fact that one of its preferred hosts, *M. namaquensis*, has also been shown to contain at least two divergent genetic clades in the region covered by our study (Chimimba, 2001; Russo *et al*. 2010).

Although our findings contribute significantly towards a better understanding of the biology of Laelapinae mites, our study highlights the need for more fine-scale sampling across a larger geographic region. Pertinent to such a study would be to include *D. incomptus* and also the four ecologically differentiated *Rhabdomys* species (du Toit *et al*. 2012), since if the species specificity of *L. giganteus* holds, several more undetected lineages may exist.

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