Litomosa chiropterorum Ortlepp, 1932 (Nematoda: Filarioidea) from a South African miniopterid: redescription, *Wolbachia* screening and phylogenetic relationships with *Litomosoides*

JUNKER K.*, BARBUTO M.**, CASIRAGHI M.**, MARTIN C.***, UNI S.****, BOOMKER J.* & BAIN O.***

Summary:

69 *Miniopterus natalensis*, type host of the onchocercid *Litomosa chiropterorum*, were collected in caves in the Western Province and Gauteng Province, South Africa. The prevalence of these filariae was about 50 %. The microfilaria is folded, as in other *Litomosa* and an *area rugosa* composed of cuticular bosses is present in the male posterior region. *L. chiropterorum* is close to the species parasitic in other *Miniopterus* spp. and some *Rhinolophus* spp. from Africa, Madagascar and Europe; it is unique with the expanded anterior extremity and the four cephalic submedian bosses. The molecular analysis of *L. chiropterorum*, the first done with *Litomosa* species from a bat, supports the hypothesis that *Litomosa* and *Litomosoides*, which have an exceptionally large buccal capsule in common, form a group in which *Litomosa* has a basal position. Interestingly, *L. chiropterorum* does not harbour *Wolbachia*, as proved with immunohistological staining and PCR screening using the 16S rDNA gene as target. This is contrary to *L. westi* from rodents and the majority of the *Litomosoides* species parasitic in bats or rodents. The absence of *Wolbachia* in a filarioid group considered ancient based on traditional and molecular approaches opens interesting scenarios on the evolution of the endosymbionts spread through filarial lineages.

KEY WORDS : DNA barcoding, *Litomosa*, *Litomosoides*, microchiroptera, morphology, *Wolbachia*.

Résumé : *Litomosa chiropterorum* Ortlepp, 1932 (Nematoda: Filarioidea) parasite de miniopterè sud-africain : redescription, recherche de *Wolbachia* et relations phylétiques avec *Litomosoides*

69 Miniopterus natalensis, *hôte type de l'onchocercidé* Litomosa chiropterorum, *ont été capturés dans les grottes de la Western Province et Gauteng Province, en Afrique du Sud. La prévalence de* L. chiropterorum *est de 50 % environ. La microfilaire est pliée, comme chez les autres* Litomosa *et l'*area rugosa *est présente chez le mâle, constituée de perles cuticulaires.* L. chiropterorum *est proche des espèces parasites d'autres* Miniopterus *spp. et de quelques* Rhinolophus *spp. d'Afrique, Madagascar et Europe ; les éléments distinctifs sont l'extrémité antérieure élargie et les quatre bosses céphaliques submedianes. L'analyse moléculaire de* L. chiropterorum, *la première du genre faite chez une espèce parasite de chauve-souris, confirme l'hypothèse que* Litomosa *et* Litomosoides, *qui ont tous deux une capsule buccale exceptionnellement grande, forment un groupe où* Litomosa *a une position basale.* L. chiropterorum *n'héberge pas* Wolbachia *(coloration immunohistologique et analyse du gène 16S rDNA) contrairement à* L. westi, *parasite de rongeurs, et à la majorité des* Litomosoides *parasites de chauves-souris et de rongeurs. L'absence de* Wolbachia *dans un groupe de filaires considéré ancien avec les approches traditionnelles et moléculaires ouvre des scénarios intéressants sur l'évolution des endosymbiontes propagés dans les différentes lignées de filaires.*

MOTS CLÉS : *ADN,* Litomosa, Litomosoides, *microchiroptères, morphologie,* Wolbachia.

INTRODUCTION

The filarial genera *Litomosa* Yorke & Maplestone, 1926 and *Litomosoides* Chandler, 1931 have in common the largest buccal capsule observed among the Onchocercidae. Each genus contains many species parasitic in microchiroptera, either from the Old World (*Litomosa*), or from the New World (*Litomosoides*). *Litomosoides* is also largely diversified in Neotropical rodents and marsupials, whereas only two species of *Litomosa* are known from rodents. Both these species are parasitic in North American geomyoid rodents and were initially assigned to *Litomosoides* but, based on morphological characters, they were transferred to *Litomosa* (Guerrero *et al.*, 2002).

The morphology and hosts of *Litomosa* and *Litomosoides* suggest a common origin of the two genera and, to obtain a more complete picture, molecular analyses were needed. Such studies have already been done with several species of *Litomosoides* from bats and murids (Casiraghi *et al.*, 2004), because of the fact that *Li. sigmodontis* Chandler, 1931 became an important murine model for filariasis (Allen *et al.*, 2008). The situation in *Litomosa* is different and molecular data from only one species, *L. westi* (Gardner & Schmidt, 1986) parasitic in North American rodents, are available (*i.e.* 12S rDNA and

* Department of Veterinary Tropical Diseases, University of Pretoria, Private Bag X04, Onderstepoort, 0110 South Africa.
** Dipartimento di Biotecnologie e Bioscienze, ZooPlantLab, Università degli Studi di Milano Bicocca, P.zza della Scienza 2, 20126 Milano, Italy.
*** Muséum National d'Histoire Naturelle, Parasitologie comparée et Modèles expérimentaux, USM 307, CP52, 61, rue Buffon, 75231 Paris, Cedex 05 France.
**** Department of Medical Zoology, Osaka City University Medical School, Osaka 545-8585, Japan.
Correspondence: Odile Bain.
Tel.: + 33 (0)1 40 79 34 97 – Fax: + 33 (0)1 40 79 34 99.
E-mail: bain@mnhn.fr

coxI gene sequences, Casiraghi *et al.*, 2004). In the molecular phylogenies generated *L. westi* was placed at the base of the *Litomosoides* group (Casiraghi *et al.*, 2004). However, molecular data on typical *Litomosa* species, such as those parasitic in Old World microchiroptera, were lacking.

One such *Litomosa* species was obtained from Natal long-fingered bats, *Miniopterus natalensis* (Smith, 1834), in South Africa, from which *L. chiropterorum* had been described (Ortlepp, 1932).

These filariae appeared to belong to the same species. We augmented the original description since more morphological characters are now used to distinguish species, such as microfilariae and the male *area rugosa*. We generated a molecular phylogenetic analysis, using mitochondrial gene sequences (*i.e.* 12S rDNA and *coxI* gene sequences) on the available *Litomosa* and *Litomosoides* representatives. Since filarial onchocercid nematodes may harbor the endosymbiont bacteria *Wolbachia* (Bandi *et al.*, 1998; Casiraghi *et al.*, 2004), we investigated its presence in *L. chiropterorum* by immunohistological staining and PCR screening, using 16S rDNA as target.

MATERIALS AND METHODS

Biological samples: collection and storage

A total of 69 *M. natalensis* were examined: 57 from the De Hoop Guano Cave in the De Hoop Nature Reserve (34° 26' S/20° 25' E), Western Cape Province, collected during September 2006 (CapeNature Permit No. AAA004-000030-0035); 12 from the Monument Park Cave, Pretoria, Gauteng Province, collected in March 2007 (no permit necessary). Worms collected from hosts from the De Hoop Guano Cave were fixed in 70 % ethanol and those from bats from the Monument Park Cave were directly transferred into absolute ethanol.

Alcohol fixed samples used for the immunohistological staining were subsequently fixed in 4 % paraformaldehyde (TAAB Laboratories Equipment Limited, 40 Grovelands Rd., Reading, Berks, England) for 8 hrs at 4° C, and then transferred to 0.1 M phosphate buffer solution, pH 7.4 and stored in a refrigerator at 4° C until they were embedded in paraffin.

Samples used for the PCR screenings were kept refrigerated at 4° C in absolute ethanol until used for molecular analysis.

Morphological study

Worms were cleared in lactophenol and drawn with the aid of a microscope equipped with a camera lucida. An apical view of the head was prepared as previously described (Guerrero *et al.*, 2002; Martin *et al.*, 2006). The male posterior part was examined with particular emphasis on the ventral cuticular ornamentation, the *area rugosa* (Bain, 1966). Spicules were dissected out for detailed analysis. Microfilariae were extracted from female uteri, near the vagina. Length and maximum external diameter of buccal capsules were measured, and capsule segments numbered according to Bain (1966). Measurements are given in µm, except for the body length, which is given in millimetres.

Immunohistological staining

Immunohistological staining was done according to the method described by Kramer *et al.* (2003). Briefly, specimens of *L. chiropterorum* were embedded in paraffin and 4 µm sections were cut and placed on Silane coated glass slides and then kept at 63° C overnight, to avoid sections detaching from the slides. A rabbit polyclonal antiserum raised against the *Wolbachia* surface protein (WSP) of the endobacteria from *B. pahangi* was used (1:2,000) to stain sections of *L. chiropterorum*. Sections of *Li. sigmodontis* were used as positive control. Negative controls were carried out by omitting the primary antibody.

Molecular analyses

Eight specimens of *L. chiropterorum*, males and females, and crude DNA preparations were obtained by proteinase-K treatment, according to Bandi *et al.* (1998). *L. chiropterorum coxI* and 12S rDNA gene sequences were generated according to the method described by Casiraghi *et al.* (2001, 2004). The amplifications obtained were gel-purified with the QIAquick® PCR Purification Kit (Qiagen) and directly sequenced using ABI technology. The *L. chiropterorum* sequences obtained have been deposited in the EMBL Data Library (accession numbers FM209527-FM209547).

PCR screening for *Wolbachia* of the *L. chiropterorum* specimens was conducted following the methods described by Casiraghi *et al.* (2001; 2004), using general *Wolbachia* primers for 16S rDNA. PCRs were performed under different conditions (see Casiraghi *et al.*, 2004) to increase the sensitivity of the screenings.

Data analysis: molecular phylogenetic reconstructions

The obtained *coxI* and 12S rDNA sequences were aligned with the available sequences of *L. westi* (*coxI:* AJ544871; 12S rDNA: AJ544851); *Li. brasiliensis* Lins de Almeida, 1936 (AJ544867; AJ544850); *Li. galizai* Bain, Petit & Diagne, 1989 (AJ544870; AJ544849); *Li. hamletti* Sandground, 1934 (AJ544868; AJ544847); *Li. sigmodontis* (AJ271615; AJ544848); *Li. yutajensis* Guerrero, Martin & Bain, 2003 (AJ544869; AJ544846) and of two Oncho-

cercidae used as outgroups, a representative of Setariinae, *Setaria labiatopapillosa* (Alessandrini, 1848) (accession numbers: AJ544872 and AJ544833) and a representative of Waltonellinae, *Ochoterenella* sp. Casiraghi *et al.*, 2004 (accession number: AJ544878 and AJ544836). The alignments generated have been analysed using distance matrix method (*i.e.* neighbour joining). Phylogenetic analyses were performed using MEGA 4.0 (Tamura *et al.*, 2007).

RESULTS

Filariae were recovered from 5 *M. natalensis* from Pretoria (numbers 252 JW to 256 JW) and 18 *M. natalensis* from De Hoop (numbers 257 JW to 273 JW). Specimens deposited in the MNHN collection are the following: one male 252 JW; one male, one entire female, anterior and posterior regions of females 253 JW; one male, one female 254 JW; one male, one female 254 JW; parts of filariae 257 JW to 262 JW grouped in a tube; three females 264 JW; one male 265 JW and one 266 JW; one male, three posterior regions of female 267 JW; two males and part of female 268 JW; two males 269 JW; posterior part of male 270 JW; 271 JW to 273 JW, a male and fragment of females grouped in a tube; two males and a female in two parts 274 JW.

Redescription of *Litomosa chiropterorum* Ortlepp, 1932

Morphology (Fig. 1) was similar in Pretoria and De Hoop samples. Widened shoulder-shaped apex and body diameter regularly decreasing from head to the oesophageal-intestinal level; head square, with four submedian bosses, well visible in apical view. Four papillae and two amphids, all similarly small and placed very anteriorly. Nerve ring often far from head. Mouth minute. Buccal capsule segmented, with segment 3 larger, its anterior aspect plane or concave; buccal cavity bottle-shaped. Oesophagus without glandular part.

Female: when gravid, coiled uteri reaching anterior extremity. Tail with two conical lappets, terminal or subterminal and ventral; in one specimen, a third smaller axial point; in another one, a crest at base of the lappets. Vulva post-oesophageal or at level of oesophageal-intestinal junction; vagina: proximal horizontal tube lined with cuticle, a bend, then a chamber lined with thick epithelium, a sphincter between two bends, then the ovejector. Microfilariae folded in the sheath; body progressively attenuated from anterior region to tail tip.

Male: *area rugosa* composed of a longitudinal band of cuticular bosses. Caudal papillae: a precloacal papilla; a group of four pairs, regularly arranged including two postcloacal pairs on a transverse line (squared disposition of papillae) or less symmetrically arranged (Fig. 1E & H). Tail extremity rounded; phasmids visible, no lappets. Spicules: right spicule with sclerotized distal part and dorsal heel; left spicule with thick handle and lamina terminated by a membranous elongated flap.

Measurements

Entire gravid female 255 JW and [parts of females 267 & 268 JW]: length 72 mm long [ND], width at mid body 178 [180 & 150]; buccal capsule length/maximum external diameter 17/23 [17/20 & 18/27]; oesophagus 528 [510 & 480] long; vulva from apex 820 [500 & 800]; tail length/width at anus 185/55 [220/80 & 142/75]. An immature female 266 JW: 45 mm long, 150 wide; buccal capsule 17/20; oesophagus 510 long; vulva 500 from apex; tail 170/55.

Microfilariae (268 JW): 100-113 long, 4.8-5 maximum width.

One male 255 JW and [four males 265-267-268-269 JW]: length 28 mm [33-33-38-41], width at mid body 120 [90-100-105-110]; buccal capsule length/maximum external diameter 16/18 [13/18-16/17-16/22-16/18]; oesophagus length 460 [440-475-550-475]; tail length 95 [106-95-170-112]; left spicule length 315 [310-322-323-320], handle 170 [170-150-172-160]; right spicule length 103 [110-98-120-115]; *area rugosa* from tip tail 800 to 2,600 [measured on two other males 720 & 700 to 2,600 & 2,650].

Wolbachia detection

Following immunohistological staining the sections of a single *L. chiropterorum* female were negative for the presence of *Wolbachia* in the female genital contents and in the lateral chords (Fig. 2A & B). PCR analysis was negative for the eight specimens.

Molecular analyses

Neighbour joining reconstructions on the representatives of the *Litomosa* + *Litomosoides* group generated the tree shown in Figure 3. In this tree *L. chiropterorum* is placed as the deepest branch in the *Litomosa* + *Litomosoides* group. The topology of Figure 3 has been generated using a concatenated alignment of *coxI* + 12S rDNA gene sequences. The same topology (data not shown) has been generated independently using *coxI* and 12S rDNA as separated alignments, and also (in the case of *coxI* alignment) using the first and second or the third positions of the codon only. The only slightly appreciable differences were in bootstrap supports.

Prevalence

L. chiropterorum prevalence was 50.9 % in the bats from the Western Cape Province, with an intensity of infection ranging from 1 to 5 (mean 2.3 ± 1.44). In Gauteng Province, the prevalence was 41.7 % and the intensity of infection ranged from 2 to 6 (mean 4.0 ± 2.31).

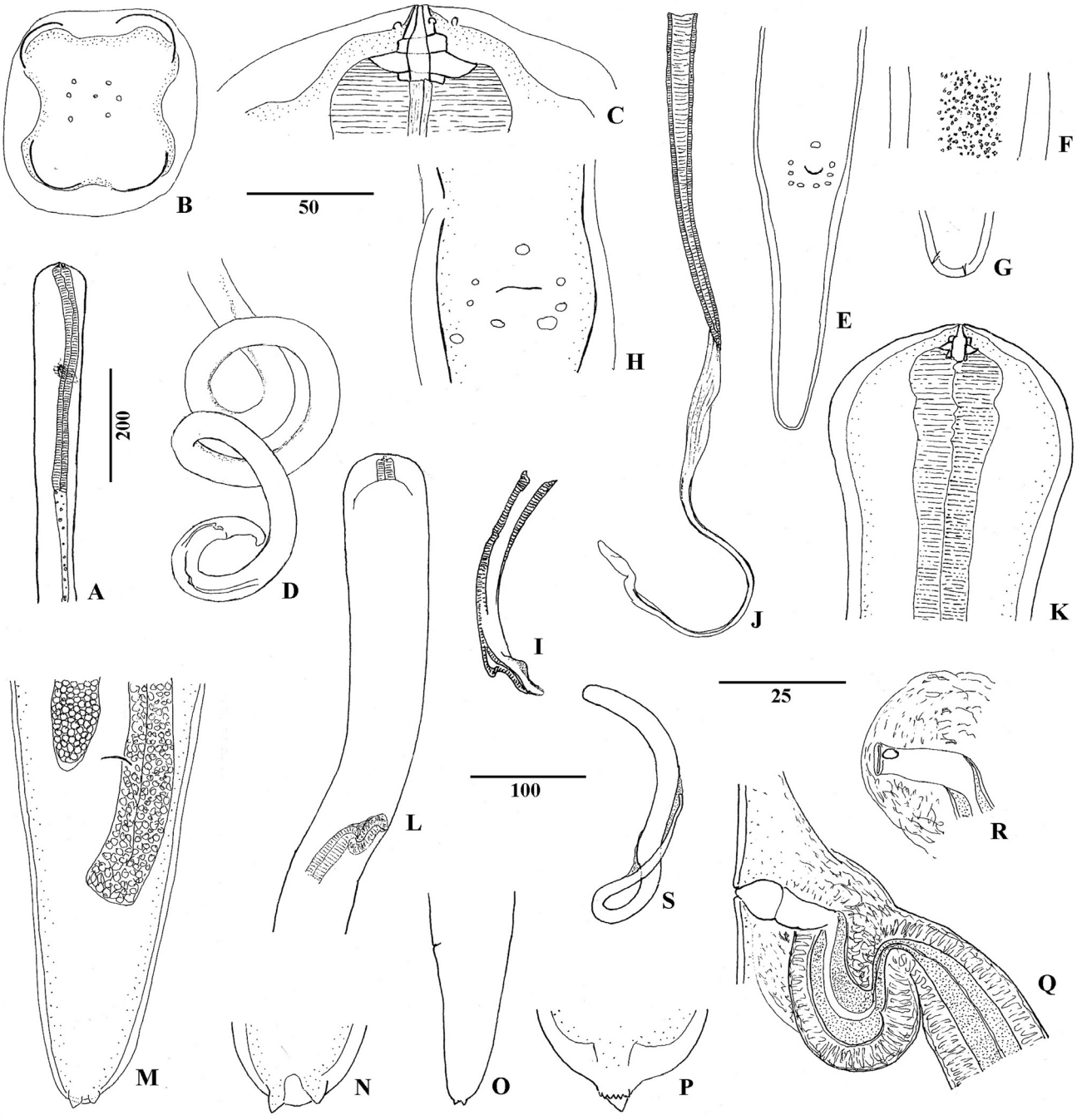

Fig. 1. – *Litomosa chiropterorum* from *Miniopterus natalensis*. A-K. Male. A. Anterior region, right lateral view. B. Head, en face view. C. Buccal capsule and head papillae, lateral view. D. Posterior region and *area rugosa*. E. Caudal region, ventral view. F. *Area rugosa*, detail at mid-length, ventral view. G. Caudal extremity and phasmids, ventral view. H. Papillae near cloacal aperture of another male, ventral view. I. Right spicule, right lateral view. J. Left spicule, dissected out. K. Cephalic region dilated. L-S. Female. L. Anterior region (only vagina and anterior limit of coiled uteri are represented). M. Tail, ventral view. N. Caudal extremity. O. Tail, left lateral view. P. Caudal extremity of another female, lateral view. Q. Vagina, left lateral view. R. Vulva and beginning of vagina, ventral view. S. Microfilaria, extracted from ovejector. Scales in µm. A, D, L, 200. B, E, F, I, J, K, M, Q, R, 50. C, G, H, N, P, S, 25. O, 100 (specimens from De Hoop, except K from Pretoria).

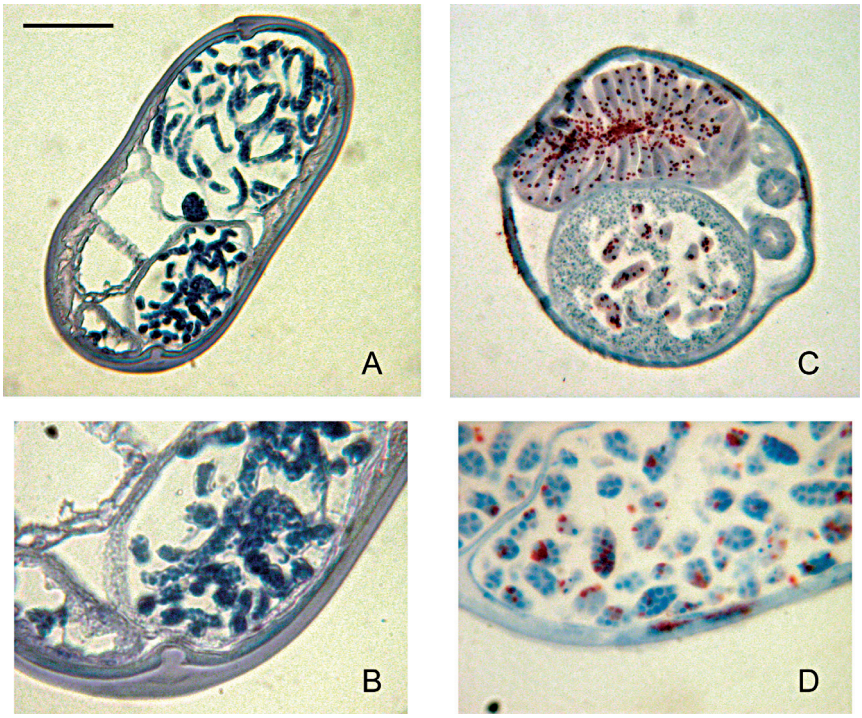

Fig. 2. – *Wolbachia* immunohistological staining. A & B. *Litomosa chiropterorum* female. A. Transverse section of body and uteri filled with microfilariae. B. Detail of a lateral chord and uterus with microfilariae. No staining is observed. C & D. *Litomosoides sigmodontis* female. C. Transverse section of body at level of ovary and beginning of uterus containing eggs and spermatozoa. Positive staining of rachis, ova and eggs. D. Detail showing lateral chords and genital tract with eggs, both positive. Scales in µm. A, C, 50. B, D, 25.

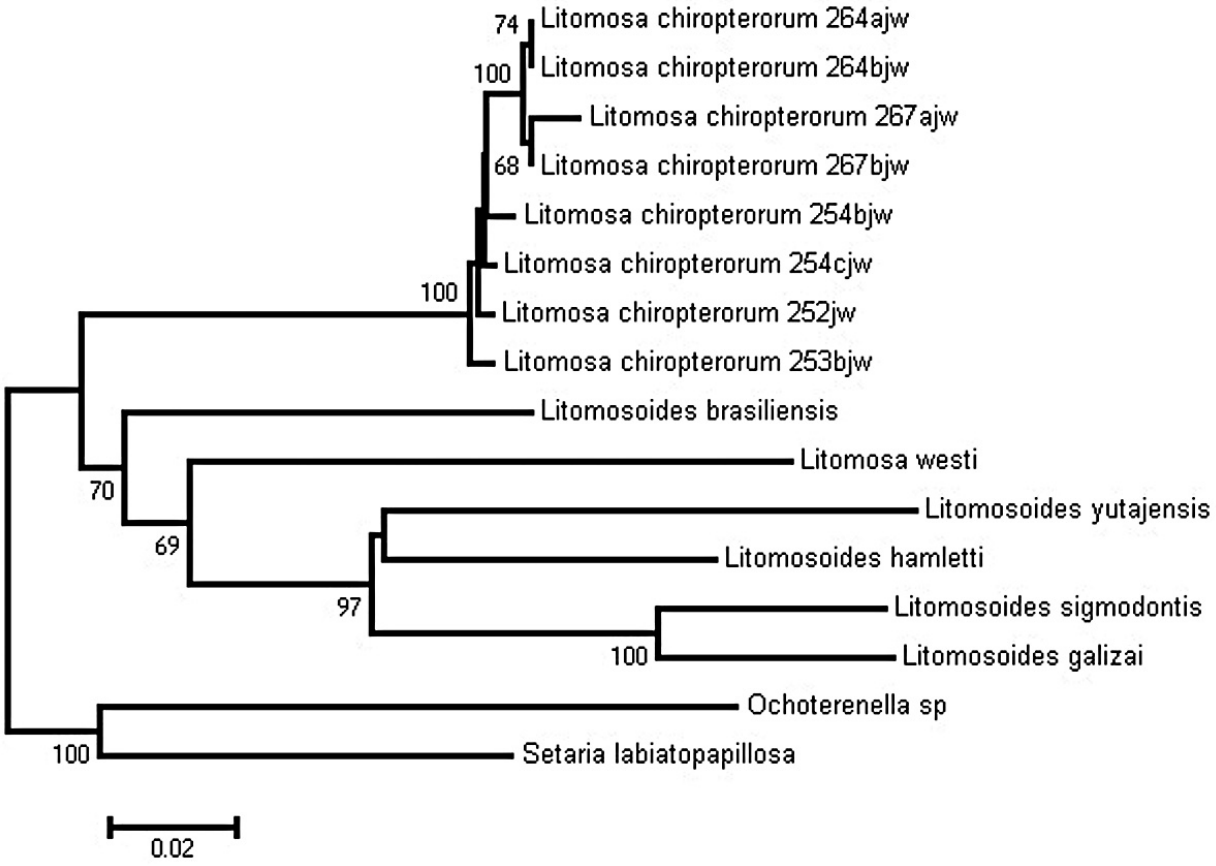

Fig. 3. – Phylogeny of filarial nematodes based on a concatenated alignment of *coxI* and 12S rDNA gene sequences. The tree has been obtained by Neighbour Joining analysis, using MEGA 4.0; numbers at the nodes are bootstrap supports after 100 replications (values below 60 are not shown). Accession numbers are given for the sequences present in the databases in Material and Methods.

DISCUSSION

RELATIONSHIPS AMONG *LITOMOSA* SPECIES

The present filariae from *Miniopterus natalensis* were easily identified as *Litomosa chiropterorum* with the measurements, the thick apex with shoulders, the shape of buccal capsule, the two conical caudal lappets oof the female (Ortlepp, 1932). The original material, composed of several males and females, was recovered from the same host, collected from the Irene caves, Pretoria. Ortlepp also recovered a single female *L. chiropterorum* from the abdominal cavity of a single specimen of *Neoromicia capensis* (Smith, 1829) [= *Eptesicus capensis*] from Onderstepoort, Pretoria. To date, *L. chiropterorum* is thus the only species of the genus recovered in South Africa and, since its description, this parasite has gone largely unnoticed. Anciaux de Faveaux (1974) listed this filaria from *M. schreibersi* (Kuhl) in South Africa, which was likely *M. natalensis*, now elevated to full species rank (Miller-Butterworth *et al.*, 2005). Lanza (1999) referred to *L. chiropterorum* from *M. schreibersi* in Turkey and the Ethiopian region. However the reports of this species outside the type region are probably erroneous since *Litomosa* is highly diverse (Martin *et al.*, 2006).

As suspected (Martin *et al.*, 2006), the *area rugosa* of *L. chiropterorum* is composed of cuticular bosses and this confirms that this species belongs to the lineage which includes the type species *L. filaria* (v. Beneden, 1872). *L. chiropterorum*, with the large segment 3 of the buccal capsule, is particularly close to five species: in the Ethiopian region, *L. Adami* Petit, 1980 (type-host *Miniopterus m. minor* Peters, Gabon), *L. goodmani* Martin *et al.*, 2006 (type-host *M. gleni* Peterson, Eger & Mitchel, Madagascar), *Litomosa* sp. Martin *et al.,* 2006 (type-host *M. manavi* Thomas, Madagascar); in the Mediterranean and European areas, *L. seurati* Martin *et al.*, 2006 (type-host *Rhinolophus ferrum-equinum* (Schreb.), Algeria) and *L. ottavianii* Lagrange & Bettini, 1948 (type-host *Myotis blythii* (Tomes), Sardinia, Europe). Since *L. ottavianii* is a common parasite of *M. scheibersi* in Europe (Lagrange & Bettini, 1948; Bain, 1966) and *L. seurati* likely a local capture from this species (Martin *et al.* 2006), the group of *Litomosa* with large segment 3 seems to have diversified with the *Miniopterus* spp. This group shows a marked reduction of the head papillae (one circle) and the persistence of the squared arrangement of caudal papillae (two postcloacal pairs on a transverse line). The South African *L. chiropterorum* from *M. natalensis* is distinct with a derived character, the gradually dilated anterior part, which is contrary to *L. adami*, that is also found in *M. natalensis* but in Zaïre (Petit, 1980). In the Ethiopian region, the *Litomosa* species described from Rhinolophoidea do not belong to this group: *L. hugoti* Petit, 1980 (type-host *Rhinolophus sylvestris* Aellen, Gabon) is remarkable with the primitive arrangement of head papillae (two circles); *L. pujoli* Bain, 1966 (type-host *Hipposideros cyclops* Temminck, Hipposideridae; later identified in three different microchiroptera in Nigeria by Edungbola, 1981) has a tubular buccal capsule, resembling that of *Litomosoides* species.

LITOMOSA AND *LITOMOSOIDES* RELATIONSHIPS

The molecular phylogenetic reconstruction indicates a basal position for *L. chiropterorum*, in the *Litomosa* + *Litomosoides* group (Fig. 3). However, *Li. brasiliensis* is positioned between the *Litomosa* species from African bats, *L. chiropterorum*, and the *Litomosa* species from North American geomyoids, *L. westi*. The peculiar morphological characteristics of *Li. brasiliensis* (caudal papillae aligned on a ventral line) had been stressed by Guerrero *et al.* (2002) and the phylogenetic reconstructions generated have not solved its positioning. Unfortunately, we could only evaluate the intraspecific molecular diversity in very few species, such as *L. chiropterorum* and *Li. sigmodontis*, while for all other species only one or very few specimens/sequences were available. This is a clear limitation to the power of our analyses. In addition, considering the total number of species included in *Litomosa* and *Litomosoides* (22 and 32 species respectively; Martin *et al.*, 2006; Bain *et al.*, 2008), we only have molecular data from a very limited number of them. Given these circumstances our reconstructions do not support a monophyletic status for either *Litomosa* or *Litomosoides*. From a molecular point of view *Litomosa* + *Litomosoides* is recognized as an undoubted and well supported cluster (see for instance Casiraghi *et al.*, 2004). Further work is necessary to elucidate the relationships among the representatives of these two filarioid genera.

It is interesting to note that, excluding *Li. brasiliensis*, two main divisions are recognizable in the *Litomosoides* group corresponding to the two lineages observed using morphological characters: the so called "sigmodontis group" (with *Li. sigmodontis*, *Li. galizai*) and the "carinii group" (with *Li. hamletti* and *L. yutajensis* (Bain *et al.*, 1989; 2003; Guerrero *et al.*, 2002; 2003).

At present, no life-cycle has been elucidated for *Litomosa* spp. Since this genus seems closely related to *Litomosoides*, the vectors might also be macronyssid acarians (Guerrero *et al.*, 2006). The prevalence of *L. chiropterorum* in *M. natalensis* is exceptionally high: around 50 %, whereas it does not exceed 10 % in the rare reports from other species (Edungbola, 1981 in Nigeria; Martin *et al.*, 2006 in Madagascar). *L. chiropterorum* in South Africa presents the optimal conditions to attempt elucidating the intermediate hosts of the genus *Litomosa*.

Wolbachia symbiosis

Interestingly, the presence of *Wolbachia* was not detectable in *L. chiropterorum*, a result contrary to the previous finding of the endosymbionts in the only other species of the genus screened, *L. westi* (Casiraghi *et al.*, 2004). The present distribution data of *Wolbachia* in filarial nematodes support a complicated picture. In closely related filarial nematodes within the same genus, *Wolbachia* is both present and absent. This opens several questions, and in particular how strong is the relationship among filarioid nematodes and *Wolbachia*? These bacteria have been claimed to be essential for filarial survival and reproduction (see for instance a review in Fenn & Blaxter, 2004). However, support for *Wolbachia* loss during filarial evolution is growing (Casiraghi *et al.*, 2004; Bain *et al.*, 2008). Even if the relationships among representatives of *Litomosa* and *Litomosoides* have not been solved by our analyses, there is support for a basal position for *L. chiropterorum* in the *Litomosa* + *Litomosoides* group (Fig. 3). This creates several scenarios: if there had been an ancestral *Wolbachia* acquisition in the Onchocercinae, a scenario for which some support is available (see Casiraghi *et al.*, 2004), *L. chiropterorum* could represent a case of *Wolbachia* loss. Other *Wolbachia* losses could have occurred in the *Litomosa* + *Litomosoides* group, for example in *Li. yutajensis*, the only member of the genus *Litomosoides* for which no *Wolbachia* has been detected (Casiraghi *et al.*, 2004; Bain *et al.*, 2008). On the other hand, if there had been separate events of *Wolbachia* acquisition, these endosymbionts could have been acquired in the *Litomosa* + *Litomosoides* group following the separation of the ancestor of *L. chiropterorum* from the evolutionary lineage. To test the different hypotheses and obtain a clearer picture, further species of the genus *Litomosa* should be analysed for the presence of *Wolbachia* endosymbionts.

ACKNOWLEDGEMENTS

We thank Prof D.S. Jacobs and Ms M.K. Mason, Department of Zoology, and Ms D. Hockman, Department of Molecular and Cellular Biology, University of Cape Town for making the bats from the De Hoop Guano Cave available and for delivering them to us. We are indebted to Prof M. van der Merwe, Department of Zoology, University of Pretoria, for collecting the bats from the Monument Park Cave. This research was made possible by a postdoctoral fellowship awarded to K. Junker by the University of Pretoria and financial support from the Department of Veterinary Tropical Diseases, University of Pretoria. The work was also supported by Hubert Curien Partnership, the Galileo exchange programme.

REFERENCES

Allen J.E., Adjei O., Bain O., Hoerauf A., Hoffmann W.H., Makepeace B.L. *et al.* Of mice, cattle and humans: the immunology and treatment of river blindness. *PLoS Neglected Tropical Diseases*, 2008, *2*, e217, 1-8.

Anciaux de Faveaux M. Parasitologie des chiroptères du continent africain. Ph.D. thesis, University of Paris, France, 1974, 282 p.

Bain O. Diversité et étroite spécificité parasitaire des filaires de chauves-souris, confondues sous le nom de *Litomosa filaria* (van Beneden, 1872). *Bulletin du Muséum National d'Histoire Naturelle*, 1966, 2è^{me} série, *38*, 928-939.

Bain O., Petit G. & Diagne M. Étude de quelques *Litomosoides* parasites de rongeurs; conséquences taxonomiques. *Annales de Parasitologie Humaine et Comparée*, 1989, *64*, 268-289.

Bain O., Casiraghi M., Martin C. & Uni S. The Nematoda Filarioidea: critical analysis linking molecular and traditional approaches. *Parasite*, 2008, *15*, 342-348.

Bain O., Guerrero R., Rodriguez B., Babayan S. & Jouvenet N. Examination of type material of *Litomosoides* spp. Filarioidea (Onchocercidae), parasites from bats; taxonomic consequences. *Parasite*, 2003, *10*, 211-218.

Bandi C., Anderson T.J.C., Genchi C. & Blaxter M.L. Phylogeny of *Wolbachia* in filarial nematodes. *Proceedings of the Royal Society of London*, 1998, B, *265*, 2407-2413.

Casiraghi M., Favia G., Cancrini G., Bartoloni A. & Bandi C. Molecular identification of *Wolbachia* from the filarial nematode *Mansonella ozzardi*. *Parasitology Research*, 2001, *87*, 417-420.

Casiraghi M., Bain O., Guerrero R., Martin C., Pocacqua V., Gardner S.L., Franceschi A. & Bandi C. Mapping the presence of *Wolbachia pipientis* on the phylogeny of filarial nematodes: evidence for symbiont loss during evolution. *International Journal for Parasitology*, 2004, *34*, 191-203.

Edungbola L.D. Parasites of house-dwelling, insectivorous bats from Alabe, Kwara State, Nigeria. *Journal of Parasitology*, 1981, *67*, 287-288.

Fenn K. & Blaxter M. Are filarial nematodes *Wolbachia* obligate mutualist symbionts? *Trends in Ecology and Evolution*, 2004, *19*, 163-166.

Guerrero R., Martin C. & Bain O. *Litomosoides yutajensis* n. sp., first record of this filarial genus in a mormoopid bat. *Parasite*, 2003, *10*, 219-225.

Guerrero R., Bain O., Attout T. & Martin C. The infective larva of *Litomosoides yutajensis* Guerrero *et al.*, 2003 (Nematoda: Onchocercidae), a *Wolbachia*-free filaria from bats. *Parasite*, 2006, *13*, 127-130.

Guerrero R., Martin C., Gardner S. L. & Bain O. New and known species of *Litomosoides* (Nematoda: Filarioidea): important adult and larval characters and taxonomic changes. *Comparative Parasitology*, 2002, *69*, 177-195.

Kramer L.H., Passeri B., Corona S., Simoncini L. & Casiraghi M. Immunohistochemical/immunogold detection and distribution of the endosymbiont *Wolbachia* of *Dirofilaria immitis* and *Brugia pahangi* using a polyclonal antiserum raised

against WSP (*Wolbachia* Surface Protein). *Parasitology Research*, 2003, *89*, 381-386.

LAGRANGE E. & BETTINI S. Descrizione di una nuova filaria, *Litomosa ottavianii* Lagange e Bettini, 1948, parassita di pipistrelli. *Rivista de Parassitologia*, 1948, *9*, 61-77.

LANZA B. I parassiti dei pipistrelli (Mammalia, Chiroptera) della fauna italiana. Museo Regionale di Scienze Naturali, Torino, 1999, 318 p.

MARTIN C., BAIN O., JOUVENET N., RAHARIMANGA V., ROBERT V. & ROUSSET D. First report of *Litomosa* spp. (Nematoda: Filarioidea) from Malagasy bats; review of the genus and relationships between species. *Parasite*, 2006, *13*, 3-10.

MILLER-BUTTERWORTH C.M., EICK G., JACOBS D.S., SCHOEMAN M.C. & HARLEY E.H. Genetic and phenotypic differences between South African Long-fingered bats, with a global miniopterine phylogeny. *Journal of Mammology*, 2005, *86*, 1121-1135.

ORTLEPP R.J. Some helminths from South African bats. 18th Report of the Director of Veterinary Services and Animal Industry, Union of South Africa, 1932, *18*, 183-196.

PETIT G. Sur les filaires du genre *Litomosa* (Nematoda : Filarioidea), parasites de chauves-souris. *Bulletin du Muséum National d'Histoire Naturelle, A* (*Zoologie, Biologie et Écologie Animales*), 1980, *2*, 365-374.

TAMURA K., DUDLEY J., NEI M. & KUMAR S. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution*, 2007, *24*, 1596-1599.

Reçu le 10 juillet 2008
Accepté le 30 septembre 2008