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MOLECULAR PHYLOGENY OF FILARIAL WORMS (NEMATODA: FILARIOIDEA)

Praphathip Eamsobhana
Department of Parasitology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand
Email: praphathip.eam@mahidol.ac.th

Phaik-Eem Lim
Institute of Biological Sciences and Institute of Ocean and Earth Sciences, University of Malaya, 50603 Kuala Lumpur, Malaysia
Email: phaikeem@um.edu.my

Hoi Sen Yong
Institute of Biological Sciences, University of Malaya, 50603 Kuala Lumpur, Malaysia
Email: yong@um.edu.my (Corresponding author)

ABSTRACT. — No fewer than 33 species from 20 genera of filarial parasites (superfamily Filarioidea) have been recorded from various Malaysian vertebrates (amphibians, reptiles, birds, and mammals). Eight species worldwide are known to use humans as their definitive host and are the causative agents of human filariasis. Filariasis can also affect domestic and farm animals, such as dogs, cattle, and sheep. Although the filarial parasites have been extensively studied, some species remain dubious and studies on molecular phylogeny indicate that both the subfamilies Dirofilarriinae and Onchocercinae appear as polyphyletic groups. The phylogenetic relationships of 50 taxa of Filarioidea, comprising 49 taxa of Onchocercidae and one of Filariidae, were determined by the nucleotide sequences of cytochrome *c* oxidase subunit I (COI) and 12S rDNA. *Dracunculus medinensis* (Dracunculoidea) and *Thelazia callipaeda* (Thelazioidea) were used as outgroups. In addition the concatenated sequences of 12 protein-coding genes (*cox*1–3, *nad*1–6, *nad*4L, *cyt*b, and *atp*6) for nine taxa with complete mitochindrial genomes were studied. All species in this study could be differentiated by the genetic markers employed. The phylogenetic relationships of 50 taxa of Filarioidea based on COI, 12S rDNA and combined COI+12S rDNA nucleotide sequences in the present analysis do not support the family status for the genus *Setaria*. Members of Dirofilariinae, Onchocercinae, and Splendidofilariinae were not well resolved and indicated polyphyly for these subfamilies. It is evident that multiple genes and more taxa are needed to elucidate the phylogeny of filarial parasites.

KEY WORDS. — phylogenetics, systematics, DNA sequences, evolutionary relationships, polyphyly, Dirofilariinae, Onchocercinae, Setariinae, Splendidofilariinae

INTRODUCTION

Filarial worms are nematodes of the superfamily Filarioidea (Anderson, 2000). No fewer than 33 species from 20 genera have been recorded from various Malaysian vertebrates (amphibians, reptiles, birds, and mammals; Yen, 1983). Eight species worldwide are known to use humans as their definitive host and are the causative agents of human filariasis. They belong to three groups based on the habitat they inhabit within the body of their host: 1) the lymphatic group – *Brugia malayi*, *Brugia timori*, and *Wuchereria bancrofti* inhabiting the lymphatic system causing lymphatic filariasis leading to the condition known as elephantiasis; 2) the cutaneous group – *Onchocerca volvulus*, *Loa loa* (the eye worm) and *Mansonella streptocerca* inhabiting the subcutaneous layer of the skin causing subcutaneous filariasis, with *L. loa* causing *Loa loa* filariasis and *O. volvulus* causing river blindness; and 3) the body-cavity group – *Mansonella ozzardi* and *Mansonella perstans* inhabiting the serous cavity of the abdomen causing serous cavity filariasis. Only two genera, *Brugia* and *Wuchereria*, are mainly responsible for human filariasis in Malaysia and the surrounding countries (Mak, 1983). The other genera (*Loa*, *Mansonella*, *Onchocerca*) have not been reported from this region.

Filariasis can also affect domestic and farm animals, such as dogs, cattle, and sheep. In dogs, *Dirofilaria immitis* causes heart filariasis; in cattle, intradermal onchocercosis is due to *Onchocerca* species (*O. dermata*, *O. dukei*, *O. ochengi*); and in horses, *Parafilaria multipapillosa* causes 'summer-bleeding' in the head and upper forelimbs. Animal filariae (various species of *Brugia*, *Dipetalonema*, *Dirofilaria*, *Onchocerca*, etc.) which are natural parasites of mammals can also cause zoonotic filariasis (human infections with

filariae of animals) worldwide (Orihel & Eberhard, 1998; Uni et al., 2010; Otranto et al., 2011; Tan et al., 2011).

The filarial parasites have been extensively studied because several species are the causative agents of human diseases. The studies involved both traditional (morphological and biological) and molecular approaches (Bain et al., 2008). Nonetheless, many of the nearly 30 known species remain dubious (Kreuger et al., 2007). At the higher taxonomic level, the genus *Setaria* has been treated as a subfamily Setariinae of Onchocercidae (Yatawara et al., 2010) as well as a distinct family Setariidae (Liu et al., 2013). Studies on molecular phylogeny indicated that both the Dirofilarriinae and Onchocercinae subfamilies appear as polyphyletic groups (Ferri et al., 2009; McNulty et al., 2012; Liu et al., 2013).

This paper reports the molecular phylogeny of nine taxa of Filarioidea based on the 12 protein-coding genes of the mitochondrial genome, and 50 taxa based on the nucleotide sequences of cytochrome *c* oxidase subunit I (COI) and 12S rRNA genes.

MATERIAL AND METHODS

Complete mitochondrial genome. — The complete mitochondrial genomes of nine taxa of filarial worms belonging to four subfamilies of Onchocercidae were accessed from the GenBank. These were: 1) Dirofilariinae – *Dirofilaria immitis* NC_005305 and *Loa loa* NC_016199; 2) Onchocercinae – *Acanthocheilonema viteae* NC_016197, *Brugia malayi* NC_004298, *Onchocerca flexuosa* NC_016172, *Onchocerca volvulus* NC_001861, and *Wuchereria bancrofti* NC_016186; 3) Setariinae – *Setaria digitata* NC_014282; and 4) Splendidofilariinae – *Chandlerella quiscali* NC_014486. *Dracunculus medinensis* NC_016019 (Nematoda: Dracunculoidea: Dracunculidae) and *Thelazia callipaeda* NC_018363 (Nematoda: Thelezioidea: Thelaziidae) were used as outgroups.

Nucleotide sequences of COI and 12S rRNA genes. — The nucleotide sequences of COI and 12S rRNA genes for 50 taxa of Filarioidea were accessed from the GenBank (Table 1). Only species with both the COI and 12S rDNA nucleotide sequences were selected, and each species was not duplicated. *Dracunculus medinensis* NC_016019 and *T. callipaeda* NC_018363 were used as outgroups.

Phylogenetic analysis. — The nucleotide sequences of individual genes and the concatenated sequences of various genes were initially aligned using BioEdit v.7.0.9.0 (Hall, 1999) with Clustal W programme (Thompson et al., 1994) and then manually aligned. Phylogenetic and molecular evolutionary analyses were conducted using *MEGA* version 5 (Tamura et al., 2011) and MrBayes v.3.1.2 (Ronquist & Huelsenbeck, 2003).

Phylogenetic trees were generated, using *MEGA* version 5, by: 1) Maximum Likelihood (ML) method based on the Tamura-Nei model (Tamura & Nei, 1993); 2) Maximum Parsimony (MP) method using the Subtree-Pruning-Regrafting (SPR) algorithm (Nei & Kumar, 2000); and 3) Neighbor-Joining (NJ) method (Saitou & Nei, 1987) with the evolutionary distances computed using the Kimura 2-parameter method (Kimura, 1980). For Bayesian Inference (BI) analyses using MrBayes v.3.1.2, Kakusan v.3 (Tanabe, 2007) was used to determine the best-fit nucleotide substitution models using the Bayesian information criterion (Schwarz, 1978).

RESULTS AND DISCUSSION

Concatenated nucleotide sequences of the protein-coding genes of mitochondrion complete genome. — The present analysis by the ML method of the concatenated sequences of 12 protein-coding genes (*cox*1–3, *nad*1–6, *nad*4L, *cyt*b, and *atp*6) for the nine taxa with complete mitochindrial genomes indicated the presence of two main clades (Fig. 1): 1) *O. flexuosa* and *O. volvulus* with *D. immitis* and *A. viteae*; and 2) *B. malayi* and *W. bancrofti* with *L. loa*. *Setaria digitata* and *Chandlerella quiscali* appear to be basal to the other filarioid taxa. The tree topology was similar for nucleotide sequences and amino acid sequences. The phylogenetic trees resulting from ML, NJ, MP, and BI methods concurred with earlier findings (McNulty et al., 2012; Liu et al., 2013) of *Onchocerca-Dirofilaria* and *Brugia-Wuchereria* clades.

As in other studies (McNulty et al., 2012; Liu et al., 2013), members of Dirofilariinae and Onchocercinae were not well resolved and indicated polyphyly for these subfamilies. The analysis by Bayesian inference of the nucleotide sequences (McNulty et al., 2012) and amino acid sequences (Liu et al., 2013) showed *A. viteae* to be a basal group (with *S. digitata*), and *C. quiscali* clustered with *B. malayi*, *W. bancrofti*, and *L. loa*.

Our analyses using NJ (Fig. 2) and MP methods also yielded slightly different tree topology from the ML tree in the grouping of the basal taxa *S. digitata* and *C. quiscali*, with *C. quiscali* being most basal. The phylogenetic tree of the nucleotide sequences generated by the BI method (Fig. 3) shows *S. digiata* as the basal species, *C. quiscali* clustered with *Brugia-Wuchereria-Loa* and *A. viteae* clustered with *Onchocerca-Dirofilaria*. This concurs with other findings based on BI method (McNulty et al., 2012; Liu et al., 2013).

COI and 12S rDNA nucleotide sequences. — The phylogenetic trees based on COI (Fig. 4) and 12S rDNA (Fig. 5) nucleotide sequences do not show good concordance. Similar results have been reported by other workers (Lefoulon et al., 2012). Nonetheless, for practical purpose, both COI and 12S rDNA appear to be suitable markers for species differentiation or DNA barcoding.

Both COI and 12S rDNA markers indicate that Dirofilariinae and Onchocercinae are not monophyletic (Figs. 4, 5). This concurs with the findings based on 12 protein-coding genes. It is also reflected by the phylogenetic tree based on concatenated COI and 12S rDNA nucleotide sequences (Fig. 6; Ferri et al., 2011) and 5S rDNA nucleotide sequences (Michalski et

Table 1. Nucleotide sequences of COI and 12S rRNA genes for 50 taxa of Filarioidea accessed from the GenBank. *Dracunculus medinensis* and *Thelazia callipaeda* were used as outgroups.

Family/Superfamily	Species	COI	12S
Onchocercidae	*Acanthocheilonema reconditum*	AJ544876	AJ544853
	Acanthocheilonema viteae	AJ272117	AJ544852
	Aproctella sp.	FR823335	FR827905
	Brugia malayi	AJ271610	AJ544843
	Brugia pahangi	AJ271611	AJ544842
	Cercopithifilaria bulboidea	AM749247	AM779779
	Cercopithifilaria crassa	AM749260	AM779791
	Cercopithifilaria japonica	AM749262	AM779794
	Cercopithifilaria longa	AM749245	AM779781
	Cercopithifilaria minuta	AM749253	AM779786
	Cercopithifilaria multicauda	AM749254	AM779799
	Cercopithifilaria roussilhoni	AM749264	AM779798
	Cercopithifilaria shohoi	AM749251	AM779797
	Cercopithifilaria tumidicervicata	AM749258	AM779787
	Chandlerella quiscali	NC_014486	NC_014486
	Dipetalonema gracile	AJ544877	AM779824
	Dirofilaria immitis	EU169124	EU182327
	Dirofilaria repens	AM749231	GQ292761
	Foleyella candezei	FR823336	FR827906
	Foleyella furcata	AJ544879	AJ544841
	Litomosa westi	AJ544871	AJ544851
	Litomosoides brasiliensis	AJ544867	AJ544850
	Litomosoides galizai	AJ544870	AJ544849
	Litomosoides hamletti	AJ544868	AJ544847
	Litomosoides sigmodontis	AJ271615	AJ544848
	Litomosoides yutajensis	AJ544869	AJ544846
	Loa loa	AJ544875	AJ544845
	Loxodontofilaria caprini	AM749237	AM779821
	Madathamugadia hiepei	JQ888272	JQ888290
	Mansonella atelensis	AM749278	AM779823
	Mansonella perforata	AM749265	AM779803
	Mansonella ozzardi	JF412346	JF412318
	Onchocerca dewittei japonica	AM749266	AM779815
	Onchocerca eberhardi	AM749268	AM779810
	Onchocerca flexuosa	NC_016172	JQ733523
	Onchocerca gibsoni	AJ271616	AY462913
	Onchocerca gutturosa	AJ271617	AY462923
	Onchocerca lupi	JX080029	JN863696
	Onchocerca ochengi	AJ271618	KC167333
	Onchocerca skrjabini	AM749269	AM779809
	Onchocerca suzukii	AM749275	AM779811
	Onchocerca volvulus	AM749285	KC167339
	Piratuba scaffi	AM749281	AM779831
	Rumenfilaria andersoni	JQ888279	JQ888296
	Setaria digitata	EF174427	EF179382
	Setaria equina	AJ544873	AJ544835
	Setaria labiatopapillosa	AJ544872	AJ544833
	Setaria tundra	AJ544874	AM779848
	Wuchereria bancrofti	AJ271612	AJ544844
Filariidae	*Filaria martis*	AJ544880	AJ544855
Dracunculoidea	*Dracunculus medinensis*	NC_016019	NC_016019
Thelazioidea	*Thelazia callipaeda*	NC_018363	NC_018363

al., 2010). In addition the subfamily Splendidofilariinae also does not appear to be monophyletic (Figs. 4–6).

Based on COI, 12S rDNA and combined COI+12S rDNA nucleotide sequences, the component species of *Setaria* form a distinct cluster grouping with i) *D. immitis* and *Onchocerca* species (COI, Fig. 4), or ii) *Piratuba scaffi* (Oswaldofilariinae) and *T. callipaeda* (Thelazioidea; 12S rDNA; Fig. 5), or iii) *Brugia-Wuchereria* (COI+12S rDNA; Fig. 6). It is evident that, unlike *Filaria martis* (Filariidae) which is distinctly separated from Onchocercidae, the genus *Setaria* is grouped together with other taxa of Onchocercidae. The phylogenetic

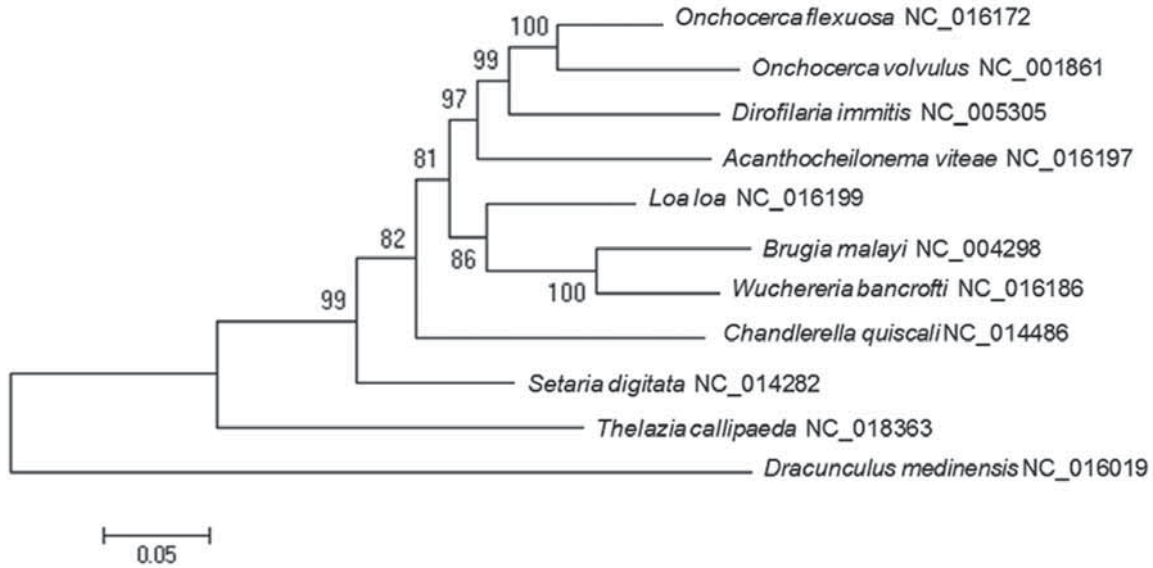

Fig. 1. Phylogenetic tree of nine taxa of Onchocercidae (with *Thelazia callipaeda* and *Dracunculus medinensis* as outgroups) generated by the Maximum Likelihood method based on 12 protein coding genes of the mitochondrial genome conducted in MEGA5. The tree with the highest log likelihood (–62978.0961) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 11 nucleotide sequences. Codon positions included were $1^{st}+2^{nd}+3^{rd}$. All positions containing gaps and missing data were eliminated. There were a total of 9989 positions in the final dataset.

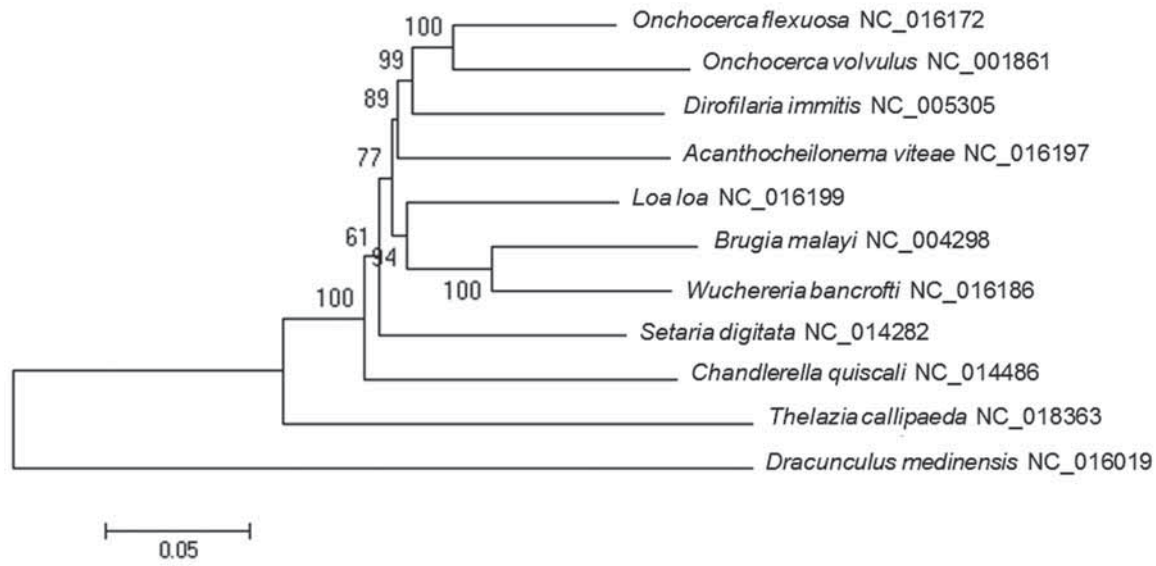

Fig. 2. Phylogenetic tree of nine taxa of Onchocercidae (with *Thelazia callipaeda* and *Dracunculus medinensis* as outgroups) generated by the Neighbor-Joining method based on 12 protein coding genes of the mitochondrial genome conducted in MEGA5. The optimal tree with the sum of branch length = 1.32972499 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein, 1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Kimura 2-parameter method (Kimura, 1980) and are in the units of the number of base substitutions per site. The analysis involved 11 nucleotide sequences. Codon positions included were $1^{st}+2^{nd}+3^{rd}$. All positions containing gaps and missing data were eliminated. There were a total of 9989 positions in the final dataset.

relationships of 50 taxa of Filarioidea based on COI, 12S rDNA, and combined COI+12S rDNA nucleotide sequences (Figs. 4–6) in the present analysis do not support the family status for the genus *Setaria*.

In the present analysis, *T. callipaeda* (Thelazioidea) appears to be related to Onchocercidae. It clusters with *Brugia-Wuchereria*, Onchocercinae (COI sequences, Fig. 4), or *P. scaffi*, Oswaldofilariinae and *Setaria*, Setariinae (12S rDNA sequences, Fig. 5), or *C. quiscali*, Splendidofilariinae (COI+12S rDNA sequencs, Fig. 6). The question remains whether *T. callipaeda* is a rogue taxon. With combined COI+12S rDNA nucleotide sequences, it forms a basal group with *C. Quiscali*, well separated from other taxa of the Onchocercidae. The exclusion of *T. callipaeda* as an outgroup does not affect the topology of the phylogenetic tree (Fig. 7). More taxa of Thelazioidea as well as some subfamilies of Onchocercidae (e.g., Oswaldofilariinae and Splendidofilariinae) are needed to elucidate the evolutionary relationships of Filarioidea. The application of multiple genes will also contribute to solving the problem.

Based on the combined COI and 12S rDNA dataset, species of the same genus in general cluster together (Fig. 6). Two exceptions are *Onchocerca dewittei japonica* which clusters with *Loxodontofilaria caprini*, and *Mansonella atelensis* with *Dipetalonema gracile*. This may be due to only a single species each (with both COI and 12S rDNA nucleotide sequences) of the genera *Loxodontofilaria* and *Dipetalonema* being available for the present study. Alternatively, the generic status of *Loxodontofilaria* and *Dipetalonema* may need re-examination.

At the subfamily level, members of Dirofilariinae are clustered in two groups: 1) the genus *Dirofilaria* clustered with *Onchocerca-Loxodontifilaria* of Onchocercinae; and 2) the genera *Loa* and *Foleyella* with *Aproctella*, *Madathamugadia*, and *Rumenfilaria* of Splendidofilariinae (Fig. 6). Whether the inclusion of multiple taxa of a genus and multiple genes will resolve this 'anomaly' and result in more concordant phylogenetic relationships, remains to be confirmed.

Phylogenetic analysis. — It is evident that the topology of the phylogenetic trees generated by different methods may differ from each other (Figs. 1–3; Morales-Hojas, 2009). In the present study, the inference and discussion are based on the Maximum Likelihood (ML) method, although analyses using other methods were also carried out, as ML trees appear to be more concordant and more commonly employed by other studies.

The topology of the phylogenetic tree is also affected by the number of taxa. In an analysis of 25 taxa of Filarioidea based on combined COI and 12S rDNA nucleotide sequences using the ML method, *Filaria martis* clustered with *Setaria* species, *L. loa* and *Foleyella furcata* with *Brugia-Wuchereria* group, and *Litomosoides-Litomosa* with *Dipetalonema-Acanthocheilonema* group (Morales-Hohas, 2009). The present analysis of 50 taxa, including more genera and subfamilies, yielded a phylogenetic tree with different evolutionary relationships (Figs. 6, 7). As stated by Morales-Hojas (2009): "... further analyses using more markers and more species should be performed in order to comprehend the evolutionary history of these organisms." In this regard, our analysis using the combined nucleotide sequences of *nad*1–6 and *nad*4L genes for the nine taxa with complete mitochondrial genomes yielded phylogenetic tree like the 12 protein-codong genes. However the combined nucleotide sequences of *cox*1–3 genes (Fig. 8) did not produce concordant results. The choice of an optimum number of suitable genes for determining accurate phylogeny needs to be explored.

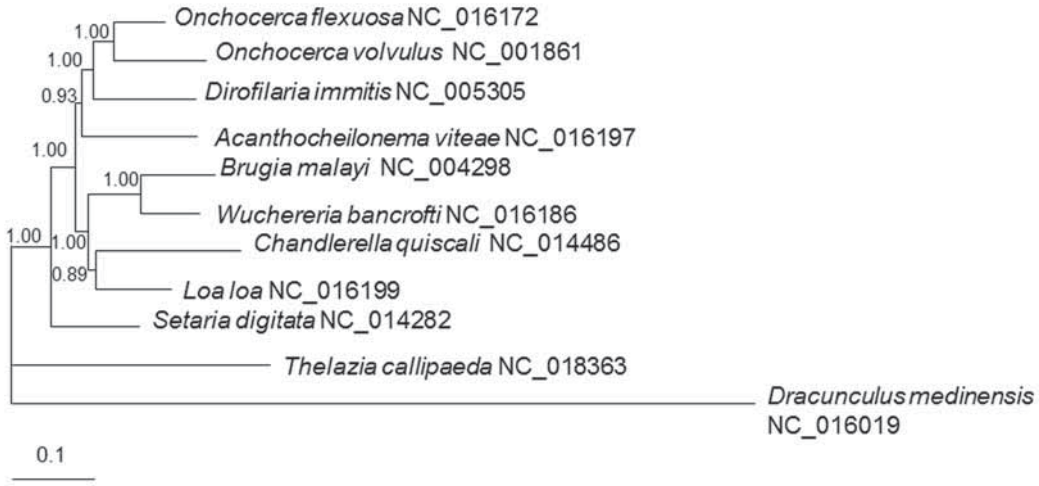

Fig. 3. Phylogenetic tree of nine taxa of Onchocercidae (with *Thelazia callipaeda* and *Dracunculus medinensis* as outgroups) generated by Bayesian Inference (BI) analyses based on 12 protein coding genes of the mitochondrial genome using MrBayes v.3.1.2 and Kakusan v.3. The program was set to run four chains of Markov chain Monte Carlo iterations for 4,000,000 generations, keeping one tree for every 100 generations. The first 4000 trees sampled were discared as "burn-in" to ensure stabilisation, based on the stationarity of ln *L* in the first 300,000 generations as assessed using Tracer v.1.5 (http://tree.bio.ed.ac.uk/software/tracer/).

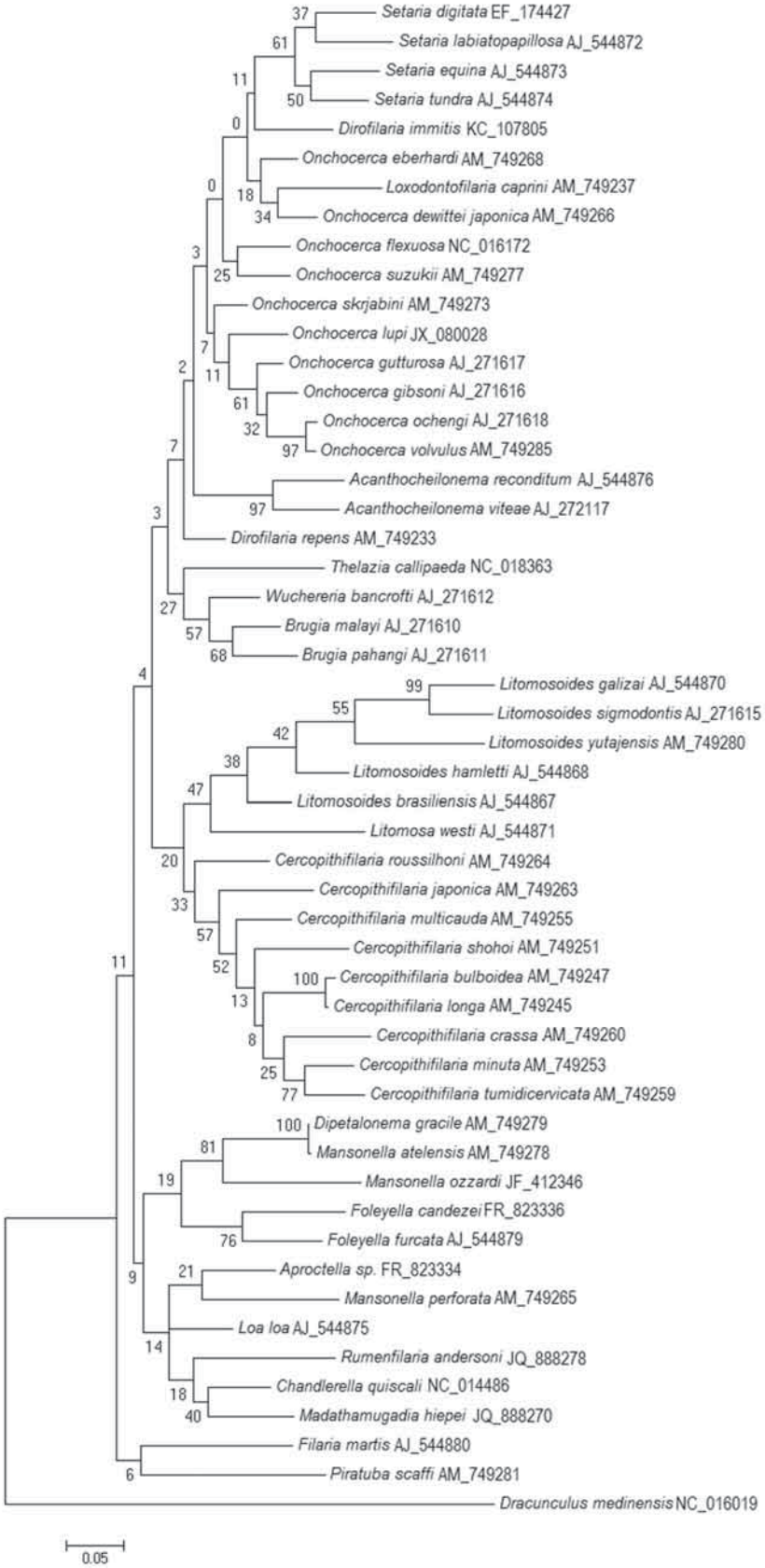

Fig. 4. Phylogenetic tree of 50 taxa of Filarioidea (with *Thelazia callipaeda* and *Dracunculus medinensis* as outgroups) generated by the Maximum Likelihood method based on partial COI nucleotide sequences conducted in MEGA5. The tree with the highest log likelihood (–7886.3023) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 52 nucleotide sequences. Codon positions included were $1^{st}+2^{nd}+3^{rd}$. All positions containing gaps and missing data were eliminated. There were a total of 399 positions in the final dataset.

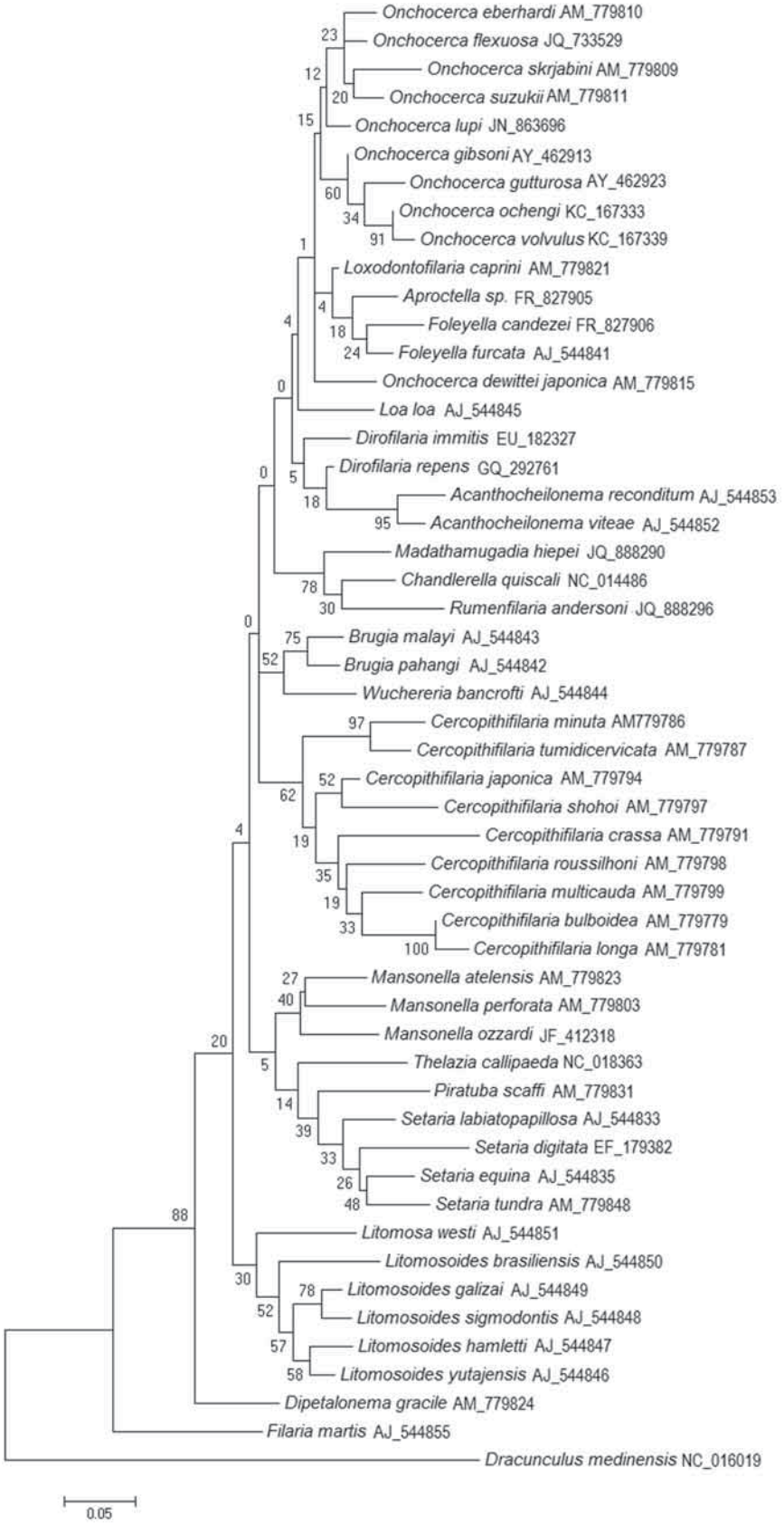

Fig. 5. Phylogenetic tree of 50 taxa of Filarioidea (with *Thelazia callipaeda* and *Dracunculus medinensis* as outgroups) generated by the Maximum Likelihood method based on 12S rDNA nucleotide sequences conducted in MEGA5. Inference of the evolutionary history was based on the Tamura-Nei model (1993). The tree with the highest log likelihood (–3983.2096) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 52 nucleotide sequences. Codon positions included were $1^{st}+2^{nd}+3^{rd}$. All positions containing gaps and missing data were eliminated. There were a total of 259 positions in the final dataset.

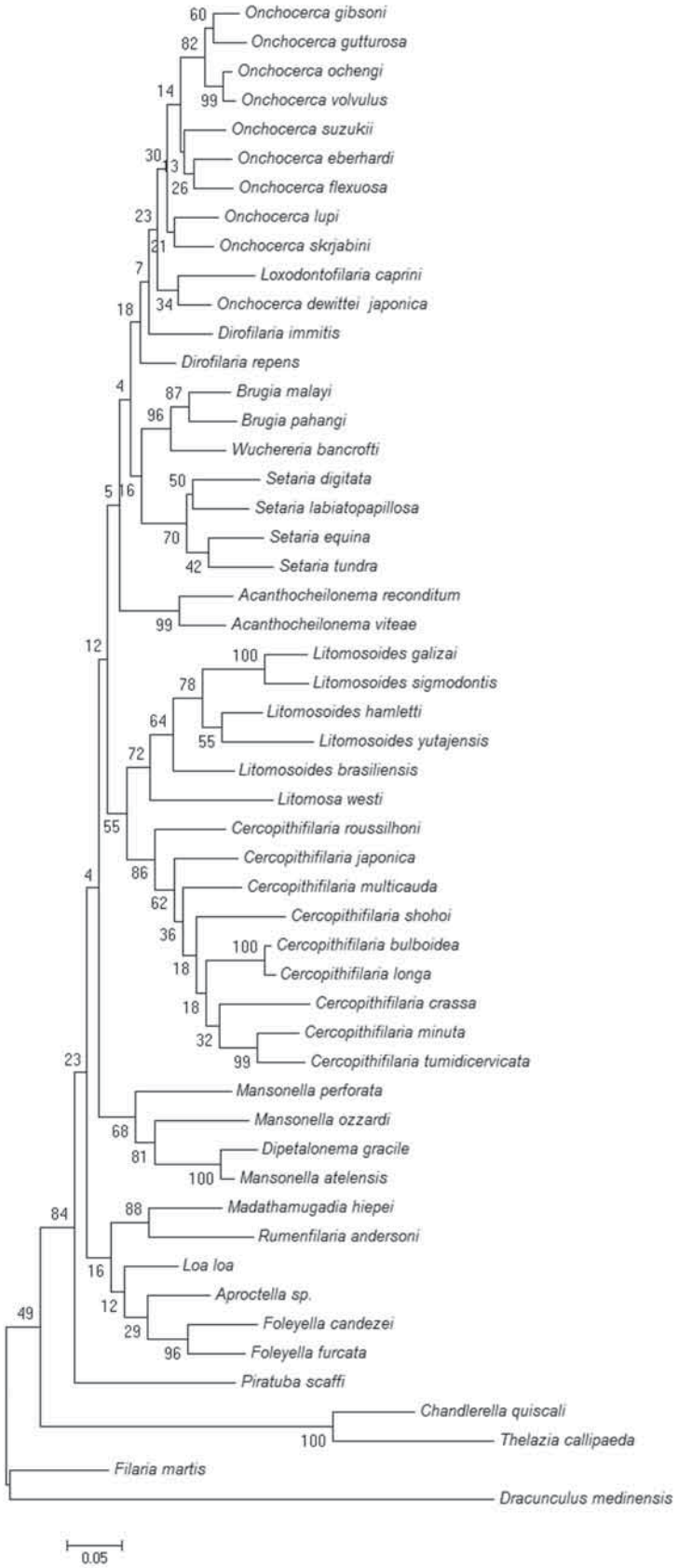

Fig. 6. Phylogenetic tree of 50 taxa of Filarioidea (with *Thelazia callipaeda* and *Dracunculus medinensis* as outgroups) generated by the Maximum Likelihood method based on combined COI+12S rDNA nucleotide sequences conducted in MEGA5. Inference of the evolutionary history was based on the Tamura-Nei model (1993). The tree with the highest log likelihood (–12594.7984) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 52 nucleotide sequences. Codon positions included were $1^{st}+2^{nd}+3^{rd}$. All positions containing gaps and missing data were eliminated. There were a total of 650 positions in the final dataset.

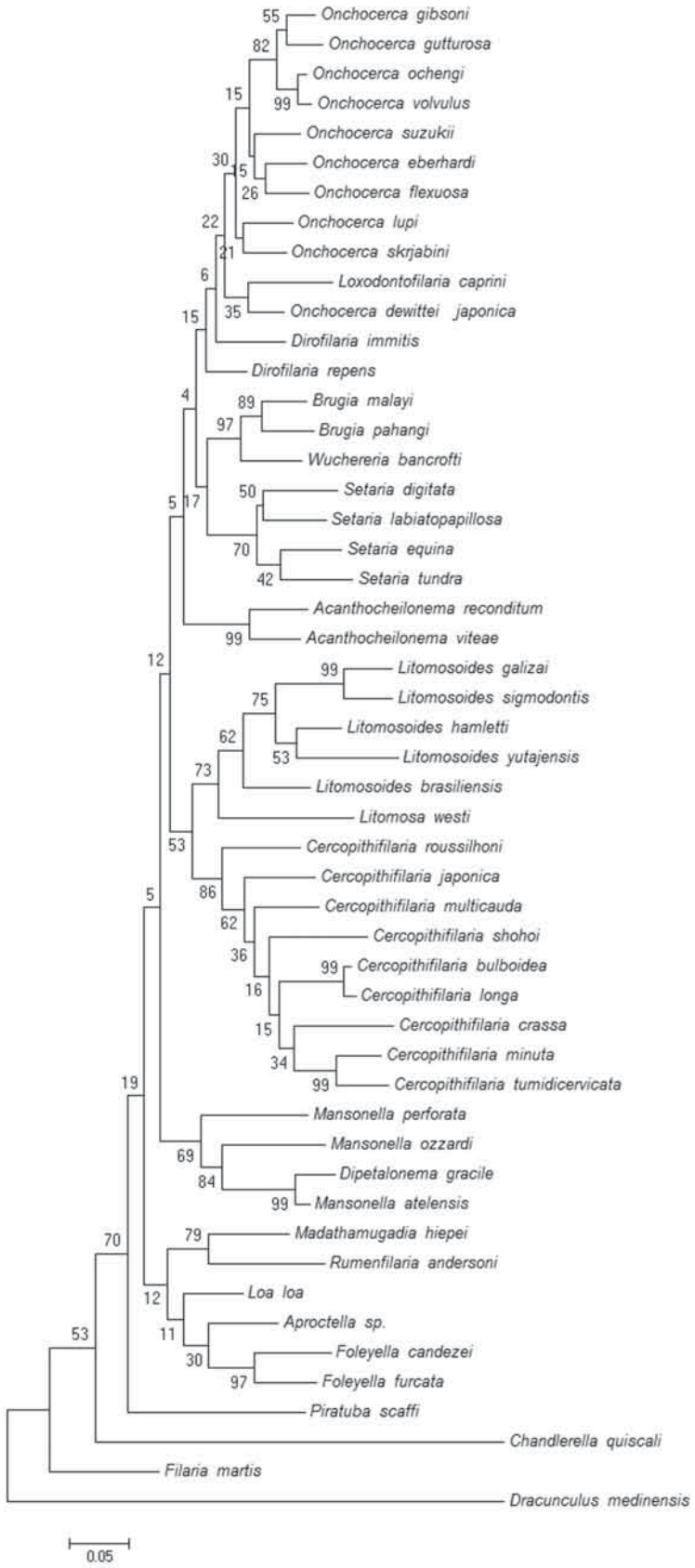

Fig. 7. Phylogenetic tree of 50 taxa of Filarioidea (with *Dracunculus medinensis* as outgroup) generated by the Maximum Likelihood method based on combined COI+12S rDNA nucleotide sequences conducted in MEGA5. Inference of the evolutionary history was based on the Tamura-Nei model (1993). The tree with the highest log likelihood (–12196.5115) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 51 nucleotide sequences. Codon positions included were $1^{st}+2^{nd}+3^{rd}$. All positions containing gaps and missing data were eliminated. There were a total of 650 positions in the final dataset.

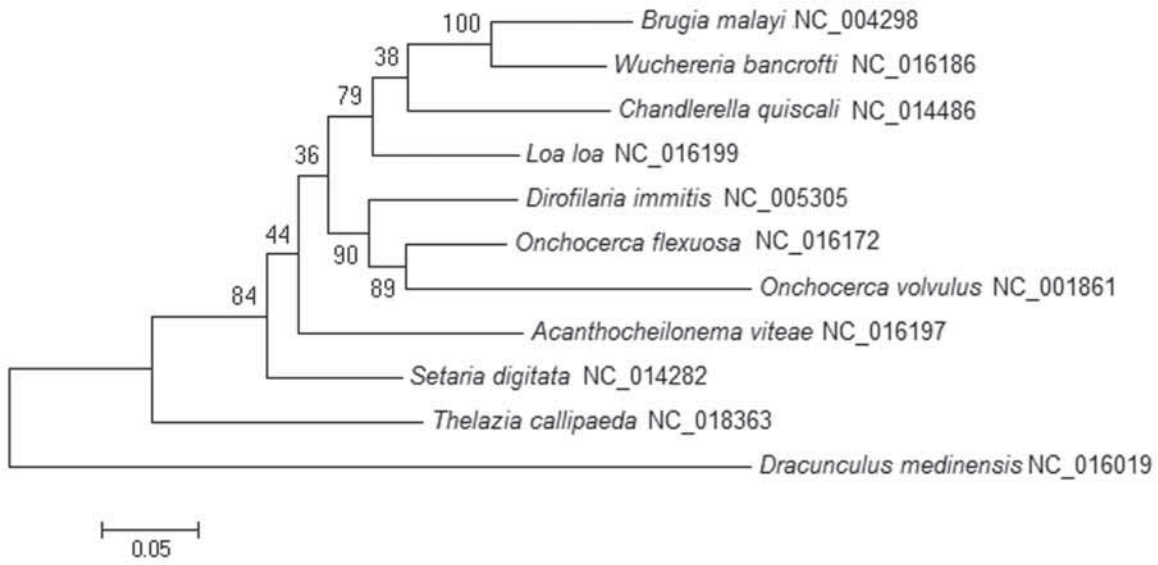

Fig. 8. Phylogenetic tree of nine taxa of Onchocercidae (with *Thelazia callipaeda* and *Dracunculus medinensis* as outgroups) generated by the Maximum Likelihood method based on combined COI+COII+COIII nucleotide sequences of the mitochondrial genome conducted in MEGA5. The tree with the highest log likelihood (–19037.5829) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 11 nucleotide sequences. Codon positions included were $1^{st}+2^{nd}+3^{rd}$. All positions containing gaps and missing data were eliminated. There were a total of 2962 positions in the final dataset.

CONCLUSIONS

Both COI and 12S rDNA appear to be suitable markers for species differentiation or DNA barcoding. The topology of the phylogenetic trees generated by different methods may differ from each other. It is also affected by the number of taxa. The phylogenetic relationships of 50 taxa of Filarioidea based on COI, 12S rDNA, and combined COI+12S rDNA nucleotide sequences in the present analysis do not support the family status for the genus *Setaria*. Members of Dirofilariinae, Onchocercinae, and Splendidofilariinae were not well resolved and indicated polyphyly for these subfamilies. In view of the unsettled questions regarding the phylogenetic relationships of various taxa of the filarial worms, further analyses using more markers and more taxa are warranted.

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