Parasitol Res (2011) 109:645–655
DOI 10.1007/s00436-011-2297-4

ORIGINAL PAPER

# Microfilariae in birds in the Czech Republic, including a note on adult nematodes *Eufilaria delicata* in a song thrush *Turdus philomelos*

**Martina Haas · Vlastimil Baruš · Václav Benedikt · Ivan Literák**

Received: 15 November 2010 / Accepted: 15 February 2011 / Published online: 12 March 2011


**Abstract** Blood samples of more than 1,100 passerineform birds of 40 species were investigated for the occurrence of microfilariae. In the year 2005, 3 out of 677 birds of 31 species (prevalence 0.4%) were infected with microfilariae during the post-nesting period. During the pre-nesting period in the year 2007, 11 out of 438 birds of 31 species were infected with microfilariae (prevalence 2.5%). Both the pre-nesting and post-nesting examinations were conducted at the same location in the northeastern part of the Czech Republic. The microfilariae of the *Eufilaria delicata* and *Ornithofilaria mavis* species were found in *Turdus merula, Turdus philomelos,* and *Erithacus rubecula* (Passeriformes, Turdidae). Single individual of *Poecile montanus* (Passeriformes, Paridae) was infected with undetermined microfilariae. The morphometric variability of microfilariae found in *T. philomelos, E. rubecula*, and *Poecile montanus* were recorded. Infections caused by microfilariae *E. delicata* were more frequent than infections caused by *O. mavis*. Seven adult nematodes *E. delicata* were found in a subcutaneous cyst on the heel joint in one *T. philomelos,* which is the first record of adult *E. delicata* nematodes in birds in the Czech Republic.

M. Haas (✉) · V. Benedikt · I. Literák
Department of Biology and Wildlife Diseases,
Faculty of Veterinary Hygiene and Ecology,
University of Veterinary and Pharmaceutical Sciences,
Palackého 1–3,
612 42 Brno, Czech Republic
e-mail: haas.2m@gmail.com

M. Haas
Institute of the High Mountain Biology, University of Zilina,
059 56 Tatranska Javorina 7, Slovak Republic

V. Baruš
Department of Zoology and Fisheries, Faculty of Agrobiology,
Food and Natural Resources, Czech University of Life Sciences,
Kamýcká 957,
165 21 Prague 6–Suchdol, Czech Republic

## Introduction

The filariid nematodes are viviparous and produce their larval stages (microfilariae) in the bloodstream (Keymer 1982). While microfilariae of *Eufilaria delicata* Supperer, 1958 and *Ornithofilaria mavis* Leiper, 1909 (Nematoda: Filarioidea) readily occur in birds in Europe, occurrence of the adult stages of these nematodes is rare (Sonin 1966; Kučera 1982; Baruš 1992; Hauptmanová 2003; Votýpka et al. 2003; Hauptmanová et al. 2004; Palinauskas et al. 2005). They are known to be transmitted by dipterans including genera *Simulium* and *Culicoides* (Anderson 2000).

Adult filariids of the genus *Eufilaria* Seurat, 1921 (Lemdaniinae) are mostly found in subcutaneous tissue around the larynx, esophagus, and crop, and also in subcutaneous tissue of the leg joints (Anderson 2000; Bartlett 2008). *E. delicata* adults and microfilariae have been recorded in passerine birds (Passeriformes) of the genus *Turdus* (Turdidae) in Austria and France (Supperer 1958; Bain 1980), in *Garrulus glandarius* (Passeriformes, Corvidae) in Austria (Supperer 1958), and *Corvus frugilegus* (Corvidae) in Moldavia (Sonin 1966). Findings of microfilariae have also been recorded in Spain in Turdidae (López-Caballero 1978a).

The adult filariids of the genus *Ornithofilaria* Gönnert, 1937 (Splendidofilariinae) have been found in birds' leg joint tissues, subcutaneous tissue, and vessel walls (Anderson 2000). Adult specimens of *O. mavis* have been found in the birds of the genus *Turdus* in England, Germany, France, Austria, Spain, and Poland (Leiper 1909; Gönnert 1937; Chabaud and Golvan 1956; Supperer 1958; López-Caballero 1978a; Okulewicz 1981, 1997), while

**Table 1** The list of all investigated and positive birds in the years 2005 and 2007

| Family | Species | Year 2005 | | Year 2007 | |
|---|---|---|---|---|---|
| | | Examined | Positive | Examined | Positive |
| *Laniidae* | *Lanius collurio* | 12 | 0 | | |
| *Aegithalidae* | *Aegithalos caudatus* | 13 | 0 | 3 | 0 |
| *Troglodytidae* | *Troglodytes troglodytes* | 9 | 0 | 8 | 0 |
| *Certhiidae* | *Certhia familiaris* | 10 | 0 | 3 | 0 |
| *Emberizidae* | *Emberiza citrinella* | 10 | 0 | 10 | 0 |
| *Fringillidae* | *Carduelis carduelis* | | | 1 | 0 |
| | *Chloris chloris* | | | 1 | 0 |
| | *Coccothraustes coccothraustes* | 1 | 0 | 9 | 0 |
| | *Fringilla coelebs* | 7 | 0 | 29 | 0 |
| | *Loxia curvirostra* | | | 1 | 0 |
| | *Pyrrhula pyrrhula* | 7 | 0 | 2 | 0 |
| *Muscicapidae* | *Ficedula albicollis* | 5 | 0 | 1 | 0 |
| | *Ficedula hypoleuca* | 21 | 0 | 13 | 0 |
| | *Muscicapa striata* | 10 | 0 | | |
| *Turdidae* | *Erithacus rubecula* | 137 | 0 | 106 | 2 |
| | *Luscinia megarhynchos* | | | 1 | 0 |
| | *Phoenicurus phoenicurus* | | | 3 | 0 |
| | *Turdus merula* | 24 | 0 | 19 | 4 |
| | *Turdus philomelos* | 38 | 3 | 15 | 4 |
| | *Turdus viscivorus* | | | 1 | 0 |
| *Paridae* | *Periparus ater* | | | 2 | 0 |
| | *Parus major* | 31 | 0 | 29 | 0 |
| | *Cyanistes caeruleus* | 18 | 0 | 2 | 0 |
| | *Poecile palustris* | 10 | 0 | 6 | 0 |
| | *Poecile montanus* | 13 | 0 | 2 | 1 |
| *Motacillidae* | *Anthus trivialis* | | | 1 | 0 |
| | *Motacilla cinerea* | 2 | 0 | | |
| *Prunellidae* | *Prunella modularis* | 28 | 0 | 20 | 0 |
| *Regulidae* | *Regulus regulus* | | | 1 | |
| *Sittidae* | *Sitta europaea* | 7 | 0 | | |
| *Sylviidae* | *Acrocephalus palustris* | 1 | 0 | | |
| | *Hippolais icterina* | 1 | 0 | | |
| | *Locustella naevia* | 2 | 0 | | |
| | *Phylloscopus collybita* | 14 | 0 | 24 | 0 |
| | *Phylloscopus sibilatrix* | 2 | 0 | | |
| | *Phylloscopus trochilus* | 2 | 0 | 6 | 0 |
| | *Sylvia atricapilla* | 220 | 0 | 113 | 0 |
| | *Sylvia borin* | 13 | 0 | | |
| | *Sylvia communis* | 8 | 0 | 3 | 0 |
| | *Sylvia curruca* | 1 | 0 | 3 | 0 |
| Total | | 677 | 3 | 438 | 11 |

**Table 2** Intensity of infection and prevalence in positive birds

| Species | Year | Prevalence | Intesity of infection | | |
|---|---|---|---|---|---|
| | | | Low | Medium | High |
| *Turdus merula* | 2007 | 20% | 4 | | |
| *Turdus philomelos* | 2005 | 8% | 2 | | 1 |
| | 2007 | 27% | 3 | | 1 |
| *Erithacus rubecula* | 2007 | 2% | 2 | | |
| *Poecile montanus* | 2007 | 50% | | 1 | |

microfilariae of this species were found repeatedly in the blood of the same host in Spain (Jimenéz-Millan and López-Caballero 1975; López-Caballero 1978b; Cano-Martil et al. 1989; López-Caballero et al. 1992). This species also has been recorded in *Coccothraustes coccothraustes* (Passeriformes, Fringillidae) in the microfilariae stage in the Czech Republic (Hauptmanová et al. 2004).

The aim of our study was to determine the prevalence and intensity of microfilarial infection in birds during post-nesting and pre-nesting periods in the Czech Republic and to detect any adult specimens of the species *E. delicata* in *Turdus philomelos*.

## Materials and methods

The birds were captured at the locality Čerťák (49° 34′ N, 17° 59′ E), near Nový Jičín in the northeastern part of the Czech Republic. This site is situated in mixed forest at the altitude of 370–400 m a.s.l. The first phase of examination was carried out in the post-nesting period during August–September 2005, and the second in the pre-nesting period during spring migration in April 2007. The birds were captured with ornithological mist nets, identified, ringed, scanned for the presence of subcutaneous cysts, and after blood collection released back into the wild.

One drop of blood was taken from the *vena ulnaris cutanea* and the blood smear was made by standard procedure. The smears were air-dried and then fixed by methanol. In the laboratory, the smears were stained with a combination of May-Grünwald and Giemsa-Romanowski stains, using a method according to Pappenheim (Lukas and Jamroz 1961). The stained smears were examined microscopically for the presence of microfilariae under ×200 magnification. If microfilariae were present, their morphology was studied under ×1,000 magnification and measurements were carried out using the QuickPHOTOMICRO 2.2 software. In microfilariae, total body length, head space, tail length, maximum width of body, head width, tail width, and distance of fixed points from anterior body end were measured. Fixed point values were expressed as percentages of the total body length. In infected birds, the total number of microfilariae in a whole blood smear was counted. Infection intensity was categorized into three levels: low (1–10 microfilariae per slide), medium (11–20 microfilariae per slide), and high (>20 microfilariae per slide).

In one individual *T. philomelos*, a cyst was recorded on the left heel joint. The cyst was slit with a scalpel at a

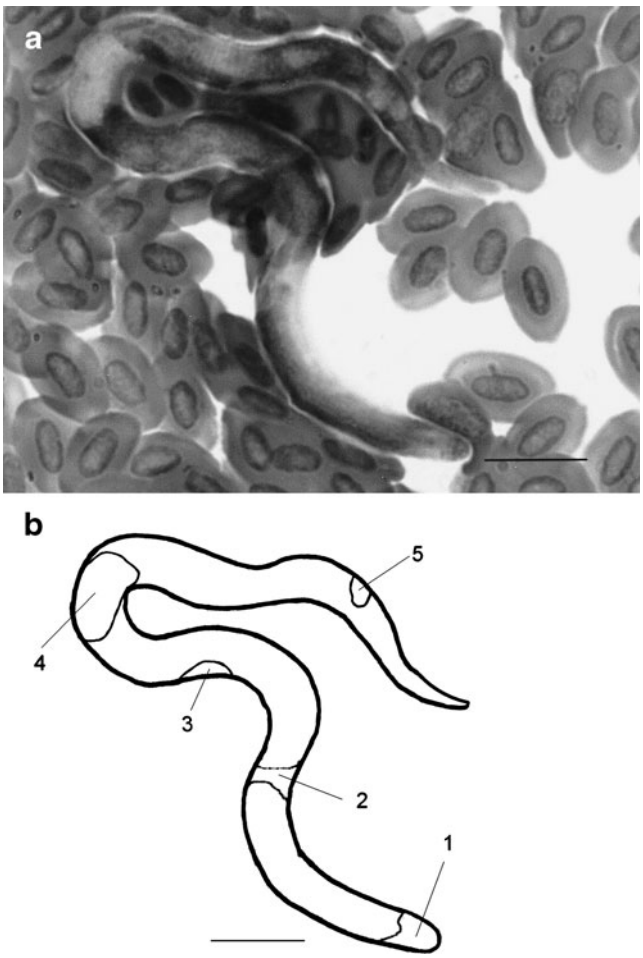


**Fig. 1** **a**, **b** Microfilaria *E. delicata* from host *T. philomelos*. *1* head space, *2* nerve ring, *3* excretory pore, *4* inner body, *5* anal pore. Scale bar=10 μm

**Table 3** Morphometric characteristics of microfilariae *Eufilaria delicata* in various bird hosts (*n*=number of all microfilariae in all infected birds in one host species)

| Host | *Turdus philomelos* | | *Turdus merula* | | *Erithacus rubecula* | | Total | |
|---|---|---|---|---|---|---|---|---|
| Parameter (μm) | *n*=30 | | *n*=20 | | *n*=2 | | *n*=52 | |
| | Range | Mean | Range | Mean | Range | Mean | Range | Mean |
| Total body length | 72–113 | 93.5 | 83–113 | 96.1 | 102–103 | 102.5 | 72–113 | 94.8 |
| Head width | 3–4 | 3.5 | 2–4 | 3.5 | 3–4 | 3.5 | 2–4 | 3.5 |
| Maximum body width | 4–6 | 5.0 | 4–6 | 5.1 | 5 | 5.0 | 4–6 | 5.0 |
| Tail width | 1–2 | 1.6 | 1–2 | 1.8 | 2 | 2.0 | 1–2 | 1.8 |
| Head space | 2–6 | 4.1 | 4–7 | 5.1 | 3–4 | 3.5 | 2–7 | 4.4 |
| Nerve ring | 13–25 | 18.9 | 17–25 | 20.7 | 23–25 | 24.0 | 13–25 | 19.8 |
| Excretory pore | 17–40 | 28.0 | 20–37 | 29.1 | 35–38 | 36.5 | 17–40 | 28.7 |
| Inner body | 40–61 | 49.7 | 41–62 | 50.2 | 55–64 | 59.5 | 40–64 | 50.3 |
| Anal pore | 55–96 | 74.0 | 67–95 | 79.4 | 83–86 | 84.5 | 55–96 | 76.5 |
| Tail length | 12–30 | 17.1 | 10–18 | 13.7 | 16–18 | 17.0 | 10–30 | 15.8 |

length of about 2 mm and the contents were removed by forceps. The incision was then disinfected with ethanol and the bird was released. No cysts were found on other parts of the body. The cyst contained nematodes, which were fixed in 96% ethanol. The nematodes were cleared with glycerin, after which they were examined using a light microscope (Olympus BX 61) equipped with differential interference contrast optics, a digital image analysis system (analySIS auto 5.0), and a drawing attachment. After examination, the specimens were stored in vials with 70% ethanol. Measurements are given in micrometers (μm), unless otherwise stated, with the means in parentheses.

**Table 4** Values of fixed points for *Eufilaria delicata* and *Eufilaria* sp. according to previous references and our findings. In our measurements were indicated statistical significant differences in head space (*n*=number of all microfilariae in all infected birds in one host species)

| | *E. delicata* | *E. delicata* | *Eufilaria* sp. | *E. delicata* | | |
|---|---|---|---|---|---|---|
| | Supperer (1958) | Bain (1980) | Cano-Martil et al. (1989) | Our findings | | |
| | *T. merula, T. viscivorus, G. glandarius* | *T. merula* | *T. philomelos* | *T. philomelos* | *T. merula* | *E. rubecula* |
| Parameter (μm, except as indicated) | *n*=not defined | *n*=5[a]<br>*n*=13[b]<br>*n*=2[c] | *n*=not defined | *n*=30 | *n*=20 | *n*=2 |
| Total body length | 163–166 | 146–170[a]<br>107–155[b]<br>158–176[c] | 151.04 | 72–113 (93.5) | 83–113 (96.1) | 102–103 (102.5) |
| Values of fixed points | % | % | % | Min–max (mean)% | Min–max (mean)% | Min–max (mean)% |
| Head space | – | – | 6.20 | 2.4–5.6 (4.3) | 3.5–6.9 (5.3) | 2.9–3.9 (3.4) |
| Nerve ring | 17.2 | – | 22.48 | 13.3–28.2 (19.4) | 17.9–26.6 (21.9) | 22.3–24.5 (23.4) |
| Excretory pore | 32.0 | 28.23 | 32.96 | 19.5–41.2 (30.2) | 20.0–34.8 (30.2) | – |
| Inner body | 51.33–62.8 | 49.9–51.20 | 52.38 | 46.5–60.4 (53.4) | 47.1–56.4 (51.9) | 53.9–62.1 (58.0) |
| Anal pore | – | – | 81.58 | 52.3–85.7 (78.3) | 77.0–91.5 (82.6) | 81.4–83.5 (82.5) |
| Tail length | – | – | 18.40 | 10.7–18.7 (15.6) | 10.6–18.4 (14.3) | 15.5–17.6 (16.6) |

[a] Measured in blood (fixed with 5% formalin and measured after 24 h)

[b] Measured on unstained smear

[c] Measured on stained smear

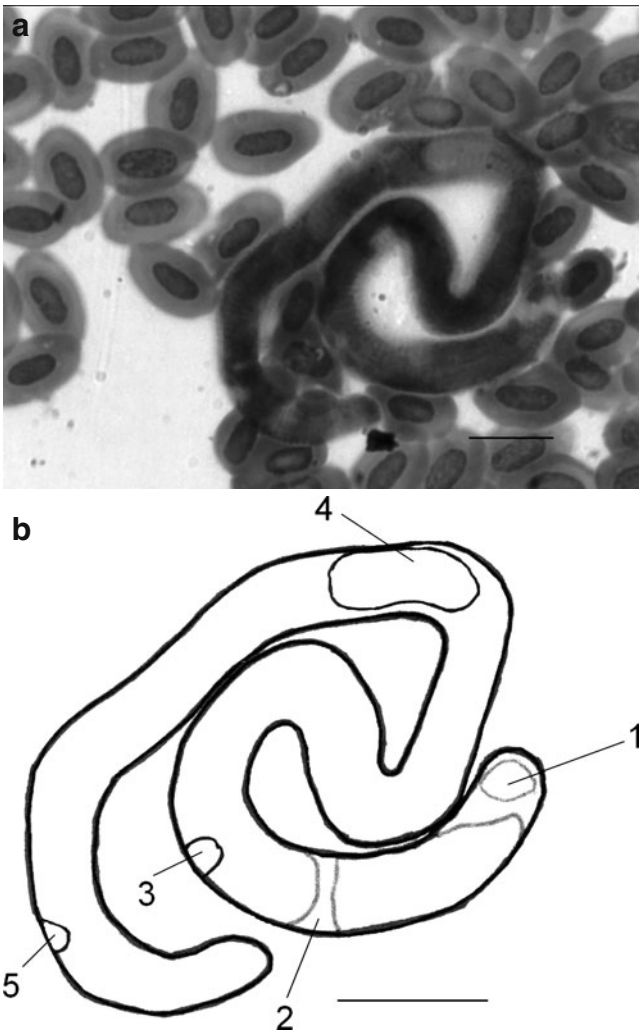


**Fig. 2 a, b** Microfilaria *O. mavis* from host *T. philomelos*. *1* head space, *2* nerve ring, *3* excretory pore, *4* inner body, *5* anal pore. Scale bar=10 μm

Morphometric data from Leiper (1909), Gönnert (1937), Chabaud and Golvan (1956), Supperer (1958), Bain (1980), and Okulewicz (1981) were used in identifying adult nematodes. Indications from Supperer (1958), López-Caballero (1978a, b), Cano-Martil et al. (1989), López-Caballero et al. (1992), and Hauptmanová et al. (2004) were used in morphometric determination of microfilariae. Reference material (voucher specimens) of the *E. delicata* was deposited in the Helminthological Collection of the Institute of Parasitology, Academy of Sciences of the Czech Republic, České Budějovice, Czech Republic (Cat. No. N-946).

Morphometric differences between microfilariae of different host species and seasons were tested by Mann–Whitney *U* test statistics.

## Results

### Prevalence and intensity of microfilarial infection

During the 2005 post-nesting period, 677 birds including 31 species were examined (Table 1). Microfilariae were found in three birds (prevalence 0.4%). Three individuals of *T. philomelos* were infected with *E. delicata*.

During the 2007 pre-nesting period, 438 birds of 31 species were examined (Table 1). Microfilarial infection was recorded in 11 birds (prevalence 2.5%). Infected individuals belonged to species: *Turdus merula* (three infections with *E. delicata,* one mixed infection with *E. delicata* and *O. mavis*), *T. philomelos* (two infections with *E. delicata,* one mixed infection with *E. delicata* and *O. mavis*, one infection with undetermined microfilariae) *Erithacus rubecula* (one mixed infection with *E. delicata* and *O. mavis*, one infection with undetermined microfilariae), and *Poecile montanus* (one infection with undetermined microfilariae). Prevalences and intesity of infections are given in Table 2.

### Morphometric characteristics of microfilariae

Microfilariae *E. delicata* (Fig. 1a, b) were found without cuticle cover, and the cuticle appears not to be transversally striated. Anterior body end (head space) is clearly rounded, pale, with slight prominence. The cephalic space is about as long as the body width. Interruption of the cellular stem indicates the location of the neural ganglion, not equally well visible in all specimens. The nerve ring is visible as a short brightening over the whole width of the body. The excretory pore is recognizable as a short and pale lateral spot. The inner body is visible as a distinct elongated homogeneous space, filling the entire inner space of the microfilarial body. The anal pore, like excretory pore, is visible only as a pale lateral spot, opening with the anal cell. The posterior end of the body is conical and ends with a sharply pointed tapering tail. The morphometric characteristics of the microfilariae are given in Table 3, and comparison of the fixed point values from our measurements with those of other authors are given in Table 4.

Statistically significant difference in the mean head space morphological parameters of *E. delicata* between host species *T. philomelos* and *T. merula* was found (Mann–Whitney $U=159.0$; $p=0.005$). Differences at other fixed points were not found. The difference in the mean body length of microfilariae *E. delicata* between *T. philomelos* and *T. merula* was statistically not significant. Statistically significant difference in the body length of *E. delicata* was found between post-nesting (2005) and pre-nesting (2007) period (Mann–Whitney $U=109.0$; $p<0.001$).

**Table 5** Morphometric characteristics of microfilariae *Ornithofilaria mavis* according to a previous reference and our findings (*n*=number of all microfilariae in all infected birds in one host species)

| Parameter (μm) | Hauptmanová et al. (2004) | | Our findings | | | | |
|---|---|---|---|---|---|---|---|
| | *Coccothraustes coccothraustes* | | *Turdus philomelos* | | *Turdus merula* | *Erithacus rubecula* | |
| | *n*=10 | | *n*=2 | | *n*=1 | *n*=2 | |
| | Range | Mean | Range | Mean | | Range | Mean |
| Total body length | 101.9–158.0 | 128.3 | 129.0–159.0 | 144.0 | 107.0 | 98.0–105.0 | 101.5 |
| Head width | 3.0–4.6 | 3.7 | 4.0–5.0 | 4.5 | 4.0 | 3.0–4.0 | 3.5 |
| Maximum body width | 3.9–5.8 | 4.5 | 5.0 | 5.0 | 4.0 | 4.0–5.0 | 4.5 |
| Tail width | 2.4–3.4 | 2.9 | 3.0 | 3.0 | 3.0 | 2.0–3.0 | 2.5 |
| Head space | 2.9–4.6 | 3.7 | 4.0–7.0 | 5.5 | 6.0 | 5.0–6.0 | 5.5 |
| Nerve ring | 14.2–27.4 | 22.0 | 19.0–30.0 | 24.5 | 17.0 | 17.0 | – |
| Excretory pore | 31.2–46.5 | 36.8 | 26.0–47.0 | 36.5 | 33.0 | 28.0 | – |
| Inner body | 58.9–92.8 | 74.3 | 71.0–88.0 | 79.5 | 38.0 | – | – |
| Anal pore | 86.6–129.8 | 112.8 | 119.0 | 119.0 | 79.0 | 85.0–89.0 | 87.0 |
| Tail length | – | – | 8.0–9.0 | 8.5 | 11.0 | 11.0–13.0 | 12.0 |

Microfilariae *O. mavis* (Fig. 2a, b) were covered with smooth cuticle, which appears not to be transversally striated. Front and rear end of the body were clearly rounded. The cephalic space is about as wide as its length. The caudal part is tapering, but narrowing is not very sharp and creating a broad, rounded tail. Nerve ring is visible as a short brightening over the whole width of the body. The excretory pore is visible as a short and pale lateral spot in the microfilaria. The inner body is a relatively clear space, varying in size, located closer to the posterior body end. The anal pore is structured like excretory pore and is located close to the tail part. The morphometric characteristics of the microfilariae are given in Table 5, and comparison of the fixed point values from our measurements with those of other authors are in Table 6.

*Microfilariae* sp. from *P. montanus* (Fig. 3) had a total body length of 69–98 μm (average 83 μm). The average width was 5.3 μm (5–6 μm). The head space was rounded and as long as wide (4 μm). Nerve ring was visible only in single individual of microfilaria and appeared as a narrow brightening across the whole width of the microfilaria, with its location 23 μm from the anterior end of the body. Excretory pore was not visible in any of the measured microfilariae. Inner body

**Table 6** Values of fixed points for *Ornithofilaria mavis* and *O. böhmi* according to previous references and our findings (*n*=number of all microfilariae in all infected birds in one host species)

| Parameter (μm) | *O. mavis* Gönnert 1937 | *O. mavis* Cano-Martil et al. 1989 | *O. mavis* Hauptmanová et al. 2004 | Mean | *O. böhmi* Supperer 1958 | *O. mavis* Our findings | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | *T. philomelos* | | *T. merula* | *E. rubecula* | |
| | *n*=not defined | *n*=not defined | *n*=10 | | *n*=not defined | *n*=2 | | *n*=1 | *n*=2 | |
| | Mean | Mean | Range | | Mean | Range | Mean | | Range | Mean |
| Head space | – | (6.80) | 2.2–3.8 | (3.0) | – | 2.5–5.4 | (3.9) | (5.6) | 5.15.7 | (5.4) |
| Nerve ring | (21.7) | (23.34) | 13.1–26.1 | (17.6) | (17.9) | 14.7–18.9 | (16.8) | (15.9) | 17.3 | – |
| Excretory pore | (32.3) | (36.35) | 21.8–35.7 | (29.1) | (1.6) | 20.2–29.6 | (24.9) | (30.8) | 28.6 | – |
| Inner body | (55.8) | (53.48) | 54.0–60.2 | (57.1) | (5.3) | 55.0–55.3 | (55.2) | (35.5) | – | – |
| Anal pore | (81.3) | (84.05) | 82.2–85.1 | (83.9) | – | 74.8–92.2 | (83.5) | (73.8) | 84.8–86.7 | (5.8) |
| Tail length | – | – | – | – | – | 5.0–7.0 | (6.0) | (10.2) | 11.2–12.4 | (11.8) |

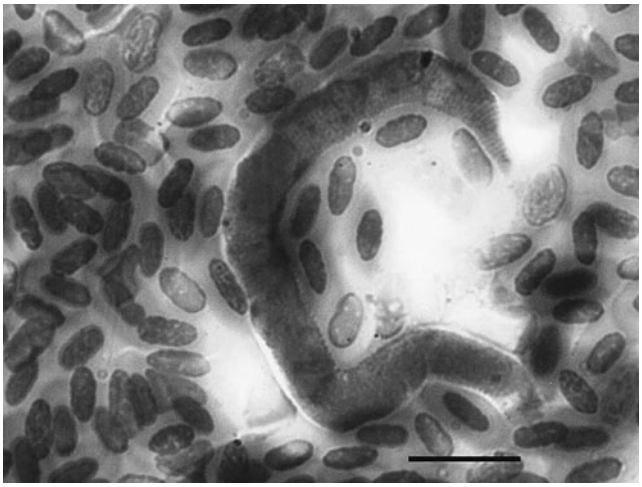

**Fig. 3** *Microfilaria* sp. from the host *P. montanus*. Scale bar=10 μm

was visible only in two microfilariae as a homogeneous space filling the whole inner space of the microfilaria body (44–54 μm, average 49 μm). Anal pore is visible as a brightening opening to the lateral side of the body (59–68 μm, average 63.5 μm) near to the posterior body end. The tail part is tapering to a cone shape with sharp peak. Length of tail is 11–12 μm (11.5 μm), width of tail end is 1–2 μm (1.7 μm). The fixed point values in our measurements and those for microfilaria from the blood of *Parus major* according to Hauptmanová (2003) and comparable measurements for *Eufilaria bartlettae* are shown in Table 7.

## Description of the adult *E. delicata* nematodes from *T. philomelos*

Seven specimens of adult nematodes (three males, two females, two without sex determination, because they were not prepared and remain in the collection of the authors) were located in the subcutaneous cyst in the heel joint in one *T. philomelos* captured July 30, 2005. A high-intensity infection with *E. delicata* microfilariae was found in the blood of the same bird. A total of 62 and 34 birds from genus *Turdus* were examined in 2005 and 2007, respectively, but no other parasitic cysts were found on these or other birds.

The anterior end of the nematodes was widely rounded; posterior end was slightly narrower, but also rounded. The oral orifice was small and rounded without lips. The cephalic papillae were rudimentary or completely lost. The oral cavity was small, extending into a thin esophagus, with unmarked transition to the gut. The cuticle was thin with faint longitudinal stripes.

Male (Table 8, Fig. 4, a–f): Male body length reached approximately half of the female body length. Maximum body width at the anterior ganglion was 83–104 μm, at the level of cloaca 67–90 μm. Maximum width of esophagus was 20–21 μm. Two weakly sclerotized spicules had a slightly different length (3–22 μm difference). The width of the proximal part of the longer spicule was 18–19 μm, shorter spicule 16–18 μm. The distal ends of the spicules were pointed. Small bulges were present laterally to the cloaca, supported by rudimentary papillae. A less pronounced and similarly rounded bulge is present above the

**Table 7** Values of fixed points for *Eufilaria bartlettae* according to previous references and values of fixed points for microfilariae from *Poecile montanus* (our findings)

| Parameter | *E. bartlettae* (Bain 1980) | *E. bartlettae* (Hauptmanová 2003) | Undetermined microfilariae |
|---|---|---|---|
| | *Turdus merula* | *Parus major* | *Poecile montanus* |
| | *n*=8 [a]<br>*n*=1 [b] | *n*=1 | *n*=3 |
| | | | Min–max (mean)% |
| Total body length (in μm) | 110.0–122.0[a]<br>116.0 [b] | 100.0 | 69.0–98.0 (83.0) |
| Values of fixed points | % | | |
| Head space | – | 4.0 | 4.9–5.8 (5.4) |
| Nerve ring | 18.96 | 26.0 | 23.0 (23.0) |
| Excretory pore | 37.09 | 34.0 | – |
| Inner body | 54.5–55.7[a]<br>56.03 [b] | 60.0 | 53.7–55.1 (54.4) |
| Anal pore | – | 84.0 | 82.9–98.5 (84.2) |
| Tail length | – | – | 14.6–15.9 (15.3) |

[a] Measured in blood (fixed with 5% formalin and measured after 24 h); [b] measured on stained smear

**Table 8** Size of *E. delicata* or *E. bartlettae* males according to previous references and our findings

| Parameter (μm, except as indicated) | Supperer (1958) *Eufilaria delicata* *n*=not defined | Bain (1980) *Eufilaria delicata* *n*=1 | Bain (1980) *Eufilaria bartlettae* *n*=4 | Our findings *Eufilaria delicata* *n*=3 |
|---|---|---|---|---|
| Total body length (mm) | 11–13 | 10.85 | 8.3–10.2 | 8.49–10.26 |
| Maximum body width | 100 | 65 | 45–68 | 235–302 |
| Length of esophagus | 230–277 | 260 | 280–400 | 565–904 |
| Nerve ganglion – anterior body end | 150–160 | 182 | 135–165 | 131–145 |
| Testes–anterior body end | 512 | 325 | 450–650 | 522–875 |
| Length of spicule (1) | 60–65 (Difference 3–4) | 58 (Difference 8) | 65–70 (Difference 11–17) | 75–96 (Difference 3–22) |
| Width of spicule (1) | 11 | – | – | 18–19 (Prox. part) |
| Length of spicule (2) | 57–61 | 50 | 52–57 | 72–74 |
| Width of spicule (2) | 7 | – | – | 16–18 (Prox. part) |
| Cloaca–posterior body end | Subterminal | 20 | 10–13 | 42–51 |
| Ampullaceous dilation of gut–posterior body end | 65 | – | – | 146–151 |
| Host | *T. viscivorus, T. merula, G. glandarius* | *T. merula* | *T. merula* | *T. philomelos* |
| Country | Austria | France | France | Czech Republic |

upper rim of the cloacal orifice (other measurements in Table 8.)

Female (Table 9, Fig. 4, g–i): Body width at the anterior ganglion 154 μm, at vulva 274 μm. The gut forms a broadened ampula approximately 290–660 μm from the posterior end. Both uterus branches are filled with microfilariae.

## Discussion

The prevalence of microfilariae in birds is dependent upon many factors, including species, sex, age of host, ethology of host, locality, and sampling period (Kučera 1981). We found the prevalence of microfilarial infection to be generally low in the Czech Republic, although in the 2007 pre-nesting period, it was higher than in the 2005 post-nesting period. In hematozoan parasites, the higher prevalence in spring was interpreted to be caused by the weakening of the organism by increased load during breeding and increased number of suitable vectors, and lower number of parasites reflecting the relative abundance of juveniles in the population (Deviche et al. 2001). Another aspect may be the migration of birds and the fact that migrants move through more terrain, which increases the likelihood of encountering a higher diversity of parasite vectors (Møller and Erritzøe 1998, Smith et al. 2004, Valkiūnas 2005).

The number of microfilariae in the smear can be generally very low and does not accurately reflect their density in blood (Kučera 1982), which can be influenced by the circadian rhythm of their occurrence (they occur regularly at night, while sampling is done predominantly during the day) (Kučera 1982; Anderson 2000). Irregular occurrence of microfilariae also negatively impacts the evaluated intensity of infection, and therefore, low levels of infection estimated in blood smears may not adequately reflect the current state of the microfilarial presence in the blood stream.

In comparison with the reports for the genus *Turdus* (López-Caballero 1978a), our findings identically confirm a higher occurrence of microfilariae in the species *T. philomelos* than in the species *T. merula*, but in contrast, our study showed more infections caused by the species *E. delicata* than *O. mavis*. This can be due to the geographic location of our trapping sites, as well as to the trapping dates. The comparable research in Spain was done between November and March (López-Caballero 1978a).

Our findings complement the description regarding the occurrence of microfilariae *E. delicata* in *T. philomelos* and *T. merula* (Supperer 1958; López-Caballero 1978a, b; Bain 1980; Cano-Martil et al. 1989), confirm the presence of this species in central Europe, and expand the known distribution of *E. delicata* northward.

We documented the first record of *O. mavis* in *E. rubecula*. In the Czech Republic, the nematode *O. mavis* had been recorded in *Coccothraustes coccothraustes* (Hauptmanová et al. 2004). Findigs of *O. mavis* in *T. philomelos* and *T. merula* are new host records for the

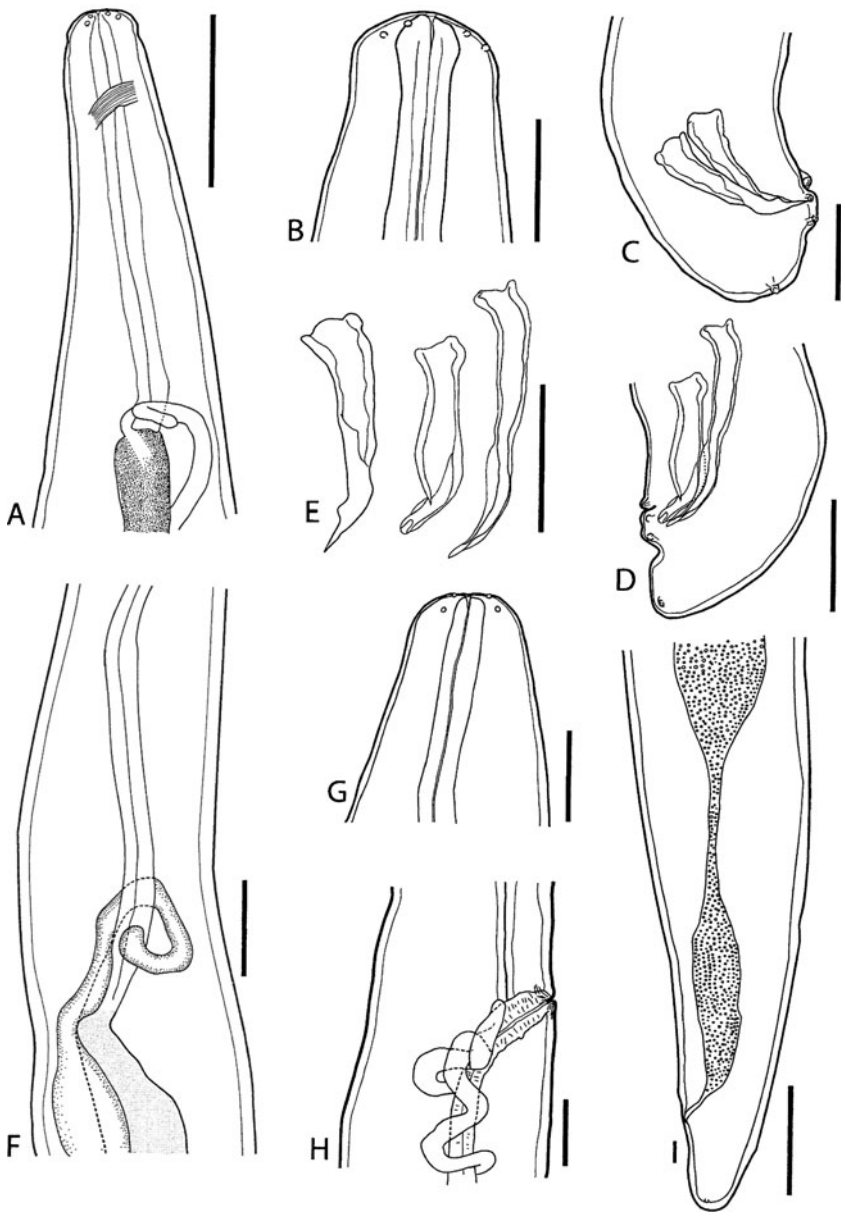


**Fig. 4** Adults of *E. delicata*. **a**–**f** (male), **g**–**i** (female). **a** anterior part of male, **b** detail of cephalic region, **c**, **d** detail of caudal part, *lateral view*, **e** spicules, **f** testis around esophagus, **g** detail of cephalic region, **h** vulva region, **i** caudal part, *lateral view*. Scale bars: *A* (200 μm), *B–H* (50 μm), *I* (100 μm)

Czech Republic. We found mixed infection with *E. delicata* and *O. mavis* microfilariae in three birds (*T. merula, T. philomelos*, and *E. rubecula*). Mixed infections of this type have previously been recorded in *T. philomelos* and *T. iliacus* in Spain (López-Caballero 1978a).

Findings of microfilariae have been rare in tits (Passeriformes: Paridae). Unspecified microfilariae have been reported in Sweden for *P. major* (Allander and Bennett 1994) and in Russia for *P. montanus* (Palinauskas et al. 2005). Hauptmanová (2003) described a single microfilaria in *P. major* and determined it as *E. bartlettae* Bain, 1980. Comparison of fixed points from that individual (Hauptmanová 2003) and our results (see Table 5) showed a high level of similarity. However, the difference in body length (our material is shorter; average length 83 μm), supports the suggestion that this may not be the same species. According to the shape of the tail (conical with sharp peak), which is an important morphological feature, we placed the microfilariae from *P. montanus* into the genus *Eufilaria*.

The finding of adult nematodes *E. delicata* completes previously described findings in the birds of genus *Turdus* (Supperer 1958; Bain 1980). Values of measurable characteristics in adult individuals of *E. delicata* by other authors (Supperer 1958; Bain 1980) show significant differences (maximum body width, esophagus length, location of testes and cloaca, ampullary gut enlargement, vaginal length, and location of the posterior ovary). Body size measurements

**Table 9** Size of *E. delicata* females according to previous references and our findings

| Parameter (μm, except as indicated) | Supperer (1958) *n*=not defined | Bain (1980) *n*=4 | Sonin (1966) *n*=not defined | Our findings *n*=1 complete female and 1 fragment of posterior part of body |
|---|---|---|---|---|
| Total body length (mm) | 20–24 | 19.1–24.9 | 22–24 | 19.50 |
| Maximum body width | 147 | 90–130 | 201–203 | 359–404 |
| Length of esophagus | 260–298 | – | – | – |
| Width of esophagus | 8 | – | 13 | 18 |
| Nerve ganglion–anterior body end | 220–265 | 150–220 | 153 | 200 |
| Maximum width of gut | 12 | – | 13–50 | 124–145 |
| Vulva–anterior body end | 796–782 | 534–800 | 691 | 739 |
| Length of vagina | 848–857 | 2,025 | 650–1,037 | 2,088 |
| Anus–posterior body end | 135–140 | 55–100 | 94–203 | 86–106 |
| Distance of the posterior ovary from the tail end | – | 450–1,670 | 676 | 1,416–1,834 |
| Host | *T. viscivorus, T. merula, G. glandarius* | *T. merula* | *Corvus frugilegus* | *T. philomelos* |
| Country | Austria | France | France | Czech Republic |

are not useful for species identification if the size variation of the available specimens is so extensive. In such cases, characteristic morphological traits are to be preferred for species identification. Unclear borders between inner organs may lead to inaccuracies of the measurements. Departures from the real values can be also caused by a small number of specimens measured or inherently high variability of body proportions, compression of the specimen (affects mostly the maximum body and gut width) during the preparation of the slide. The comparison of the morphological characteristics of adult males and females found in our material with the data in the descriptions from already named authors demonstrates that adult nematodes from the host *T. philomelos* are conspecific with *E. delicata* (Supperer 1958). It was also possible to identify microfilariae present in the blood of this bird.

In France, Bain (1980) described a new species of filariae named *E. bartlettae* only on the basis of the material from male specimens collected from *T. merula.* This taxon is morphologically very close to *E. delicata*, but it differs slightly in morphology and topography of the pericloacal bulges, as well as in its morphometrics. Considering the intraspecific variability of morphometric characteristics of males and females, we suggest the taxon *E. bartlettae* to be valid, probably as a sibling species to *E. delicata.*

Sites in the avian hosts occupied by adult *Eufilaria* nematodes are generally subcutaneous connective tissues of the head and neck, connective tissue around the trachea, esophagus and crop, and occasionally subcutaneous connective tissue of the groin or legs (Anderson 2000; Bartlett 2008). Subcutaneous connective tissues in birds have been reported as parasite locations for the nematode *E. delicata* (Supperer 1958; Bain 1980). We have observed the leg joint to be an occasional location of the nematode *E. delicata* in *T. philomelos*. Such localization is common in birds of the family *Turdidae* for the filarids *O. mavis* and *O. böhmi* Supperer, 1958, from which *E. delicata* morpholocically is very different. The species *O. böhmi* was considered by Sonin (1966) to be a synonym of the previous.

The adults, as well as microfilariae of *E. delicate* in *T. philomelos*, represent the first documented finding of this nematode in the Czech Republic.

**Acknowledgments** This study was funded by Grants MSM6215712402 and MSM 604607091 from the Ministry of Education, Youth and Sports of the Czech Republic. We would like to thank Dr. Martin Lukáň for help with statistical analysis.

## References

Allander K, Bennett GF (1994) Prevalence and intensity of haematozoan infection in a population of Great Tits *Parus major* from Gotland, Sweden. J Avian Biol 25:69–74

Anderson RC (2000) Nematode Parasites of Vertebrates: their Development and Transmission, 2nd edn. CABI Publishing, Wallingford

Bain O (1980) Deux filaires du genre *Eufilaria* chez le merle: développement chez *Culiciodes nubeculosus*. Ann Parasitol (Paris) 55:583–590

Bartlett CM (2008) 2006. Filarioid Nematodes. In: Atkinson CT, Thomas NJ, Hunter DB (eds) Parasitic Diseases of Wild Birds. Wiley-Blackwell Publishing, Ames, Iowa, pp 439–462

Baruš V (1992) List of filariids (Nematoda: Filiariata) parasitic in vertebrates of Czech and Slovak Federative Republic (systematics, hosts, comment to biology, bibliography). Helminthologia 29:101–107

Cano-Martil S, Vallejo-Ruiz D, Coy-Otero A, López-Caballero EJ (1989) Demonstración de la actividad de fosfatasa ácida en microfilarias hemáticas de *Turdus philomelos* Brehm (Aves: Turdidae). Rev Iber Parasitol 49:349–355

Chabaud AG, Golvan Y (1956) Nouvelle filaire des grives en France. Ann Parasit Hum Comp 31:405–413

Deviche P, Greiner EC, Manteca X (2001) Seasonal and age-related changes in blood parasite prevalence in dark-eyed juncos (*Junco hyemalis*, Aves, Passeriformes). J Exp Zool 289:456–466

Gönnert R (1937) Zur Frage der Artzugehörigkeit von *Filaria mavis* Leiper, 1909. In: Festschrift Bernhard Nocht zum 80. Geburtstag. Institut für Schiffs- und Tropenkrankheiten in Hamburg, Hamburg

Hauptmanová K (2003) Hematologie a krevní parazité volně žijících ptáků (Haemathology and blood parasites in wild birds, in Czech). Dissertation Thesis, University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic

Hauptmanová K, Baruš V, Literák I, Benedikt V (2004) Haemoproteids and microfilariae in hawfinches in the Czech Republic. Helminthologia 41:125–133

Jimenéz-Millan F, López-Caballero EJ (1975) Encuesta sobre parasitismo por nematodos hematicos y tisulares en aves Españolas. Ardeola 21:1111–1117

Keymer JF (1982) Parasitic Diseases. In: Petrak ML (ed) Diseases of Cage and Aviary Birds, 2nd edn. Lea & Febiger, Philadelphia, pp 536–552

Kučera J (1981) Blood parasites of birds in central Europe.1. Survey of literature. The incidence in domestic birds and general remark to the incidence in wild birds. Folia Parasitol 28:13–22

Kučera J (1982) Blood parasites of birds in central Europe. 4. *Trypanosoma*, *Atoxoplasma*, microfilariae and other rare haematozoa. Folia Parasitol 29:107–113

Leiper RT (1909) Description of *Filaria mavis* n. sp. from the trush. Zoologists 13:337–339

López-Caballero EJ (1978a) Incidencia del parasitismo por formas de la superfamilia filarioidea (Nematoda: Spirurina) en aves del genero *Turdus*. Bol Estac Central Ecol 7:67–71

López-Caballero EJ (1978b) Utilization de un metodo numerico para interpretar el crecimiento de la microfilaria hematica de *Splendidofilaria mavis*. Rev Iber Parasitol 38:193–205

López-Caballero EJ, Vallejo D, Cano-Martil S, del Valle-Portilla MT (1992) Descriptión de estructuras cefálicas y corporales de microfilarias (Nematoda, Filarioidea) de aves españolas, mediante técnicas histoquímicas. Bol R Soc Esp Hist Nat (Sec Biol) 88:179–187

Lukas AM, Jamroz C (1961) Atlas of Avian Hematology. Agriculture monograph 25, United States Department of Agriculture, Washington

Møller AP, Erritzøe J (1998) Host immune defense and migration in birds. Evol Ecol 12:945–953

Okulewicz A (1981) *Ornithofilaria mavis* (Leiper, 1909) Gönnert, 1937 in blackbird (*Turdus merula* L.) in Poland. Wiad Parazytol 27:669–671

Okulewicz A. (1997) Katalog Fauny Parazytniczej Polski (Catalogus Faunae Parasiticae Poloniae). Czesc IV. Parazyty Ptaków (Parasiti Avium). Zeszyt 2B. Niciene-Nematoda. Polskie Towarzystwo Parazytologiczne, Komisja Faunistyczna. Warsaw

Palinauskas V, Markovets MY, Kosarev VV, Efremov VD, Sokolov LV, Valkiûnas G (2005) Occurrence of avian haematozoa in Ekaterinburg and Irkutsk districts of Russia. Ekologija (Lithuania) 4:8–12

Smith RB, Greiner EC, Wolf BO (2004) Migratory movements of Sharp-shinned Hawks (*Accipiter striatus*) captured in New Mexico in relation to prevalence, intensity, and biogeography of avian hematozoa. Auk 121:837–846

Sonin MD (1966) Osnovy Nematodologii – Tom 17. Filjariaty Životnych i Cheloveka i Vyzyvajemije imi Zabolevanija. Chast I. – Aproctoidei. Osnovy Nematodologii – XVII. Izdat. Nauka, Moscow

Supperer R (1958) Zwei neuen Filarien (S.L.), *Eufilaria delicata* spec. nov. und *Ornithofilaria böhmi* spec. nov. aus der Misteldrossel, *Turdus viscivorus* L. Z Parasitenkunde 18:312–319

Valkiūnas G (2005) Avian malaria parasites and other haemosporidia. CRC Press, Boca Raton

Votýpka J, Šimek J, Tryjanowski P (2003) Blood parasites, reproduction and sexual selection in the red-backed shrike (*Lanius collurio*). Ann Zool Fenn 40:431–439