Parasitol Res (2016) 115:1235–1244
DOI 10.1007/s00436-015-4860-x

ORIGINAL PAPER

First evidence of the European wildcat (*Felis silvestris silvestris*) as definitive host of *Angiostrongylus chabaudi*

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Received: 18 November 2015 / Accepted: 27 November 2015 / Published online: 5 December 2015

Abstract *Angiostrongylus chabaudi* (Strongylida, Angiostrongylidae) is a parasitic nematode described for the first time last century from the pulmonary arteries of six European wildcats (*Felis silvestris silvestris*) in central Italy. Since then, this parasite remained practically unknown until recently, when immature *A. chabaudi* have been reported from one wildcat in Germany and two domestic cats (*Felis silvestris catus*) in Italy. The present report describes the first record of *A. chabaudi* in Greece and, most importantly, the first known case of patent infection by *A. chabaudi*. The necropsy of a road-killed *F. s. silvestris* found near the lake Kerkini, in the municipality of Serres (Macedonia, Greece), revealed the presence of nematodes of both sexes in the right ventricle and the pulmonary artery of the heart. All parasites were mature adults and numerous eggs were present in the uteruses of females. The morphological characteristics of the parasites were consistent with those of *A. chabaudi*. Moreover, *Angiostrongylus*-like first stage larvae (L1) were present in the faeces of the animal that was negative for any other cardio-pulmonary parasite. Genetic examination of adult parasites and L1 confirmed the morphological identification as *A. chabaudi*. Histopathological examination of the lungs showed severe, multifocal to coalescing, chronic, interstitial granulomatous pneumonia due to the presence of adult parasites, larvae and eggs. These findings demonstrate for the first unequivocal time that this nematode reproduces in the European wildcat which should be ultimately considered a definitive host of *A. chabaudi*. Finally, the L1 of *A. chabaudi* are described here for the first time, opening new prospects for further studies on this neglected parasite.

Keywords *Angiostrongylus chabaudi* · Angiostrongylosis · Wildcat · *Felis silvestris silvestris* · Morphology · Histology · Feline

Introduction

In general, very few parasites infect the cardiovascular system of felids. *Dirofilaria immitis*, i.e. the dog heartworm, is the most common and may sometimes occur in domestic and wild felids, especially in areas with high prevalence of infection in canine populations (Bowman and Atkins 2009). Two metastrongyloid nematodes belonging to the genus *Angiostrongylus* have been recorded in wild felids. *Angiostrongylus chabaudi* was described for the first time in 1957 from the pulmonary arteries and the right heart of European wildcats (*Felis silvestris silvestris*) in central Italy (Biocca 1957) and more recently, *Angiostrongylus felineus* was found in the pulmonary arteries of a yagouaroundi (*Puma yagouaroundi*) in Brazil (Vieira et al. 2013). Indeed, knowledge on felid angiostrongylosis is rather poor. In particular, *A. chabaudi*, since its first description, remained practically unknown until recently, when immature

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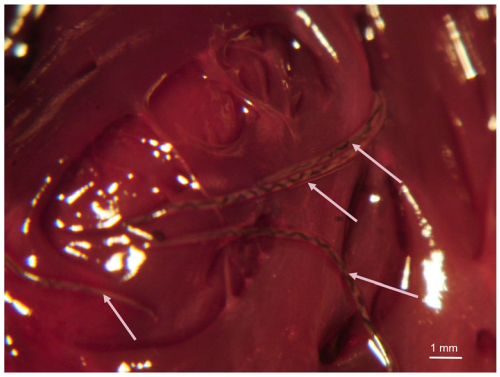

Fig. 1 *A. chabaudi* (*arrows*) on the endothelium of the right ventricle of a wildcat in Northern Greece

adults were reported from two domestic cats (*Felis silvestris catus*) in Italy (Varcasia et al. 2014; Traversa et al. 2015). Additionally, there is also an unconfirmed report of *A. chabaudi* in a wildcat from Germany (Steeb et al. 2014) and a report of nematodes of the genus *Angiostrongylus* in a wildcat from Italy (Veronesi et al. 2015).

The life cycle of *A. chabaudi* is unknown but, in analogy to the other species of the genus (Spratt 2015), the first stage larvae (L1) are supposed to be shed to the environment via the faeces of the definitive host and to develop to the infective stage in an intermediate host, possibly a terrestrial pulmonate gastropod. Interestingly, the L1 of *A. chabaudi*, i.e. the diagnostic stage, has never been described and the definitive host, although assumed to be the European wildcat, is not known. In fact, L1 are not mentioned in Biocca's first description of *A. chabaudi* (Biocca 1957) and only immature or unfertilized specimens with no eggs or L1 were described in the recent reports (Steeb et al. 2014; Varcasia et al. 2014; Traversa et al. 2015).

Infections by *A. chabaudi* and other little known cardio-respiratory nematodes in felids, especially in domestic cats (*F. s. catus*), have recently become a priority in veterinary parasitology for a range of reasons, such as the increasing prevalence of detection for some of these parasites and the need for clarification of their pathogenetic role in their hosts (Di Cesare et al. 2015c). Considering the important gaps existing in our knowledge on *A. chabaudi*, there is significant merit to add new crucial data on the infection caused by this almost unknown nematode. There is also the need of a better knowledge on this infection in domestic cats because the recent findings in these animals have raised questions about the ability of the parasite to reach genetic maturation and its potential to cause clinical disease in *F. s. catus* (Varcasia et al. 2014; Di Cesare et al. 2015c; Traversa et al. 2015).

The present paper reports on the first record of mature male and female *A. chabaudi* in a European wildcat in Greece and, most importantly, describes for the first time the L1 of the parasite. In addition, the first evidence of the pathogenic potential of *A. chabaudi* is here presented, based on the histological examination of lung lesions caused in the infected animal.

Materials and methods

A road-killed, male wildcat was found in the area of Kerkini Lake National Park, municipality of Serres (41.35400°N, 023.35288°E, 73 m alt.). The animal was identified as pure European wildcat (*F. s. silvestris*) according to morphological and morphometric characteristics, as well as molecular mitochondrial markers (Ragni 1981; Ragni and Possenti 1996; Randi et al. 2001; Mattucci et al. 2013). At necropsy, nine slender nematodes were recovered from the right ventricle (n=7) of the heart (Fig. 1) and the proximal pulmonary artery (n=2). The parasites were put into saline, examined under a stereomicroscope and an optical microscope, and then preserved in alcohol. No parasites were found in the trachea, bronchi and bronchioles of the animal. The nematodes were microscopically identified on the basis of their morphological and morphometric features (Biocca 1957; Varcasia et al. 2014; Traversa et al. 2015).

Pulmonary lavage was performed with saline and the fluid was collected, centrifuged and the sediment was examined under an optic microscope. In addition, parasitological faecal examination (i.e. flotation, sedimentation and Baermann method) was performed on the contents of the large intestine

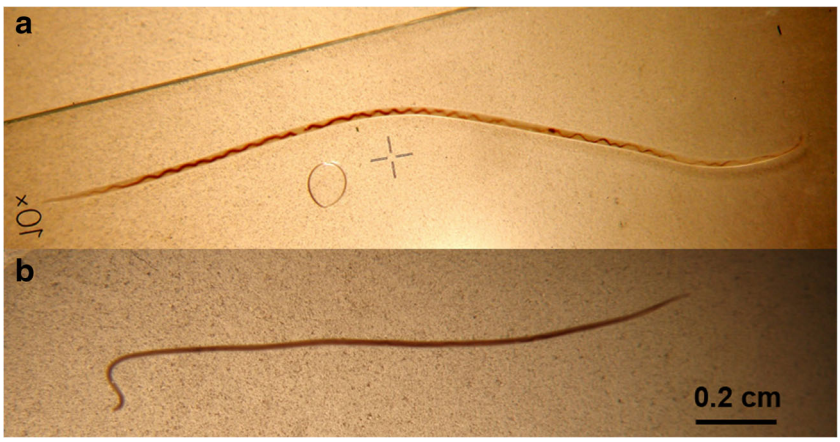

Fig. 2 Female (**a**) and male (**b**) *A. chabaudi* specimens from a wildcat in Northern Greece

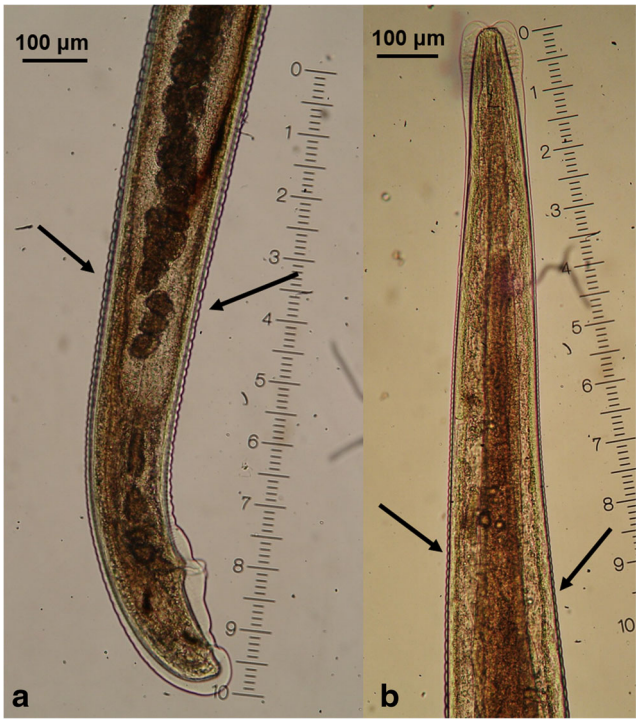

Fig. 3 Posterior (**a**) and anterior (**b**) extremity of a female *A. chabaudi*, showing transversal striation of the cuticle (*arrows*)

(MAAF 1986; Thienpont et al. 1986). Metastrongyloid first stage larvae (L1) were found both in the pulmonary lavage and the faeces (by all three examination methods). The adult nematodes and the larvae were subjected to molecular analyses as recently described (Traversa et al. 2015).

Selected parts of the lungs and the heart were fixed in 10 % neutral buffered formalin for 72 h, embedded in paraffin, sectioned at 5 µm and stained with hematoxylin and eosin (H&E) for standard subsequent histological evaluation.

Results

Parasite description

The parasites recovered were six female (21–22 mm in length and 260–270 µm in width) and three male (14–18 mm in length and 193–200 µm in width) nematodes (Fig. 2). Their cuticle showed transversal striation throughout the whole body that was more intense at both anterior and posterior extremities (Fig. 3). The anterior extremity was tapered, bearing a cephalic vesicle (Fig. 4). The oral aperture was circular, surrounded by six labial papillae, i.e. "perityles" in Biocca 1957. Four pairs of sensory setae, distributed in equal distances between them around the nematode body, were visible at approximately 17 µm from the anterior end (Fig. 4). The oesophagus was 330–360 µm long (Fig. 5) and 22 µm and 45–55 µm wide at its narrowest (anterior) and at its wider (posterior) level, respectively. The excretory pore was opening at a distance of 410–430 µm from the cephalic extremity (Fig. 5).

The female specimens had the characteristic "barber-pole" appearance, with the intestine intertwined with the genital tube (Figs. 2 and 6). Their posterior extremity was ventrally curved, with an ~25-µm-thick marginal space between the end of the body and the cuticle. The anal opening was at a distance of 67–72 µm and 43–45 µm from the end of the body including and not including the cuticle margin, respectively. The vulvar opening was at 135–142 µm distance from the anal opening and at 180–185 µm distance from the end of the body, not including the cuticle margin. The uterus at its distal part was full of eggs, measuring 50–60×36–38 µm (Fig. 7).

The males had a uniform colouration throughout their body and a slightly spiral posterior end (Fig. 2). The copulatory bursa was relatively small and possessed two symmetrical lateral lobes and a smaller dorsal lobe. The lateral lobes showed two shallow indentations corresponding to the medio-lateral and postero-lateral rays, and a deeper indentation corresponding to the ventro-lateral ray (Fig. 8). The dorsal ray was short with a wide conical base and two short, lateral, distal protuberances. The externo-dorsal ray was robust and straight, with a slightly enlarged apex. The three lateral rays had a common base, the medio-lateral and postero-lateral were closer associated and slightly thinner than the externo-lateral ray. The ventral rays had a long common trunk and were distally separated from each other. The ventro-ventral ray bore a slender, bristle-like protuberance at its tip (Fig. 8). Spicules were long, both starting at equal distance from the end of the body (proximal extremity, Fig. 9a), but at their distal extremity

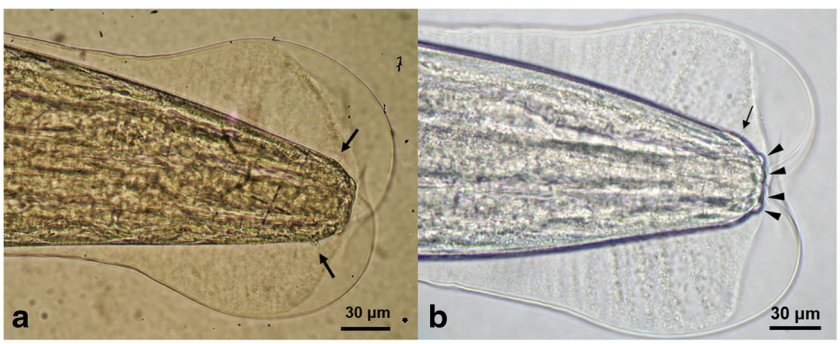

Fig. 4 Anterior end of *A. chabaudi*, female, showing sensory setae (**a**, **b**: *arrows*), and labial papillae (**b**: *arrowheads*)

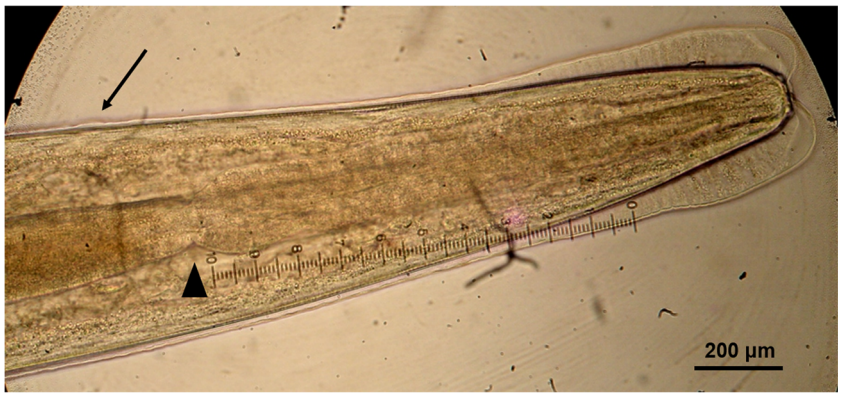

Fig. 5 Anterior part of adult *A. chabaudi* male, showing the excretory pore (*arrow*) and the oesophageal-intestinal junction (*arrowhead*)

one spicule was slightly longer than the other (long spicule, 588–610 µm; short spicule, 576–597 µm) (Fig. 9b). Their proximal extremity was slightly dilated and the distal extremity was undulated, S-shaped, transversely striated, ending with a light hyaline expansion that surrounded a yellowish-brown central part (Fig. 9). All parasites found in the right ventricle of the heart and the pulmonary arteries of the wild cat were identified as *A. chabaudi* according to these morphological characteristics. Measurements of *A. chabaudi* specimens found in the present study are presented in detail in Table 1 and compared with the measurements provided by Biocca (1957). Varcasia et al. (2014) and Traversa et al. (2015).

Pulmonary lavage and faecal examination

The larvae found in the pulmonary lavage and in the faeces of the animal had a typical *Angiostrongylus*-like morphology, measured 362–400 µm in length and 15–18.5 µm in width, and had a kinked tail with dorsal spine and notch (Fig. 10).

The L1 collected in this wildcat revealed morphological differences both in the posterior and the anterior extremity (Fig. 11), with other common metastrongyloid L1 that can be found in the faeces of Felidae, i.e. *Aelurostrongylus abstrusus* and *Troglostrongylus brevior*.

Molecular identification

The ribosomal sequences of the adult nematodes and of the larvae displayed 99–100 % identity with the *Angiostrongylus* sp. found in a European wildcat from Germany (GenBank Accession Number: KM216825.1) and with *A. chabaudi* isolate N. 1 from Italy (GenBank Accession Number: KM009115.1).

Gross lesion and histological examination

Lungs were heavy and swollen with cobblestone appearance and pulmonary parenchyma was multifocally consolidated. The histological examination revealed that the pulmonary interstitium was expanded and partially effaced by multifocal to coalescing granulomatous foci. The inflammatory foci were composed of macrophages, fibroblasts, lymphocytes and plasma cells, which were arranged concentrically around numerous parasitic larvae, embryonated and unembryonated eggs (Fig. 12). The larvae had a thin eosinophilic cuticle, numerous basophilic nuclei and an eosinophilic cytoplasm. The thin-walled eggs were ovoid, 50–60 µm in diameter, and contained multiple blastomeres. A cross section of an adult female parasite was found in the lumen of a vessel (Fig. 13) showing a thin eosinophilic cuticle, pseudocoelom, prominent lateral

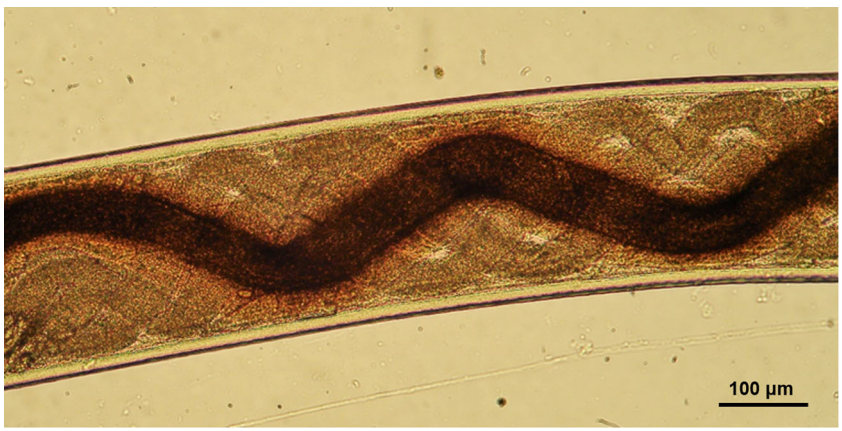

Fig. 6 Mid body of a female *A. chabaudi*, showing the intestine (*dark coloured tube*) intertwined with the genital tube (*light coloured tube*)

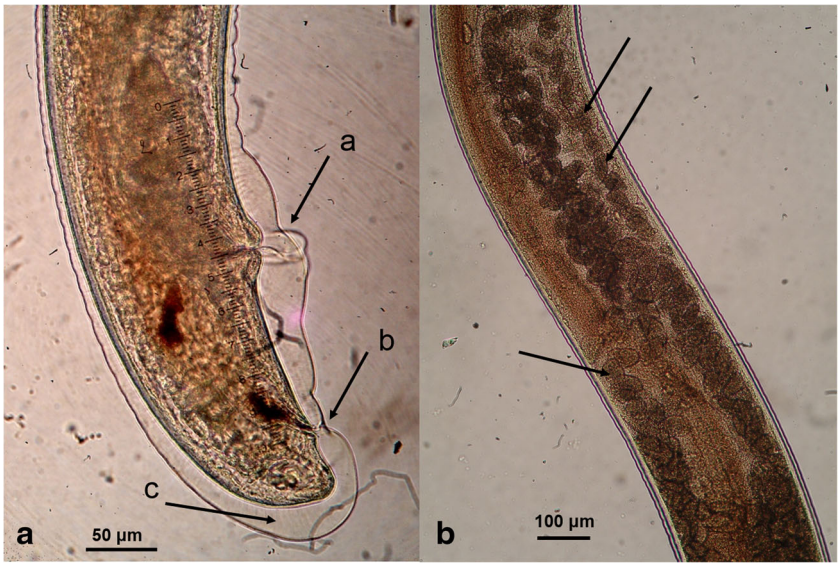

Fig. 7 Female *A. chabaudi*. **a**. Posterior end, showing the vulvar opening (*a*), the anal opening (*b*), and the margin between the body end and the cuticle (*c*). **b** Uterus filled with eggs (*arrows*)

cords, coelomyarian/polymyarian musculature, intestinal tract lined by cuboidal epithelial cells and uterus with eggs. Multifocal dilatation of the pulmonary artery branches was also noted, with the tunica media severely thickened due to smooth muscle hypertrophy (Fig. 14). These histopathological features were consistent with a severe, multifocal to coalescing, chronic, interstitial granulomatous pneumonia. No histological lesions were found in the heart.

Discussion

This report corresponds to the first description ever of a patent infection of a European wildcat by *A. chabaudi*. It thus represents a clear demonstration that the European wildcat serves as definitive host for this almost unknown nematode parasite.

The scientific interest on pulmonary and cardiovascular parasitic nematodes affecting cats has dramatically increased in the past few years (Di Cesare et al. 2015c). Reports of these infections in different hosts in various parts of the world have brought to light new epizootiological aspects and have provided new information on the biology, ecology and pathogenesis of both well-known and neglected parasites (Brianti et al. 2012; Traversa and Di Cesare 2013; Colella et al. 2015).

There is evidence that specific epizootiological drivers may favour bridging infections with extra-intestinal nematodes between the European wildcat (*F. s. silvestris*) and domestic cat (*F. s. catus*) and vice versa (Falsone et al. 2014; Traversa et al. 2015; Veronesi et al. 2015). For example, the "cat lungworm" *A. abstrusus* is considered the most prevalent and thus most important lung parasite of domestic cats (Traversa and Di Cesare 2013). whilst there is evidence that it is not a common parasite of wildcats (Jeżewski et al. 2013; Falsone et al. 2014;

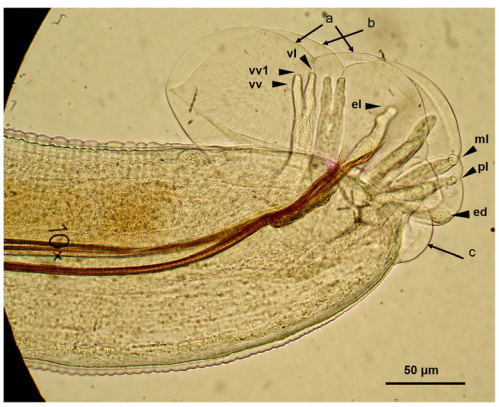

Fig. 8 Copulatory bursa of a male *A. chabaudi*: right lateral lobe (*a*), left lateral lobe (*b*), dorsal lobe (*c*), ventro-ventral ray (*vv*) and bristle like protuberance (*vv1*), ventro-lateral ray (*vl*), externo-lateral ray (*el*), medio-lateral ray (*ml*), postero-lateral ray (*pl*), externo-dorsal ray (*ed*)

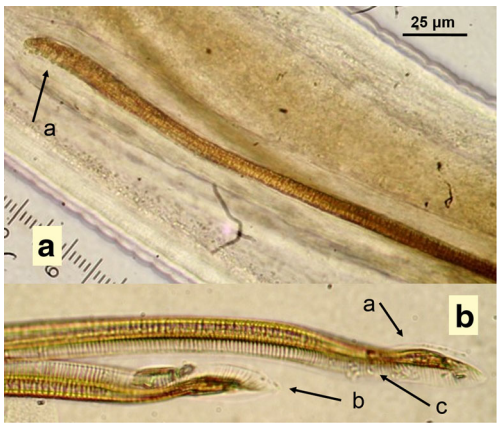

Fig. 9 Spicules of a male *A. chabaudi*. **a** Proximal extremity, showing slight dilatation (*a*). **b** Distal extremity (note the transversal striations), long spicule (*a*), short spicule (*b*), hyaline expansion (*c*)

Table 1 Measurements of *A. chabaudi* specimens found in a wildcat in Northern Greece, in comparison with the measurements obtained from specimens previously recorded in wildcats and domestic cats. Measurements are in micrometre unless otherwise stated

	Biocca 1957		Varcasia et al. 2014		Traversa et al. 2015		Present study	
	Male	Female	Male	Female	Male	Female	Male	Female
Total body length	14.6–16.3 mm	19.8–24.1 mm	–	16.9 mm	9.0 mm	19.0 mm	14–18 mm	21–22 mm
Body width	185.0–225.0	245.0–298.0	–	195.8	79.0	103.7	193–200	260–270
Cephalic vesicle width	–	–	–	–	–	–		125
Cephalic vesicle length	–	–	–	–	–	–		200
Cuticle thickness			–				6	6.25
Oesophagus length	300.0–345.0	345.0–380.0	–	276.6	275.0	338.0	330	360
Oesophagus width at the vulva	–	–	–	–	–	–	45	50–55
Distance excretory pore to cephalic extremity	335.0–405.0	395.0–470.0	–	361.5	–	370.0	370	410–430
Distance vulva to posterior extremity	–	170.0–210.0	–	160.6	–	264.1	–	204–210
Distance vulva to end of the body (without cuticle margin)	–	–	–	–	–	–	–	180–185
Distance vulva to anus	–	–	–	–	–	–	–	135–142
Spicules length	510.0–555.0	–	–	–	503.3–515.1	–	Longer: 588–610 Shorter: 576–597	–
Distance anus to caudal end			–	43.4	–	64.9	–	67–72
Distance anus to end of the body (without cuticle margin)	–	–	–	–	–	–	–	43–45

Napoli et al. 2015). However, there are some recent reports of aelurostrongylosis in *F. s. silvestris* (Veronesi et al. 2015). indicating that some environmental conditions facilitate the passage from domestic to wild cats. On the other hand, the lungworm *T. brevior* was until recently affiliated exclusively to wild felids (Traversa and Di Cesare 2013). Indeed, *F. s. silvestris* is the natural reservoir of *T. brevior*, as shown by the high percentage of infection (up to 71.4 %) in wildcats in Italy (Falsone et al. 2014; Veronesi et al. 2015). However, in the last few years, *T. brevior* has been increasingly reported from domestic cats in different parts of Southern Europe (Jefferies et al. 2010; Brianti et al. 2012, 2013, 2014; Di Cesare et al. 2014a, 2014b; Diakou et al. 2014, 2015) showing that this neglected parasite may also infect domestic cats, mostly in areas where the natural wild reservoir is present (Di Cesare et al. 2015b). but also in territories where wildcats are not being recorded (Diakou et al. 2015).

Fig. 10 First stage larva (L1) of *A. chabaudi*. **a** Whole larva showing anterior (*a*) and posterior extremity (*b*). **b** Anterior extremity. **c** Posterior extremity

Similarly, *A. chabaudi* has been considered a parasite of wildcats, as, in the first description, it was found in six out of seven (85.7 %) examined animals and, at the same time, it was not found in hundreds of other species (i.e. domestic cats, dogs, red foxes and badgers) examined from the same area in the same period (Biocca 1957). However, L1 were neither described nor mentioned in any kind of biological material, leaving a gap in the morphological description of the diagnostic stage of the parasite and an unanswered question of whether the parasite can complete its life cycle in wildcats.

It is hard to explain why since the description of Biocca (1957) there were not any reports of this nematode until pretty recently, when parasites identified as *A. chabaudi* were

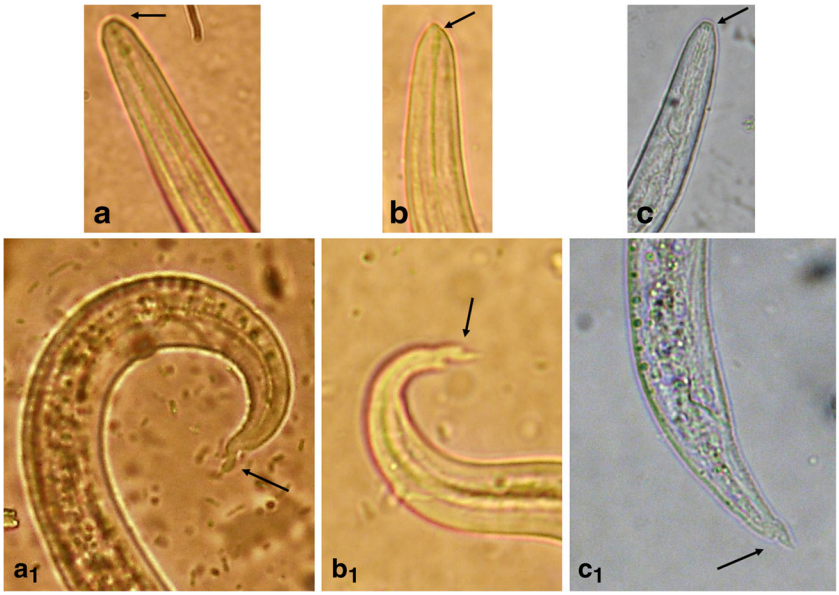

Fig. 11 Morphological comparison of first stage larvae (L1) of three metastrongylid nematodes affecting felids: *A. abstrusus* (*a*, a_1), *T. brevior* (*b*, b_1), *A. chabaudi* (*c*, c_1). **a**, Anterior extremity of *A. abstrusus* showing apical "plateau" and a terminal oral opening (*arrow*); **b**, *T. brevior* showing a pointed head and the sub-terminal oral opening (*arrow*); **c**, *A. chabaudi* showing a narrower plateau than *A. abstrusus* and a terminal oral opening (*arrow*). a_1, S-shaped posterior extremity of *A. abstrusus* showing a prominent dorsal kink, deep dorsal and ventral incisures and terminal knob-like endings. b_1, S-shaped posterior extremity of *T. brevior* with a shallow dorsal kink, deep dorsal (*arrow*) and shallow ventral (*arrowhead*) incisures and straight endings. c_1, Posterior extremity of *A. chabaudi* with a small dorsal spine and notch, and short sigmoid ending

unequivocally found in two domestic cats in Italy (Varcasia et al. 2014; Traversa et al. 2015). More specifically, 57 years after its first description, one specimen of *A. chabaudi* was found in a road-killed domestic cat in Sardinia (Varcasia et al. 2014). The genetic analysis of the parasite showed 99 % identity with a specimen of *Angiostrongylus* sp. recovered from the pulmonary artery of a wildcat in Germany (Steeb et al. 2014). Importantly, this specimen was considered an immature or unfertilized female and no first stage larvae of the parasite were found in the faeces of that cat (Varcasia et al. 2014).

One year later, a natural mixed infection by *A. abstrusus*, *T. brevior* and *A. chabaudi* in a domestic cat was described in central Italy (Traversa et al. 2015). In that case, one male and one female parasite, morphologically and genetically identified as *A. chabaudi*, were collected from the proximal pulmonary arteries of the animal. The ribosomal sequences of these nematodes displayed 99–100 % identity with those of the parasites previously found in Germany and Italy. Once more, no L1 were detected in the faeces of the animal, leaving unanswered the question whether *A. chabaudi* reaches sexual maturity and mate in domestic cats (Traversa et al. 2015).

Conversely, a high number of L1 were found in the lung parenchyma (i.e. histological examination), in the pulmonary lavage and in the faeces of the animal of the present report from Greece. These data fill an important gap in the morphological description of *A. chabaudi* diagnostic stage and

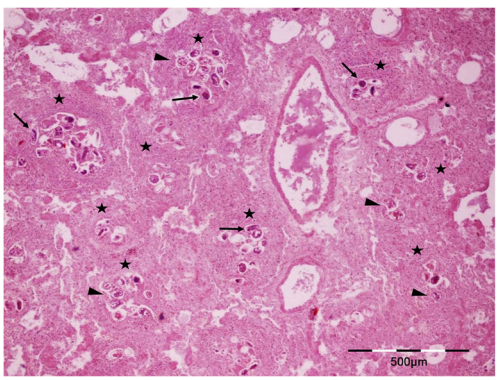

Fig. 12 Histological examination (haematoxylin-eosin staining) of the lung of the wildcat infected with *A. chabaudi*. Numerous granulomatous foci (*asterisks*) around numerous parasitic larvae (*arrowheads*) and eggs (*black arrows*)

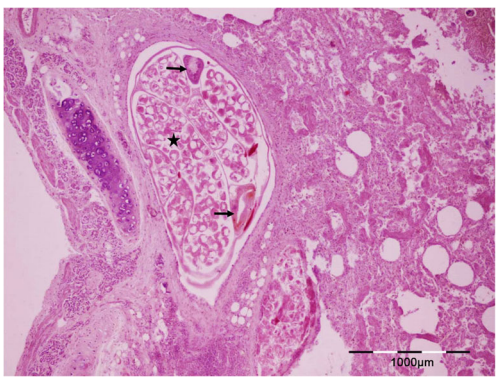

Fig. 13 Histological examination (haematoxylin-eosin staining) of the lung of the wildcat infected with *A. chabaudi*, adult parasite in the lumen of a vessel. Note the uterus (*asterisk*) and the digestive track (*arrows*)

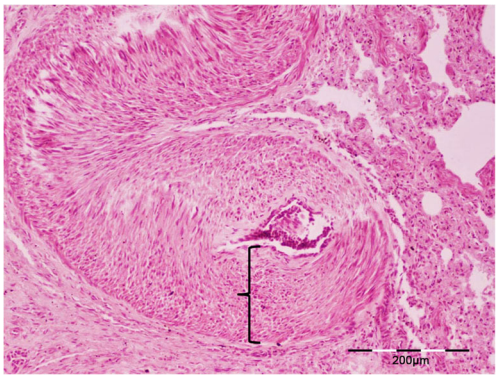

Fig. 14 Histological examination (haematoxylin-eosin staining) of the lung of the wildcat infected with *A. chabaudi*. Artery presenting severe thickening (*bracket*) due to smooth muscle hypertrophy

provide evidence on the completion of the parasite's life cycle in *F. s. silvestris*, which should be now considered ultimately a definitive host of this parasite.

The morphological description of *A. chabaudi* L1 brings important information for the differentiation of these larvae from other larvae with overlapping characteristics, such as *A. abstrusus* and *T. brevior* that can be found in feline samples. It is important to note that metastrongyloid L1 share common general morphological features, such as transparent body and sigmoid tail. However, the differentiating characteristics of the L1 of these species are (a) the body length, although similar or, in some cases, overlapping, i.e. mean length 372.5–399.1 μm for *A. abstrusus*, 323.5–339.3 μm for *T. brevior* (Brianti et al. 2012; Di Cesare et al. 2014a) and 362–400 μm for *A. chabaudi* (present work), and most importantly (b) the morphology of the posterior end in combination with the characteristics of the anterior end (Fig. 11). More specifically, the anterior extremity of *A. abstrusus* has an apical "plateau" and terminal oral opening, the anterior extremity of *T. brevior* is pointed with a sub-terminal oral opening, while *A. chabaudi* has a narrower plateau than *A. abstrusus* and a terminal oral opening. Moreover, the posterior extremity of *A. abstrusus* is S-shaped, with a prominent dorsal kink, deep dorsal and ventral incisures and terminal knob-like endings. The posterior extremity of *T. brevior* is also S-shaped, with shallow or even non-existent dorsal kink, deep dorsal and shallow ventral incisures and straight endings. The posterior extremity of *A. chabaudi* has a more "clean-cut" appearance, showing a small dorsal spine and notch, and short sigmoid ending (Fig. 11).

In general, the morphometric and morphological characteristics of the adult parasites here retrieved were in accordance with those originally described by Biocca (1957) (Table 1). However, in that first description, transversal striation of the cuticle was not described, whilst a longitudinal striation was reported. Nonetheless, this characteristic was particularly visible in fixed specimens, so it could be either an artefact or indiscernible in fresh specimens (Biocca 1957).

Until now, the pathogenicity of *A. chabaudi* has not been established. The post mortem findings of Biocca (1957) and Varcasia et al. (2014) did not report any pathological or clinical evaluation of the infected animals, and no histological examination of the lungs and the heart of these animals was performed. Also, in the case reported by Traversa et al. (2015). the pathogenic potential of *A. chabaudi* could not be determined because there were no pathological findings consistent with angiostrongylosis. In the present case, although a clinical evaluation was not possible, the histological examination of the lung tissue revealed several lesions ascribed to the presence of adult nematodes, eggs and migrating larvae of *A. chabaudi*. In this context, the marked hypertrophy and thickening of the arterial wall (Fig. 14) is most likely a result of the pulmonary hypertension caused by the presence of nematodes in the pulmonary arteries (Borgeat et al. 2015). as in canine angiostrongylosis by *Angiostrongylus vasorum* (Helm et al. 2010). As no other cardiopulmonary parasites were found and the heart showed no anatomical or other lesions, the pathological findings in the lungs and the pulmonary arteries should be exclusively attributed to *A. chabaudi*. This suggests that the parasite can play a relevant pathogenic role in infected wildcats.

Although the present findings confirm that the European wildcat (*F. s. silvestris*) is a definitive host of *A. chabaudi*, there are still aspects of the biology and epizootiology of this parasite to be clarified. Remarkably, *A. chabaudi* was recorded in only 1 of 37 wildcats examined post mortem in different sites of Central and Southern Italy in the past few years (Falsone et al. 2014; Veronesi et al. 2015). and no *Angiostrongylus*-like larvae were found in 121 faecal samples of wildcats examined in Sicily (Napoli et al. 2015). This implies that this parasite occurs occasionally even in its definitive host. It would be thus important to investigate the infection prevalence in wildcat populations from various geographic areas, in order to achieve a sound conclusion on how this parasite may impair health and welfare of the European wildcat and if it bears affiliations with specific geographic regions. Moreover, the complete life cycle of *A. chabaudi* is unknown; thus, further investigations in terrestrial molluscs, i.e. the most probable intermediate host, would eventually indicate the species that are involved in the transmission of the parasite and could confer information on their geographic distribution and their link to the dispersal of the disease.

The information currently available casts shadows on the ability of *A. chabaudi* to infect also the domestic cat. In fact, parasite specimens recently described in domestic cats (Varcasia et al. 2014; Traversa et al. 2015) were immature or unfertilized, not fully developed and smaller than those recovered in wildcats (Table 1). Additionally, no microscopic nor genetic evidence of *A. chabaudi* was demonstrated in hundreds of recently examined domestic cats in Italy, even in areas where this parasite had been recorded in the wildcats

(Di Cesare et al. 2015a, b). In this context, the presented description of the L1 of *A. chabaudi* is pivotal for future studies aiming at evaluating whether also the domestic cat acts as definitive host of *A. chabaudi* and whether this parasite truly causes a cardiopulmonary disease in domestic hosts.

Acknowledgments The authors would like to thank Prof. Bernardino Ragni (University of Perugia, Italy) for his contribution in identifying the species of the animal here examined.

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