

Infection with *Rhabdias tokyoensis* (Nematoda: Rhabdiasidae) in European captive-bred swordtail newts, *Cynops ensicauda* (Amphibia: Salamandridae)

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Abstract. Lungworm infections in amphibians are a major cause of die offs in private collections. To date, only four species of the family Rhabdiasidae are known to occur within the order Caudata. We here describe the occurrence of *Rhabdias tokyoensis*, a Far Eastern species, causing intracoelomic and especially intrapulmonary lesions in the natural host *Cynops e. ensicauda* under captive conditions. This is – to the best of our knowledge – the first report on a *Rhabdias tokyoensis* infection in captive-bred *Cynops ensicauda* and on the occurrence of this parasite in European private collections.

Key words. Amphibia, Caudata, Salamandridae, *Cynops ensicauda*, *Rhabdias tokyoensis*, lung worm, rhabdiasis, Europe.

Introduction

Lungworm infections due to members of the nematode genus *Rhabdias* STILES & HAS-SAL, 1905 occur frequently in captive-bred as well as free-living amphibians (WRIGHT & WHITAKER 2001). Their direct life-cycle, which is often complicated by the parasite's geohelminthic heterosexual phase, leads to an enrichment of infectious larvae in the surroundings of the amphibians and therefore poses harm due to superinfections under captive conditions (FRANK 1985). In herpetocultural medicine heavily infested amphibians are regularly presented with clinical signs of rhabdiasis including poor body condition, skin lesions, secondary infections, open mouth-breathing, or sudden death (WRIGHT & WHITAKER 2001).

The alteration of two generations seems to be a prerequisite for most *Rhabdias* spp. and at least for all members occurring in amphibians. During their life cycles a hermaphroditic generation of adult worms is living in the lungs or body cavities of their definite hosts, amphibians and reptiles (CHU 1936, BAKER 1978). Large numbers of parasites are generally not found in the lungs of wild-caught

amphibians, thus raising the possibility that there may be some regulation of the number of worms invading the lungs at any given time from a subadult pool remaining in the body cavity (reviewed by ANDERSON 2000). The adult stages shed their thin walled eggs with embryonated larvae, which are transported by mucous membranes to the upper respiratory tract. They are released orally or frequently swallowed and shed with the faeces. First stage larvae hatch during passage through the intestinal tract or shortly thereafter. From these, larvae reach maturity as males and females outside the host and establish a second gonochoristic generation (FRANK 1985), which in turn gives rise to the hermaphroditic generation. The developing larvae moult twice before reaching the definite host as infective third stage larvae. They usually enter the amphibian by penetrating its skin (transcutaneous route of infection) or by ingestions of a paratenic host (e.g. snails; REICHENBACH-KLINKE 1961). The potential role of such paratenic (= cumulative) hosts in transmission of *Rhabdias* has not been fully clarified, yet, but infective larvae of *R. bufonis* and *R. americanus* will invade and survive in tissues of snails (reviewed by ANDERSON

2000). *Rhabdias* species in reptiles, on the other hand, probably infect the host orally. Within the definite host another two or three moults complete the ontogenetic transformation during the migration towards the lung (WRIGHT & WHITAKER 2001). *Rhabdias* spp. thereby display a relatively strict host specificity (KUZMIN et al. 2001) with individuals of a certain species never parasitize hosts representing multiple orders (RAUSCH et al. 1984). Whereas two members of *Rhabdias* spp. parasitizing Caudata have been known from the Palaearctic for a long time two additional species were recently described from the Nearctic (KUZMIN et al. 2001, 2003).

Two swordtail newts, *Cynops ensicauda ensicauda* (HALLOWELL, 1861), from a private collection were presented for necropsy after their sudden deaths. They were said to have been raised from imported eggs, but were eventually kept together with presumably infected captive-bred conspecifics from a prior sale. The latter originated from imported pet-trade animals and had been in contact with the environment (plants, stones) of their parents. The growing newts were fed a diet gathered from German ponds from time to time. Here we demonstrate with relevance to herpetoculture that under appropriate semi-natural conditions in a terrarium the successful cycle-development of allochthone *Rhabdias* spp. is possible, causing death even in captive-bred offspring.

Material and methods

Pathologic examination was carried out using a dissecting microscope (M3B, Wild, Heerbrugg, Switzerland) with cold-light-device (KL 1500, Schott). For further parasitological studies nematodes were collected, rinsed with tap-water, conserved in 70 % ethanol and mounted on slides using Eukitt (Vogel, Giessen, Germany). Fecal analyses were undertaken with a Diaplan microscope (Leica, Wetzlar, Germany) using magnifications of 25 to 1000-times. Lengths of parasitic details

were taken with a scale-ocular (Leica) with an accuracy of 0.5 μm .

For the species diagnosis of the adult worms the following features were assessed (reference values for *Rhabdias tokyoensis* according to Table 1): body length, body width, buccal capsule width, buccal capsule depth, oesophagus length, posterior bulb of oesophagus width and tail length.

Results

During necropsy, the only abnormal findings were numerous (up to twelve) adult nematodes measuring between 7-12 mm within the newts' body cavities and lungs (Fig. 1). The worms were already dead, because the newts had been frozen prior to submission. According to the characteristic shape of the worms' oesophagus (rhabditiform style in first stage larvae and parasitizing adults) they could be differentiated as belonging to the family Rhabdiasidae. Measurements of certain structures on the basis of table 1 revealed comparable values, especially with respect to the width and depth of the buccal capsule (Fig. 2). We randomly selected five of the isolated worms, which revealed a buccal capsule width of 30-32.5 μm and a depth of 20-21.6 μm . These results are consistent with *Rhabdias tokyoensis* (table 1) and differentiated these worms from other known *Rhabdias* spp. of Caudata (KUZMIN et al. 2003), from

Tab. 1. Measurements of *Rhabdias tokyoensis*, parasitic adults, eight specimens from *Cynops e. ensicauda* (Okinawa, Japan) according to Y. KUZMIN (pers. comm.).

parameter	length
body length	12.45-17.25 mm
body width	506-664 μm
buccal capsule width	30-34 μm
buccal capsule depth	16-20 μm
oesophagus length	548-714 μm
posterior bulb of oesophagus width	96-132 μm
tail length	174-232 μm

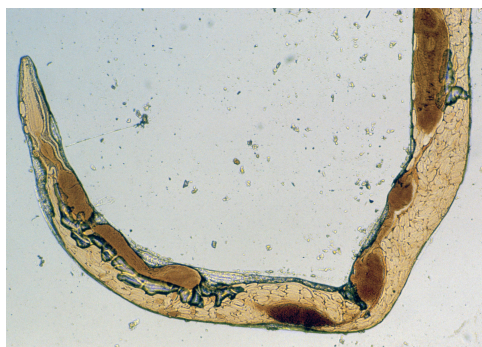


Fig. 1. General view of a parasitic adult *Rhabdias tokyoensis* isolated from the body cavity of a *Cynops e. ensicauda* (fixed sample; anterior end at the left; 25 ×). Photo: T. EISENBERG.

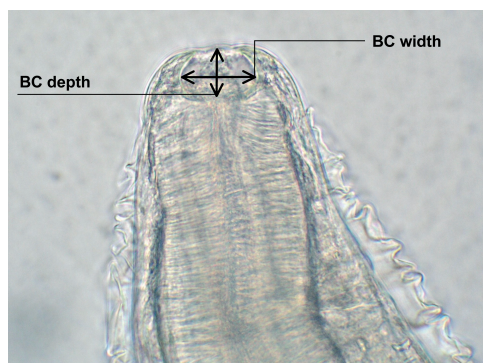


Fig. 2. Anterior end of *Rhabdias tokyoensis* from *Cynops e. ensicauda* with the remarkably large buccal capsule (BC), which distinguishes it from other *Rhabdias* species (600 ×). Photo: N. PANTCHEV.

those of anurans in Europe (the buccal capsule of e. g. *R. bufonis* is just 10–12 μm wide; Y. KUZMIN, pers. comm.) as well as those parasitizing South and North American anurans (MORAVEC & KAISER 1995).

Discussion

Of the roughly 60 currently known species of the family Rhabdiasidae, 22 affect reptiles (colubrids, vipers, chameleons, agamid, anguid, and scincid lizards, KUZMIN & TKACH: “*Rhabdias*” [[dias/ – last accessed 5 January 2008\]; authors’ unpubl. data\) whereas the majority utilize amphibians as hosts. The vast minority is parasitic to the order Caudata with only four species being involved. Of these, *Rhabdias ambystomae* KUZMIN, TKACH & SNYDER, 2001 and *Rhabdias tarichae* KUZMIN, TKACH & SNYDER, 2003 are restricted to North America and *Rhabdias bermani* RAUSCH, RAUSCH & ATRASHKEVICH, 1984 and *Rhabdias tokyoensis* WILKIE, 1930 to the eastern Palearctic region. The latter is known from Japan, identical in distribution to *Cynops ensicauda*, but seems to occur also in other areas, as it was recently described from imported red-tailed knobby newts \(*Tylototriton kweichowensis*; PASMANS et al. 2008\). While the predominant *Rhabdias* spp. in Europe is *R. bufonis*, which is known to infest anuran hosts, reports on parasitism due to lungworms in salamanders and newts are scarce. Interestingly, the experimental infection with *Rhabdias* is also possible in tadpoles \(reviewed by ANDERSON 2000\), but – to our knowledge – not yet reported for eggs. Therefore we assume that this infection resulted from maintenance with infected conspecifics, which were in direct contact to contaminated environmental structures like plants in the terrarium.](http://izan.kiev.ua/ppages/rhab-</p>
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Oral administration of fenbendazole (20 mg/kg three times at one week intervals) was found to be effective against the *Rhabdias tokyoensis* infection in imported red-tailed knobby newts (*Tylototriton kweichowensis*; PASMANS et al. 2008).

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