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STUDY OF BIOLOGY, MORPHOLOGY AND TAXONOMY OF THE NEMATODE *STEPHANOFILARIA ASSAMENSIS* (FILARIINA, STEPHANOFILARIIDAE)

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Study of Biology, Morphology and Taxonomy of the Nematode *Stephanofilaria assamensis* (Filariina, Stephanofilariidae). Saparov K. A., Akramova F. D., Azimov D. A., Golovanov V. I. — Biological characters of *Stephanofilaria assamensis* Pande, 1936, a parasite of cattle, were studied under environmental conditions of Uzbekistan with the emphasis on morphology of all developmental stages. Bloodsucking flies *Haematobia atripalpis* Bezzi, 1895, *Lyperosia titillans* Bezzi, 1907 and *L. irritans* Linnaeus, 1758 were established as intermediate hosts. Prevalence of the nematode larvae in the flies constituted 2.1, 1.5, and 1.2 %, respectively. Characters for the species identification were revised. On that basis *S. zaheeri* Singh, 1958 is regarded as a synonym of *S. assamensis*. The place of *Stephanofilaria* genus in the system of Stephanofilariidae is discussed.

Key words: Stephanofilaria, biology, morphology, taxonomy, dipteran, infective larva, microfilaria.

Исследование биологии, морфологии и таксономии нематоды *Stephanofilaria assamensis* (Filariina, Stephanofilariidae). Сапаров К. А., Акрамова Ф. Д., Азимов Д. А., Голованов В. И. — Исследованы особенности биологии *Stephanofilaria assamensis* Pande, 1936 — паразита крупного рогатого скота, в условиях Узбекистана с акцентом на морфологию всех фаз развития. В качестве промежуточных хозяев нематоды *S. assamensis* установлены кровососущие мухи: *Haematobia atripalpis* Bezzi, 1895, *Lyperosia titillans* Bezzi, 1907 и *L. irritans* Linnaeus, 1758. Заражённость мух личинками нематод *S. assamensis* составила 2,1; 1,5 и 1,2 % соответственно. Выявлены признаки для видовой дифференциации, на основании которых *S. zaheeri* Singh, 1958 рассматривается в качестве синонима *S. assamensis*. Ведётся полемика о месте рода *Stephanofilaria* в системе Stephanofilariidae.

Ключевые слова: стефанофилярия, биология, морфология, таксономия, двукрылые насекомые, инвазионная личинка, микрофилярия.

Introduction

The problem of biology and life cycles of parasites still is one of the main issues of general parasitology. This problem applies to the nematode genus *Stephanofilaria* Ihle et Ihle-Landenberg, 1933 as well. About ten species are numbered in the genus currently (Wehr, 1935; Anderson, 1958; Sonin, 1977; Johnson, 1989; Mercky, 2002). Some species validity is dubious due to the lack of their detailed descriptions. There are disagreements in species composition of the genus *Stephanofilaria* and eligibility of the family Stephanofilariidae. These assume investigations of some species biology with an emphasis on morphological characters of *Stephanofilaria* in all ontogenic stages and ascertainment of the genus *Stephanofilaria* and the family Stephanofilariidae place in the system of the suborder Filariina.

The objectives of provided investigations are to study the biology and life cycle of *Stephanofilaria assamensis* Pande, 1936 nematodes in terrestrial coenoses of Uzbekistan and to determine the taxonomical value of diagnostic characters in all stages of ontogenesis for the progress in clarification of the problem.

Material and methods

Data for the work were collected during field and experimental investigations of *S. assamensis* biology and life cycle. Insects, that could be potential intermediate hosts of the nematodes, were sampled in locations of cattle herds on farms of the north-eastern and southern parts of Uzbekistan in 2009–2012. More than 5000 specimens of dipterans were collected and analyzed according to the standard technique (Agrinsky, 1962). Insects were trapped in the points of animal concentration outdoors during summer season, from May to October (fig. 1).

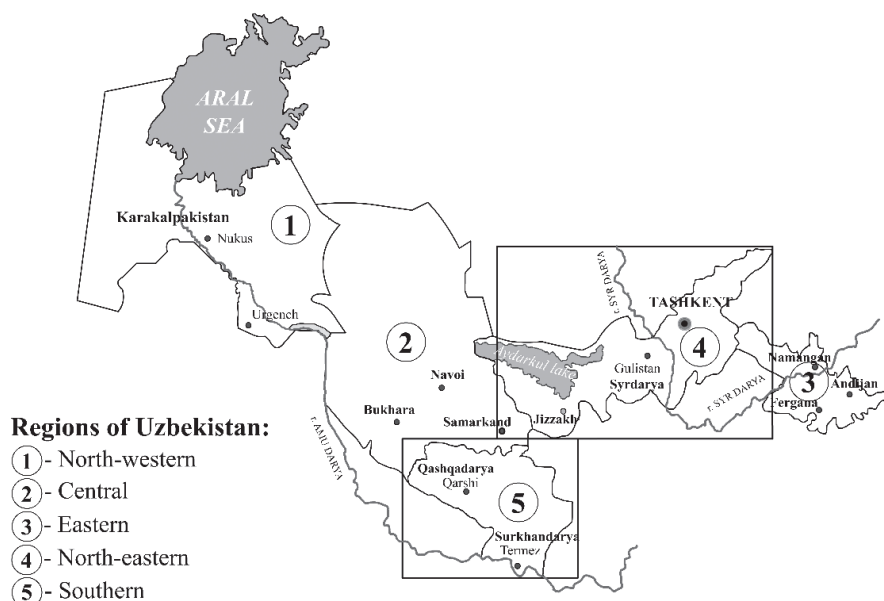


Fig. 1. Map of Uzbekistan: places of material collection.

Рис. 1. Карта Узбекистана: места сбора материала.

Captured insects were killed by ether and placed into the Petri dishes or watch glasses. After identification of species¹ each specimen was transferred on a microscope slide in a drop of normal saline solution, then dissected using preparation needles and examined under light microscope to detect *Stephanofilaria* larvae. The compression method was also used. Accordingly, five or six specimens of dipterans (without legs and wings) were mounted on a microscope slide, a drop of warm (20–25 °C) normal saline solution was added, then a cover glass was set with a slight pressure and microscopically examined. The major part of detected nematode larvae were studied alive and the other part of larvae were fixed in 1.5–2.0 % formaldehyde solution. Larvae were placed on a microscope slide in a drop of normal saline solution and immobilized by warming over a spirit-lamp, then covered by a cover glass and examined under the microscope. The methods developed by different researchers (Rahman, 1957; Nelson, 1959, 1960; Nelson, Pester, 1962; Patnaik, Roy, 1966, 1968; Kabilov, 1983; Johnson, 1989; Anderson, 2000) were used for identification of infective larvae. *Stephanofilaria assamensis* larvae collected from bloodsucking flies were used to infect two calves (steers number 8 and 9). Infective larvae were injected intradermally at multiple sites of the calf neck, 275 larval specimens per each calf. The steer number 10 was a control one. Experimental infection of animals was carried out on June 25, 2010. The experimental animals and animals of control groups were kept under the same environmental conditions. Specimens of 66 adult nematodes (35 ♂, 31 ♀) and 48 larvae (microfilariae — 28, infective larvae — 20) were the subject of the morphometric study.

Results and discussion

Examination of collected insects had revealed contamination by *S. assamensis* Pande, 1936 larvae only in the species of bloodsucking flies from the family Muscidae: *Haematobia atripalpis* Bezzi, 1895 *Lyperosia titillans* Bezzi, 1907 and *L. irritans* Linnaeus, 1758 (table 1).

Infestation of the insects by *Stephanofilaria* larvae was found in warm season, from May to October. Maximum infection rate was registered in late May and early September. This implies that cattle are infected by *Stephanofilaria* in spring, summer and early autumn. Prevalence of nematode larvae was the largest in the flies *H. atripalpis* (2.1 %), followed by *L. titillans* (1.5 %) and *L. irritans* (1.2 %) (table 1).

Body measurements of *S. assamensis* infective larvae were the following: length 0.810–1.120 mm and width 0.040–0.050 mm. The larva body is narrowing towards anterior and posterior ends. Head end with small projection in a shape of a crown, aciculose, with 23–24 spines. Well defined nerve-ring is about 0.026–0.048 mm from the anterior end of the

¹ Species identification of dipterans was carried out by A. Sh. Hamraev and D. B. Daminova.

Table 1. Infection rate of blood-sucking flies by the *Stephanofilaria assamensis* larvae under natural conditions of Uzbekistan**Таблица 1.** Заражённость кровососущих мух личинками *Stephanofilaria assamensis* в природных условиях Узбекистана

Species of bloodsucking flies	Examined		
	Total, number of specimens	Infected number of specimens	Intensity of infection
<i>Stomoxys calcitrans</i>	1005	—	—
<i>Haematobia stimulans</i>	1002	—	—
<i>Haematobia atripalpis</i>	1010	21 (2.1 %)	5–103
<i>Lyperosia irritans</i>	1009	12 (1.2 %)	3–15
<i>Lyperosia titillans</i>	1007	16 (1.5 %)	3–9

body (fig. 2). Our data conform to those of other researchers (Rahman, 1957; Patnaik, Roy, 1968; Shamsul, 1971; Kabilov, 1983; Duut, 1970, l. c.: Anderson, 2000).

Thus, different species of bloodsucking flies: *Musca conducens*, *M. planiceps*, *Lyperosia irritans*, *L. titillans* and *Haematobia atripalpis*, were found to be the intermediate hosts in the natural habitats of *S. assamensis*. Hence, microfilariae can penetrate into the fly's organism during sucking, with a blood from skin lesions of infected cattle individuals. In the bloodsucker's body, microfilariae undergo a series of developmental stages that are accompanied by transformation of organs and systems. Larvae molt twice and become infective. Under repeated sucking, *Stephanofilaria* infective larvae get from the proboscis of flies into the tissues of appropriate definitive host where they develop up to puberty. The results of our examinations support this view.

Observations shown bleeding ulcerations of outer auricle in infected calves number 8 and 9 55–65 days after infection. Mature nematodes (males and females) and numerous microfilariae, as well as some eggs of the parasite, were revealed embedded in the skin reticular layer of the studied animals. Dissected out of the lesions mature nematodes were quite thin, females were larger than males (fig. 3, table 2).

Field and experimental investigations revealed that the life cycle of *S. assamensis* involves the blood-sucking flies *H. atripalpis*, *L. titillans* and *L. irritans* under environmental conditions of Uzbekistan. According to the published data, bloodsucking flies *Musca planiceps*, *M. autumnalis*, *Lyperosia titillans* and *L. irritans* are responsible for the transmission of *S. assamensis* (Rahman, 1957; Patnaik, Roy, 1968; Shamsul, 1971; Kabilov, 1983; Johnson, 1987, 1989; Mercky, 2002). The list of intermediate hosts of *S. assamensis* is supplemented by us with the additional species, *Haematobia atripalpis*.

Data analysis of the original study and publications on morphology and biology of the nematode *S. assamensis* let to assess taxonomical value of the complex of characters in different developmental stages.

The diagnostic characters of males appeared to be the most suitable for the species identification. Particularly, the spicules possess more or less wide range of specific morphological characters that satisfy the requirements of the morphological criterion of species.

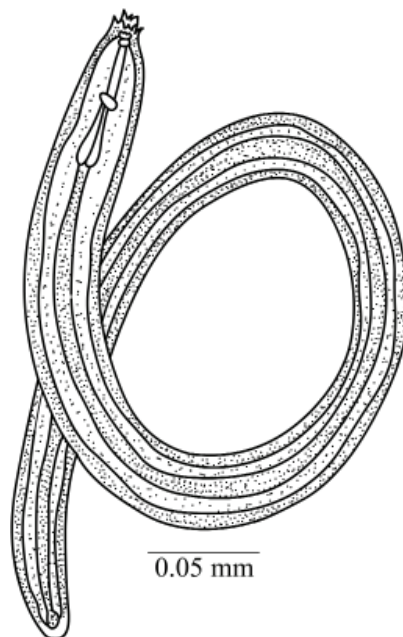


Fig. 2. *Stephanofilaria assamensis*, infective larva isolated from *Haematobia atripalpis*.

Рис. 2. *Stephanofilaria assamensis*, инвазионная личинка, выделенная из *Haematobia atripalpis*.

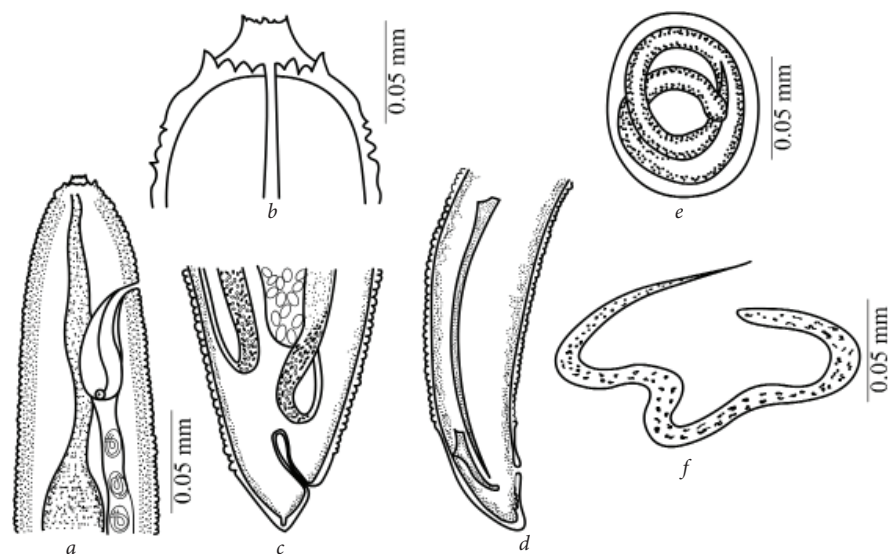


Fig. 3. *Stephanofilaria assamensis*: a — anterior end of female, lateral view; b — head end; c — tail end of female, lateral view; d — tail end of male; e — egg with larva; f — microfilaria.

Рис. 3. *Stephanofilaria assamensis*: a — передний конец самки, латерально; b — головной конец; c — хвостовой конец самки, латерально; d — то же самца; e — яйцо с личинкой; f — микрофилярия.

The whole complex of characters has uneven taxonomic value. The arrangement and number of spines and caudal papillae, both in males and females, revealed most reliable characters regarding their diversification and taxonomic value. Taxonomic application of metric characters of males, females and microfilariae is limited due to high level of their variability associated with the host organisms and geographical distribution.

Table 2. General characteristics of *Stephanofilaria assamensis* by original data (sizes in millimeter)

Таблица 2. Общая характеристика *Stephanofilaria assamensis* по оригинальным данным (размеры приведены в миллиметрах)

Characters	Ranges
Male	
Body length	2.5–6.0
Body width	0.08–0.12
Esophagus	0.12–0.20
Left spicule length	0.133–0.230
Right spicule length	0.042–0.063
Distance from the anterior end to nerve-ring	0.060–0.080
Postcloacal papillae	2–3 pairs
Precloacal papillae	6–8 pairs
Peribuccal spines	14–18
Cephalic spines	18–24
Female	
Body length	7.0–13.6
Body width	0.10–0.21
Esophagus	0.16–0.45
Distance to the vulva from the anterior end	0.075–0.113
Distance to nerve-ring from the anterior end	0.063–0.099
Peribuccal spines	14–18
Cephalic spines	16–24
Microfilariae	0.11–0.14
Definitive host	<i>Bos taurus</i>
Intermediate hosts	<i>Haematobia atripalpis</i> , <i>Lyperosia titillans</i> , <i>L. irritans</i>
Place of distribution	Uzbekistan

The presence of stable amount of cephalic spines in infective larvae makes them suitable for the species diagnostics. Despite the extensive studies on *S. assamensis* morphology and biology, the validity of this nematode taxon is under consideration: the views of researchers are inconsistent or sometimes are alternative. It concerns the species *S. assamensis* and *S. zaheeri*. Under some views (Singh, 1958; Patnaik, 1964) both species are valid. Other researchers (Patnaik, 1964; Patnaik, Roy, 1968) ascertain that *S. zaheeri* is a probable synonym of *S. assamensis*, since the number of spines on the anterior end and length of female bodies are the same in both species. However, the lateral alae have been never registered in *S. assamensis*. Extra morphological examination of *S. assamensis* parasitizing buffalo and domestic goats allowed Patnaik, Roy (1968) to reduce *S. zaheeri* to the synonyms of *S. assamensis*. Nevertheless, Johnson (1987) assumed *S. assamensis* as one of the synonyms of *S. dedoesi*. In the later publication, Johnson (1989), based on the data of *S. dedoesi* cephalic ornamentation study using scanning electron microscopy and analysis of morphological characters, had recognized as valid the following species: *S. dedoesi* Ihle et Ihle-Landenberg, 1933; *S. zaheeri* Singh, 1958; *S. dinniki* Round, 1964 and *S. stilesi* Chitwood, 1934. Even so, this researcher considers *S. assamensis* as a synonym of *S. dedoesi*. At the same time, structure and size of the left spicule and number of cephalic spines differ in the named nematode species. This provides an evidence of erroneous statements of Johnson (1987, 1989). Generally, the validity of the species *S. assamensis* and *S. dedoesi* in the system of the genus *Stephanofilaria* is supported by the data of numerous examinations carried out by different researchers (Ihle, Ihle-Landenberg, 1933; Patnaik, Roy, 1968; Shamsul, 1971; Sonin, 1977; Mercky, 2002).

According to our point of view, some publications are inconsistent in application of the priority principle adopted in the "International Code of Zoological Nomenclature" (1999) with regard to acceptance of *S. zaheeri* as a valid taxon name in the system of the genus *Stephanofilaria*. The fact is that *S. zaheeri* was described as a new taxon by Singh (1958). Later the name was transferred (Patnaik, Roy, 1968) to the synonym of the valid species *S. assamensis* Pande, 1936. In the examined case *S. assamensis* described in 1936 (Pande, 1936) should be recognized as senior taxon under the priority principle of the Code. Therefore, the validity should be preserved for *S. assamensis*, and *S. zaheeri* becomes a synonym of the above mentioned. The authors adhere to this view.

Besides, noteworthy are the various interpretations of the genus *Stephanofilaria* place in the system of the suborder Filariina. The genus *Stephanofilaria* is transferred from one family to the other under hypothesis of different researchers. Without going into details, we recognize the statement of Wehr (Wehr, 1935) as optimum, according to which the genus is assigned to a separate family Stephanofiliariidae. The Wehr's statement is based on the morphobiological characters and localization of the species *Stephanofilaria* in the definitive host. Thus, evolvement of the specific morphological characters and unique ontogeny of Stephanofiliariidae, distinguishing it from other known families of the suborder Filariina, contributed to ecological isolation of nematodes from this family.

Conclusions

Bloodsucking flies of *Haematobia atripalpis*, *Lyperosia titillans* and *L. irritans* were established as intermediate hosts of the nematode *S. assamensis* in Uzbekistan. Studied flies were infected by *S. assamensis* larvae (prevalence of infection 2.1, 1.5 and 1.2 %, respectively) in locations of cattle concentration under environmental conditions of the republic. Maximum infestation was observed in summer.

The life cycle of the nematode *S. assamensis* was experimentally reproduced by infection of the sampled cattle (calves) with infective larvae collected from the bloodsucking flies. *Haematobia atripalpis* was added to the list of intermediate hosts of *S. assamensis*.

The characters with taxonomical value in different developmental stages were specified on the basis of the data analysis of the original study and publications on morphology and biology of the nematode *S. assamensis*. Most reliable characters for the species identifica-

tion are the spicule structure of males, the number and arrangement of spines and caudal papillae, both in males and females, and the number of cephalic spines of infective larvae.

Results of the study clarify the understanding of the nematodes *S. assamensis* and *S. zaheeri* position in the system of the genus *Stephanofilaria*. Conforming to the views of other researchers (Anderson, 1958, 2000; Patnaik, Roy, 1968; Sonin, 1977) *S. zaheeri* is regarded as a synonym of *S. assamensis* in the present publication.

As for the place of the genus *Stephanofilaria* and the family Stephanofiliariidae in the system of the suborder Filariina, without going into analysis of existing interpretations, the opinion of Wehr (1935), Chabaud and Choquet (1953), Skrjabin and Ivashkin (1968) is supported in the present publication: the genus *Stephanofilaria* is considered as a part of the valid family Stephanofiliariidae. Morphological and biological characters of Stephanofiliariidae representatives differ them clearly distinguish them from Setariidae, Onchocercidae and Filariidae; these species are worth to be segregated and classified in the rank of separate family within the suborder Filariina.

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