INTERSPECIFIC VARIABILITY OF FILARIOID NEMATODES OF THE GENUS *SETARIA* VIBORG, 1795 OCCURRING IN WILD RUMINANTS IN EUROPE: A REVIEW*

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Nematodes of the genus *Setaria* (Spirurida, Filarioidea) are parasites of vertebrates except fish. Dangerous are their larvae (microfilariae) that may migrate to the central nervous system of hosts causing serious neuropathic disorders of vertebrates including human. *Setaria cervi*, *S. labiatopapillosa*, and *S. tundra* are potential parasites in wild ruminants in Europe. The most recent studies of variability among *Setaria* spp. are based on a combination of morphometric and molecular methods. Although there is no clear consistency in the morphological structures used by different authors, a morphological key was compiled based on the body length, the arrangement of peribucal crown, and the number of papillae in the caudal part of the body. The barcoding system of *Setaria* genus is based on sequences of conserved gene polymorphisms such as *COX1* and rDNA genes. The published sequences of *COX1* gene in *Setaria* populations were analysed using *in silico* phylogenetic analysis by the maximum likelihood method (Tamura-Nei model). This analysis confirmed that the *COX1* nucleotide polymorphisms genes are species-specific and represent the theoretical basis for the development of markers enabling barcoding system in the genus *Setaria*.

Setaria, cervidae, morphology, rDNA, *COX1*, species discrimination

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INTRODUCTION

Filarioid viviparous nematodes of the genus *Setaria* Viborg, 1795 occur in the tissues, blood, and peritoneal cavity of all vertebrates except fish. They are common in cattle and other ungulates and horses (Anderson, 2000; Tung et al., 2003; Nakano et al., 2007) and represent the most important species of filarioids found in ruminant cloven-hoofed game (Rehbein, 2014). They can occur freely in the peritoneal cavity, on the surface of the intestines and mesentery, on the peritoneal wall, lungs, liver, heart, urinary bladder, uterus and fascia (Davoodi, 2014; Sundar, D´Souza, 2015), in the subcutaneous ligaments (Husak et al., 1986) and are easily visible when removing organs of the wild game (Chroust, Forejtek, 2010).

Six *Setaria* species have been registered in Europe (Gibson et al., 2014). The occurrence of *S. cervi*, *S. tundra*, *S. transcaucasica,* and *S. labiatopapillosa* has been proven in wild ruminants (Schwangart, 1940; Liang-Sheng, 1959; Shoho, 1959; Blazek et al., 1968; Kostyaeva, Kostyaev, 1969; Shol, Drobishchenko, 1973; Kotrla, 1984; Husak et al., 1986; Fei et al., 1992; Barus, 1994; Hai et al., 1995; Jaervis, 1995; Aguirre et al., 1999; Anderson, 2000; Rehbein, Visser, 2007; Laaksonen et al., 2009a; Chroust, Forejtek, 2010; Kowal et al., 2013; Rehbein et al., 2014;

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Table 1. Recent species of the genus *Setaria* (Filaroidea) occurring worldwide

Species	Species
Setaria africana **(Yeh, 1959)**	*Setaria kabargi* **Kadenazii, 1948**
Setaria bernardi **Railliet & Henry, 1911**	**Setaria labiatopapillosa* **(Alessandrini, 1848)**
Setaria bicoronata **(Linstow, 1901)**	*Setaria lamyfortensis* Troncy, Graber & Thal, 1968
Setaria bidentata **(Molin, 1858)**	*Setaria longicauda* **Chabaud & Rousselot, 1956**
Setaria boulengeri **Thwaite, 1927**	*Setaria loveridgei* **Sandground, 1928**
Setaria castroi **Ortlepp, 1964**	*Setaria machadoi* **Desset, 1966**
Setaria cervi* **(Rudolphi, 1819)	*Setaria marshalli* **(Boulenger, 1921)**
Setaria congolensis **Railliet & Henry, 1911**	*Setaria pillersi* **Thwaite, 1927**
Setaria cornuta **(von Linstow, 1899)**	*Setaria poultoni* **Thwaite, 1927**
Setaria digitata* **(Linstow, 1906)	*Setaria rodhaini* **Van Den Berghe & Vuylsteke, 1936**
Setaria dipetalonematoides **Chabaud & Rousselot, 1956**	*Setaria saegeri* **Le Van Hoa, 1962**
Setaria dubosti **Desset, 1966**	*Setaria sandersoni* **Baylis, 1936**
Setaria effilaria **(Linstow, 1897)**	*Setaria scalprum* **(Linstow, 1908)**
Setaria equina* **(Abildgaard, 1789)	*Setaria shohoi* **Desset, 1965**
Setaria gagarini **Mamedov, 1970**	*Setaria southwelli* **Thwaite, 1927**
Setaria gaillardi **Desset, 1966**	*Setaria thomasi* **Sandosham, 1954**
Setaria graberi Shoho in Troncy, Graber & Thal, 1976	*Setaria thwaitei* **Mönnig, 1933**
Setaria hartwichi **(Yeh, 1959)**	**Setaria transcaucasica* **Assadov, 1952**
Setaria hornbyi **Boulenger, 1921**	*Setaria transversata* **(Linstow, 1907)**
Setaria hyracis **Baylis, 1932**	**Setaria tundra* **Isaichikov & Rajewskaja, 1928**
Setaria indica (Dutt, 1963)	*Setaria yehi* **Desset, 1966**
Setaria javensis **Vevers, 1923**	*Setaria yorkei* **Thwaite, 1927**

*species that can be considered as potential parasites of vertebrates in Europe (Gibson et al., 2014)

Demiaszkiewicz et al., 2015; Rajsky, 2015; Angelone-Alasaad et al., 2016; Enemark et al., 2017). *S. digitata* and *S. equina* are parasites of other animals.

As significant determination characteristics can be considered the arrangement of the peribucal crown and the caudal body end (Becklund, Walker, 1969; Shoho, Uni, 1977; Zdarska, Scholl, 1978; Nakano et al., 2007; Kowal et al., 2013; Sundar, D'Souza, 2015; Kumar, Kumar, 2016; Enemark et al., 2017).

For unambiguous taxonomic classification of the genus *Setaria* a combination of morphometric methods and molecular techniques is essential.

Taxonomy of the *Setaria* genus

The genus *Setaria* Viborg, 1795 belongs to the order Spirurida and the superfamily Filarioidea (Gibson et al., 2014). Forty-four species of the genus *Setaria* are valid at this time occurring worldwide (Table 1). The rather problematic discrimination of the species according to morphology suggests that some synonyms may be present in this large species spectrum (Burger et al., 2006; Taylor et al., 2015), however, molecular approaches will help to resolve this problem in the future.

Variability of wild ruminants parasitised by *Setaria* species in Europe

According to Gibson et al. (2014), six *Setaria* species have been registered in Europe (see Table 1).

The relatively broad European occurrence of *Setaria cervi* has been proven in roe deer (*Capreolus capreolus*), red deer (*Cervus elaphus*), caucasian deer (*Cervus elaphus maral*), sika deer (*Cervus nippon*) and in fallow deer (*Dama dama*) (Schwangart, 1940; Blazek et al., 1968; Kostyaeva, Kostyaev, 1969; Shol, Drobishchenko, 1973; Husak et al., 1986; Aguirre et al., 1999; Rehbein, Visser, 2007; Chroust, Forejtek, 2010; Rehbein et al., 2014; Demiaszkiewicz et al., 2015; Rajsky, 2015).

Setaria labiatopapillosa is the most widespread congener in Europe, and usually can be detected in cattle, sheep and goats (Soulsby, 1982; Pietrobelli et al., 1995; Anderson, 2000; Khedri et al., 2014), but the occurrence has been proven also in

deer (*Cervus* spp.), sika deer, and Formosan sika deer (*C. nippon taiouanus*) (Fei et al., 1992; Hai et al., 1995; Anderson, 2000).

The next broadly occurring *Setaria* species is *S. tundra* which is broadly distributed in reindeer (*Rangifer tarandus*), roe deer and in moose (*Alces alces*) (Liang-Sheng, 1959; Shoho, 1959; Laaksonen et al., 2009a; Chroust, Forejtek, 2010; Kowal et al., 2013; Demiaszkiewicz et al., 2015; Rajsky, 2015; Angelone-Alasaad et al., 2016; Enemark et al., 2017).

The species *Setaria transcaucasica* was also sometimes referred in deer game in Europe including Czech and Slovak Republics (Kotrla, 1984; Barus, 1994; Jaervis, 1995). However, Enemark et al. (2017) stated that the taxonomic status of this parasite is highly ambiguous as only scarce information can be found in the literature about its morfology, vectors, or mode of transmission. The authors hypothesised that *S. transcausica* may be the junior synonym of *S. tundra*. It was not possible to find any molecular data in international nucleotide databases for this species and it was therefore not included in current molecular survey.

The remaining two species are parasites of other animals: *Setaria equina* in horses, mules, and donkeys (Gawor, 1995; Sotiraky et al., 1997; Oge et al., 2003; Boch, Bauer, 2006; Marzok, Desouky, 2009) and *Setaria digitata* in cattle predominantly in Asia (Wijesundera et al., 1996; Jayasena et al., 1999; Subhachalat et al., 1999; Shin et al., 2002; Tung et al., 2003; Nakano et al., 2007; Yatawara et al., 2007; Kim et al., 2010; Dehkordi et al., 2015; Kaur et al., 2015; Sundar, D'Souza , 2015; Kumar, Kumar, 2016; Liu et al., 2017).

Lifecycle of *Setaria* ssp. and variability of their vectors

Adult viviparous nematode females inhabiting the peritoneal (abdominal and thoracic) cavities daily produce thousands of larvae (microfilariae) into the bloodstream of their hosts (Nelson, 1966). By sucking blood, microfilariae get over into the mosquito vectors (Culicidae), where, after double shedding of the cuticle (two moults), they develop into the infective L3 larval stage. When the infected mosquito is feeding again, the third-stage larva break out from the vector into the definitive host, in which it develops through two additional moults penetrating into his abdominal or thoracic cavity (Anderson, 2000; Laaksonen, 2009a).

The vector organisms are mosquitos (Culicidae) of the species *Aedes vexans, Ochlerotatus annulipes, O. sticticus, O. cantans, O. rusticus, Coquillettidia richiardii, Anopheles hyrcanus, and A. claviger* (Czajka, 2012; Kemenesi et al., 2015), *Culex* spp., *Armigeres* spp. (Tung et al., 2003), but also representatives of the family Simuliidae, and the Muscidae genus *Stomoxys* (Baldacchino et al., 2013). The most common vector of *S. labiatopapillosa* is the mosquito *Aedes caspius;* congeneric species *A. claviger* and *A. maculipennis* also contribute to nematode transmission, but their rare incidence reduces their epidemiological significance (Cancrini et al., 1997). *Setaria tundra,* abundantly occurring also in Finland, is most often transmitted by mosquitoes of the genus *Aedes*. Warm summers obviously support the transfer and the genesis of disease that favours the development of nematodes in vectors by forcing the reindeer to accumulate in wetlands rich in mosquitoes (Laaksonen et al., 2009b).

Variability of morphological and biometric traits in *Setaria* species found in European wild ruminants

The nematodes *Setaria* spp. are thin whitish worms, whose females reach up to 156 mm, the males up to 80 mm in length (Blazek et al., 1968; Zdarska, Scholl, 1978; Wee et al., 1996; Nakano et al., 2007; Bednarski et al., 2010; Kim et al., 2010; Ruminsky, 2015; Singh et al., 2015; Sundar, D´Souza, 2015; Enemark et al., 2017).

As significant determination characteristics of the *Setaria* spp. can be considered, in particular, the arrangement of the peribucal crown (ventral and dorsal projections, lateral lips, the shape of the mouth opening) and the caudal end (Becklund, Walker, 1969; Shoho, Uni, 1977; Zdarska, Scholl, 1978; Nakano et al., 2007; Kowal et al., 2013; Sundar, D'Souza, 2015; Kumar, Kumar, 2016; Enemark et al., 2017).

Also, the shape and location of tail appendages is species-specific. In *S. tundra,* as compared to other species, the appendages are less pronounced (Enemark et al., 2017). The male caudal end is blunt while the females have thorns at the end of the tail (Subhachalat, Adachi, 1997; Nakano et al., 2007; Kim et al., 2010; Singh et al., 2015; Sundar, D'Souza, 2015). The thorns can change their shape or disappear. The caudal part ended with a round 'knob' is typical for females of *S. labiatopapillosa* and *S. tundra* while *S. cervi* tails studded with spines (Blazek et al., 1968; Zdarska, Scholl, 1978; Subhachalat, Adachi, 1997; Sundar, D'Souza, 2015; Kumar, Kumar, 2016; Enemark et al., 2017). Scarce information can be found in the literature about morphology for *S. transcaucasica.* It is possible to assume morphological features similar to *S.tundra* (Rajsky, 2010; Enemark et al., 2017).

Scanning electron microscopy is needed for detection of the number and location of the precloacal, cloacal, postcloacal, dorsolateral and tail papillae, observed on the caudal body end, and also for the description of the shape of mouth opening and the localization of deirids, amphids, and phasmids

Table 2. Morphological key for identification of *Setaria* species referred from European wild ruminants

Morphometric trait	Sex	*Setaria cervi*	*Setaria labiatopapillosa*	*Setaria tundra*	*Setaria transcaucasica*
Body length (mm)	♀	76–142	84–150	46–80	55–80
	♂	43–76	80	28–37	28–42
Dorsal and ventral projections shape	♀ ♂	bifurcated	bifurcated	bifurcated	not specified
Lateral lips	♀	non-bifurcated	non-bifurcated	non-bifurcated	not specified
		crescentic	rectangular	no cuticularized	not specified
	♂	non-bifurcated	non-bifurcated	non-bifurcated	not specified
		crescentic	rectangular	no cuticularized	not specified
Mouth opening shape	♀ ♂	not specified	not specified	rounded	rounded
Count of precloacal papillae pairs	♂	4	3	3	not specified
Count of postcloacal papillae pairs	♂	4	4	1	not specified

(Zdarska, Scholl, 1978; Almeida et al., 1991; Subhachalat, Adachi, 1997; Nakano et al., 2007; Kowal et al., 2013; Kumar, Kumar, 2016; Enemark et al., 2017).

It should be noted that the abovementioned authors do not use a uniform terminology for the description of morphological body structures and their conclusions are based on the evaluation of different morphological traits. However, a key was designed to identify the *Setaria* species parasitising on wild ruminants in Europe (Table 2). This identification key was compiled on the basis of morphological studies (optical and scanning electron microscopy) carried out by Shoho, Uni (1977), Zdarska, Scholl (1978), Kotrla (1984), Wee et al. (1996), Subhachalat, Adachi (1997), Favia et al. (2003), Nakano et al. (2007), Bednarski et al. (2010), Kim et al. (2010), Kowal et al. (2013), Singh et al. (2015), Sundar, D'Souza (2015), Kumar, Kumar (2016), Enemark et al. (2017).

Variability of *Setaria* spp. determined by molecular methods

Molecular data have become widely used to aid rapid assessment of species diversity of different organisms. DNA barcoding involves sequencing of one or a number of genes from several representatives of a species, as well as comparisons of these sequences within and between species. The performance of DNA barcoding with different parameters was compared measuring the strength of correlation between morphological and molecular identification approaches and different combinations of data handling were compared in order to provide a stronger tool for easy identification of filarioid worms (Ferri et al., 2009).

This review was focused only on *Setaria* species that are referred from Europe, without regard to the nematode hosts. However, no molecular data about the species *S. transcaucassica* are available. Totally 122 records characterising nucleotide sequences of *Setaria* species saved in the database of the National Center for Biotechnology Information (NCBI) were analysed. Most records represent conserved gene sequences, such as nuclear and mitochondrial rDNA genes or *COX1* gene for mitochondrially encoded cytochrome c oxidase I. The minimum nucleotide record refers to the structural genes, for example, the *IFP* gene for intermediate filament protein (Takesue et al., 2015), the gene encoding heat shock protein 70 (Jayasena et al., 1999), *MLC-3* gene for alkali myosin light chain (Muruganan than et al., 2009), gene for actin (Chandrasekharan et al., 1998), *SXP-1* gene encoding hypodermally expressed SXP/RAL2 protein (Sasisekhar et al., 2005), *rpb1* gene for RNA polymerase II large subunit (Lefoulon et al., 2015), *MyoHC* gene encoding myosin heavy chain (Lefoulon et al., 2015), gene for NADH dehydrogenase subunit 4 (Laaksonen et al., 2005), gene encoding phosphoglycerate kinase (Kumar et al., 2015), gene for H2B histone (Yadav et al., 2012a), gene encoding galectin (Yadav et al., 2012b), gene for acid phosphatase (Yadav et al., 2012c), gene encoding hexokinase (Arya et al., 2003). Only two records published in NCBI database represent tandem repeat sequences (Wijesundera et al., 1996).

Because most DNA barcoding systems are based on sequential polymorphisms of conserved genes, this study was focused on the use of nuclear and mitochondrial rDNA genes in taxonomy of the *Setaria* genus (Table 3). Although there is a relatively large number of rDNA sequential records in the NCBI database, it should be noted that the published sequences often have a different origin and character.

Table 3 shows that the data used here came not only from the European populations. The different character of the sequences results, in particular, from their length variability. The vast majority of the sequences

Table 3. Sequences of nuclear and mitochondrial rDNA genes used for study of the *Setaria* genus variability

Species	Nuclear rDNA genes for 18S rRNA-ITS1-5.8S rRNA-ITS2-28S rRNA			Nuclear rDNA gene for 5S rRNA		
	author	host/vector	origin	author	host/vector	origin
Setaria cervi				Wijesundera et al. (2000a)	not specified	not specified
Setaria digitata	Chamuah et al. (2014) Yatawara et al. (2007)	gayal shep, goat, horse	India Sri Lanka	Wijesundera et al. (2000b)	not specified	not specified
Setaria equina	Saparov et al. (2015a)	horse	Uzbekistan			
Setaria labiatopapillosa	Saparov et al. (2015b)	horse	Uzbekistan	Favia et al. (2000) Wijesundera et al. (2000c)	not specified not specified	Italy not specified
Setaria tundra	Laaksonen et al. (2009) Lefoulon et al. (2015)	reindeer reindeer	Finland Finland			
	Mitochondrial rDNA gene for 12S rRNA			Mitochondrial rDNA gene for 16S rRNA		
Setaria digitata	Ferri et al. (2009) Chamuah et al. (2014) Liu et al. (2017) Yatawara et al. (2007) Yatawara et al. (2010)	cattle gayal buffalo, cattle sheep, goat, horse buffalo, cattle	Japan India China Sri Lanka Sri Lanka	Liu et al. (2017) Yatawara et al. (2007) Yatawara et al. (2010)	buffalo, cattle sheep, goat, horse buffalo, cattle	China Sri Lanka Sri Lanka
Setaria equina	Casiraghi et al. (2004) Nabie, Spotin (2015)	horse human	Italy Iran			
Setaria labiatopapillosa	Casiraghi et al. (2004) Lefoulon et al. (2015)	cattle cattle	Italy Cameroon			
Setaria tundra	Al-Sabi et al. (2016) Casiraghi et al. (2004) Czajka et al. (2012) Ferri et al. (2009) Lefoulon et al. (2015)	roe deer roe deer vector *Aedes vexans* roe deer reindeer	Denmark Italy Germany France, Italy Finland			

presented in Table 3 represent partial sequences of the rRNA genes. Complex sequences were obtained only in projects aimed at sequencing the entire mitochondrial genome (Yatawara et al., 2007, 2010; Liu et al., 2017). Sequences of rDNA genes were obtained with different intent, for example, for phylogenetic studies, the identification of mature specimens obtained from dissections or from preserved collections, or the diagnosis of microfilariae in hosts and vectors.

What is the information impact of the sequences published in the database for the study of interspecific and intra-species variability of the *Setaria* genus? To answer this question, this study was targeted on the mitochondrial *COX1* gene which is most commonly used for barcoding (Table 4). Because the obtained *COX1* gene sequences in Table 4 represent variable regions, this review was focused on the 573 bp region which was acquired by all authors.

The MAFFT version 7 software (Katoh, Toh, 2008) was used for multiple sequence alignment (G-INS-i iterative refinement method). In aligned haplotype sequences only one *S. cervi* haplotype, six *S. digitata* haplotypes, one S. *equina* haplotype, two *S. labiatopapillosa,* and eight *S. tundra* haplotypes were revealed. We hypothesised that if the haplotype sequences have a sufficient interspecific polymorphism, the *Setaria* species will be separated into separate clades in the phylogenetic tree. The software jModelTest 2.1.10 v20160303 (Darriba et al., 2012) was used for an optimal substitution model selection. Subtree Pruning and Regrafting method (SPR) was used as a tree topology search operation. The best substitution model was chosen according to Akaike Information Criterion (AIC) (Akaike, 1973), Bayesian Information Criterion (BIC) (Schwarz, 1978) and Decision Theory Performance-Based Selection (DT) (Minin et al., 2003). Phylogenetic analysis was conducted with PhyML 3.0 (Guindon, Gascuel, 2003). The evolutionary history was inferred by using the maximum likelihood method based on the Tamura-Nei model (TrN + G) (Tamura, Nei, 1993). A discrete Gamma distribution was used to model evolutionary rate differences among sites. The bootstrap consensus tree, inferred from 1000 replicates, was used to represent *Setaria* species evolutionary history. *COX1* gene sequence (Ghedin et al., 2007) of *Brugia malayi* was used as outgroup. Branches corresponding to partitions reproduced in less than 60% bootstrap replicates were collapsed. The result of the *in silico* analysis is a phylogram presented in Fig. 1, which clearly shows clades corresponding with studied *Setaria* species.

It was confirmed, that molecular markers based on *COX1* gene polymorphisms discovered by authors cited in Table 3 are suitable tools for reliable differentiation of *Setaria* species. In aligned haplotypes based on sequences cited in Table 3 were discovered 62 SNPs which have a potential to be used as the barcoding system of *Setaria* genus based on polymerase chain

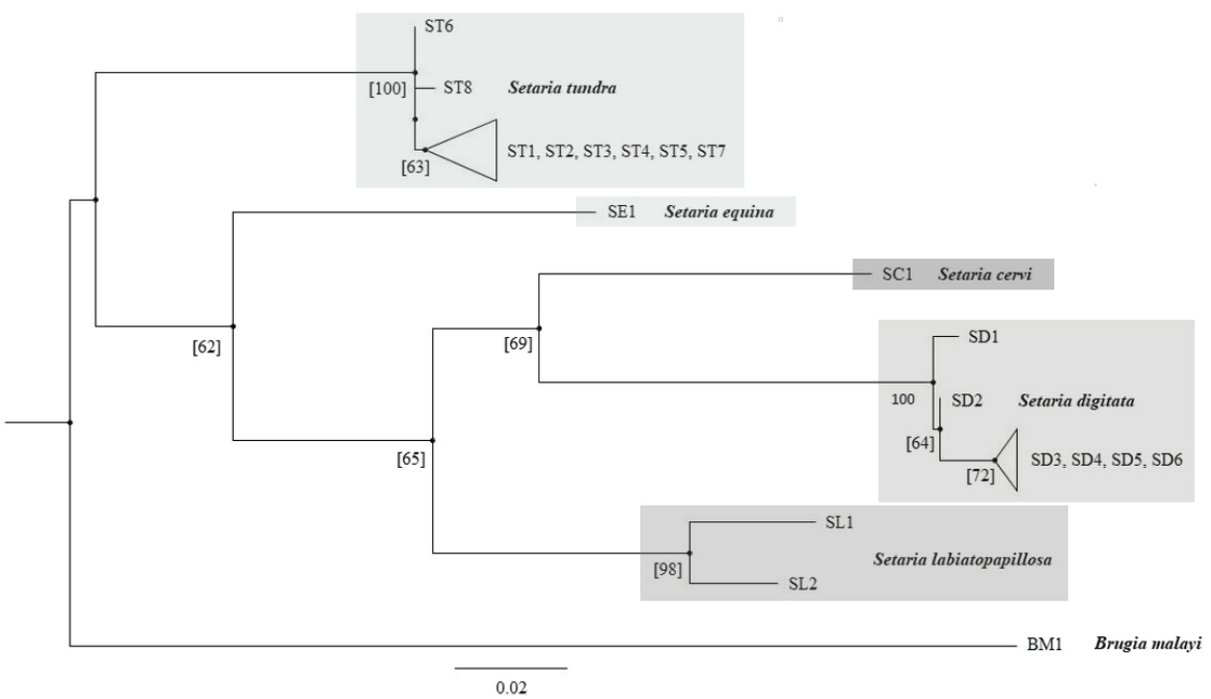

Fig. 1. Phylogram as a result of in silico analysis clearly showing the cap corresponding to the studied species of Setaria

Table 4. Sequences of mitochondrial *COX1* gene used for study of the *Setaria* genus variability

Species	Haplo-type	Author	NCBI accession number	Aligned region (bp)	Host/vector	Origin
Setaria cervi	SC1	Alasaad et al. (2012)	JF800924	49–622	red deer	Italy
Setaria digitata	SD1	Ferri et al. (2009)	AM749298	31–603	cattle	Japan
	SD2	Liu et al. (2017)	KY284626	319–891	buffalo	China
	SD3	Yatawara et al. (2007)	EF174423	50–622	cattle	Sri Lanka
	SD3	Yatawara et al. (2007)	EF174424	50–622	cattle	Sri Lanka
	SD4	Yatawara et al. (2007)	EF174425	50–622	cattle	Sri Lanka
	SD5	Yatawara et al. (2007)	EF174426	50–622	cattle	Sri Lanka
	SD3	Yatawara et al. (2007)	EF174427	50–622	cattle	Sri Lanka
	SD3	Yatawara et al. (2007)	EF174428	50–622	cattle	Sri Lanka
	SD6	Yatawara et al. (2010)	GU138699	2570–3142	buffalo, cattle	Sri Lanka
	SD6	Yatawara et al. (2010)	NC_014282	2570–3142	buffalo	Sri Lanka
Setaria equina	SE1	Casiraghi et al. (2004)	AJ544873	36–608	horse	Italy
Setaria labiatopapillosa	SL1	Casiraghi et al. (2004)	AJ544872	34–606	cattle	Italy
	SL2	Lefoulon et al. (2015)	KP760208	3–575	cattle	Cameroon
Setaria tundra	ST1	Al-Sabi et al. (2016)	KU508982	20–592	roe deer	Denmark
	ST2	Al-Sabi et al. (2016)	KU508983	20–592	roe deer	Denmark
	ST3	Al-Sabi et al. (2016)	KU508984	20–592	roe deer	Denmark
	ST3	Al-Sabi et al. (2016)	KU508985	20–592	roe deer	Denmark
	ST4	Angelone-Alasaad et al. (2016)	KX599455	30–602	roe deer	Spain
	ST4	Angelone-Alasaad et al. (2016)	KX599456	30–602	roe deer	Spain
	ST5	Casiraghi et al. (2004)	AJ544874	40–612	roe deer	Italy
	ST6	Zittra et al. (2015)	KM452922	6–578	vector *Aedes vexans*	Hungary
	ST6	Kronefeld et al. (2014)	KF692103	60–632	vector *Aedes vexans*	Germany
	ST7	Kronefeld et al. (2014)	KF692104	60–632	vector *Aedes vexans*	Germany
	ST8	Kronefeld et al. (2014)	KF692105	60–632	vector *Aedes vexans*	Germany
	ST7	Kronefeld et al. (2014)	KF692106	60–632	vector *Aedes vexans*	Germany
Brugia malayi	BM1	Ghedin et al. (2007)	AF538716	2566–3137	human	Asia and Indonesia

reaction (PCR) amplicon sequencing or SNaPshot™ Multiplex method.

CONCLUSION

The conclusions resulting from this review can be summarised as follows: (1) The occurrence of six *Setaria* species was demonstrated in Europe, but only *S. cervi*, *S. labiatopapillosa*, and *S. tundra* can be considered as potential parasites of wild ruminants. The validity of the other species *S. transcaucasica*, referred sometimes from European deer, need further evaluation. (2) The study of interspecific variability using morphometric traits is based on the description of three-dimensional structures in the cephalic and caudal part of the body of adult individuals. (3) For unambiguous taxonomic classification of *Setaria* spp. a combination of morphometric methods and molecular techniques is essential. Barcoding system is based, in particular, on the evaluation of SNPs of conserved genes. (4) It was demonstrated that the published sequences of mitochondrial *COX1* gene are suitable for the development of various types of markers aimed at the study of *Setaria* interspecific variability.

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