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DOI 10.1515/helmin-2015-0066

HELMINTHOLOGIA, 53, 1: 47 – 54, 2016

Taxonomic status of *Cyathostoma* nematodes (Nematoda: Syngaminae) parasitizing respiratory tracts of birds of prey and owls in Europe and North America: how many species are there?

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Article info

Received May 14, 2015
Accepted September 30, 2015

Summary

So far, the identity of *Cyathostoma (Hovorkonema)* nematodes collected from respiratory tracts of birds of prey (Accipitriformes, Falconiformes) and owls (Strigiformes) in Europe and North America is extremely inconsistent. Our results, based on analyses of ITS-2 sequences suggest that the *Cyathostoma (Hovorkonema)* nematodes found in the birds of prey and owls from Central Europe and North America probably belong to the same species , *C.* (*Hovorkonema) americana* Chapin, 1925. We are convinced, that described in recent literature high ITS-2 divergence among *C. (Hovorkonema)* nematodes collected from Europe, has occurred as a result of invalid synonimisation of some *C. (Hovorkonema)* species. In our opinion *C.* (*Hovorkonema) americana* (typically parasites of tracheae and air sacs of raptors) and *C.* (*Hovorkonema) variegatum* (Creplin, 1849) (typically parasites of tracheae of cranes and storks) are valid molecular and morphologically distinct species.
Keywords: Nematoda; Syngamidae; *Cyathostoma (Hovorkonema)*; birds of prey; owls; ITS-2

Introduction

The subfamily Syngaminae Baylis & Daubney, 1926 comprising parasites of respiratory tracts of birds (genera *Boydinema*, *Cyathostoma*, *Syngamus*) and mammals (genera *Mammomonogamu*s and *Rodentogamus*). The largest (more than 20 species) is genus *Cyathostoma*, a taxa with a long and confused history. This genus was established by Blanchard (1849) for *Cyathostoma lari*, species described from the orbital cavity of the black-headed gull *Chroicephalus ridibundus*. Over a period of years, the validity of *Cyathostoma* was widely discussed: some authors recognized *Cyathostoma* as a synonym of *Syngamus* (e.g., Skryabin, 1915; Yamaguti, 1961), but others accepted this taxon as correct. T However, the definition and taxonomic position of *Cyathostoma* has been modified several times in relation to different evaluation of morphological features (e.g., Chapin, 1925; Ryzhikov, 1949; Hovorka & Macko, 1959; Turemuratov, 1963; Ryzhikov, 1967; Lengy, 1969; Ali, 1970; Baruš & Tenora, 1972). In the most recent system of Syngaminae proposed by Lichtenfels (1980) the genus *Cyathostoma* is divided into two subgenera in relation to the structure of copulatory bursa and spiculae length: *Cyathostoma* (*Cyathostoma*) (Blanchard, 1849), in which the dorsal ray extends beyond the end of the copulatory bursa to form characteristic thorn-like projections and spicules that measure 0.08 – 0.4 mm, and *Cyathostoma* (*Hovorkonema*) Turemuratov, 1963 in which the dorsal ray does not extend beyond the end of the copulatory bursa and spicules measure 0.45 – 0.8 mm. In the systems of Syngamidae created by Ryzhikov (1967) and Baruš and Tenora (1972) *Cyathostoma* and *Hovorkonema* are treated as independent genera.

Occurrence of *Cyathostoma* nematodes have been reported in a variety species of birds from several orders (Kanarek *et al*., 2013). Indisputably, *Cyathostoma* infection has great significance as a potential pathological factor in poultry production and many aspects of conservation of vulnerable or endangered avian species (for details

see e.g., Fernando & Barta, 2008 and references therein). Usually, occurrence of these nematodes is subclinical, but heavy infections are often associated with bacterial and/or fungal infections. Several reports of morbidity and mortality of birds, particularly birds of prey and owls caused by infection of *Cyathostoma* are available (e.g., Hunter *et al*., 1993; Lavoie *et al*., 1999; Krone *et al*., 2007; Vaughan-Higgins *et al*., 2013). Thus, understanding of all aspects of ecology and taxonomy of these syngamid nematodes has great practical importance for veterinary and conservation reasons in general. Currently, four species of these nematodes have been recorded from birds of prey (Accipitriformes, Falconiformes) and owls (Strigiformes): *C. (Hovorkonema) americana* Chapin, 1925; *C. (Hovorkonema) brodskii* Sultanov, 1946; *C. (Hovorkonema) variegatum* (Creplin, 1849) and *C. (Cyathostoma) lari* Blanchard, 1849. Moreover, several reports on the occurrence of *Cyathostoma* nematodes determined only to the genus level in birds of prey and owls are available (e.g., Mumcuoglu & Müller, 1974; Hunter *et al*.,1993; Lavoie *et al*., 1999; Vaughan-Higgins *et al*., 2013). Among the mentioned species, *C.* (*Cyathostoma) lari* is a typical parasite of gulls, only occasionally reported from birds of prey (Simpson & Harris, 1992). However, when delimitation of *C. (Cyathostoma) lari* from other mentioned *C. (Hovorkonema)* species recorded in birds of prey and owls regarding characteristic habitat (*C. (Cyathostoma) lari* occurred in nasal and orbital cavities, while other species are detected in tracheae, bronchii and air sacs) and morphological features typical for nominal subgenus *Cyathostoma* (structure of copulatory bursa, spiculae length) are rather trivial, thus morphological boundaries between closely related *C.* (*Hovorkonema) americana*, *C.* (*Hovorkonema) brodskii* and *C. (Hovorkonema) variegatum* are still contentious. Moreover, several revisions and doubtful synonimisations (e.g., Vogel, 1928; Ali, 1970; Ryzhikov, 1980) increased confusion. In our opinion, the crucial problem in contemporary taxonomy of *Cyathostoma* (including parasites of raptors) is poorly recognized morphological variability among/within species and disputable host specificity, resulting with large number of species with questionable validity. Thus, final confirmation of the validity of several *C. (Hovorkonema)* species, irrespective of evaluation of different morphological, morphometric and ecological data, required molecular methods. Unfortunately, up to the present, analyses of taxonomic and phylogenetic relationships among subfamily Syngaminae based on molecular markers are very scarce (Krone *et al*., 2007; Kanarek *et al*., 2013). So far, only Krone *et al*. (2007) analyzed molecular status and genetic diversity of nematode identified as *Hovorkonema variegatum*, collected from respiratory tracts of several birds species (including birds of prey) from Germany and based on the divergence of the compared ITS-2 sequences recognized existence of two distict groups of *Cyathostoma (Hovorkonema) variegatum* nematodes. On this base, the autors speculated about a occurrence of subspecies or a cryptic species, which are morphologically indistinguishable. In light of this, further studies regarding validity and molecular diversity of these nematodes occurring in birds of prey and owls are required.

Therefore, the main objective of the presented research was molecular identification and analyzes of SSU ribosomal gene diversity of *C. (Hovorkonema)* nematodes, collected from respiratory tracts of several species of raptors from Central Europe and North America. The obtained data are presented and discussed, and additionally, a hypothesis regarding validity of the avian *C. (Hovorkonema)* species is suggested.

Material and Methods

Sampling protocols, necropsy procedures and morphological identification

In spring of 2014, two subadult snowy owls *Bubo scandiacus* were found dead in Rhode Island, USA, and were transported to the Connecticut Veterinary Medical Diagnostic Laboratory (CVMDL). Necropsy revealed the presence of medium-sized, red nematodes in the air sacs and on the surface of the lung, identified visually as *Cyathostoma* sp. Worms were washed in tap water, fixed and stored in 70 % ethanol. Additionally, during standard helminthological analysis of carcasses of birds of prey and owls, obtained from the Rescue Station for Animals, Bartošovice na Moravě, Czech Republic, nematodes initially identified as *Cyathostoma* sp. were found in air sacs of single Eurasian sparrowhawk *Accipiter nisus*, long-eared owl *Asio otus* and Eurasian eagle-owl *Bubo bubo*. The nematodes were washed in tap water, fixed in 70 % ethanol and stored in the same medium for further examination.

Detailed analysis of sampled material, showed that all collected nematodes were adult females. Unfortunately, the lack of males prevented precise morphological determination to the species level. Voucher specimens have been deposited in the Polish Collection of Parasitic Helminths, Museum of Natural History, Wrocław University, Coll. No. 154285 (specimen ex *Asio otus*),154286 (specimen ex *Bubo bubo*), 154287 (specimen ex *Accipiter nissus*) and154288 (specimens ex *Bubo scandiacus*).

Molecular analysis

Material for the molecular analysis consisting of fragments (ca. 1 cm long) of the nematodes obtained from four host species (*Accipiter nissus, Asio otus, Bubo bubo, B. scandiacus*) was fixed as above and stored in 70 % ethanol. Total genomic DNA was extracted using DNeasy Blood and Tissue Kit (Qiagen, Düsseldorf, Germany) according to the manufacturer's protocol. Amplification of ITS1-5.8S rDNA-ITS2 fragments was done using the following primers (van der Auwera *et al*., 1994) – NLF/NLR (5'-TTTGyACACACCGCCCGTCG-3'/5'-ATATGCTTAArTTCAGCGGGT-3') in the following thermocycling conditions: 95 °C/3 min. – initial denaturation; 95 °C/30 sec., 48 °C/30 sec., 72 °C/45 sec. – 40 cycles; 72 °C/7 min – final extension. The PCR reaction (25 µl) was obtained: 4 µl of genomic DNA, 10mM Tris-HCl, 50mM KCl, 1.5 mM MgCl2, 200 µM of each dNTP, 150 pmol of each primer and 2 units of Taq polymerase (EurX, Gdańsk, Poland). The amplification product was purified using QIAquick PCR purification kit

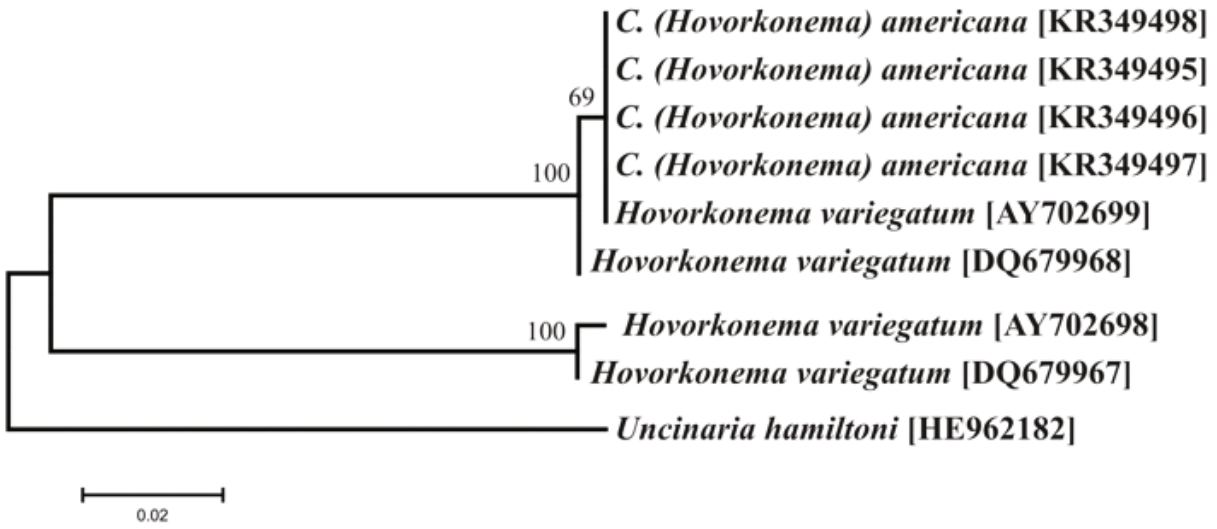

Fig. 1. Phylogenetic analysis of ITS1 and ITS2 sequences performed using Maximum Likelihood method, after fitting the best model (Tamura 3-parameter); bootstrap support calculated on basis of 500 replicates

(Qiagen, Düsseldorf, Germany) and sequenced in both directions (Genomed S.A., Poland). The obtained sequences were deposited in GenBank under following accession numbers KR349495-KR349498.

In order to elucidate any homologies with previously deposited sequences in GenBank, a BLAST search (http://blast.ncbi.nlm.nih.gov/Blast.cgi) was conducted. The multiple alignment was done by the use of CLUSTAL W using MEGA 5.0 package (Tamura *et al*., 2011). Phylogenetic analysis of *C. (Hovorkonema)* was inferred using the Maximum Likelihood method.

Results

As a result of molecular analysis, four 929 – 934 bp long sequences of ITS complex (ITS1, 5.8S rDNA and ITS2) for *C. (Hovorkonema*) sp. were obtained. In the overlapping fragment (930 bp) all four sequences were identical and no genetic diversity was observed. In addition, the comparison of our sequences with those deposited in Gen Bank showed none (AY702699) or a single nucleotide difference (DQ679968) with the sequences described as "*Hovorkonema variegatum*". At the same time, our sequences showed quite large genetic variability with the two latter sequences of *H. variegatum* deposited under accession numbers: AY702698 (38 sites out of 297 bp long alignment – 12.8 %) and DQ679967 (36/297 bp – 12.1 %). The ML phylogenetic analysis (based on ITS2) performed for our sequences and the sequences of *H. variegatum* deposited in Gen Bank showed the presence of two clades, one including six identical or almost identical (1 nucleotide difference) sequences of syngamid nematodes from birds of prey (KR349495-KR349495) and sequences previously deposited by Krone *et al*. (2007) (AY702699 and DQ679968) (Fig. 1). The second clade comprises two sequences (AY702698 and DQ679967) of *H. variegatum.*

Discussion

As mentioned previously, *C. (Hovorkonema)* nematodes detected in respiratory tracts of raptors have been identified usually as *C. (Hovorkonema) americana* Chapin, 1925; *C (Hovorkonema) brodskii* Sultanov, 1946 and *C. (Hovorkonema) variegatum* (Creplin, 1849). *C. (Hovorkonema) americana* was described by Chapin (1925), based on material collected from a host determined as the red-tailed hawk *Buteo borealis* (actually synonym of *Buteo jamaicensis* (Gmelin, 1788)) in Virginia, USA. Since description, this species has been detected in a wide range of birds of prey and owls and are considered by several authors (e.g., Borgsteede & Okulewicz, 2001; Borgsteede *et al*., 2003; Sitko & Okulewicz, 2010) as a rare, typical parasite of respiratory tracts of raptors in the Holarctic Region. However, Ryzhikov (1980) on the basis of experimental infection contested the validity of *C.* (*Hovorkonema) americana* and *C.* (*Hovorkonema) brodskii* and regarded both as synonym of *C. (Hovorkonema) bronchialis* (Mühling, 1884) widely recognized as typical parasite of Anseriformes. These conclusions were questioned by Borgsteede and Okulewicz (2001), who on the basis of morphological data and a critical review of existing literature data consider *C.* (*Hovorkonema) americana* as a valid taxa. Our results confirmed these statements: clearly demonstrating the existence of the same species of *C. (Hovorkonema)* in air sacs in birds of prey in Europe and North America – *C.* (*Hovorkonema) americana*. Obtained sequences clearly showed almost 100 % homogeneity among nematodes sampled from birds of prey and owls from the Czech Republic, the snowy owl from North America and,

Table 1. Comparison of selected biometrical and morphological characteristics of species of *C.(Hovorkonema)* nematodes recorded in birds of prey: all measurements in millimeters

Species name	*C. (Hovorkonema) americana*		*C. (Hovorkonema) brodskii*	*C. (Hovorkonema) variegatum*			
Author	Chapin (1925)	Borgsteede and Okulewicz (2001)	Sultanov (1946)	Vogel (1928)	Okulewicz (1984)	Hartwich (1994)	Krone *et al*. 2007
Country	USA, North America	Holland, Europe	Tajikistan, Asia	Germany, Europe	Poland, Europe	Germany, Europe	Germany, Europe
Host	*Buteo borealis*	*Accipiter gentilis, Buteo buteo, B. lagopus*	*Circus aeruginosus*	*Grus sp., Balearica pavonina*	*Ciconia ciconia*	Anseriformes, Ciconiiformes Gruiformes	*Accipiter gentilis, A. nisus, B. buteo,C.aeruginosus, Haliaeetus albicilla*
Number of teeth	6 – 7		7	7		6 – 7	6 – 7
Head collar	absent			absent		absent	absent
Male: Body length	12	10.815 – 13.420	13.26 – 13.30	7.1 – 9.8 (8.7)	8.11 – 8.43	4 – 14	12.9
Body width		0.487 – 0.545	0.501		0.31 – 0.35	0.25 – 0.6	0.409
Oesophagus length	0.73	0.721 – 0.745	0.714		0.51 – 0.58		0.728
Buccal capsule (width x depth)	0.185 x 0.185	0.175 – 0.187 x 0.165 – 0.174	0.232 x 0.276 – 0.280	0.105 – 0.125 (0.114) x 0.095 – 0.11 (0.106)	0.101 – 0.105 x 0.095 – 0.10	0.065 – 0.170 x 0.055 – 0.166	0.201 x 0.184
Spicules length	0.47 – 0.49	0.49 – 0.511	0.512	0.58 – 0.77 (0.63)	0.64 – 0.67*	0.320 – 0.870	0.522
Gubernaculum	present, 0.067 long	present, 0.070 – 0.074 long	absent	present	?	?	?
Female: Body length	up to 30	26.7 – 28.95	18.5 – 19	15.3 – 30.5	25.95	4.9 – 46	29.3
Body width		0.975 – 1.4	1.02		0.68	0.55 – 1.0	1.04
Oesophagus length	0.96	0.908 – 0.952			1.09	0.55 – 1.25	0.935
Buccal capsule (width x depth)	0.37 x 0.28	0.36 – 0.37 x 0.275 – 0.283	0.408 – 0.544	0.393 – 0.275	0.31 x 0.25	0.11 – 0.56 x 0.08 – 0.368	0.525 x 0.371
Vulvar distance from anterior end	"…vulva just before to the middle of the body…"	13.07 – 14.375	before middle of the body	36 – 40.5 (38.2) % of the body length	10.22	14 – 16	16.94
Tail length		0.350 – 0.377	0.360	0.19 – 0.25	0.22	0.16 – 0.45	0.467
Eggs	0.072 x 0.042	0.074 – 0.076 x 0.042 – 0.044	0.092 – 0.096 x 0.048	0.070 – 0.088 x 0.046 – 0.055	0.072 – 0.080 x 0.049 – 0.051		0.082 x 0.043

* in original text 0.64 – 6.67 – probably typographic error

Table 2. Comparison of selected biometrical and morphological characteristics of *C.(Hovorkonema) bronchialis* (Mühling, 1884); all measurements in millimeters

Author	Chapin (1925)	Griffiths *et al*. (1954)	Ryžikov and Zavadil (1958)	Hernandez-Rodriguez *et al.* (1978)
Country	USA, North America	USA, North America	Ukraina, Europe	Espania, Europe
Host	domestic geese, swans	domestic geese	*Anser anser* dom.	*Anser anser*
Number of teeth	?	6 – 7	7	?
Head collar	absent	absent	weakly differentiated	?
Male: Body length	4 – 5.8	9.5 – 10	9.5 – 10.7	10 – 12
Body width		0.25 – 0.328	0.32 – 0.33	0.367 – 0.402
Oesophagus length	0.35	0.560 – 0.570		0.630 – 0.690
Buccal capsule (width x depth)		0.09 – 0.13 x 0.14	0.145 – 0.15 x 0.109 – 0.132	0.117 – 0.123 x 0.091 – 0.105
Spicules length	0.51 – 0.62	0.54 – 0.70	0.58 – 0.72	0.56 – 0.70
Gubernaculum	?	?	present, 0.08 long	?
Female: Body length	16 – 31	16 – 26	25 – 40	22 – 28
Body width	0.7 – 0.9	0.75 – 0.86	0.62 – 0.83	0.376 – 0.779
Oesophagus length		1.0 – 1.14	1.12 – 1.15	1.210 – 1.350
Buccal capsule (width x depth)	0.325 x 0.205	0.33 – 0.42 x 0.325	0.41 – 0.56 x 0.304 – 0.368	0.262 – 0.297 x 0.290 – 0.320
Vulvar distance from anterior end	"…vulva just in front to the anterior end…"	"…posterior part of the anterior third of the body…"	12 – 14.5 mm from anterior end	in first 1/3 length of the body
Tail length	0.16 – 0.30	0.075	0.272 – 0.336	0.230 – 0.263
Eggs	0.074 – 0.083 x 0.049 – 0.062	0.068 – 0.085 x 0.043 – 0.058	0.073 – 0.083 x 0.052 – 0.056	0.082 – 0.086 x 0.059 – 0.065

surprisingly, with only part of the sequences deposited by Krone *et al*. (2007) and described as "*Hovorkonema variegatum"* (Fig. 1).
In our opinion, sequences obtained by Krone *et al*. (2007) and identified as "*H. variegatum"*, clearly consist of two molecularly distinct species and should be treated with considerable caution. Indisputably, as we mentioned previously, some of these represent C. (*Hovorkonema) americana*. But what about the others? *C.* (*Hovorkonema) variegatum* was described by Nathusius (1837) based on material collected from the black stork *Ciconia nigra* in Germany under the name *Strongylus trachealis.* Over a period of time, *C.* (*Hovorkonema) variegatum* was recognized as a typical parasite of storks and cranes (Vogel, 1928; Ryzhikov, 1949; Okulewicz, 1984), and also reported from the Indian peafowl, *Pavo cristatus,* in India (Ali, 1970). Moreover, this species was reported frequently in several species of birds of prey in Germany (Lierz *et al*., 1998; Krone, 2000; Krone *et al*., 2002; Lierz *et al*., 2002; Krone *et al*., 2007), Finland (Krone *et al*., 2006) and Spain (Sanmartín *et al*., 2004), but most of the cited authors do not give any morphological and morphometric data about these findings. According to detailed data, included only in the paper of Krone *et al*. (2007) *Cyathostoma* nematodes collected from Ciconiform, Falconiform and Gruiform birds were identified according to Hartwich (1994). However, Hartwich (1994), based on existing literature data, synonimised three species of *C. (Hovorkonema)*: the author agreed with the hypothesis formulated by Ryzhikov (1980) and treated *C.* (*Hovorkonema) americana* as synonym of *C.* (*Hovorkonema) bronchialis*. Furthermore, according to Vogel (1928) and Ali (1970), Hartwich also regarded *C.* (*Hovorkonema) bronchialis* as a synonym of *C.* (*Hovorkonema) variegatum*. Thus, *C.* (*Hovorkonema) variegatum* sensu Hartwich (1994) consisted of three species: *C.* (*Hovorkonema) americana*, *C.* (*Hovorkonema) bronchialis* and *C.* (*Hovorkonema) variegatum* sensu stricto. In light of this, high level of morphometric variability given by Hartwich (1994) as key features for *C.* (*Hovorkonema) variegatum*, especially the spiculae length (range 0.320 – 0.870 mm, Table 1) are not reliable. In several species of *Cyathostoma* spiculae length tends to be a constant feature, characterized by relatively low variability (Kanarek, 2009), and seems to be a good differentiating feature with key significance. According to available literature data (see Table 1), morphology of *C.* (*Hovorkonema) americana* and *C.* (*Hovorkonema) variegatum* are closely related, but not identical; despite almost identical structure of copulatory bursa (for details and comparison see e.g., Chapin, 1925; Vogel, 1928; Ali, 1970; Okulewicz, 1984; Borgsteede & Okulewicz, 2001) *C.* (*Hovorkonema) variegatum* also possess distinctly longer spiculae (0.58 – 0.77 mm) than *C.* (*Hovorkonema) americana* (0.47 – 0.511 mm) (Table 1). Other observed morphometric differences, such as dimensions of buccal capsule which is slightly larger in *C.* (*Hovorkonema) variegatum* (Table 1) are, in our

opinion, not conclusive. What is important, nematodes identified as "*H. variegatum"* Krone *et al*. (2007) recorded not only in birds of prey (*Accipiter gentilis*, *A. nissus*, *Buteo buteo*, *Circus aeruginosus*, *Haliaeethus albicilla*), but also in common crane *Grus grus*, typical host of *C.* (*Hovorkonema) variegatum* sensu stricto. Unfortunately, sequences of ITS2 gene deposited by Krone *et al*. (2007) were not adequately described (lacking the name of the hosts), similar to the presented morphological data (lacking such basic facts as name of the hosts and range of the measurements). However, during preparation of the reviewed version of this manuscript the sequence data of Krone *et al*. (2007) have been updated (last modification 9th of September 2015). Thus, in the light of presented facts, we are convinced, that some sequences deposited in GenBank as "*H. variegatum"* by Krone *et al*. (2007) are clearly distinct from sequences obtained in the present work of *C.* (*Hovorkonema) americana* and represent sequences of *C.* (*Hovorkonema) variegatum* sensu stricto (DQ679967 and AY702698). In our opinion *C.* (*Hovorkonema) americana* (typical parasites of tracheae and air sacs of raptors) and *C.* (*Hovorkonema) variegatum* (typical parasites of tracheae of cranes and storks) are valid, molecularly and morphologically distinct species. Although, we cannot exclude the possibility that *C.* (*Hovorkonema) variegatum* can accidentally parasitize birds of prey and owls (e.g. DQ679967 – *Accipiter nisus* and AY702698 – *Buteo buteo*) but this results do not confirm that this phenomenon is common and this issue requires further study. On the other hand, literature data clearly showed, that *C.* (*Hovorkonema) variegatum* in typical hosts, such as storks and cranes occurs exclusively in trachea (Vogel, 1928; Okulewicz, 1984), while *C.* (*Hovorkonema) americana* in raptors were recorded mainly in air sacs, less frequently in tracheae (Hunter *et al*., 1993; Borgsteede & Okulewicz, 2001; Krone *et al*., 2007), but this issue regarding further, detailed analysis.

Another interesting issue is the validity of *C.* (*Hovorkonema) bronchialis*, a species reported mainly in geese, but also detected in Casuariiformes. Some authors (Ryžikov & Zavadil, 1958; Ali, 1970; Baruš *et al*., 1978) recognized *C.* (*Hovorkonema) boularti* (Megnin, 1884) and *C.* (*Hovorkonema) bronchialis* as synonym of *C.* (*Hovorkonema) variegatum*. However, Borgsteede and Okulewicz (2001) recognized *C.* (*Hovorkonema) bronchialis* as a valid species. Based on literature data (e.g., Griffiths *et al*., 1954; Ryžikov & Zavadil, 1958; Hernandez-Rodriguez *et al*., 1975) the structure of copulatory bursa, morphology and morphometry of *C.* (*Hovorkonema) bronchialis*, especially spiculae length are closely related to *C.* (*Hovorkonema) variegatum* (for details and comparison see e.g., Chapin, 1925; Vogel, 1928; Ryžikov & Zavadil, 1958 and Tables 1 and 2). However, spiculae length provided by Chapin (1925) for *C.* (*Hovorkonema) bronchialis* (0.51 – 0.62 mm), differ slightly from measurements provided by Griffiths *et al*. (1954) (0.54 – 0.70 mm), Ryžikov and Zavadil (1958) (0.58 – 0.72 mm) and Hernandez-Rodriguez *et al*. (1975) (0.56 – 0.70 mm) (Table 2). Based on this, it is quite possible that *C.* (*Hovorkonema) bronchialis* is an synonym of *C.* (*Hovorkonema) variegatum*, but this issue requires further research based on detailed molecular and morphological analysis of material collected from typical hosts (Anseriformes).

The last species of *C. (Hovorkonema)* recorded in birds of prey, *C.* (*Hovorkonema) brodskii* was described on the basis of nematodes collected from the Western marsh-harrier *Circus aeruginosus*, sampled near Tashkent, Uzbekistan, Central Asia (Sultanov, 1946) and has not been recorded since. Therefore its validity is still doubtful. In fact, *C.* (*Hovorkonema) brodskii* and *C.* (*Hovorkonema) americana* are almost identical, except slightly smaller body dimensions, larger eggs and buccal capsule in *C.* (*Hovorkonema) brodskii* (Table 1), but in our opinion these differences are not significant. What is important, spicuale length in *C.* (*Hovorkonema) brodskii* and *C.* (*Hovorkonema) americana* are the same; the only one, well-articulated differentiating feature is absence of gubernaculum in *C.* (*Hovorkonema) brodskii* (Table 1). Among Strongyloidea, absence/presence of gubernaculum is recognized as an important feature with significant taxonomic implications e.g., Ali (1970) based his arrangement of subfamily Cyathostominae on the presence/absence of gubernaculum. However, this structure (when it occurs) in *Cyathostoma* is small, delicate and very easy to overlook, especially in well-cleared material. In fact, it is difficult to resolve whether differences in absence/presence of gubernaculum in morphologically close related species of *C. (Hovorkonema)* are key differentiating features, or whether they result from variability among specimens or misapprehension in the original descriptions. On the other hand, two *C. (Cyathostoma)* species occurring in respiratory tract of fish-eating avian hosts, *C.* (*Cyathostoma) phenisci* Baudet, 1937 and *C.* (*Cyathostoma) verrucosum* Hovorka & Macko, 1959 are almost identical, with the exception of the presence of gubernaculum in *C. (Cyathostoma) verrucosum*; however, molecular analysis showed the identical nature of both species (Kanarek *et al*., 2013). On this basis, we tend to recognize *C. (Hovorkonema) brodskii* as a synonym of *C. (Hovorkonema) americana*.

The present work shows that in the tracheae, bronchii and air sacs in analyzed birds of prey and owls from Europe and North America, only one species of *C. (Hovorkonema)* occurs, *C. (Hovorkonema) americana*. However, incidental occurrence of other *C. (Hovorkonema)* species, typical for other groups of avian hosts, is possible. The result obtained also emphasizes the necessity for careful and critical interpretation of the existing data, regarding morphology and systematics of both subgenera among *Cyathostoma*. Moreover, this demonstrates the need to use both morphological and molecular methods in the contemporary ecology and systematics of syngamid nematodes.

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